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Hella Kehlenbeck, Udo Heimbach und Michael Zellner

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Internationale Fachtagung zum Forschungsprogramm über den Westlichen Maiswurzelbohrer, 14.-16. November 2012, Berlin

Proceedings

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Preface

Vorwort

The western corn rootworm *Diabrotica virgifera virgifera* is one of the most significant corn pests. Since it was introduced accidentally into Europe in 1992 near Belgrade in Serbia, it has spread and invaded many important corn growing areas in Europe. In Germany, mainly the federal states of Bavaria and Baden-Württemberg are affected. Hesse, North Rhine-Westphalia, Rhineland-Palatinate and Saxony have detected individual specimens but the beetle has not yet established there. Therefore, effective control measures are required to slow down the further spread of the beetle in Germany to protect corn production in the areas concerned in the long term. To achieve these aims, an extensive research programme was established in 2008 by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) and the Bavarian State Ministry of Food, Agriculture and Forestry (StMELF) with the participation of the federal state of Baden-Württemberg. This programme aimed at gaining knowledge and enabling scientific recommendations regarding necessary measures for containing and controlling the western corn rootworm.

23 projects were sponsored within the research programme. They considered the most varied aspects, from the biology of the beetle, efficacy and environmental compatibility of different control measures for beetles and larvae to economic impacts. The *Diabrotica* website of the research programme (http://diabrotica.jki.bund.de) provides general information on the beetle, descriptions of the projects and a list of publications containing about 90 publications up to now which are a result of the research programme. Moreover, the website allows access to a simulation model for a crop-specific prediction of the beetle's population dynamics (the model is hosted by www.ISIP.de). The research programme was completed in 2012 and the results were presented at the International Conference on the German Diabrotica Research Program organised by BMELV at the Julius Kühn-Institut in Berlin. More than 80 participants from 10 European countries attended the event and several *Diabrotica* experts from different European countries contributed with scientific presentations. Almost all contributions are presented within this special issue of the Julius-Kühn-Archiv, either as short or extended abstracts or as full scientific papers.

The conference provided the most up to date knowledge and the basis for Germany to improve measures to contain and control this significant pest. Maintaining crop rotation is the most suitable and successful way of controlling the beetle and hindering its further spread. The replacement of maize with other crops is so successful because *Diabrotica* larval development on alternative host plants such as grasses and cereals is not possible or very limited. In addition to crop rotation, chemical control measures against *Diabrotica* using insecticides are effective and should therefore be made available as an additional option. Effects on honey bees were assessed and key factors for risk mitigation identified. Data to improve seed treatment and spray applications to reduce environmental effects were presented. Other methods such as controlling *Diabrotica* larvae with entomopathogenic nematodes proved to be very effective in many studies and should be optimised further to make biological methods available for practical control of *Diabrotica*. The basic principles and prototypes for forecasting models have been developed, which now have to be validated and improved. The economic impact of the beetle and of the official control measures proved to strongly depend of regional conditions and farming types. A consequent eradication of single small new *Diabrotica* outbreaks showed to be still economically very appropriate when considering the overall impact for Germany. The spread situation in Bavaria and Baden-Württemberg, however, could in the long term lead to *Diabrotica* becoming a "normal" pest that needs IPM control measures with threshold concepts.

Several remaining knowledge gaps were identified that need further studies to cope with *Diabrotica*. Though relevant environmental aspects of chemical seed treatment are now understood, still more research is needed to get such insecticides registered for control of *Diabrotica*. Resistant plants cultivars would support sustainable maize production, but such research still needs support. Practical application of entomopathogenic nematodes needs more improvement and should be accompanied by other biological alternatives like entomopathogenic fungi. When the newly developed models on population development and spread of *Diabrotica* will be validated with data already available from infested areas (e.g. Hungary, Romania, Austria), the federal states in Germany could plan their monitoring activities and control strategies much more efficiently. Results on egg density in the soil could be integrated as parameters into these models, making them more reliable and providing a first approach for infestation-damage relationships which still offer a large research potential for the future. In order to minimise income losses for farms in infested areas with crop rotation restrictions, regional studies on possible alternative plant species for crop rotation (e.g. for fodder or bioenergy) should be carried out.

For the future it is expected that most results will be of practical use for growers. Future research is needed to ensure an efficient and sustainable maize production for food, feed and energy in Germany despite the presence of *Diabrotica*.

Clemens Neumann

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Januar 2013

Keynote Presentation

Einleitendes Referat

20 years of *Diabrotica* in Europe, present and future challenges

20 Jahre Diabrotica *in Europa – aktuelle und zukünftige Herausforderungen*

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The lodged maize plants due to larval damage of western corn rootworm, (WCR, *Diabrotica virgifera virgifera* LeConte) and WCR adults were first detected in Europe in a maize field near Belgrade, Serbia, in 1992 (Baca, 1993). Though the first introduction of the WCR into this area is likely to have occurred between 1979 and 1984 (Szalai *et al.*, 2011), the visible maize plant damage and adult beetle presence on the field has raised serious concerns among authorities and scientists obviously since 1992. By 2012, the WCR has spread to almost all important maize growing areas in Europe and resulted in well established populations in many regions.

The first detection and the continued spread of the WCR coincided with several important and even "historical" changes in Europe, namely the enlargement of the EU, developing the principles and implementing integrated pest management, the changes in the spectrum of registered insecticide active ingredients and the ongoing revision of plant protection products in the EU etc. that have made the monitoring, control and management of this pest very challenging.

The detection of WCR larval damage and the specific situation in the region in the 1990's required immediate actions, i.e. monitoring, control and range of quarantine measures in the countries concerned. Experiences on the biology, control and management of the WCR from the USA were used and adapted to European conditions to prevent or reduce yield losses and population build up as well as to slow the spread of the WCR. US experts together with European scientists were supported by international bodies (FAO of UN, IWGO of IOBC, EPPO, etc.) that has initiated and established a broad international cooperation since the middle 1990's.

The first EU-5 framework multi-country project (WCR ecology and management in Europe) related to the biology, behavior, economy of control and quarantine measures as well as the control and management of the WCR started in 2000 as an important milestone for IPM development for this pest. In addition, from 2000 on an FAO donor-assisted project involving 7 Central European countries introduced a novel approach, the participatory training of farmers, for IPM development in maize based on local ecosystems. This activity significantly contributed to a broader IPM development in maize and in arable cropping systems in these countries. In addition to these projects, later other EU-funded projects on the WCR were also successfully completed. Significant national, bilateral and regional activities have started in several Member States and broader regional cooperation is being developed nowadays as well (including development, testing and application of biological control agents, population modelling etc.).

Session 1: Present situation of *Diabrotica* in European countries

The *Diabrotica virgifera virgifera* situation in Hungary: Modelling plant protection regulations on landscape level

Die Befallssituation mit Diabrotica virgifera virgifera *in Ungarn: Räumliche Modellierung von Pflanzenschutzregelungen*

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Diabrotica virgifera virgifera spread into Hungary in 1995, reached all maize production areas of the country by 2003, and is currently considered a well-established and well known pest within the pest species complex of maize. A discrete spatiotemporal simulation model was developed to investigate the effectiveness of maize rotation strategies against *D. virgifera virgifera* within the current framework of Hungarian integrated pest management legislations as well as for other potential legislations. The modelled agricultural landscape was simplified into a lattice, encapsulated into a torus, with fields as cells. The population dynamics of *D. virgifera virgifera* was simulated as an interacting particle system of the cells considering the inter-maize-field movements of adults and the presence of subsequent generations exclusively in continuous maize. The yearly update of cells according to different rotation strategies was applied for ten consecutive years, and was determined by the proportion of maize in the modelled agricultural landscape, the proportion of first year maize among all maize fields (% of rotation), and the presence/absence of legislations to grow maize for not more than 2, 3, 4 or 5 consecutive years. The model output was the proportion of maize fields at risk of reaching *D. virgifera virgifera* densities above a defined economic threshold.

Variance based global sensitivity analysis was conducted to identify the key input factors of the model. These factors were the percentage of rotation and the generational growth rate of *D. virgifera virgifera*. The proportion of maize in the modelled landscape was not a key input factor in the investigated range being typical for European maize growing areas, i.e. between 20% and 60%. In our simulations a 100% rotation of maize was not necessary to keep *D. virgifera virgifera* populations below the threshold level in the majority of maize fields of the landscape. Presence of the aforementioned legislations could decrease the proportion of maize fields with pest populations above economic threshold levels. The simulation model is suggested to be used by rural development policy makers at regional level decisions as well as for recommendations for appropriate integrated pest management guidelines.

This study was supported by the fund of 'Improvement of Research and Education Standard of Szent István University', TÁMOP-4.2.1.B-11/2/KMR-2011-0003.

Survey for *Diabrotica* extension and management in France

Erhebung der Diabrotica-*Ausbreitung und Bekämpfungsmaßnahmen in Frankreich*

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The western corn root worm, *Diabrotica virgifera virgifera*, has become a major maize pest in Europe. After its first establishment in areas of intensive maize cultivation near airports in Serbia and Italy, outbreaks were observed in less intensive maize growing areas, close to airports in Western Europe, mainly around Paris but extending to the Ile de France region. In 2006 the beetle was successfully eradicated. Current spread of *Diabrotica* in France happens by road transport, with establishment close to motorways and transit areas for trucks. The regions Rhone-Alpes, Alsace, and to a lower extent the Burgundy region, had the highest numbers of beetle captures in 2011. Few individuals were detected in the Aquitaine region, as well as in the alpine valleys of Provence-Alpes-Côte d'Azur (PACA) in 2011. In 2012, the situation was similar to 2011 except a new outbreak in the Rhone Valley, along the motorway to the Mediterranean Sea, between Avignon and Valence.

Pest management was first carried out with insecticides against adults and regional crop rotation. Crop rotations have now been extended in combination with the use of insecticides against larvae. Taking economic consequences of management measures into account, including increased mycotoxin values in wheat planted in rotation with maize, a diverse range of control options for pest management seems to be the key for a sustainable control of *Diabrotica*. Although eradication and avoidance of new outbreaks are very ambitious aims, they still remain viable within specific regions. However, for other regions the delay of spread of the beetle seems to be the only realistic option available. Beetle captures in Alsace for example were seventeen times lower compared to those on the German side of the Rhine Valley in 2011. This may show that the management strategy applied in France could contribute to slow down spread with strategies that have an acceptable economical impact on growers.

The infestation situation of the western corn rootworm (*Diabrotica virgifera virgifera*) in Germany

*Die Befallssituation mit dem Westlichen Maiswurzelbohrer (*Diabrotica virgifera virgifera*) in Deutschland*

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In Germany, 6,217 beetles of the western corn rootworm were caught in pheromone traps in 2012 in total. The highest number of beetles was detected in Baden-Wuertemberg in the containment zone with 5,818 beetles. Compared to the previous year (2011: 6,119) this number represented a reduction of about only of 5%. In contrast, in Bavaria in 2012 the number of captured beetles was only slightly higher with 370 beetles in the containment zone (2011: 173 beetles) and it was still on a low infestation level. Nevertheless, the containment zone was extended in the western part of Bavaria due to new outbreaks next to the zone. Further new outbreaks were registered in Baden-Wurttemberg in one location with 17 beetles, in Bavaria in two locations with one and four beetles, respectively, and in Rhineland-Palatinate in two locations with two and four beetles, respectively. Additionally a new outbreak was registered in one location in Saxony next to Dresden with one beetle. The eradication measures in North Rhine-Westphalia in two locations, in Hesse in one location, in Rhineland-Palatinate in one location and in Baden-Wuerttemberg in one location were successful and no beetles were detected in these areas anymore. After two years without any beetles captured the eradication measures were finalized and stopped in North Rhine-Westphalia in two locations. The map in Figure 1 shows the situation of infested areas, containment areas and eradication spots for 2012 in Germany.

Situation of *Diabrotica* 2012

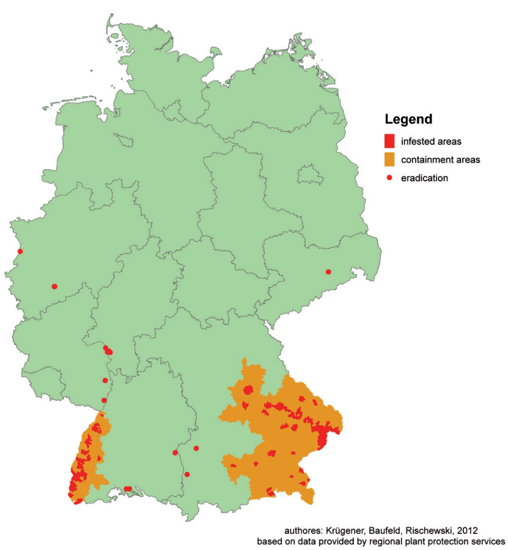

Fig. 1 Situation of infested areas, containment areas and eradication spots in Germany in 2012.

***Abb. 1** Situation für Befallsgebiete, Eingrenzungsgebiete und Ausrottungsflächen in Deutschland 2012.*

Diabrotica virgifera virgifera LeConte wing shape variation reveals multiple populations across the European expansion front

Unterschiedliche Flügelausprägungen bei Diabrotica virgifera virgifera *LeConte offenbaren das Vorhandensein multipler Populationen entlang der europäischen Ausbreitungsfront*

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The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is subject to much research because of the economic damage it causes to maize crops in the USA and recently in Europe. Understanding levels of genetic variability of introduced populations is important for investigating the adaptive potential of such populations in their new invaded environments e.g. WCR in Europe. To enhance the management and control of the WCR, an understanding of the temporal and spatial genetic structure, number of genetic clusters and pattern of gene flow is required. Croatia was the country economically affected first by the range expansion of the WCR from Serbia (where the beetle was detected for the first time in Europe) and it is an important part of the invasion puzzle in central Europe. The WCR core set of microsatellite markers was used to investigate populations from Croatia collected in 1996 (introduction) and again in 2009 (expansion). Using the program STRUCTURE, low to no genetic differentiation was found between populations by pairwise comparisons in 1996, with a greater level of differentiation found between populations sampled in 2009. A single genetic cluster was found for putative populations sampled in 1996 (ΔK1996=1) and in 2009 (ΔK09=1). However, two genetic clusters were detected when the 1996 and 2009 data were combined (ΔK=2), suggesting that the genetic structure and thus pattern of alleles changed over time either in response to genetic drift, increased gene flow or a single or multiple admixture event(s). Although the use of classical population genetic analyses has provided much needed information on the population genetic structure and gene flow of WCR in Croatia, its use to completely understand how populations have changed over time is limited. Thus, further population and diagnostic markers were investigated that could provide a more complete understanding of how WCR populations had changed over time and during the invasion of Croatia. Specifically 14 wing venation landmarks were used to assess the variation in wing shape and size for WCR populations shown to be genetically homogenous using microsatellites. The results showed that asymmetry and allometry did not occur in WCR, however sexual dimorphism was significant for both size and shape. Sexual dimorphism in wing morphology was found to be the result of females having generally larger and longer wings, a result consistent with a greater capacity for migratory flight, probably reflecting the role that gravid females play in range expansion. Significant pairwise differences in 2011 were noted between all sampled sites. Otok had the largest pairwise Mahalanobis distances with all sites investigated and was thus recognized as a distinct population. In addition, an isolation by distance pattern in the population structure was found throughout the sites investigated. This study found that a significant amount of variation in wing morphology exists in WCR across their invaded range in Croatia. The results indicate that the use of wing morphology can provide valuable additional information on population structure when used with microsatellite markers especially in suspected genetically homogenous geographic locations.

First occurrence of western corn root worm beetles in the federal states Hesse and Rhineland-Palatinate (Germany), 2011

Erstauftreten des Westlichen Maiswurzelbohrers in den Bundesländern Hessen und Rheinland-Pfalz (Deutschland), 2011

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Summary

In 2011, western corn root worm beetles were detected in the federal states Hesse (Groß-Gerau) and Rhineland-Palatinate (Bodenheim) for the first time. Control measures based on commission decision 2003/766/EG (Byrne, 2003) were conducted after detection in PAL-traps. Focus and safety zones were established. In Hesse, both focus and safety zones were treated with the insecticide Biscaya, due to the high number of 50 beetles which were detected in the PAL-traps. Since in Rhineland-Palatinate, only one beetle had been captured, only the focus zone was treated with the insecticide. After insecticide treatment, new PAL-traps were arranged like a close grid over the infested areas in both federal states. In each maize field in the focus- and safety zone further traps were placed and checked weekly until September 30th by supporting staff. Until the end of the monitoring in 2011 (September 30th) further beetles were detected in the south of the area (district of Groß-Gerau, Hesse), were the first infestation had been discovered. However, in Rhineland-Palatinate no further beetles were detected that year. By the end of the monitoring 354 beetles in Hesse and one beetle in Rhineland-Palatinate had been captured in total. Subsequently the demarked zones in Hesse were extended. Taking into account the local circumstances, the new focus zone was delimited to include all the areas where beetles had been detected as well as the surrounding maize fields. In the focus zones the cultivation of maize was forbidden for the consecutive two years and a crop rotation with at least 50 percent maize was established in the safety zones. In 2012 no further beetles were captured in the infested region.

Key words: *Diabrotica virgifera virgifera* LeConte, first occurrence, Hesse, Rhineland-Palatinate, eradication

Zusammenfassung

Der Westliche Maiswurzelbohrer (*Diabrotica virgifera virgifera* LeConte) trat in den Bundesländern Hessen (Groß-Gerau) und Rheinland-Pfalz (Bodenheim) erstmalig im Jahr 2011 auf. Die in der Kommissions-Entscheidung 2003/766/EG vorgeschriebenen Bekämpfungsmaßnahmen (Byrne, 2003) wurden umgehend durchgeführt. Befalls- und Sicherheitszonen wurden eingerichtet. In Hessen wurde wegen der hohen Zahl von 50 gefangenen Käfern sowohl die Befalls- als auch die Sicherheitszone mit dem Insektizid Biscaya behandelt. Da in Rheinland-Pfalz nur ein Käfer gefunden wurde, ist hier ausschließlich die Befallszone mit dem Insektizid behandelt worden. Im Anschluss an die Insektizidbehandlung wurden PAL-Fallen in den Befallsgebieten beider Länder engmaschig platziert- in jedem Maisfeld wurden PAL-Fallen aufgehängt. Alle Fallen wurden bis zum 30. September 2011 wöchentlich durch zusätzlich eingestellte Hilfskräfte kontrolliert. Bis zum Ende des Monitorings wurden südlich von Groß-Gerau, dem Ort des Erstfundes, weitere Käfer gefunden, sodass in Hessen insgesamt 354 Käfer festgestellt worden sind. Auf Rheinland-Pfälzischer Seite sind keine Käfer mehr hinzu gekommen. Die hessische Befallszone wurde unter Berücksichtigung der örtlichen Verhältnisse so ausgeweitet, dass alle Befallsstandorte einschließlich der umliegenden Nachbarmaisflächen eingeschlossen waren. Als Auflage darf in den Befallszonen beider Länder in den Jahren 2012 und 2013 kein Mais angebaut werden. In den Sicherheitszonen darf kein Mais nach Mais folgen. Im Jahr 2012 traten in den Befallsgebieten der Länder Rheinland Pfalz und Hessen, in denen 2011 Maiswurzelbohrer festgestellt wurde, keine Käfer mehr auf.

Stichwörter: Maiswurzelbohrer, Erstauftreten, Hessen, Rheinland-Pfalz, Ausrottung

1. Introduction

Diabrotica virgifera virgifera LeConte has been monitored in **Hesse** and **Rhineland-Palatinate** since 1999. According to Commission Decision 2003/766/EG (Byrne, 2003) the intended regular monitoring was conducted every year from July to October. Pheromone traps (PAL) were placed in endangered areas as airports, train and truck terminals, ports, central markets, motorway stations and especially in maize fields. In 2011 186 traps have been used for the regular monitoring in Hesse and 106 traps in Rhineland-Palatine. The presence of *Diabrotica virgifera virgifera* LeConte was detected for the first time in Hesse on August 24th and 25th 2011in a cornfield located in Groß-Gerau, Wallerstädten. 50 beetles were identified in pheromone traps as a result of the survey. A possible source of introduction could be tourism, because a camping site is located near the outbreak area. There are no other high risk areas nearby like truck companies, ports, motorway stations etc. where the organism could have been introduced. In the local authority region of Groß-Gerau, the region where the beetle was detected first, 14 pheromone traps had been placed every year. As the safety zone in Hesse extended into the area of Rhineland-Palatinate a more intense survey at the Rhine riverbank was established. In this context one beetle was detected on May 5th 2011 in the district of Bodenheim (Rhineland-Palatinate). In the following methods section, measures applied are described in more detail.

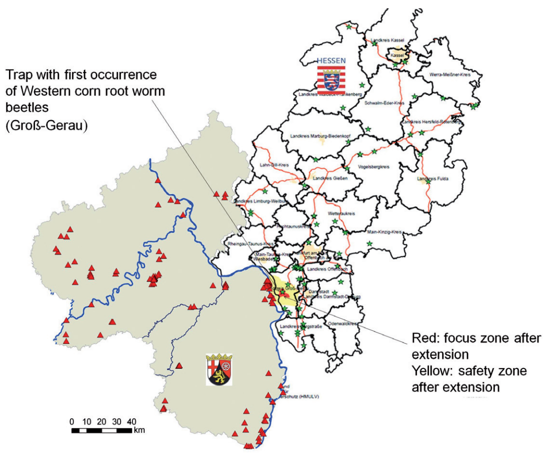

Fig. 1 Distribution of trap locations (2011) in Hesse (stars) and Rhineland-Palatinate (triangles).

Abb. 1 *Verteilung der Fallenstandorte (2011) in Hessen (Sterne) und Rheinland-Pfalz (Dreiecke)* .

2. Methods

From August 31st to September 6th, 2011, 216 ha maize were treated in the focus and safety zone in Hesse with Biscaya (application dose 0.3 l/ha) by a contractor. Both silage maize for biogas plants and grain corn were treated. Because of the high number of captured beetles, the safety zone was also treated. At this time sweet corn had already been harvested.

The district of Bodenheim in Rhineland-Palatinate is a traditional region of grape-vine with a minor percentage of maize and other agricultural crops. For this reason only 17 ha maize fields in the focus zone were treated with Biscaya (application dose 0.3 l/ha). The treatment was applied on September 9th 2011 by a contractor. A large part of silage maize had already been harvested at this time.

Traps (PAL traps) were arranged like a grid over the infested areas in both federal states. In each maize field in the focus- and safety zone further traps were placed and checked weekly until September 30th by supporting staff. During this auxiliary monitoring another 173 traps in Hesse and 30 traps in Rhineland-Palatine were added to the traps of the basic monitoring.

In the focus zones the cultivation of Maize was completely forbidden for the consecutive two years and a crop rotation with at least 50 percent maize percentage was established in the safety zones.

Until September 30th further beetles were caught in the grid of pheromone traps in the south of the area (in the district of Groß-Gerau) were the first infestation was discovered.

After one beetle was found on September 5th 2011in Rhineland-Palatinate in a PALI-trap, one part of the safety zone of Rhineland-Palatinate extended into hessian territory. The area was included into the Hessian survey. No more beetles were detected here, neither in the infested area of Rhineland-Palatinate.

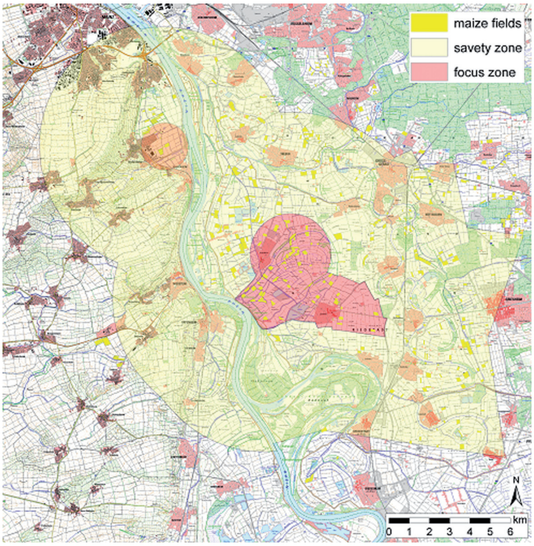

Fig. 2 Infested area of Hesse and Rhineland-Palatinate 2011.

Abb. 2 *Befallsregion von Hessen und Rheinland-Pfalz 2011.*

3. Results and discussion

By the end of the monitoring 354 beetles in Hesse and one beetle in Rhineland-Palatinate had been captured in total. Subsequently the demarked zones in Hesse were extended. Taking into account the local circumstances, the new focus zone was delimited to include all the areas where beetles had been detected (31) as well as the surrounding maize fields. The new safety zone in Hesse was established around the new focus zone in a 5 km radius and extended then into the district of Darmstadt–Dieburg. No further eradication measures were applied in the new demarked zones because of the treatment in the first safety zone. The area of the extended focus zone had also been treated already. Since harvest was almost completed no further areas had to be treated. The established zones in Rhineland-Palatinate were not extended, due to no further captures of beetles.

It is expected, that eradication can be achieved in the infested areas of both federal states. Particularly the guidelines for crop-rotation established in these areas will hopefully prove to be effective to eradicate this serious maize pest. However, in 2012 no further captures were observed in the infested region. Although the aims of the control measures in the infested area of Groß-Gerau and Bodenheim were achieved in 2012, a worrying situation was determined for southern located areas in the Upper Rhine Valley of Rhineland-Palatinate: three more focus and safety zones had been established in the districts of Leimersheim, Dannstadt-Schauernheim and Hördt due to overall seven new trap captures of western corn root worm beetles in 2012 (data not presented).

References

BYRNE, D., 2003: Commission decision of 24 October 2003 on emergency measures to prevent the spread within the Community of *Diabrotica virgifera* LeConte (notified under document number C (2003) 3880) (2003/766/EC).Official journal of the European Union L 275/**49**.

Session 2: Biology, monitoring, control, scenarios of *Diabrotica*

Effect of different temperatures on the development and fitness of western corn rootworm

Einfluss unterschiedlicher Temperaturen auf Entwicklung und Fitness des Westlichen Maiswurzelbohrers

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Summary

The objective of this study was to obtain data that could be used to develop a model for forecasting the occurrence of *Diabrotica virgifera virgifera*. The model currently available is mainly based on results from American studies using non-diapausing beetles, but the biological performance (fitness and mobility) of American non-diapausing beetles and those of European origin could differ, which would affect the accuracy of this model when used for forecasting in Europe. Thus, it is important to know whether the biological performance of *D. virgifera virgifera* of European origin differ from that of non-diapausing American beetles.

The following aspects of the effect of temperature on the biology of *D. virgifera virgifera* occurring in Europe were studied and compared with those of a non-diapausing strain from the US:

- pre-diapause development of eggs,
- post-diapause development of eggs,
- effect of different temperatures and densities on the development of larvae.

The results indicate that the American data based on studies of non-diapausing strains of *D. virgifera virgifera* are of only limited use for forecasting the occurrence of this pest in Europe. In particular, the results obtained for the development of eggs, larvae and adults provide information about this species' biology that could be used to increase our understanding of the population dynamics of this pest in Europe.

Keywords: *Diabrotica virgifera virgifera,* population dynamics, forecasting

Zusammenfassung

Ziel dieser Studie war die Entwicklung eines Modells zur Vorhersage des Auftretens von *Diabrotica virgifera virgifera*. Das derzeit verfügbare Modell basiert im Wesentlichen auf amerikanischen Studien, die sich auf nicht diapausierende Käfer stützen. Die biologische Leistung (Fitness und Mobilität) amerikanischer nicht diapausierender Käfer und europäischer Käfer könnte jedoch unterschiedlich sein. Das hätte Einfluss auf die Aussagegenauigkeit des Modells, wenn es für Vorhersagen in Europa benutzt werden würde. Aus diesem Grund muss festgestellt werden, ob sich die biologische Leistungsfähigkeit von *D. virgifera virgifera* europäischen Ursprungs von der amerikanischer nicht diapausierender Käfer unterscheidet.

Die folgenden Auswirkungen der Temperatur auf die Biologie von europäischen und nicht diapausierenden US-amerikanischen Stämmen von *D. virgifera virgifera* wurden untersucht und verglichen:

- Entwicklung der Eier vor der Diapause,
- Entwicklung der Eier nach der Diapause,
- Wirkung unterschiedlicher Temperaturen und Besiedlungsdichten auf die Entwicklung von Larven.

Die Ergebnisse zeigen, dass die Studien an amerikanischen nicht diapausierenden Stämmen von *D. virgifera virgifera* sich nur begrenzt für die Vorhersage des Auftretens dieses Schadorganismus in Europa eignen. Insbesondere die Ergebnisse zur Entwicklung von Eiern, Larven und Adulten enthalten Informationen über die Biologie der Art, die unser Verständnis von der Populationsdynamik des Schadorganismus in Europa verbessern helfen.

Stichwörter: *Diabrotica virgifera virgifera,* Populationsdynamik, Prognose

1. Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte 1868, is one of the most serious pests of maize. Management options for controlling WCR are to target either the root feeding larvae by crop rotation, application of soil insecticides or planting varieties of transgenic *Bt*-maize, or adult beetles by the application of insecticides.

Concerns over unintended side-effects, the development of resistance to some insecticides and behavioural adaptation to crop rotation in parts of the US corn-belt stimulated renewed interest in a better understanding of the population dynamics of WCR.

Therefore, the aim of this study was to provide data on (i) the embryonic development of eggs, (ii) the post-diapause development of eggs and (iii) the biological performance of larvae reared at different temperatures and densities. The objective is to use these data to develop a new model for forecasting the occurrence of *D. virgifera virgifera*. The forecasting model currently available is mainly based on results from American studies using non-diapausing beetles, but the biological performance of American non-diapausing beetles and those of European origin could differ, which would affect the accuracy of this model in Europe. Thus, in order to increase the understanding of its biology these parameters were determined for *D. virgifera virgifera* of European origin.

2. Material and Methods

2.1 Test organisms

Beetles of three different origins were used: a non-diapausing strain (USDA-NCARL, Brookings, USA; Branson 1976) reared since 2006, a diapausing strain from Northern Italy and a second diapausing strain from Hungary. Eggs of the last strain were kindly delivered by Dr. Stefan Töpfer (CABI). The rearing-methods are those described by Branson *et al.* (1975) and Jackson (1986). Larvae were reared on maize seedlings cv. Tassilo (KWS Saat AG, Einbeck, Germany) and adult beetles were fed fresh leaves of young maize plants, maize pollen and combs, zucchini, and pieces of apple, lettuce and water. The rearing of the *Diabrotica* strains took place in isolated rooms registered for quarantine proposes.

2.2 Plant material

The rearing of maize plants for maintaining the cultures of the different strains of *Diabrotica* and for use in the experiments was done in an air-conditioned green house kept at 20 °C, 65 ± 10% RF and under long-day conditions (16:8 h D:N). If the natural light intensity went below 10.000 lux additional light was provided by 400 W Philips Son-T Agro sodium-vapour lamps. The adjustment of the conditions in each cabinet (temperature, humidity, light etc.) was done using a ‚climate computer' and constantly recorded. The seeds of maize cv. Tassilo used were not coated with plant protection substances.

2.3 Experiments

Pre-diapause development of eggs

For the determination of the pre-diapause egg hatch the diapausing strains from Hungary and Italy were used. The eggs were laid beginning on the 11.09.09 in Hungary (HU) and in the laboratory of BTL (IT). The periods for which the females of *Diabrotica* laid eggs differed; for those from Hungary it was 9 days and Italy 21 days. Prior to the start of this experiment the females were placed in a container with wet sand that was previously passed through a sieve with a 200 µm mesh. Fifty two days after the eggs were laid they were washed from the sand with the help of a sieve (250 µm mesh size), floated in 1.25 M $MgSO_4$ and surface sterilized with 5' 0.05% NaOCl + 2' 0.25% peracetic acid. The eggs were then transferred in to a 0.15% dilution of agar and pipetted onto 30 mm discs of filter paper. Thirty mm diameter Petri dishes with perforated lids, which allowed air exchange (10 mm hole covered with 100 µm metal gauze), were used as test containers. The number of eggs of Italian origin was smaller than of Hungarian origin (Tab. 1). The Petri dishes with eggs of different origins were regularly monitored and the number of larvae that had hatched recorded. Larvae and empty egg shells were removed to avoid growth of fungi. Eighty days after beginning the experiments monitoring of Petri dishes ceased.

Tab. 1 Production of eggs by the two diapausing strains of *Diabrotica*.

Tab.1 *Eiablage von zwei Diabrotica-Stämmen mit Diapause.*

Population	Hungary (Kardoskut)	Italy (Brescia)
Beginning of oviposition	11.09.09	11.09.09
End of oviposition	20.09.09	02.10.09
Storage temperature (°C)	16	25
Egg age (max) at start of experiments (d)	52	52
Eggs total	≈ 2000	≈ 400
Eggs/Petri dish	64.2	31.6
Petri dishes	30	10

Post-diapause development of eggs

The study of post-diapause development was carried out on eggs that completed their diapause development in February. These eggs start to develop at temperatures above 11 °C. Thus, the beginning of development of the eggs of the different strains of *Diabrotica* was timed from when they emerged from diapause. Therefore, in spring the eggs of the strains from Hungary and Italy were extracted from the sand using the floatation technique described above and surface sterilized with NaOCl and peracetic acid.

Biological performance of larvae reared at different temperatures and densities

After obtaining records of the soil temperature at a depth of 5 and 10 cm at 12 stations of the German Weather Service (DWD) we decided to use the following three temperatures 15, 20 und 25 °C in the experiments on the effect of temperature on the development of larvae.

The speed of development of larvae of the non-diapausing strain was analyzed first at 20±1 °C. For this 50 recently hatched larvae were transferred with the help of a very fine brush to a plastic pot containing 15 to 20 maize seedlings. The larvae were extracted from the soil and plants of four pots on each sampling date by carefully searching by hand and then transferring the remaining material into a MacFayden extraction apparatus. Heat extraction in this apparatus was carried out over a period of three days. The head capsule widths (HCW) of the larvae were measured using a microscope (Olympus SZX 12), a CCD-camera (ColorView III, Olympus) and the software cell^D (Olympus). After drying for at least 48 h at 40 °C the larvae were weighed using a microbalance (Mettler-Toledo XP 26, d=1 µg).

As speed of larval development is much slower at low temperatures the larvae were sampled more often, at 15 °C, up to 13 times, that is, up to 72 days after transfer of larvae into the pots, at 20 °C 8 times and 25 °C 6 times (Tab. 2). Measurements of the HCW and dry weights (as described above) were used as indicators of the fitness of the larvae at different stages in their development.

To determine the effects of competition and shortage of food on the development of the larvae of the non-diapausing strain they were reared at 20 °C at two densities, 40 and 80 larvae and those of the diapausing strain from Hungary at densities of 30 and 80 larvae. Details of the design of this experiment are presented in Table 2.

Tab. 2 Intervals in days at which the larvae were sampled and duration of experiments on the development of larvae of a non-diapausing (USDA) and diapausing strain (Hungary) of *Diabrotica* that were reared at different temperatures and larval densities.

Tab. 2 *Beprobungsintervalle in Tagen an denen Larven beprobt wurden und Dauer der Experimente zur Entwicklung von* Diabrotica*-Larven eines Stammes ohne Diapause (USDA) und eines Stammes mit Diapause aus Ungarn (HU), die bei unterschiedlichen Temperaturen und Larvendichten aufgezogen wurden.*

	USDA				**Hungary**			
Temperature	15 °C	20 °C	20 °C	25 °C	15 °C	20 °C	20 °C	25 °C
N larvae/pot	40	40	80	40	30	30	80	30
Intervals in days at which was sampled	13	7	7	5	12	8	8	6
Duration of experi-ments (d)	72	22	21	14	63	27	26	19

The adult beetles that resulted from rearing the larvae at different temperatures and densities were used to determine the effects of temperature and larval density on their fecundity. The young females were isolated immediately after they emerged from pupae and were mated with males that developed under the same conditions. The number of eggs laid within nine days at 20 °C in Petri dishes filled with sand was determined by washing as described above.

2.4 Statistical Analysis

All the basic statistical analyses were done using the computer programme SYSTAT, version 10.0.

3. Results and Discussion

Pre-diapause development of eggs

The experiments on the embryonic development of unchilled *Diabrotica* eggs show that none of the eggs of European origin developed.

Post-diapause development of eggs

The hatching of larvae from eggs from both European origins occurred synchronously at 66 days. At 75 d the percentage that had hatched was greater for the Hungarian strain (see different slopes of hatching curves, Fig. 1A). The mean percentage egg hatch (± SD) of the eggs of the Hungarian strain is significant greater (77.2 ± 9.4%) than those of Italian origin (44.3 ± 11.6%; Mann-Whitney Test; Fig. 1B). In addition, 50% of the eggs produced by the Hungarian strain hatched after 90 days whereas for the Italian strain it was after more than 133 days. The end of hatching could not be recorded because of a strong fungal infection, which caused the experiment to be discontinued after 140 days. There was little variation recorded in both trials; so the use of 10 Petri dishes for each was sufficient. Similar results are reported in the US. Chiang *et al.* (1972) show that egg hatch of a field strain started 6-8 weeks after egg laying. They record a percentage egg hatch of 85% after 20–24 weeks. George and Ortman (1965) report a lower percentage egg hatch of 60% within 189 days. They record that the beginning of egg hatch occurred 44 days after egg laying.

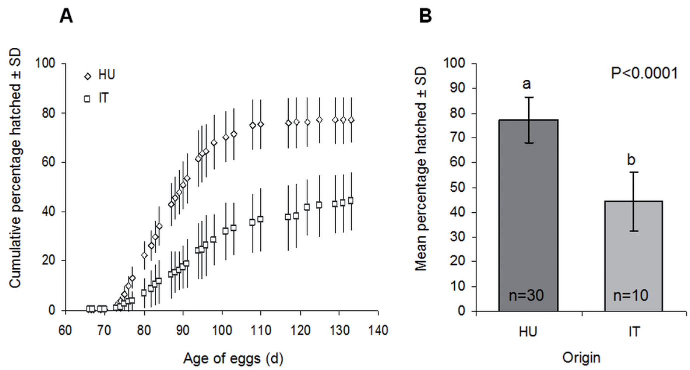

Fig. 1 Percentage of eggs of *D. virgifera virgifera* from Hungary (HU) and Italy (IT) that hatched in relation to **A** = the age of the eggs, **B** = origin of the beetles.

Abb. 1: *Prozentanteil von* Diabrotica-*Eiern aus Ungarn (HU) und Italien (IT), aus denen Käfer in Abhängigkeit von* ***A*** *dem Alter der Eier und* ***B*** *der Herkunft der Käfer schlüpften.*

Biological performance of larvae reared at different temperatures and densities

Access to constant temperature cabinets was an essential pre-condition for doing the experiments on the development of larvae at different temperatures. The stability of the conditions in the cabinets was monitored and adjusted using data loggers (Escort, iLog). Loggers with external sensors enabled us to monitor the temperature of the soil in the pots and provided a precise documentation of the degrees accumulated in each trial.

The HCW of larvae of different developmental stages do not overlap (Fig. 2). Exceptions are the pre-pupae, which are late L3 and have a typical hooked shape and therefore easily identified, whose development is not preceded by moulting. Therefore, these developmental stages cannot be separated using head capsule width.

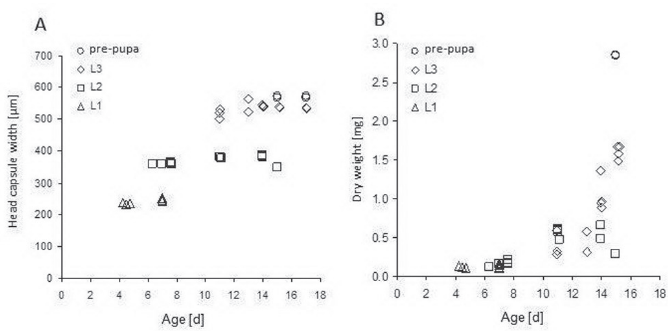

Fig. 2 A = Mean head capsule width; B = mean dry weight of different aged larvae of the non-diapausing *Diabrotica* strain reared at 20 °C.

Abb. 2 *A = Mittlere Kopfkapselbreite; B = mittleres Trockengewicht verschieden alter Larven eines* Diabrotica-*Stammes, aufgezogen bei 20 °C.*

The clear separation of the different larval stages (L1-L3) indicates that HCW is a strongly conserved and rather constant character, which cannot be used to characterize the fitness of larvae. On the other hand there are unpublished results that indicate HCW is affected by other external influences (e.g. food quality of host plants).

Obviously the weights of the different larval stages overlap and increase exponentially with age (Fig. 2B). There is a strong relationship between fresh and dry weight (R^2=0.98, curve not presented here). In spite of the time and effort need to dry larvae until a constant weight is achieved the precise measurement of dry weight is technically more simple then weighing fresh larvae as their weight constantly decreases as a result of evaporation. Therefore, the fitness of larvae is expressed in terms of their dry weight.

There is a strong relationship between HCW and dry weight (determination coefficient R^2=0.85) indicating a strong exponential relationship in both parameters (Fig. 3). Therefore, dry weight could be used to define the fitness of the different larval stages in future studies.

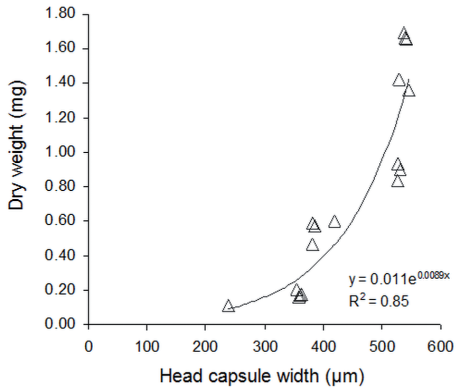

Fig. 3 The relationship between dry weight and head capsule width of the larvae of the non-diapausing strain of *Diabrotica* reared at 20 °C.

***Abb. 3** Zusammenhang zwischen Trockengewicht und Kopfkapselbreite bei Larven eines* Diabrotica-*Stammes ohne Diapause, aufgezogen bei 20 °C.*

The dry weight of larvae increases differently at the different temperatures, with the larvae of the non-diapausing strain reaching their maximum weight at 15 °C after more than 70 days and those reared at 25 °C after less than 15 days (Fig. 4). At 20 °C the weight of larvae of this strain was greater than at 15 °C. At the higher density the larvae achieved a slightly higher dry weight, but at the end of the experiment the differences were insignificant. At 20 °C larvae of the diapausing strain from Hungary reached their maximum weight after more than 20 d (Fig. 5). The larval weight of this strain was greater at 25 °C, if the weights at the same developmental times are compared. Because the duration of this experiment was reduced these larvae might not have reached their maximum weight. After more than 60 days at 15 °C the larvae were lighter than those reared at 20 and 25 °C. Those reared at the higher density were lighter after 10 days, but at the end of the experiments the difference in weight was insignificant.

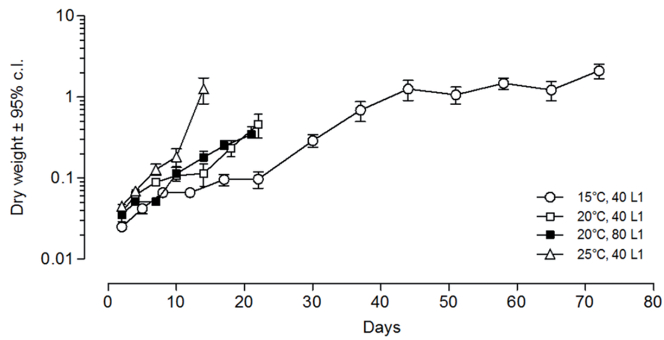

Fig. 4 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the non-diapausing strain of *Diabrotica* from USDA.

Abb. 4 *Einfluss von Temperatur und Dichte während der Larvenaufzucht, auf den Anstieg des Trockengewichtes von Larven eines* Diabrotica*-Stammes ohne Diapause vom USDA.*

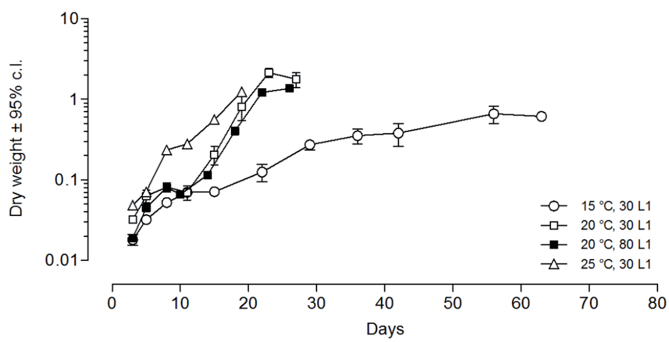

Fig. 5 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the diapausing strain of *Diabrotica* from Hungary.

Abb. 5 *Einfluss von Temperatur und Dichte während der Larvenaufzucht, auf den Anstieg des Trockengewichtes von Larven eines* Diabrotica*-Stammes mit Diapause aus Ungarn.*

The same is recorded if the dry weight is related to the larval stage (Fig. 6 and 7). There is an exponential increase in growth independent of the origin of the larvae, but the dry weight of the third larval stage of the diapausing strain from Hungary was greater when reared at 20 °C than that of the non-diapausing strain reared at 15 °C. Thus, as it is likely larvae grow and increase in weight within each larval stage there is some variation in weight depending on when they were weighed.

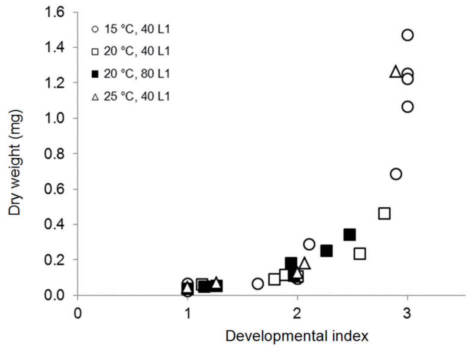

Fig. 6 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the non-diapausing strain of *Diabrotica* from the USDA in relation to the developmental index (developmental index: mean of developmental stages (L_1=1, L_2=2, L_3=3, Pre-pupa=4, Pupa=5)).

***Abb. 6** Einfluss von Temperatur und Dichte während der Larvenaufzucht, auf den Anstieg des Trockengewichtes von Larven eines* Diabrotica-*Stammes ohne Diapause vom USDA im Vergleich zum Entwicklungsindex (Entwicklungsindex: Mittelwert der Entwicklungsstadien (L_1 = 1, L_2 = 2, L_3 = 3, Vorpuppe = 4, Puppe = 5)).*

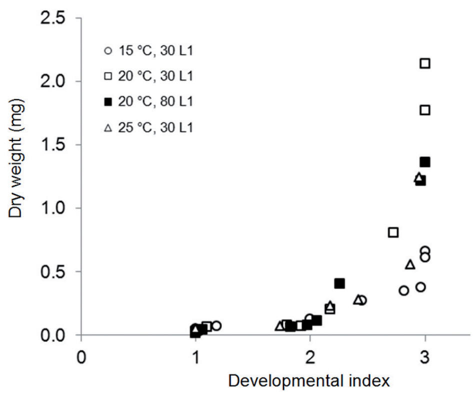

Fig. 7 Effect of temperature and density at which larvae were reared on the increase in dry weight of larvae of the diapausing strain of *Diabrotica* from Hungary in relation to the developmental index (developmental index: Mean of developmental stages (L_1=1, L_2=2, L_3=3, Pre-pupa=4, Pupa=5)).

***Abb. 7** Einfluss von Temperatur und Dichte während der Larvenaufzucht auf den Anstieg des Trockengewichtes von Larven eines* Diabrotica-*Stammes mit Diapause aus Ungarn im Vergleich zum Entwicklungsindex (Entwicklungsindex: Mittelwert der Entwicklungsstadien (L_1 = 1, L_2 = 2, L_3 = 3, Vorpuppe = 4, Puppe = 5)).*

To compensate for the dependency of weight on time Fisher (1921) used the „mean relative growth rate", which is termed MRGR and calculated using the following equation:

MRGR = (lnW2 - lnW1)/D

where W1 is the weight of a larva at $time_x$, W2 is the weight of a larva at $time_{x+1}$ and D the number of days between $time_x$ and $time_{x+1}$.

MRGR is the increase in weight per unit of time and includes the effects of environmental conditions (e.g. food quality of host plant) for a specific taxon of animals. The effects of rearing the larvae of both the non-diapausing strain and diapausing strain from Hungary at different temperatures and larval densities are shown in Fig. 8 and 9. The largest increase in weight was recorded for the larvae of the diapausing strain. At 15 °C, however, the larvae of the non-diapausing strain had a greater MRGR.

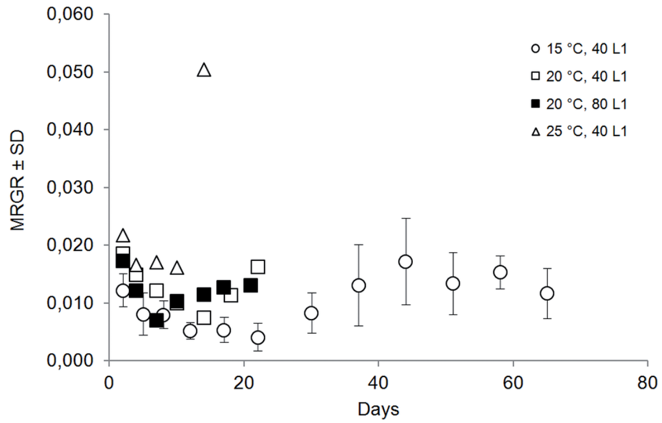

Fig. 8 Effect of temperature and density on the mean relative growth rate (MRGR) of *Diabrotica* larvae of the non-diapausing strain from USDA.

Abb. 8 *Einfluss von Temperatur und Besiedlungsdichte auf die mittlere relative Wachstumsrate (MRGR) von* Diabrotica-*Larven eines Stammes ohne Diapause vom USDA.*

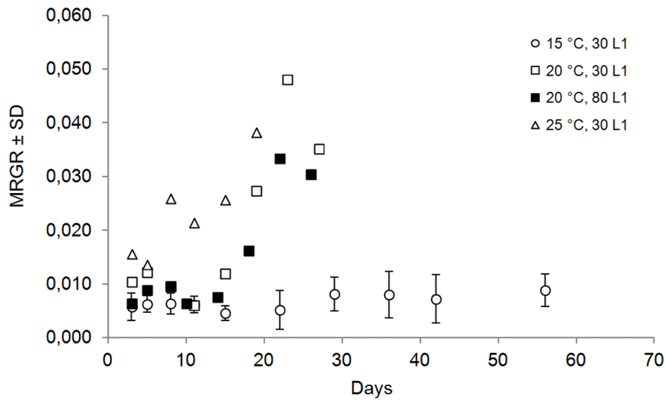

Fig. 9 Effect of temperature and density on the mean relative growth rate (MRGR) of *Diabrotica* larvae of a diapausing strain from Hungary.

Abb. 9 *Einfluss von Temperatur und Besiedlungsdichte auf die mittlere relative Wachstumsrate (MRGR) von* Diabrotica-*Larven eines Stammes aus Ungarn mit Diapause.*

The head capsule width is used to separate larval stages. There are small differences between the head capsule widths of the larvae of the non-diapausing laboratory strain and those of the diapausing field strain from Hungary. Comparisons of our results with those published show that the larvae of the Italian strain are similar to those of the non-diapausing strain in terms of their morphometric characters (Tab. 3). The larvae of both strains have wider head capsules than those of Hungarian origin. It should be mentioned that the head capsule width may be affected by food supply (Agosti *et al.,* 2009). These authors argue that in wet years the host plants are of a better food quality and as a result the larvae have larger head capsules.

Head capsule widths at the different stages of development of the larvae of the non-diapausing strain were larger in our experiments (Fig. 10). Temperature did not appear to affect the HCW of first instar larvae, but that of second instar larvae is greater when they were reared at 20 and 25 °C than at 15 °C. The third instar larvae had wider heads at 15 and 25 °C. Rearing the larvae at a high density had no effect on the head capsule width of larvae of the non-diapausing strain. In contrast the head capsule width of the second and third instar larvae of the diapausing strain from Hungary was larger at 20 and 25 °C (Fig. 10). First and second instar larvae of this strain were affected by rearing them at high density as they had smaller head capsules.

Tab. 3 Head capsule width (µm) of *Diabrotica* larvae of the non-diapausing strain (USDA) and field strain from Hungary (HU) that were reared at 20 °C and data of other authors (1 Agosti *et al.* (2009); 2 George and Hintz (1966); 3 Hammack *et al.* (2003)).

Tab.3 *Kopfkapselbreiten (µm) von* Diabrotica-*Larven eines Stammes ohne Diapause (USDA) und eines Stammes mit Diapause aus Ungarn (HU), die jeweils bei 20 °C aufgezogen wurden sowie Daten anderer Autoren (1 Agosti* et al. *(2009); 2 George and Hintz (1966); 3 Hammack* et al. *(2003)).*

Larval stage	USDA	HU	Field strain1 Italy	Field strain 2 USA	Field strain 3 USA
	Mean ± SE (n)	**Mean ± SE (n)**	**Mean ± SE (n=2063)**	**Mean, min-max (n)**	**Mean ± SE (n =>150)**
1	225 ± 0.4 (451)[a]	221 ± 0.5 (373)[b]	225 ± 3	200, 200–225 (55)	216 ± 1
2	343 ± 0.7 (478)[a]	336 ± 0.6 (538)[b]	350 ± 2	325, 300–350 (14)	332 ± 1
3	522 ± 1.7 (220)[a]	508 ± 0.9 (437)[b]	524 ± 1	500, 450–550 (18)	501 ± 1

[a,b] values with different letters within a row are significantly different, Mann-Whitney-test, p<0.05

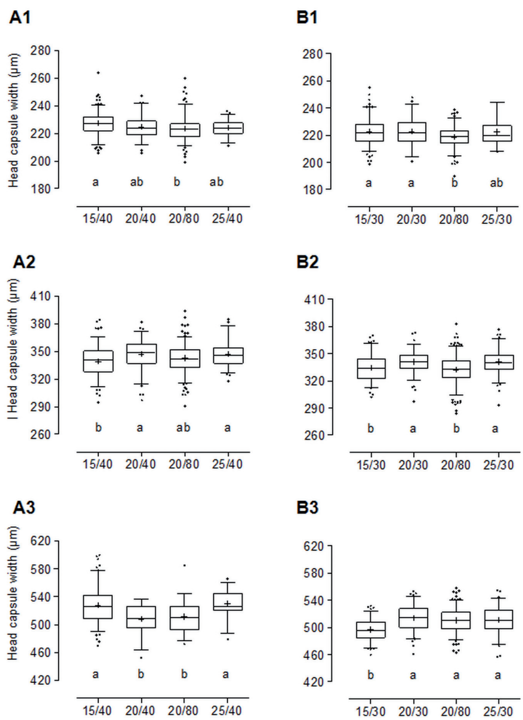

Fig. 10 *Head capsule width of different larval stages (1 L1, 2 L2, 3 L3) reared at different temperatures and larval densities (e.g. 15/40=15 °C, 40 L1).* ***A*** *= non-diapausing laboratory strain.* ***B*** *= diapausing strain collected in the field in Hungary (different letters indicate significant differences, p<0.05).*

Abb. 10 *Kopfkapsel-Breiten unterschiedlicher Larvenstadien (1 L1, 2 L2, 3 L3), aufgezogen bei verschiedenen Temperaturen und Larvendichten (z. B. 15/40 = 15 °C, 40 L1).* ***A*** *= Labor-Stamm ohne Diapause,* ***B*** *= Stamm mit Diapause, gesammelt auf Feldern in Ungarn (unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede, p<0.05).*

The data presented indicate that the experimental procedure adopted here is appropriate for future experiments on the response to temperature of other strains of *Diabrotica* of different origins. It must be pointed out, however, that temperature also affects the growth of maize and that 15 °C is close to the bottom of the temperature range tolerated by maize.

The live weight and selected morphometric characters (length of elytra, width of pronotum, head capsule width, and length of hind tibia) of male and female beetles reared in these experiments were recorded and are presented in Table 4. The adults of the non-diapausing strain are in most cases the heaviest with the exception of those reared as larvae at 25 °C. There is a direct relationship between larval weight and adult weight with the heavier larvae of the non-diapausing strain developing into larger adults with a greater fecundity. The largest number of eggs was produced by adults of the non-diapausing strain. Taking into consideration also those pairs of beetles the females of which died before the end of the experiment, the beetles that were reared at 20 °C were the most fecund (615 eggs/female) followed by those reared at 25 °C (484 eggs/female). Beetles reared at 15 °C were the least fecund (372 eggs/female).

Tab. 4 Effect of temperature (°C) and density at which the larvae were reared on the mean (± SD) weight (mg) and selected morphometric characters (mm) of adult beetles of the diapausing Hungarian field strain (H) and non-diapausing strain (U) of *Diabrotica*. (M males, F females, FG fresh weight, E elytra, Pn pronotum, HCW head capsule width, HT hind tibia).

Tab. 4 *Einfluss von Temperatur (°C) und Besiedlungsdichte während der Larvenaufzucht auf das mittlere (± SD) Gewicht (mg) und ausgewählte morphometrische Charakteristika (mm) erwachsener Käfer des* Diabrotica*-Stammes mit Diapause aus Ungarn (H) und des Stammes ohne Diapause (U). (M männliche, F weibliche, FG Feuchtgewicht, E Deckflügel, Pn Pronotum, HCW Kopfkapselbreite, HT hintere Tibia).*

	Temp	Density	Sex	FG	E- length	E-width	Pn-width	HCW	HT-length	(n)
H										
	15	30	M	7.54	3.66	1.83	1.22	0.98	1.53	1
	20	30	M	6.62 ±1.30	3.64 ±0.25	2.06 ±0.19	1.27 ±0.09	1.03 ±0.12	1.46 ±0.16	29
	20	80	F	7.20 ±1.89	3.76 ±0.32	2.17 ±0.21	1.34 ±0.11	1.03 ±0.09	1.53 ±0.15	21
			M	8.54 ±2.89	3.87 ±0.28	2.20 ±0.18	1.33 ±0.11	1.08 ±0.08	1.56 ±0.15	78
	25	30	M	6.00 ±1.32	3.76 ±0.22	2.03 ±0.16	1.26 ±0.06	1.11 ±0.09	1.32 ±0.13	9
U										
	15	40	F	9.20 ±1.54	4.17 ±0.09	2.30 ±0.04	1.38 ±0.07	1.18 ±0.04	1.61 ±0.09	3
			M	9.17 ±1.34	4.05 ±0.25	2.27 ±0.15	1.39 ±0.08	1.22 ±0.11	1.66 ±0.16	5
	20	40	F	10.66 ±1.93	4.31 ±0.19	2.60 ±0.09	1.48 ±0.07	1.24 ±0.04	1.75 ±0.09	3
			M	9.82 ±2.43	3.92 ±0.94	2.35 ±0.24	1.43 ±0.11	1.19 ±0.11	1.72 ±0.20	16
	20	80	F	9.37 ±7.27	4.09 ±0.41	2.33 ±0.31	1.45 ±0.13	1.13 ±0.12	1.71 ±0.26	9
			M	9.29 ±2.26	3.98 ±0.40	2.29 ±0.30	1.40 ±0.16	1.17 ±0.10	1.68 ±0.18	14
	25	40	F	7.96 ±1.34	3.94 ±0.20	2.30 ±0.12	1.41 ±0.07	1.14 ±0.04	1.55 ±0.10	9
			M	4.81 ±3.23	3.78 ±0.51	2.22 ±0.28	1.36 ±0.11	1.12 ±0.07	1.63 ±0.14	25

Based on the fecundity of those pairs in which the females survived to the end of the experiment those beetles that were reared at 20 °C were the most fecund (615 eggs/female) followed by those reared at 15 °C (559 eggs/female). Those reared at 25 °C were the least fecund (484 eggs/female). In addition, beetles reared at a high density at 20 °C were less fecund (322 eggs/female).

Very few adults developed in the experiments that used the diapausing strain from Hungary. Therefore, fecundity was recorded only for the few females, which developed at 20 °C. The fecundity of this strain is clearly lower. If fecundity is calculated only for those pairs in which the females survived to the end of the experiment the fecundity is 96 eggs/female. This might not only be due to their smaller size but also to their greater activity (e.g. walking speed, take off time etc.), which was not recorded.

4. Conclusion

The results indicate that a non-diapausing strain from USDA and diapausing strains of *D. virgifera virgifera* from Europe differ in both their morphometric characters and performance. Therefore, data based on studies of American non-diapausing strains of *D. virgifera virgifera* are of only limited use for forecasting the occurrence of this pest in Europe. In particular, the results reported above on the development of eggs, larvae and adults provide information that could be used to increase our understanding of the population dynamics of this pest in Europe.

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A review of different trapping methods and purposes for *Diabrotica virgifera virgifera* LeConte

Eine Übersicht unterschiedlicher Fangmethoden und –ziele für Diabrotica virgifera virgifera *LeConte*

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The western corn rootworm has spread in Europe from the first half of the 1990s, and Hungary is in a special position neighboring Serbia, the country of the first appearance, where hardly any opportunity was available for research and development in the after war period. Hungary was the optimal place for the research of different monitoring methods of *Diabrotica* at low population densities in the early years, and the circumstances were optimal for the development of control methods 5-10 years later, when the beetle population was built up.

In this special position equipped with the scientific knowledge achieved in the chemical ecology of the western corn rootworm by researchers from the US (i.e. Prof. J.H Tumlinson, Prof. R.L.-Metcalf and colleagues) our team could develop and optimize several trapping devices for capturing the new pest using two bait types:

(1) the pheromone baits, which attract exclusively males, and

(2) the floral baits, which attract more females than males.

With these trapping methods available we investigated the most suitable ones and found that in real-life monitoring situations, their performance is different depending on the trapping purposes. The pheromone baited sticky traps (PAL) are the most suitable for catching the first male specimens, while floral attractant baited sticky traps (PALs) can catch the first females. Further, non-saturable, large catch capacity traps (KLP) are the most suitable for quantitative comparisons with both floral and/or pheromone baits.

The presented results were all derived from the evaluation of the trap types developed in our lab, which have become members of the commercially available CSALOMON® trap family. The Europe-wide use of the CSALOMON® traps and the conclusion of the EU research project DIABROTICA (QLK5-CT-1999-01110), which recommended to use PAL traps baited with pheromone as the standard detection tool for *D. virgifera virgifera* in Europe, shows a strong demand for trapping methods.

Our work on *Diabrotica virgifera* resulted in some interesting findings. Some of them are different from the common consensus, including the low to zero attractiveness of the yellow hue resulting mainly in captures by chance (despite of their wide use in the US) and the results on the circadian activity of males and females.

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Western corn rootworm: Experiments on the improvement of monitoring at low population densities

Westlicher Maiswurzelbohrer: Untersuchungen zur Verbesserung des Monitorings bei geringen Populationsdichten

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Summary

An efficient monitoring of the western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte, 1868 (Chrysomelidae, Coleoptera) is the most important precondition for convenient measures of eradication or containment. This research project aimed to assess the monitoring methods, especially at low population densities as currently observed in Germany. For this purpose, large-scaled field trials were designed in Bavaria and Upper Austria. The studies focused on the catchability, costs and by-catches of the pheromone-trap PAL in comparison to other trap types. In another experiment, different allocations of PAL traps in the corn field were examined. Finally, traps and pheromones often remain unused at the end of a monitoring season. Therefore the catchability of stored PAL traps in the next monitoring period was investigated.

PAL traps captured beetles constantly on a high level. In addition to its low costs and reduced by-catches, the trap type PAL is recommended for the monitoring of western corn rootworm in Germany. Changes in locations of PAL traps within the corn field did not result in a higher catchability. At present, traps are placed in the fifth to eighth corn row and the results confirm to maintain this practice. Comparing the catchability of stored PAL traps to the catchability of fresh traps, stored traps resulted in comparable catches than fresh ones. It is concluded that traps and pheromones, which were stored dark and deep-frozen, can be used in the next season.

Keywords: *Diabrotica virgifera virgifera*, monitoring, PAL-trap, pheromone traps

Zusammenfassung

Ein effektives Monitoring des Maisschädlings Westlicher Maiswurzelbohrer (*Diabrotica virgifera virgifera* LeConte 1868, Chrysomelidae, Coleoptera) ist die wichtigste Voraussetzung für zielgerichtete Ausrottungs- bzw. Eingrenzungsmaßnahmen. Im vorliegenden Forschungsprojekt wurde das bestehende Monitoring speziell unter geringen Befallsdichten, wie sie derzeit in Deutschland vorherrschen, überprüft. Dazu wurden großräumige Freilandversuche in Bayern und Oberösterreich angelegt. Untersuchungsschwerpunkt war die Überprüfung der Fängigkeit, Kosten und Beifänge des aktuell verwendeten Pheromon-Fallentyps PAL im Vergleich zu anderen Fallentypen. Ein weiterer Versuchsteil untersuchte verschiedene Anordnungen der PAL-Fallen im Maisfeld. Am Ende einer Monitoringsaison bleiben oft ungebrauchte Fallen und Pheromone übrig. Deshalb wurde die Fängigkeit von eingelagerten PAL-Fallen in der nächsten Monitoringperiode untersucht.

Der Fallentyp PAL erwies sich als durchgehend sehr fängig. In Verbindung mit seinem verhältnismäßig günstigen Preis und seinen reduzierten Beifangraten kann er für das Monitoring des Westlichen Maiswurzelbohrers in Deutschland empfohlen werden. Eine veränderte Position der PAL-Fallen im Feld ergab keine verbesserte Fängigkeit. Die aktuell praktizierte Anbringung der Fallen in der fünften bis achten Maisreihe sollte beibehalten werden. Bei den eingelagerten PAL-Fallen konnte keine nachlassende Fängigkeit nachgewiesen werden. Dunkel gelagert bzw. mit tiefgekühlt aufbewahrtem Pheromon sind die Fallen ohne Fängigkeitsminderung in der nächsten Saison einsetzbar.

Stichwörter: *Diabrotica virgifera virgifera*, Monitoring, PAL, Pheromonfalle

1. Introduction

The western corn rootworm *Diabrotica virgifera virgifera* is the most dangerous pest infesting corn fields. This harmful leaf beetle was introduced from North America to Europe during the Balkan wars and was first detected in 1992 near to the airport of Belgrade (Bača, 1993). In North America the beetle causes every year lots of plant protection expenses and received for this the nickname "Billion dollar bug". From Belgrade the WCR population extended the following years like ripples in a pond to the surrounding countries. In 2007 the first *Diabrotica* individuals in Germany could be found in Baden-Wuerttemberg and in Bavaria (Boegel, 2007).

The trap type PAL was one of the first traps which was specifically produced for the WCR. It works on the base of female sex pheromone and attracts mainly male individuals (Tóth *et al.,* 1996). Several other traps were designed in the following years, partly not using the sex pheromones but other attractants (Tóth *et al.,* 2006).

In Bavaria there is the special situation that *Diabrotica* populations show low to very low abundances all over the federal state. Therefore the catchability of the different trap types mentioned above was investigated under these specific conditions in Bavaria within the German Diabrotica Research Program.

Fig. 1 Examination of a yellow panel trap for *Diabrotica virgifera virgifera.*

Abb. 1 *Untersuchung einer Gelbtafel auf* Diabrotica virgifera virgifera.

2. Material and Methods

The trap type PAL, produced and delivered by Csalomon in Hungary, is currently used in most of the European countries and therefore part of the official monitoring on western corn rootworm in Bavaria. Four main issues with some subquestions should be answered in the course of this Bavarian project:

1) Do other trap types catch significantly better than the trap type PAL? Which trap of them could be recommended for a monitoring in the case of low population densities of WCR?

2) Ordinary yellow panel traps are much cheaper than the PAL trap. Is it possible to run an efficient monitoring by using yellow traps instead of PAL?

3) Up to now the PAL traps are placed within the fifth to eighth row in a corn field. Would it be better to change the position of the PAL-traps within the corn field to different rows? Should they be placed more into the center of the field or vice versa more to an outer row?

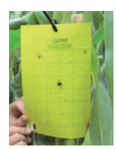
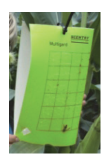
4) In case that not all PAL traps were used during a season is there any loss of quality by storing them? Is it possible to use them still in the next year?

For answering these questions large field-scale trials were designed both in the district Passau in Lower Bavaria and in the district Perg in Upper Austria. Locations were selected where corn was grown following corn.

To assess whether the PAL trap could be replaced by another trap type, in 2009 to 2011 ten different trap types were examined in comparison to PAL (Table 1). In 2009 the tests were performed in Lower Bavaria and Upper Austria, in 2010 only in Lower Bavaria and in 2011 again in both regions.

Tab. 1 Tested traps and their characteristics.

Tab. 1 *Untersuchte Fallenarten und ihre Charakteristika.*

Name of Trap	Bait	Coloured (sheet)	Funnel or hat	Killing the catch by	Photo
PAL	pheromone	transparent	---	glue	
PALx	pheromone	yellow	---	glue	
PALs	kairomone (floral)	yellow	---	glue	
KLPfero+	pheromone	yellow	hat	insecticide	
KLPflor+	kairomone (floral)	yellow	hat	insecticide	
Unitrap	pheromone	green/transparent	funnel	insecticide	
Deltatrap	pheromone	green	---	glue	
Deltax	pheromone	transparent	---	glue	
Pherocon AM	---	yellow	---	glue	
Multigard Green	---	yellow	---	glue	
Multigard Yellow	---	yellow	---	glue	

The traps were placed into the fields at a distance of at least 30 metres between each trap (effects between traps could therefore be almost avoided). After 14 days the traps were substituted and in addition the position of a trap within the corn field was changed like in a circle. For estimating the catchability of the different trap types the result of the PAL trap was defined as the reference (100 percent).

Ordinary yellow panel traps like Pherocon AM are with 0,98 € much cheaper than PAL traps with 6,10 €. For the regular Bavarian monitoring which needs about 3,500 PAL traps per year the replacement of PAL traps by the cheaper yellow panel trap would result in an economic benefit (savings of almost 18,000 € per year) if the efficiency would be comparable. Therefore in 2010 in Lower Bavaria (with a very low *Diabrotica* infestation level) 717 PAL traps and 717 Pherocon AM traps were compared to each other with respect to their efficacy. In Upper Austria (with a moderate *Diabrotica* level) 360 PAL traps and 360 Pherocon AM traps were included in the assessment.

At present PAL traps are fixed onto the plants in a height of about 1.50 meters in the fifth to eighth row of a corn field. If monitoring results could be enhanced by only changing the position of the trap to another row the improvement could be achieved without any additional costs. In 2010 and 2011 altogether in 95 test fields in Lower Bavaria and Upper Austria the PAL traps were fixed in the first, the 7th and the 14th row with a space of 30 metres in between. Every seven days the traps were substituted and according to this substitution each position of a trap was changed within the rows.

Finally the loss of quality of PAL traps was of interest in case that not all of them were used within one season. Is it possible to use the gluey panels and the pheromones in the following season if they are stored in a cool and dark place? For this test the panels were stored at a temperature lower than +5 °C and the pheromones were frozen to a temperature of –18 °C. PAL traps of the season 2010 were stored and used for testing in 2011 in each of the 25 corn fields in Lower Bavaria and Upper Austria. The stored PAL traps of 2010 as well as new PAL traps bought in 2011 and exposed shortly to the same temperature conditions were checked for their catchability.

3. Results

In comparison to the PAL trap only the PALx trap, which is a combination of a PAL trap with a yellow sheet, showed satisfying results in all cases. In Lower Bavaria as well as in Upper Austria the PALx was 2011 the most catchable trap, in 2010 it was almost as successful as the PAL trap. But the difference between PAL and PALx was not significant. Only one of the hat traps, KLPfero+ proved to be significantly the best trap in 2009 in Upper Austria. The traps containing the floral bait as well as the two traps in delta form and the funnel trap showed almost no catchability at lower population densities of *Diabrotica virgifera virgifera*. The three different yellow sheets with no bait did absolutely not attract WCR but instead many other arthropods which respond primarily to the colour yellow.

The comparative test between PAL trap and an example of a yellow panel trap (Pherocon AM) led to a highly significant result. Table 2 shows the numbers of *Diabrotica* catches in the traps.

Tab. 2 Comparison of PAL trap with yellow panel trap Pherocon AM.

Tab. 2 *Vergleich von PAL-Fallen mit Pherocon AM Gelbtafeln.*

	Total number of traps tested	Traps with WCR	WCR catches (number of beetles)
Lower Bavaria			
PAL	717	12	22
Pherocon AM	717	0	0
Upper Austria			
PAL	360	168	1,739
Pherocon AM	360	6	7

The number of positive traps with WCR catches – irrelevant of how many individuals were caught – was significantly higher for the PAL trap. That conclusion can be drawn for both regions - for Lower Bavaria, a region with a very low *Diabrotica* population density level, and as well for Upper Austria, a region with a moderate infestation level. In addition the total number of WCR catches, especially in Upper Austria, underpins the result that the PAL trap is much more attractive than an ordinary yellow panel.

Within the experiments with respect to changing of rows where traps should be placed numbers of WCR catches in Bavaria were too small to perform a statistical analysis. Table 3 therefore only shows the Austrian results. Only complete data sets were considered – in case that one trap had to be excluded the whole data set was deleted.

Tab. 3 Effect of PAL trap position within the corn field – results of Upper Austria.

Tab. 3 *Einfluss der PAL-Fallenposition im Maisfeld – Ergebnisse aus Oberösterreich.*

Year	Row	Data sets	Traps with WCR	Percentage of traps	WCR catches	Percentage of catches
2010	1	234	60	25.6	504	29.5
	7	234	63	26.9	592	34.7
	14	234	75	32.1	612	35.8
2011	1	119	69	58.0	1,783	31.1
	7	119	80	67.2	2,076	36.2
	14	119	81	68.1	1,883	32.7

The differences in the numbers of positive traps with WCR as well as the difference in the total numbers of WCR catches were statistically not significant.

Results for the comparison of stored PAL traps to fresh traps are shown in Table 4.

Tab. 4 Catchability of stored PAL traps in comparison to fresh PAL traps.

Tab. 4 *Fängigkeit von gelagerten und frischen (ungelagerten) PAL-Fallen.*

Region	Condition	Data sets	Traps with WCR	Percentage of traps	WCR catches	Percentage of catches
Bavaria	stored	372	17	4.6	22	52.4
	fresh	372	10	2.7	20	47.6
Upper Austria	stored	351	268	76.4	8,701	59.8
	fresh	351	223	63.5	5,853	40.2

The difference in the catchability was statistically not significant, but especially in Austria the stored PAL traps appeared to have a slightly better catchability.

4. Conclusions

According to the above described results the two trap types PAL and PALx are the only traps which could be recommended for a WCR monitoring at low population densities. Since the other types of traps did only catch some few individuals of *Diabrotica virgifera virgifera* if the population had already reached a certain extent, they cannot be recommended for a WCR monitoring in a non-infested area or at the outer range of a *Diabrotica* region due to the risk of too low catchability.

Yellow panel traps without bait are not suitable for an effective WCR monitoring. The catchability was negligible low in comparison to PAL traps. In summary, yellow panel traps cannot replace the PAL trap type – despite the low price.

The results showed that for a monitoring the PAL traps could be placed in corn rows 1, 7 or 14 without significant differences in the catchability. For an efficient monitoring it is even more important to hide the traps within the field in order that nobody would remove them. The first row should for this reason not be used. In addition traps may not be found if they are placed too deep within the corn field thus giving as well a disadvantage to the 14th row. Therefore the current standard of placing the traps within the fifth to eighth row should be maintained.

Stored PAL traps did not prove to have a decreased quality in catchability compared to fresh PAL traps – in the test 2011 they even seemed to work better. The producer confirmed that the composition of the pheromone element is the same for years. Only the composition of the glue changed in small details from 2010 to 2011. Maybe this other glue had a little influence on the catchability, but nevertheless it was statistical of no significance. The recommendation is now that stored PAL traps and frozen pheromones from the previous year can be used without problems in the subsequent year.

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GPS-assisted monitoring of *Diabrotica* and other pests and diseases using smartphones

GPS-unterstütztes Monitoring von Diabrotica *und anderen Pflanzenschädlingen und -krankheiten mit Hilfe von Smartphones*

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In case of occurrence of the quarantine pest *Diabrotica virgifera virgifera*, a comprehensive monitoring has to be established. Around a place of finding, up to several hundred pheromone traps have to be set up in a dense grid and to be examined on a weekly basis. By the conventional procedure, where the trap locations are marked on printed maps, an exact positioning is as difficult as the relocation, especially by persons wo did not set up the traps themselves.

In order to make this process more efficient, the Central Institution for Decision Support Systems in Crop Protection (ZEPP) and the Information System for Integrated Plant Production (ISIP) have jointly developed a mobile assistant for the monitoring of the *Diabrotica*. Using the GPS capabilities of a modern smartphone, the trap locations are determined and in the next step relocated again. The date and the number of trapped beetles can be entered directly into the device and transferred online to the server. For technological reasons a native Android application has been developed.

The process of GPS-assisted monitoring is divided into two steps.

At first, a systematic grid of trap locations is generated around the place of finding. When setting up the traps, these 'theoretical' trap locations are replaced by the actual locations according to the local conditions. The trap is then fixed to the maize plant, its coordinates are retained from the smartphone's GPS receiver and it is labelled with a system generated identifier. These data are saved to the SD card of the device ensuring that no permanent internet connection is needed. Finally, the data are sent to the ISIP server, in the ideal case directly via mobile internet, otherwise later when the device is online again.

In the second step, the traps have to be revisited to record the catch results. When starting the application, the traps to be monitored are provided in a drop-down list. The relocation of the trap is supported by maps for an overview as well as a radar-like tool for the last meters. When a trap has been visited, its identifier is deleted from the list and this continues until all traps have been assessed. The order in which the traps were visited is saved thus incrementally optimizing the route for the next monitoring cycles. The monitoring results are again stored on the SD card and can be sent to the ISIP server whenever online. On the server, the data are stored and – if desired – forwarded into the reporting chain. Back in the office, the user can log in to the ISIP system and access the trap locations and latest monitoring results displayed in a WebGIS. Apart from the mere presentation, a tool for trap management was integrated to distribute trap data among other users of the system. This includes the opportunity that one group of people will set up the traps while another can do the monitoring.

The system was tested experimentally in 2010 in Rhineland-Palatinate and has been used under practical conditions in 2011 both in Rhineland-Palatinate and North Rhine-Westphalia. In 2012 the federal state of Saxony joined this monitoring approach and contributed to the total number of 447 managed traps by September 20th. Due to the successful introduction of the system, further developments aim at (1) making the mobile application independent of the operating system of the smartphone and (2) extending the range of monitored pests and diseases.

Forecasting of root damage, plant lodging and yield loss caused by western corn root worm larval feeding based on larval population density

Vorhersage von Wurzelschäden, Lagerschäden und Ertragsverlusten durch Larvenfraß des Westlichen Maiswurzelbohrers auf der Grundlage der Larvenpopulationsdichte

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Various authors in the USA investigated the forecasting of root damage, plant lodging and yield loss caused by western corn rootworm (WCR) larvae. Depending on the year, WCR larvae are causing damages in Croatia and in Central Europe at different intensity. The total damage is the result of various factors whereof the most important are the WCR larval population and the weather conditions within specific years and at particular localities. For better understanding how larval population and weather conditions are influencing the damage, investigations under conditions with severe infestations of WCR should be carried out. Overall increase of WCR population in Croatia and in Central Europe enabled us to conduct studies under conditions of natural WCR infestation. The aim of the research was to develop a model for damage forecast based on established WCR larval population densities. Therefore we analysed the relationship between WCR larval population established at the time of maximal larval feeding and subsequent root damage, plant lodging and yield loss.

Experiments were conducted in three regions of Croatia during three field seasons (2007-2009). Within each season five continuous maize fields in each region (i.e., 15 fields per year) were involved. The WCR larval population was established in June at the Julian day (JD) 170 (in 2007), 163 (in 2008) and 167 (in 2009). Ten plants in each of four randomly selected rows in the field were dug (i.e., 40 plants per field) to assess the average number of WCR larvae per plant. By using the same methodology 40 roots per field were dug and scores on the Node Injury Scale (NIS, 0-3) were recorded at JD 203 (in 2007 and in 2009) and JD 206 (in 2008). Plant lodging was estimated at the JD 261 (in 2007), 267 (in 2008) and 265 (in 2009). Lodging was measured using 100 plants in 5 randomly selected rows in each field (i.e., 500 plants per field). The plants were grouped as follows: upright, partially and fully lodged. In each field from each grouping, three samples containing ten plants were harvested. The average yield was determined for each category and based on the ratio of each category in a particular field the average yield was calculated. Yield loss was calculated by comparing obtained yields with the yields of upright plants in each field. All relationships were assessed by using a linear regression model which involved interaction with the year as an indicator of weather conditions. Significant interactions between WCR larval population and year for forecasting root damage rate were determined. The year resulting with the steepest slope was chosen for establishing damage threshold. The infestation with 1.08 WCR larvae per plant predicts an average of 0.75 node injury score. Significant interactions between the WCR larval population and year for the relationship between WCR larval population and partially and fully lodged plants were not determined. Fully lodged plants are not suitable to forecast damage according to WCR larval population. An average of 10.9% of yield reduction could be forecasted if one WCR larvae per plant was established. Weather conditions did not affect the relationship significantly. A significant interaction between the year and upright plants in the field could be determined if the root damage rate was used as a forecast tool. An average of10% of partially lodged and an average of 10% fully lodged plants could be forecasted if the root damage rate of 0.62 and 2.14 were established, respectively. Weather had low impact on the relationship between root damage rate and yield; different years had similar weather conditions. An average of 17.5% of yield reduction could be forecasted if a root damage rate of 0.75 was established. Results from this study are important for better understanding the relationship between the final damage caused by WCR larval population and weather conditions. Therefore results could be used for creating guidelines for an integrated maize production.

Spreading predictions of the western corn rootworm in Germany until 2021

Ausbreitung des Westlichen Maiswurzelbohrers in Deutschland bis 2021

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The western corn rootworm (*Diabrotica virgifera virgifera*) is one of the most important pests of maize in the world and endemic to North America. In 1992, the first beetles of the western corn rootworm were first detected in Europe close to the Airport of Belgrade. Since then the beetle has actively spread through Europe. In 2007, first beetles were caught in pheromone traps in southern Germany (Bavaria and Baden-Württemberg). By now, the western corn rootworm has also been detected in North Rhine-Westphalia (only in 2010), Hesse (only in 2011) as well as in Rhineland-Palatinate (2011 and 2012). In future, it is expected, that the beetle will spread further in Germany.

The previous spread of *Diabrotica* in Europe as well as in North America has varied from year to year and from region to region and ranged from a couple of kilometers up to 80 km per year. This shows that dispersal is effected by regional conditions. Hence, a dispersal model was developed which integrates all relevant regional conditions.

The model consists of the following four components: situation of *Diabrotica*, regional spread, long distance flights and global spread.

The model component "situation of *Diabrotica*" includes the population development of the western corn rootworm under regional conditions. The second component of the model, "regional spread", contains all flights over short distances. The direction of the flights depends on corn growing because the western corn rootworm follows maize over short distances. Additionally, barrier cells, like cities and forests, are integrated into this component. The western corn rootworm, however, does not follow maize directly when the beetle flies over long distances and no barriers exist. This behavior is considered in the component "long distance flights".

Furthermore, the western corn rootworm was detected far away behind the established spread line because of hitchhiking on various means of transport. This fact is taken into account in the component "global spread".

Several scenarios of the western corn rootworm spread without control measures as well as considering different control measures were presented and discussed.

This project was financially supported by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Research Program on *Diabrotica*.

Population Model of the western corn rootworm

Populationsmodell für den Maiswurzelbohrer

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A web-based simulation model of the western corn rootworm was developed to plan the monitoring of the pest and to find optimum treatment dates. Based on comprehensive literature research and expert consultation within the joint German research project on *Diabrotica* all relevant impact factors with respect to the occurrence of western corn rootworm (reproduction, mortality and ontogeny) were identified and weighted. Interrelationships between these factors and the most important processes of population dynamics of the pest were quantified and combined to a deterministic simulation model. This model is part of the subproject "Simulation and Predictive Model".

The model was coded with the Java language and called "DiaSim". It allows to predict the first occurrence of the larvae stages (L1, L2, L3), the pupae stage and the hatch of the beetle depending on temperature and rainfall data.

Since spring 2011, the first version of the simulation model has been presented on the *Diabrotica* microsite of the Julius Kühn-Institut (http://diabrotica.jki.bund.de). The user gets predicted dates for a certain field through the use of simple GIS functions like map view, some navigation functions (zooming, moving) and site selection. Furthermore, the model offers a daily updated risk map of Germany's constituent states Bavaria and Baden-Württemberg and since 2012 of Hesse, Saarland, Rhineland-Palatinate and North Rhine-Westphalia, too. It is thus possible to estimate the development over the last seven days. The web-based model needs no installation, does not depend on any computer systems and may be used on mobile devices (like smartphones or a tablet pc).

For the further application of the model, a comprehensive monitoring is of enormous importance for the validating of the model. In case of higher population densities of the beetle a monitoring of larval stages would be desirable in order to identify and solve mistakes within the model. With the end of the project "DiaSim" will be handed over to ISIP (Information System on Integrated Plant Production) to be available on their website (www.isip.de).

This project was financially supported by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Research Program on *Diabrotica*.

Population dynamics studies of the western corn rootworm - experiments in isolation cages

Versuche zur Populationsdynamik des Westlichen Maiswurzelbohrers in Isolationskäfigen

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1. Introduction

Maize is a profitable crop for Central European growers. Many farmers prefer maize for feeding cows or pigs and for the maintenance of biogas plants. In case of official measures in place to prevent further spreading of the western corn rootworm *Diabrotica virgifera virgifera* the required crop rotation may lead to economic impacts for growers.

In the experiments presented here the population dynamics of *Diabrotica* was studied in isolation cages in the field. It is expected that the results may contribute to a review of the official measures and the further development of damage thresholds for the beetle.

2. Material and methods

Experiments were carried out in cages especially designed for an isolated development of *Diabrotica* beetles in the field. Cages were of 2 m^2 ground size and 2.5 m height. Maize was planted with 20 plants per cage (Figure 1). Maize plants were infested artificially by releasing beetles into the cages (in 2009 equal numbers of male and female beetles). Finally a population density of 0–1–3–5 beetles/plant was established as an artificial first generation. Hatching of beetles in the subsequent generation was recorded on a regular basis within the years following the release of beetles from 2010 to 2012. Plots within the cages usually were dug by spade in autumn 2009 and 2010 and no tillage was applied in 2011.

Fig. 1 Isolation cages sized 1.4 m x 1.4 m x 2.5 m (photo: Johann Robier).

Abb. 1 *Isolierkäfige in der Größe von 1,4 m x 1,4 m x 2,5 m (Foto: Johann Robier).*

3. Results

Table 1 summarizes the results for the number of hatched beetles recorded in the years 2010, 2011 and 2012. The number of beetles after artificial infestation in 2009 initially declined and then remained at low levels in 2011 until in 2012 the population seemed to recover slightly. Damage symptoms like goose neck symptoms with lodging were first visible in 2011. The assessment of root damage according to the traditional IOWA-scale (Hills and Peters) corresponded to the observed population density.

Tab. 1 Population dynamics of the western corn rootworm – recorded number of beetles in isolation cage experiments.

Tab.1 *Populationsdynamik des Westlichen Maiswurzelbohrers – beobachtete Anzahl Käfer in den Versuchen mit Isolationskäfigen .*

	Average number of hatched beetles per cage			Average number of hatched beetles per plant			Goose neck affected maize plants 2012 in%
Release 2009 – beetles/plant	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**	**2012**
0	0	0	2	0	0	0.1	0
1	72	117	119	3.6	5.85	5.95	7.5
3	331	21	164	16.55	1.05	8.2	8.75
5	172	44	125	8.6	2.2	6.25	3.75

No correlation was found between yield and population density. The reasons for this were based on the growing conditions: damage by slugs and a delayed development of reseeded maize in 2011 were mainly responsible for the observed differences in yields.

It is assumed that a damage threshold under Styrian conditions with sufficient rain is higher compared to dry areas and it may be reached with a number of five or even more beetles per plant and season. Large scale observations in commercial maize have shown that damage with root pruning and plant lodging only occurs under dry conditions. Since test year 3 (2011) and in particular within test year 4 (2012), symptoms appeared expectedly at higher densities of hatched beetles/ plant.

Silk clipping symptoms were measurable in 2012 predominantly at higher beetle numbers. These results are not included in the statistics shown in table1.

A few visible and measurable symptoms caused by western corn rootworms were observed in the third and fourth year of the experiments – see figures 2 and 3.

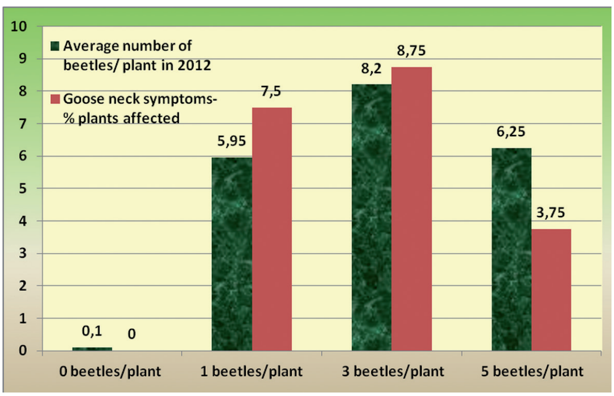

Fig. 2 Number of beetles inserted into cages in 2009 (0-5 per plant), number of beetles hatched and goose neck symptoms in 2012 after mono cropping of maize.

Abb. 2 *Anzahl freigelassener Käfer je Käfig im Jahr 2009 (0-5 je Pflanze), Anzahl geschlüpfter Käfer und Gänsehals-symptome im Jahr 2012 nach Maismonokultur.*

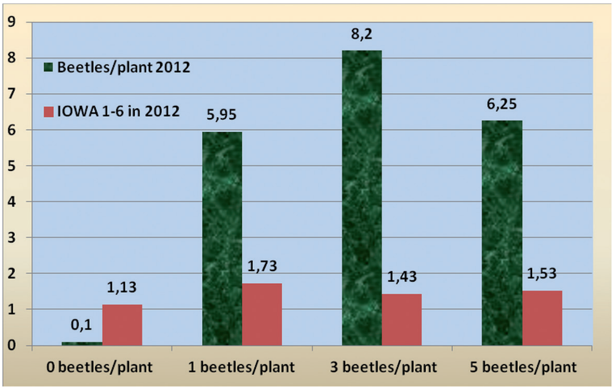

Fig. 3 Number of beetles inserted into cages in 2009 (0-5 per plant), number of beetles hatched and IOWA 1–6 scale rating of root damage in 2012 after mono cropping of maize.

Abb. 3 *Anzahl freigesetzter Käfer je Käfig im Jahr 2009 (0-5 pro Pflanze), Anzahl geschlüpfter Käfer und Wurzelschäden im Jahr 2012 nach Maismonokultur gemäß IOWA Scale (1-6).*

4. Conclusion

The results in 2011 and 2012 (representing the years 3 and 4 after release of beetles) show, that larval damage by root pruning according to the IOWA-Scale (1-6) corresponded well with affiliated population densities present, however did not correlate with populations inserted in 2009. Gooseneck symptoms differed measurably between population densities within the experimental years 2011 and in particular in 2012. Silk clipping damages differed only in 2012. In 2010 and 2011 no significant yield effects with respect to differences between population densities were observed.

From these data of plant damage of *Diabrotica* under the semi-moist conditions in Styria it is very likely that a threshold can be assumed to be five or more beetles per plant.

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Field survival analysis of adult *Diabrotica virgifera virgifera*

Analyse der Überlebensrate von Diabrotica virgifera virgifera *im Feld*

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In order to develop models on the population dynamics of the maize pest *Diabrotica virgifera virgifera*, survival rates of its eggs, three larval instars, pupae and adults need to be known. In contrast to the already studied and reported survival rates for the immature stages of this pest, the survival of *D. virgifera virgifera* adults had not been entirely clarified under field conditions. Particularly the understanding of the likely main period for oviposition of *D. virgifera virgifera* populations in the field (after a pre-oviposition period) would help to plan the timing for interventions by direct control measures against the adults. Therefore, the survival of *D. virgifera virgifera* adults was studied in two field sites in Hungary between 2009 and 2011. Between 8 and 22 large gauze cages (ca. 4 x 2 x 2 m) were placed into each of the two study fields, and about 50 newly emerged female and 50 male adults were released in each cage (usually in mid-July) of each year. Survival was recorded weekly until no beetles were found any more, i.e. usually in September. The populations of adult *D. virgifera virgifera* rapidly decreased with time following an ln-like curve. Male and females had comparable survival patterns. Survival analysis is currently developed and discussed for their use in understanding and modelling population dynamics of *D. virgifera virgifera*.

This study is funded by the Bavarian State Ministry of Food, Agriculture and Forestry.

Differences between independently invading and crossed populations of the alien maize pest *Diabrotica virgifera virgifera*

Unterschiede zwischen unabhängig einwandernden und gekreuzten Populationen des neuen Maisschädlings Diabrotica virgifera virgifera

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The chrysomelid beetle *Diabrotica virgifera virgifera* is an emblematic example of a highly successful invader. From the 1980s until early 2000s, *D. virgifera virgifera* has been introduced several times from North America into Europe resulting in several genetically differentiated outbreaks. A major outbreak currently spreads throughout central and southeastern Europe and another one throughout north-western Italy.

This study aimed to compare the neutral genetic as well as phenotypic traits between pure and crossed *D. virgifera virgifera* populations in order to understand the role of genetic variability in shaping phenotypes in *D. virgifera virgifera* and its invasion success. There are only very few hints that crossed invading populations may be different from independently invading or from source populations. Usually, the traits of the crossed populations appeared similar or the average of those of the parental populations. However, the overall phenotypic coefficient of variation of all measured traits was slightly larger in crossed populations than in parental populations. Also the allele richness was found to be slightly elevated in crossed populations compared with the parental populations (not proven for heterozygosity).

The found slightly higher variability in crossed populations may increase adaptability and may therefore render the invasion of this alien maize pest in Europe more successful and therewith more problematic. However, also the independently invading populations are highly invasive despite their comparatively lower genetic and phenotypic variability than the crosses of invading populations or than the source populations. Conclusively, there might exist more relevant factors behind an invasion success than variability, e.g. the lack of specific natural enemies (enemy release theory).

This research was funded by the French "l'Agence Nationale de la Recherche " (ANR-06-BDIV-008).

Session 3: Plant protection control in eradication and containment regions

On the influence of different host plants and of insecticide treatments on the population development of the western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)

Zum Einfluss verschiedener Wirtspflanzen und Insektizid Behandlungen auf die Populationsentwicklung des Westlichen Maiswurzelbohrers Diabrotica virgifera virgifera *LeConte (Coleoptera: Chrysomelidae)*

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Summary

D. virgifera virgifera is classified as a quarantine pest in Germany, therefore the trials, presented in this paper, were performed in the western part of Romania, where the pest is well established since more than ten years. The field tests were carried out in close collaboration with Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara.

On sites highly infested by *D. virgifera virgifera,* 1 m^2 plots in four replications per variable were planted with maize for the chemical treatments and alternative crops (cereals and weeds). Gauze covered hatch cages were used for weekly counts of the emerged adult *Diabrotica* during the hatch period from mid of June to mid of August.

It could be asserted that matured cereals are no host-plants for the western corn rootworm. In four years of trials not even one adult beetle hatched in the related cages.

Setaria viridis and *Digitaria sanguinalis* reduced the number of hatched imagines significantly, nevertheless some individuals survived. Therefore an effective herbicide management against grass weeds in maize is recommended to limit the chance of survival of the pest.

Clothianidin and Tefluthrin are effective against *D. virgifera virgifera*. 20% to 100% efficiency was assessed in the trials, strongly depending on precipitation and soil moisture in time of application. The insecticides decreased the maize root injury caused by larvae of the western corn rootworm significantly.

Keywords: Insecticides, crop rotation, host-plants, hatch cages

Zusammenfassung

Da der Westliche Maiswurzelbohrer in Deutschland noch als Quarantäneschädling eingestuft wird, wurden die Untersuchungen in Westrumänien durchgeführt, wo der Schädling bereits seit mehr als 10 Jahren etabliert ist. In Zusammenarbeit mit Banat's University of Agricultural Sciences and Veterinary Medicine, Timisoara, erfolgte die Anlage und Auswertung der Feldversuche.

Auf Versuchsflächen mit hohem *Diabrotica* Besatz wurden 1 m^2 große Parzellen (in vierfacher Wiederholung) mit Mais, für die Durchführung der Insektizidanwendungen, sowie mit alternativen Kulturen (Getreide und Ungräser) bestellt. Schlupfkäfige, die über die Parzellen gestellt wurden, dienten der wöchentlichen Zählung der aus dem Boden schlüpfenden Käfer während der Schlupfperiode, von Mitte Juni bis Mitte August.

Es konnte festgestellt werden, dass die weit entwickelten Getreidearten keine Wirtpflanze für den Westlichen Maiswurzelbohrer darstellten. In den vierjährigen Versuchen konnte kein Käfer in den entsprechenden Käfigen gefunden werden.

Setaria viridis and *Digitaria sanguinalis* reduzierten die Anzahl der gefangenen adulten *D. virgifera virgifera* signifikant, einige Exemplare überlebten jedoch auch. Eine wirksame Ungras Bekämpfung im Mais und den nachfolgenden Kulturen wird deshalb empfohlen um die Überlebensmöglichkeiten für *D. virgifera virgifera* zu begrenzen.

Clothianidin und Tefluthrin reduzierten die Anzahl der schlüpfenden Imagines. Wirkungsgrade von 20% bis 100% konnten ermittelt werden, weitgehend unabhängig von der Formulierung. Dabei war die Wirksamkeit der Insektizide sehr stark abhängig von der Bodenfeuchte bei und kurz nach der Anwendung. Die eingesetzten Präparate verminderten die Schäden an den Maiswurzeln signifikant.

Stichwörter: Insektizide, Fruchtfolge, Wirtspflanzen, Schlupfkäfige

1. Introduction

The western corn rootworm is one of the most destructive pests in maize. Since 2007 *D. virgifera virgifera* is present in Germany. In order to improve and adapt control measures, including chemical and non chemical options for German conditions a research program was generated in 2008, funded by the German Federal Ministry of Food, Agriculture and Consumer Protection and the Bavarian State Ministry of Food, Agriculture and Forestry. This study is part of this program.

As the population density of the western corn rootworm in Germany was not high enough to achieve reliable results, trials were performed in countries where the pest was already well-established. It was supposed that the findings could be applied to Bavarian conditions in this case.

The influence of insecticidal treatments on the population development of *D. virgifera virgifera* was often tested in different countries in previous years. A summary of elder investigations in the USA is given by van Rozen and Ester (2007). Results from seed dressing experiments to control *Diabrotica* larvae were obtained from trials in Hungary e.g. (Tóth and Hatala Zsellér, 2007) and Italy (Breitenbach *et al.*, 2007).

The aim of the study presented here was to confirm the well-known results from other countries and to test the insecticides already commercially available in Germany at that time, or those which were expected to be registered soon by quantifying the impact of potential host-plants and chemical treatments on the *Diabrotica* populations.

Crop rotation is a common measure to contain the western corn rootworm population in quantity and spreading (Bertossa *et al.*, 2009). Many studies were carried out to get information about other host-plants than corn. In laboratory tests it was shown that *Diabrotica* larvae even developed on barley, wheat and rice (Branson and Ortman, 1967, 1970). On the other hand it was indicated that the insect did not develop on wheat under field conditions (Breitenbach *et al.* 2005, 2006 and 2008).

The main goal of the trials performed from 2009 till 2012 in Romania was to confirm the results on the host-plant suitability of wheat and barley. Additionally two grass weeds (*Setaria viridis* and *Digitaria sanguinalis*) were also included in the tests.

2. Material and methods

On a field naturally infested with *D. virgifera virgifera,* small plots of 1 m^2 ground surface, were planted with maize (for the chemical treatments) and with cereals or weeds (for the host-plant suitability test).

Hatch cages as described in Tab. 1, were set up on the plots (see also Fig.1 and Fig. 2). A yellow sticky trap was placed inside the cages.

Tab. 1 Description of the hatch cages used in the test.

Tab. 1 *Beschreibung der verwendeten Schlupfkäfige* .

Soil-surface area	1.00 m^2 (1.00 m x 1.00 m)
Height	1.40 m
Timber frame	1.06 m x 1.06 m (outside), 0.15 m height; (thereof 0.13 m below soil-surface). Since 2010 the timber frame was replaced by a ferrule (0.05 m high)
Clothing	Gauze, mesh width 0.2 mm
Timber pile	0.08 m diameter, 1.80 m high (thereof 0.40 m below surface), in the center
Opening	1.0 m length, closed by hook-and-pile fastener

Weekly counts of hatched adults in the cages were carried out throughout the hatching period between mid of June and mid of August.

Fig. 1 Maize plot with hatch cage.

Abb. 1 *Maisschlag mit Schlupfkäfig.*

Fig. 2 Trial design (hatch cages with quadratic width).

Abb. 2 *Versuchsdesign (Schlupfkäfige mit quadratischem Grundriss).*

The assessment of the maize-root damage caused by *D. virgifera virgifera* larvae was performed by the end of August or beginning of September. To describe the intensity of destruction the Root-Node-Injury Scale was applied (Oleson *et al.,* 2005), 0=no injury, 3=roots totally damaged.

The trial plan is shown in Tab. 2

Tab. 2 Trial plan.

Tab. 2 *Versuchsplan.*

Entry	Crop	Product	Active Ingredient	Formulation	Year 2009	2010	2011	2012
1	Maize	untreated			X	X	X	X
2	Maize	Poncho Pro	Clothianidin 62.5 g/unit	seed dressing	X	X	X	X
3	Maize	Force 1.5 G	Tefluthrin 180.0 g/ha	granulate	X	X	X	X
4	Maize	Santana	Clothianidin 84.0 g/ha	granulate	-	X	X	X
5	Winter wheat				X[1)]	-	X	X
6	Spring barley				X	X	X	X
7	*Setaria viridis*				-	X	X	X
8	*Digitaria sanguinalis*				-	X	X	X

1)=spring wheat, -)=variant not applied

3. Results

The number of adult *D. virgifera virgifera* fluctuated significantly from year to year (Fig. 3). Due to strong precipitation in spring 2010 (end of May > beginning of June) the test site was partly flooded during several days. The *Diabrotica* population decreased significantly. As no sufficient number of beetles could be expected for the next season, the trial was moved to another field, where the western corn rootworm could build up an adequate population even in 2010.

In the cereal plots no adult *Diabrotica* was found. *S. viridis* and *D. sanguinalis* reduced the number of hatched imagines significantly, nevertheless some individuals survived (Fig. 3).

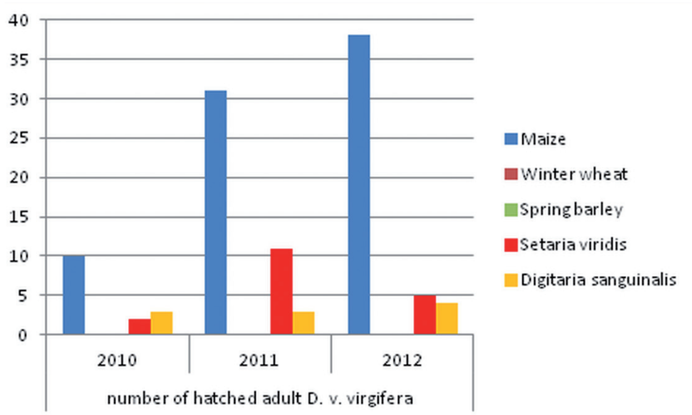

Fig. 3 Number of hatched adult *D. virgifera virgifera* per entry throughout the whole hatch period.

Abb. 3 *Anzahl gefangener adulter* D. virgifera virgifera *je Variante während des gesamten Fangzeitraums.*

The efficiency of the insecticides included in the test varied between 20–100%. In the trials presented, the seed dressing was slightly more effective than the granulate formulations (Fig. 4).

Dry periods after sowing reduced the insecticidal efficacy significantly, more or less independent of the formulation. The insecticide treatments reduced the damage to maize roots (Fig. 5).

A linear correlation between the number of emerged adult *Diabrotica* and maize root damage could not be confirmed (r^2=0,036); possibly, the sample size was too small to perform a proper analysis.

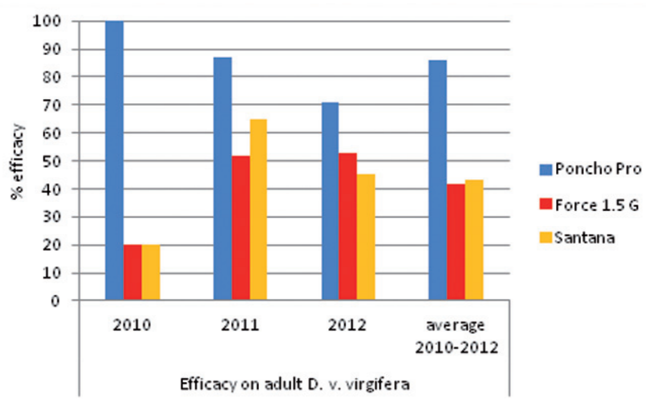

Fig. 4 Efficacy of insecticide treatments to adult *D. virgifera virgifera* with regard to the number of imagines in the hatch cages.

Abb. 4 *Die Wirkung von Insektitizidbehandlungen gegen adulte* D. virgifera virgifera *anhand der Anzahl der Imagines in den Fangkäfigen.*

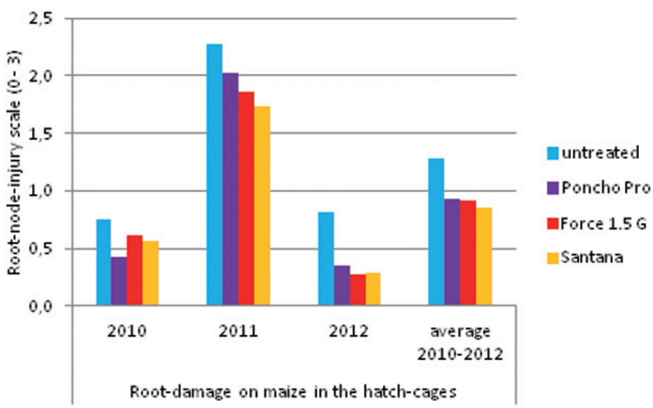

Fig. 5 Impact of insecticide treatments on maize root damage in the hatch cages.

Abb. 5 *Der Einfluss von Insektizidbehandlungen auf Maiswurzelschäden in den Fangkäfigen.*

4. Discussion

The number of adult *D. virgifera virgifera* fluctuated significantly from year to year. Due to strong precipitation in spring 2010 (end of May > beginning of June) the test site was partly flooded during several days. A survey performed in Kansas (Riedell and Sutter, 1995) showed, that water saturated soils during egg hatch can adversely impact western corn rootworm populations and subsequent larval feeding damage. It is well known, that the egg hatch period in Romania is in accordance with the time when the high precipitation happened.

On the other hand a study performed in Austria (Grabenweger *et al.*, 2010) shows, that the physical soil characteristics like permeability for water and air affect the mobility of *D. virgifera virgifera* larvae. Reduced mobility increases the possibility of drying up. That's what took place in the years 2011 and 2012, when it was extremely dry between June and September.

In the cereal plots no adult *Diabrotica* was found.

It has to be considered, that in Romania spring barley and winter wheat are already widely matured mid of June (harvest time is end of June/beginning of July). Therefore they may no longer be suitable to feed the western corn rootworm. This could be different in case of volunteer cereals. Several studies show that the insect did not develop on wheat under field conditions (Breitenbach *et al.*, 2005, 2006 and 2008). Cereals are an important part in the crop rotation to contain the *D. virgifera virgifera* population (Breitenbach *et al.*, 2006). This is also widely confirmed by practical experiences in countries with high *Diabrotica* infestation.

S. viridis and *D. sanguinalis* reduced the number of hatched imagines significantly, nevertheless some individuals survived. Therefore an effective herbicide management against grass weeds in maize is recommended to limit the chance of survival of the pest. These findings are confirmed by former studies performed in the same area in Romania (Breitenbach et. al, 2005, 2008).

The efficiency of the insecticides included in the test varied between 20 – 100%. In the trials presented, the seed dressing was slightly more effective than the granulate formulations.

Dry periods after sowing reduced the insecticidal efficacy significantly, more or less independent of the formulation. The insecticides decreased the maize root injury caused by larvae of the western corn rootworm significantly.

These findings are confirm with studies from Italy (Breitenbach et. al, 2007), which showed an efficacy of Clothianidin seed dressing of about 50% against hatched adult *D. virgifera virgifera*. The active ingredient was used in two concentrations but they showed similar results. Maize-root damages could obviously be reduced. The author stated that, regarding a low to moderate infestation with western corn rootworm seed dressing could be a suitable method within an IPM-strategy to control or to reduce the population.

In fields highly infested with *D. virgifera virgifera* in Yugoslavia (Tóth and Hatala Zseller, 2007) and Hungary (Ripka *et al.*, 2007) insecticidal seed treatments with Clothianidin were not able to reduce the damage caused by western corn rootworm larvae under the economic threshold (<3 of Iowa scale value).

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Biological control of the western corn rootworm (*Diabrotica virgifera virgifera*) by entomoparasitic nematodes

*Biologische Bekämpfung des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera*) mit entomoparasitischen Nematoden*

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1. Introduction

Entomoparasitic nematodes of the species *Heterorhabditis bacteriophora* are known to be highly virulent against larval stages of the western corn rootworm (WCR) under controlled laboratory conditions (Töpfer, 2005). However, a practical and sustainable plant protection strategy has to be efficient also under various field conditions. In addition the application of nematodes has to be easy to practice in order to be accepted by the farmers. Therefore only those application methods were used in our experiments that can be carried out in one step together with sowing of maize grains. To show the reliability of such control measures several field experiments were designed between 2009 and 2011 in the framework of the German Diabrotica research program (http://diabrotica.jki.bund.de/).

2. Material and Methods

During this project several field experiments were carried out in Deutsch Jahrndorf - 4 of them are reported here. The location is situated in the most Eastern part of Austria (17°-6'-4" N – 48°-0'-3" E) and exhibits a long history of WCR occurrence and monoculture of irrigated maize that leads to high natural pest populations. The region shows pannonian climate which is characterized by high summer temperatures and low precipitation. Preliminary experiments had shown a clustered distribution of WCR eggs in the field. To ensure even numbers of WCR larvae experimental plants were infested artificially with WCR eggs at various rates. These eggs had been derived from adults, caught in the former summer season and held under laboratory conditions with nutrition and soil for egg deposition. To ensure natural winter conditions, the oviposition substrate containing the eggs was buried in autumn at the experimental field site. After hibernation, the substrate was brought back to the lab and the eggs counted in subsamples to estimate their number per g of substrate. The egg containing soil was then applied in a furrow 20 cm apart from 6 maize plants in the center of each plot, at rates from 0 to 3000 eggs per plant.

The insect parasitic nematodes belonging to the species *Heterorhabditis bacteriophora*, were produced by e-nema GmbH, Schwentinental, Germany, and were applied as granules, as liquid formulations or as seed dressings in application rates ranging from 1.3×10^9 to 2.7×10^9 infective juveniles per ha.

Each plot had a size of 200 m^2. The experiments included a Clothianidin-treated control [25 g a.i./50 000 grains or in 50 g a.i./ha in granules], an untreated control and were carried out in five replications each. Efficacy of the treatment was evaluated by recording the emergence of adults in weekly intervals in cages put over the infested plants at the beginning of adult hatch and by rating the damage of maize roots according the "node injury scale" (Oleson *et. al.*, 2005) in August. Additionally the persistence of applied nematodes was evaluated at different times after the application. For this purpose *Tenebrio molitor* - larvae were buried in mixed soil samples for each plot and used as living baits. After storage for one week at 25 °C, infested larvae were submersed in water for validation. The statistical analysis of the experiments included ANOVA and was carried out with the help of SPSS.

3. Results and Discussion

In the experiments described herein the maize plants were infested artificially with various numbers of WCR eggs per plant. Fig. 1 shows the number of hatched beetles in untreated control groups as a function of numbers of infested eggs. The infestation in the experiment Hutweide 2009 gave extraordinary good results (datapoints marked with X in Fig. 1) – yet it is very likely that additional natural infestation may have played a major role in this case. The maximum number of beetles developing on a plant after artificial infestation appears as the upper limitation of the datapoints in Fig. 1. Up to 100 hatched beetles per plant could be achieved by the herein described artificial infestation under good conditions. The optimum number of WCR eggs per plant for infestation seems to be 300-400. It was not possible to achieve more hatching adults than 100 beetles per plant by infesting the maize plants with high numbers of 1000 or 3000 WCR eggs. It is very likely that this was the result of behavior of larvae which avoid such overcrowded roots or of density dependent mortality caused e.g. by predators like the staphylinid beetle *Platystethus sinosus* which feeds frequently on WCR eggs (Friedl S., pers. Communication, 2009).

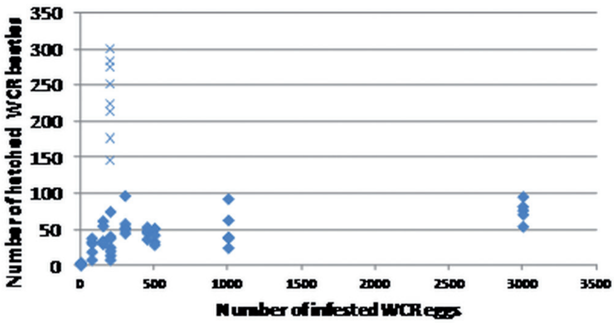

Fig.1 Number of hatched WCR beetles per plant in several untreated control groups after artificial infestation with various numbers of eggs. These pooled results are derived from various experiments in Deutsch Jahrndorf between 2009 and 2011. Datapoints marked with "X" refer to an experiment (field site Hutweide 2009) with artificial and natural infestation.

Abb. 1 *Anzahl geschlüpfter WCR-Käfer je Pflanze in mehreren unbehandelten Kontrollgruppen nach künstlicher Freisetzung unterschiedlicher Mengen von Eiern. Die Ergebnisse wurden aus unterschiedlichen Versuchen in Deutsch Jahrndorf zwischen 2009 und 2011 abgeleitet. Die mit einem „X" gekennzeichneten Datenpunkte beziehen sich auf einen Versuch (Standort Hutweide 2009) mit künstlichem und natürlichem Befall.*

The evaluation of efficacy was carried out by counting hatched beetles and by rating of damaged maize roots. Whereas exact results were achieved only by adult counts (time consuming), the root ratings were less time consuming, were not so exactly and were carried out mainly to facilitate comparison with experimental work of other authors. The results of these 4 experiments may be seen from Fig. 2, Fig. 3, Fig. 4 and Fig. 5:

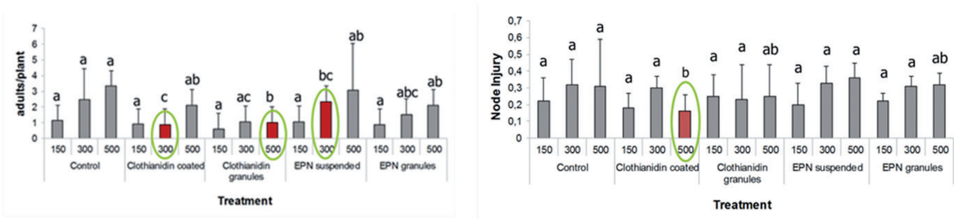

Fig. 2 The effect of the application of entomoparasitic nematodes (EPN, 1.3 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with various numbers (150, 300, 500) of WCR eggs per plant. The experiment was carried out in the field sites Karlhof (sports ground) and Fischwasser in 2010.

***Abb. 2** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 1,3 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung unterschiedlicher Anzahlen (150, 300, 500) von WCR-Eiern je Pflanze. Die Versuche wurden an den Standorten Karlhof (Sportplatz) und Fischwasser im Jahr 2010 durchgeführt.*

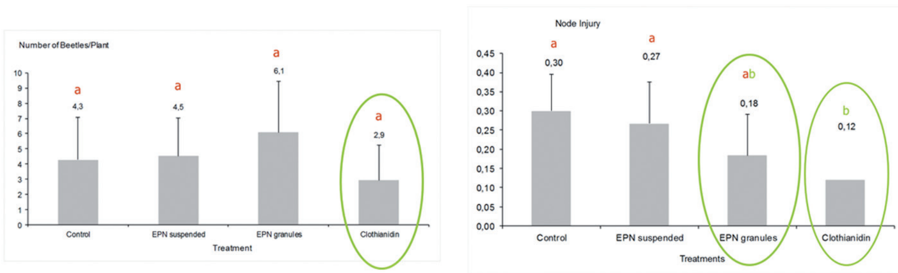

Fig. 3 The effect of the application of entomoparasitic nematodes (EPN, 1.3 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with 300 WCR eggs per plant. The experiment was carried out in the field site Karlhof (Sportplatz) in 2010.

***Abb. 3** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 1,3 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung von 300 WCR-Eiern je Pflanze. Der Versuch wurde am Standort Karlhof (Sportplatz) im Jahr 2010 durchgeführt.*

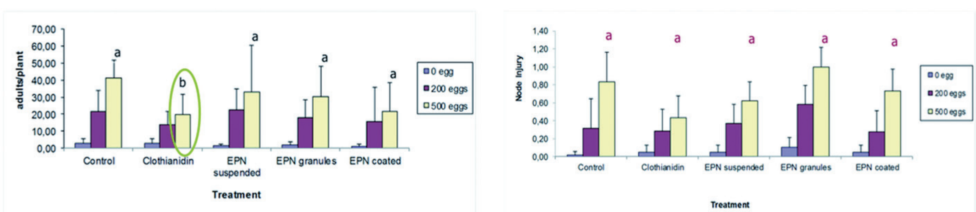

Fig. 4 The effect of the application of entomoparasitic nematodes (EPN, 2 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) after artificial infestation with various numbers (0, 200, 500) of WCR eggs per plant. The experiments were carried out in the field site Fischwasser in 2011.

***Abb. 4** Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 2 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach künstlicher Freisetzung unterschiedlicher Anzahlen (0, 200, 500) von WCR-Eiern je Pflanze. Die Versuche wurden am Standort Fischwasser im Jahr 2011 durchgeführt.*

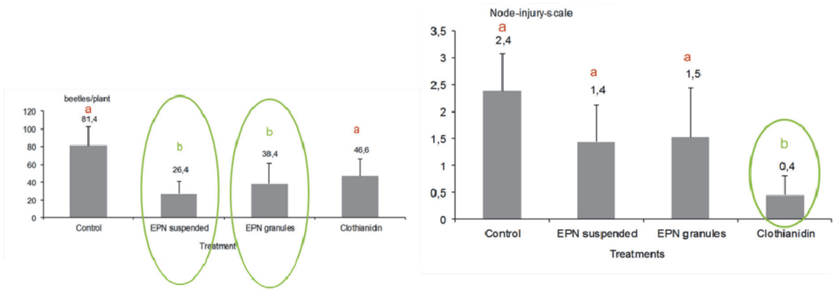

Fig. 5 The effect of the application of entomoparasitic nematodes (EPN, 2.7 x 10^9 *Heterorhabditis bacteriophora* per ha) on the hatching of adult WCR beetles (left diagram) and on corn roots (right diagram) under natural infestation and additional artificial infestation with 300 WCR eggs per plant. The experiment was carried out in the field site Hutweide in 2009.

Abb. 5 *Einfluss der Anwendung entomoparasitischer Nematoden (EPN, 2,7 x 10^9* Heterorhabditis bacteriophora *je ha) auf den Schlupf adulter WCR-Käfer (Diagramm links) und auf Maiswurzeln (Diagramm rechts) nach natürlichem und künstlichem Befall mit 300 WCR-Eiern je Pflanze. Der Versuch wurde am Standort Hutweide im Jahr 2009 durchgeführt.*

Clothianidin showed good efficacy against WCR larvae (according to adult hatch and root-rating). The application of EPNs during sowing leads to varying degrees of efficacy (according to adult hatch) ranging from poor to very good results. This variation was partially due to formulation type or application rate of the EPNs. Suspension of Nematodes in water showed high efficacy in some experiments (Fig. 5). So did granular formulations and seed coating (Fig. 4) in other cases. High concentrations of EPNs (2.7 x 10^9/ha) applied during sowing of the maize reduced the numbers of hatched adults significantly and reduced also root damages (Fig. 5). They showed equal efficacy in respect to hatched adults as Clothianidin treated seeds (Fig. 3). Low concentrated EPNs (1.3 x 10^9/ha), irrespective of their formulation were inappropriate for an efficient control (hatched beetles) in 2010. Further trials (Fig. 2, Fig. 4) with a moderate concentration (2 x 10^9/ha) lead to similar results.

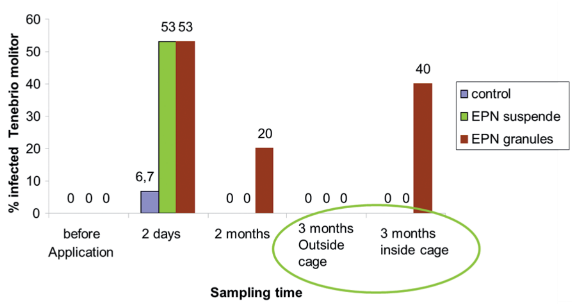

Fig. 6 The persistence of *Heterorhabditis bacteriophora* during the field experiment (Karlhof (sports ground), Fischwasser , 2010) was evaluated by exposition of Tenebrio molitor larvae in mixed soil samples from the field sites in the laboratory at 25 °C for one week.

Abb. 6 *Die Überlebensfähigkeit von* Heterorhabditis bacteriophora *im Freilandversuch (Karlhof (Sportplatz) und Fischwasser, 2010) wurde bewertet, indem Larven von* Tenebrio molitor *in Bodenmischproben von den Standorten im Labor für eine Woche bei 25 °C gehalten wurden.*

The persistence of EPN nematodes (Fig. 6) applied during sowing in April 2010 in the field sites Fischwasser and Karlhof, 2010 were sufficient to ensure parasitation of WCR larvae which lived approximately 6 weeks later. The entomoparasitic nematodes were able to multiply within the larvae and gave rise to another generation which could be detected 3 months after the initial release. This part of the project work has already been published separately (Pilz *et al.,* 2012).

4. Conclusions

Entomoparasitic nematodes in high concentrations of about 2.7 x 10^9 EPN/ha were able to reduce the emergence of adult corn rootworms in a significant way, regardless of the nematode formulation. Seed treatment with Clothanidin showed best results with respect to the prevention of root damage (according to node injury scale) in most experiments. A total eradication of WCR larvae was not possible - neither by EPNs nor by Clothianidin. None of the tested measures showed influence on the yield: this was most probably the result of irrigation used on the experimental sites, which enabled maize plants to compensate the loss of roots. Evaluation of the persistence of applied nematodes showed that they were able to survive as long as WCR-larvae were present in the soil despite their early application at sowing time.

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Successful application of entomopathogenic nematodes for the biological control of western corn rootworm larvae in Europe – a mini review

Erfolgreiche Applikation entomopathogener Nematoden zur biologischen Bekämpfung des Westlichen Maiswurzelbohrers in Europa – ein Mini-Review

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Summary

10 years of joint efforts in research and development have led to a nematode-based biological control solution for one of the most destructive maize pests, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae). Commercially mass-produced *Heterorhabditis* species of beneficial entomopathogenic nematodes are ready to use. They can be applied into the soil during sowing of maize for controlling the subsequently hatching larvae of *D. virgifera virgifera* thus preventing root feeding and damage to maize. Policy bodies, decision makers and farmers are advised to consider biological control as one of the alternatives to synthetic pesticides in maize production, and according to the EC Directive on the sustainable use of pesticides and implementation of integrated pest management.

Key words: *Diabrotica virgifera virgifera;* Chrysomelidae; insect parasitic nematodes; inundative biological control; *Zea mays*

Zusammenfassung

Nach 10 Jahren gemeinsamer Anstrengungen und Entwicklungen wurde eine biologische nematodenbasierte Möglichkeit zur Bekämpfung eines der gefährlichsten Maisschädlinge, des Westlichen Maiswurzelbohrers *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), gefunden. Nützliche entomopathogene Nematoden von *Heterorhabditis*-Arten wurden in Massenproduktion hergestellt und stehen zur Anwendung bereit. Sie können mit der Maisaussaat in den Boden eingebracht werden und dann die schlüpfenden Larven von *D. virgifera virgifera* bekämpfen und so Wurzelfraß und Maisschäden verhindern. Wir empfehlen politischen Gremien, Entscheidungsträgern und Landwirten die biologische Bekämpfung als eine Alternative zu synthetischen Pflanzenschutzmitteln im Maisanbau und entsprechend EU-Richtlinie über die nachhaltige Anwendung von Pflanzenschutzmitteln und die Umsetzung des integrierten Pflanzenschutzes zu prüfen.

Stichwörter: *Diabrotica virgifera virgifera*; Chrysomelidae; insektenparasitäre Nematoden; inundative biologische Schädlingsbekämpfung; *Zea mays*

1. Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most destructive pests of maize in North America (Levine and Oloumi-Sadeghi, 1991). It is a univoltine species with eggs that overwinter in the soil (Chiang, 1973). After maize has germinated, the eggs hatch, and its three larval life stages feed on maize roots, often causing plant lodging and yield losses. Adults can occasionally reduce yields through intensive silk feeding.

Over the last 25 years, *D. virgifera virgifera* has moved into Europe causing problems in maize (Cioisi *et a*l., 2008; Ciosi *et al*., 2011; Szalai *et al*., 2011). In an effort to eradicate or contain the species, legislation has been put into place, which forces farmers to rotate their fields (thereby interrupting the life cycle of *D. virgifera virgifera*) and to apply granular soil insecticides or to use insecticide-coated maize seeds (to target the root feeding larvae) (Carrasco *et a*l., 2010; DLZ, 2011). Additionally, broad-spectrum foliar insecticides are sprayed against the adults using high clearance spraying machinery (Rozen and Ester, 2010). However, the soil insecticides and seed coatings are potentially problematic due to non-target effects resulting in public concerns and in a partial ban on their use in maize (Gill *et al.,* 2012; Cressey, 2013). Also, the synthetic foliar insecticides are usually broad spectrum knock-down contact pesticides with considerable non-target effects. In an attempt to reduce insecticide usage against this invader, biological control options have been recommended for this pest (Babendreier *et al*., 2006; DLZ, 2011; Knuth *et a*l., 2011), and for European maize production in general (European Commission, 2009).

10 years of joint efforts in research and development for a nematode-based biological control solution are reviewed here and outcomes are presented.

2. Material and methods

Between 2004 and 2008, CABI, the University of Neuchâtel in Switzerland, the farmer association fenaco (UFA-Samen Beneficials) in Aesch in Switzerland, the Plant Protection Directorate in Hodmezovasarhely in Hungary, Agroscope Reckenholz-Tänikon in Switzerland, the University of Kiel in Germany, and the nematode producers e-nema at Schwentinenthal in Germany and Andermatt Biocontrol at Grossdietswil in Switzerland, laid the scientific base for nematode-based biological control products against *D. virgifera virgifera* (CABI, 2008). Between 2007 and 2008, a number of institutions reviewed biological control options against rootworms and proposed them to the European Commission (Diabr-Act, 2007; CABI, 2008). Between 2009 and 2012, the Landwirtschaftliches Technologiezentrum Augustenberg, Karlsruhe in Germany, Cult-tec Ltd. in Freiburg in Germany, the Austrian Agency for Health and Food Safety in Vienna in Austria, the Cereal Research Station in Szeged in Hungary, SAGEA Centro di Saggio S.r.l. in Italy, CABI, and others improved application technologies aiming for the farmer-friendliest and least-costly method (CABI, 2012).

3. Results and discussion

Review of biological control options against *D. virgifera virgifera*

Several natural enemy species or groups appeared promising candidates for control strategies with different ecological rationales. Research was proposed to pursue: (1) developing inundative biological control products, particularly mass-produced entomopathogenic nematodes and fungi, (2) understanding specific natural enemies of Diabroticina larvae throughout the Americas and of adults particularly in higher altitudes of Central America or northern South America including potential classical biological control agents against *D. virgifera virgifera*; (3) enhancing natural enemies through cultural practices, i.e. reduced tillage, reduced weed control, cover crops, diversified crop rotations or soil amendments. For complete lists of natural enemies of Diabroticina and further details refer to: Kuhlmann and Burgt, 1998; Diabr-Act, 2007; Toepfer *et al*., 2009; Pilz *et al.,* 2008).

Nematode screening in laboratory

Screenings experiments in Petri dishes on filter paper or in sand, as well as bioassays in containers with sand or soil and maize revealed that *Heterorhabditis bacteriophora, H. megidis, Steinernema feltiae, St. arenarium,* and *St. kraussei* are highly virulent against *D. virgifera virgifera* larvae. *St. abassi* was found intermediate. *St. carpocapsae* and *St. glaseri* appeared less virulent. For details refer to: Journey and Ostlie, 2000; Jackson, 1995; Toepfer *et al*., 2005, Kurtz *et al.,* 2009; Hiltpold *et al.,* 2009.

Nematode screening in the field

Plant scale field experiments with artificial *D. virgifera virgifera* infestation and into-soil applications of fluids of different nematode species during sowing in April or into and onto soil later in June, revealed that *H. bacteriophora* and *H. megidis* are highly effective against *D. virgifera virgifera* larvae (i.e. up to 81%), and in preventing damage to maize roots (i.e. up to 80%) (Fig. 1). *S. feltiae* appeared moderately effective. For comparisons with pesticides and further details refer to: Jackson, 1996; Jackson, 1997, Toepfer *et al.,* 2008; Pilz *et al*., 2009; Hitpold *et al*., 2009.

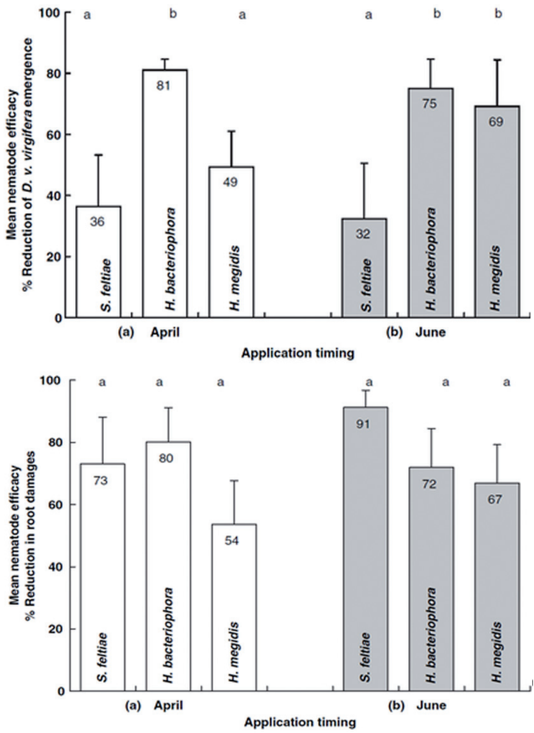

Fig. 1 Results extracted from Toepfer *et al*. (2008) on the efficacy of entomopathogenic nematodes in plant scale field trials in southern Hungary at reducing *D. virgifera virgifera* and at preventing root damage. Percent reduction of adult emergence and root damage (Node Injury scale-based) in comparison to the controls; application of 0.21 -0.26 Mill nematodes per row meter (ca. 2.8 – 3.5 x 10^9/ha) as a solid stream into the soil during sowing of maize (A) or onto the soil and along rows of young maize plants (B); Letters on *D. virgifera virgifera* bars indicate differences according to the LSD PostHoc test after ANOVA; letters on root damage bars indicate differences according to the M - Witney U test; Error bars=SEM).

Abb. 1 *Ergebnisse aus Töpfer et al. (2008) zur Wirksamkeit entomopathogener Nematoden in Feldversuchen in Südungarn zur Bekämpfung von* D. virgifera virgifera *und der Verhinderung von Wurzelschäden. Prozentuale Verringerung der Adulten und von Wurzelschäden (anhand der Knotenverletzung) im Vergleich zur Kontrolle; Anwendung von 0,21-0,26 Mio Nematoden je Reihenmeter (ca. 2,8 – 3,5 x 10^9/ha) als Flüssigkeit in den Boden während der Maisaussaat (A) oder auf den Boden entlang der Reihen junger Maispflanzen (B); die Buchstaben an den* D. virgifera virgifera*-Säulen zeigen Unterschiede gemäß LSD Post-Hoc-Test nach ANOVA; die Buchstaben an den Wurzelschädensäulen zeigen Unterschiede gemäß M – Witney U-Test; Standardfehler = SEM).*

3.1 Scientific pre-requisites

Instar susceptibility of target

Bioassays with different life stages of *D. virgifera virgifera* and different nematodes revealed that all larval instars and even pupae are effectively killed by *H. bacteriophora*, *H. megidis* and *St. feltiae*. Adults appeared less susceptible. For details refer to: Kurtz *et al*., 2009.

Orientation of nematodes to target

Nematodes were found to orient towards *D. virgifera virgifera* -damaged maize roots using the root-emitted organic volatile compound (E)-β-caryophyllene as an orientation cue to find *D. virgifera virgifera* larvae. Caryophyllene might be particularly important for *H. megidis* and less for *H. bacteriophora*. Other authors mention that caryophyllene is of little to no importance for nematodes. For details refer to: Rasmann *et al*., 2005; Hiltpold and Turlings, 2008; Kneight, 2010; Anbesse and Ehlers, 2013.

Maize hybrid importance

There are hardly any hints that the choice of maize hybrids is important for biological pest control with entomopathogenic nematodes. Some hybrids have lost the capability to emit the nematode-attracting (E)-β-caryophyllene; however, most European maize hybrids emit caryophyllene upon larval feeding. For details refer to: Rasmann *et al*., 2005; Hiltpold and Turlings, 2008; Hiltpold, 2010.

Establishment and persistence of nematodes

Field experiments revealed that applied nematodes establish at relatively low rates in the soil of maize fields; but, that they survive more than two months, which is long enough to effectively kill all three larval instars. Nematodes were found to propagate on *D. virgifera virgifera* larvae in the field, which is a big advantage over pesticides. For details refer to: Kurtz *et al*., 2007; Pilz *et al*., 2011a.

Soil importance

Field trials showed that *H. bacteriophora* can effectively kill *D. virgifera virgifera* larvae in a wide range of soils in maize fields. As *D. virgifera virgifera* larvae are usually most damaging in dense soils, control efficacies of nematodes were also often found higher in dense soils than in light, e.g. sandy, soils. For details refer to: Grabenweger *et al.,* 2010; Toepfer *et al*., 2010d; Pilz *et al.,* 2011a.

Non-target effects

Entomopathogenic nematodes are restricted to arthropods, thus there is no danger to plants or humans. Nematodes are known to be only slightly host specific on certain insect groups. However, field experiments revealed only minor effects of treatments on non-target populations, suggested to be a result of the generally poor arthropod diversity in soils of intensive field crops such as maize, as well as of the application of nematodes into relatively narrow soil areas and close to the target. For details refer to: Georgis *et al.,* 1991; Gaugler, 2002; Babendreier *et al*., 2014.

3.2 Application of nematodes

Where

Nematodes were successfully applied through fluid solid stream sprays, microgranules or seed coating into soil at sowing, or through fluid solid stream sprays or granules into soil next to young maize plants, or through fluid narrow flat sprays applied with lots of water over rows of small plants. For details and more options refer to: Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c; Knuth *et al*., 2011; Hiltpold *et al*., 2012.

When

Nematodes were successfully applied into soil at sowing (Mid April to early May in Central Europe), this is, a few weeks before *D. virgifera virgifera* eggs hatch; as well as into or onto soil along rows of young maize plants (mid to late May in Central Europe). Field applications against adults, i.e. in July or August, have never been attempted. For details refer to: Toepfer *et al.,* 2010c.

Formulation

Nematodes can be applied against *D. virgifera virgifera* larvae preferably just diluted in water. Microgranules, seed coatings, capsules and other options may need further research. For details refer to: Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c; Knuth *et al*., 2011; Hiltpold *et al*., 2012.

Need of water

Field experiments revealed that the need of water during application is variable and depends on the soil type, wheather conditions, and application techniques. Currently a minimum of 200 to 400 litres water/ha are advised for fluid stream sprays of nematodes into soils, and a minimum of 800 to 1000 litres for narrow stream sprays onto the soil or plants. Details through SAGEA Centro di Saggio S.r.l. (2010, pers. comm.) or in Toepfer *et al*., 2010a; Toepfer *et al*., 2010b; Toepfer *et al.,* 2010c.

3.3 Farmer friendly application techniques

Fluid and micro-granular applications as well as seed coating with nematodes appeared technically possible with the available farmer machineries; and all can achieve control of *D. virgifera virgifera* larvae as well as some root damage prevention. Currently most promising and most used is the fluid stream spray application into soil at sowing, using sowing machines with applicators that apply nematodes behind the sowing wheel and prior the soil-closing wheels (Fig. 2). For details refer to: Toepfer *et al.,* 2010c, SAGEA Centro di Saggio S.r.l, 2010, pers. comm.; Cult-tec 2012; Knuth *et al.,* 2011.

Fig. 2 Currently most promising and most used application technique for entomopathogenic nematodes against *D. virgifera virgifera* larvae: fluid stream spray application into the soil at sowing using sowing machines with applicators that apply nematodes behind the sowing wheel and prior the soil-closing wheels. Examples from left to right: LIQ-Inject M1 (Cult-tec company, Germany) on a Monosem NG Plus sowing machine (USA); Self-made application tube for fluids on Pneumasem sowing machine of Nodet Gugis(France); John Deer with fluid applicator on White sowing machine (USA).

Abb. 2 *Derzeit vielversprechende und meist genutzte Applikationstechnik für entomopathogene Nematoden gegen Larven von* D. virgifera virgifera*: Flüssigstrahlapplikation in den Boden bei der Aussaat mit Hilfe einer Sämaschine mit einer Vorrichtung zur Applikation von Nematoden hinter dem Särad und vor dem Bodenschließrad. Beispiele von links nach rechts: LIQ-Inject M1 (Cult-tec company, Deutschland) an einer Sämaschine Monosem NG Plus (USA); selbstgebautes Applikationsrohr für Flüssigkeiten an der Sämaschine Pneumasem von Nodet Gugis (Frankreich); John Deer mit Flüssigapplikator an einer White-Sämaschine (USA).*

3.4 Field scale efficacy and dosage-efficacy response

Field scale trials using farmer machinery revealed that *H. bacteriophora* is able to reach control efficacies of *D. virgifera virgifera* larvae to the same extent and at similar variability as soil insecticides and insecticide seed coatings. On multiple year, site and machinery average, control efficacies are approximately between 30 and 80%. Nematodes can also prevent root damage, on levels close to soil insecticides and insecticide seed coatings. A dose-efficacy response curve is not finally established, but preliminary results suggest that the optimal dose of nematodes might be somewhat between 2 and 3 billion per hectare maize field depending on local conditions. For details refer to: Pilz *et al*., 2009; Toepfer *et al*., 2010b; Pilz *et al*., 2011b.

3.5 Products

Heterorhabditis bacteriophora- and *H. megidis*-based products are available from several biocontrol companies, and can be applied, without restrictions, in countries where entomopathogenic nematodes do not need registrations and where the products consider species that are native, e.g. currently in Germany. One of the products (Dianem ™) is, for example, also registered in Austria.

3.6 Legislation

With the banning of several insecticides for seed coatings due to their bee toxicity, and with recent discussions on a number of soil pesticides in maize, farmers need alternative products. Moreover, the European Directive on sustainable use of pesticides requests from EU member countries to prefer alternative pest control options. Entomopathogenic nematodes are exceptionally safe biocontrol agents; thus they are exempted from registration in many European countries; in some they need registration. For details refer to: Ehlers, 2003; European Commission, 2008, 2009; Delos *et al*., 2011; Gill *et al*., 2012; Cressey, 2013.

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The successful development of a biological control solution for western corn rootworm was a 10-year collaborative effort of many partners (please refer to lists of partners on: CABI, 2008; 2012). The research and development largely relied on public funding (Swiss Commission for Technology and Innovation of Federal Office for Professional Education and Technology; a Specific Support Action 'Policy Oriented Research' through the ECs 6th RTD Framework Programme; the Ministry for Rural Areas and Consumer Protection of the State of Baden-Württemberg, Germany; and the Federal Ministry of Agriculture of Germany), and to some extent on funding from UFA-Samen Beneficials, Switzerland, e-nema GmbH Germany, in-kind contributions of farmers, and many others.

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Optimization of application techniques and dosages of *Heterorhabditis bacteriophora* for biologically controlling the larvae of the western corn rootworm

Optimierung von Ausbringungstechnik und Konzentration von Heterorhabditis bacteriophora *zur Bekämpfung der Larven des Westlichen Maiswurzelbohrers*

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In many European countries, the chemical control of the western corn rootworm is questioned. Seed coatings with neonicotinoids were reported to cause significant non-target effects on bees which led to a ban of such products in many European regions. Also the use of granular Tefluthrin-based soil insecticides is, due to their requested ban by the European Commission, only in few European regions still allowed. Entomopathogenic nematodes of the species *Heterorhabditis bacteriophora* are known to well-parasitize the rootworm larvae and can therefore reduce the damage of this pest to the maize roots.

Our study aimed to clarify which application techniques and which nematode concentrations are most feasible in terms of availability, practicability and costs. In 2011, field trials were conducted in southern Hungary to compare the application of nematodes via fine granules, fluid stream sprays, and seed coatings, and this versus standard pesticides. All three application techniques appeared to be technically possible. The fluid application is however most advanced in terms of the related state of the art knowledge and technical development. In 2012, five nematode concentrations were applied using a fluid stream spray of nematodes into soil at maize sowing in Hungary. Results revealed a good efficacy of the applied medium and high concentrations of nematodes at reducing western corn rootworm, and efficacies of less extent at reducing root damage. It is advised to repeat these experiments as dose 'efficacy trials' need to be conducted at different locations under different environmental conditions.

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Emission of pesticides during drilling and deposition in adjacent areas

Emission von Pflanzenschutzmitteln während der Aussaat und Deposition in benachbarten Arealen

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Summary

In seven experiments seeds of maize, oil seed rape and barley, treated with neonicotinoids, were sown using pneumatic drilling equipment with deflectors attached in case of pneumatic suction systems. Directly adjacent to the drilled area of usually about 50 m width were replicated areas with bare soil as well as with crops. During maize (*Zea mays*) drilling flowering oil seed rape (*Brassica napus*) and during drilling of barley (*Hordeum vulgare*) and oil seed rape flowering white mustard (*Sinapis alba)* was adjacent. The amount of residues in the adjacent non crop areas in Petri dishes being distributed on the bare soil declined only slowly from 1 to 20 m distance from the area drilled. Seed batches with more abrasion and higher content of active substances in the dust resulted in higher residues off crop. After drilling of maize in four experiments in Petri dishes in adjacent non crop areas in 1-5 m distance between 0.02 and 0.40 g a.s./ha of neonicotinoids and in the adjacent oil seed rape a total of 0.05–0.80 g a.s./ha were detected. After drilling oil seed rape or barley these values were only 0.02–0.06 g a.s./ha in Petri dishes in non crop areas and 0.03-0.08 g a.s./ha in total in adjacent white mustard. In gauze net samplers installed vertically in 3 m distance in non crop areas up to seven times higher values were detected compared to Petri dishes.

Key words: neonicotinoid, seed treatment, dust, drift, adjacent crop

Zusammenfassung

In sieben Feldversuchen wurde die Abdrift von neonicotinoidhaltigen Stäuben bei der Aussaat von behandeltem Mais, Winterraps bzw. Gerste mit pneumatischen Drillmaschinen bei Nutzung einer Saugtechnik mit Deflektoren zur Abdriftminderung untersucht. Auf den zum etwa 50 m breiten Drillbereich mit echten Wiederholungen benachbarten Flächen blühte Raps bei der Maisaussaat bzw. Senf bei den beiden anderen Kulturen. Staubbürtige Neonicotinoide wurden vergleichend in den blühenden Nachbarkulturen im Pflanzenbewuchs inklusive der Bodendeposition im Bestand und auf offenem Boden in Petrischalen sowie vertikal aufgespannten Gazenetzen analysiert. Die Wirkstoffgehalte in Petrischalen im offenen Bereich und in den Nachbarkulturen fielen bis zu einer Entfernung von 20 m zum Drillbereich nur langsam ab. Saatgutchargen mit höheren Abriebwerten bzw. einem höheren Wirkstoffgehalt im Abriebstaub hatten auch höhere Rückstände im off crop zur Folge. Die nach der Maisaussaat in vier Versuchen ermittelten Wirkstoffgehalte lagen im Abstand von 1 – 5 m in den Petrischalen auf dem Boden im offenen Bereich zwischen 20 - 400 mg/ha, aber als Gesamtsumme deutlich höher in der Nachbarkultur Raps bei 50 – 800 mg/ha. Nach der Aussaat von Gerste (n = 1) bzw. Raps (n = 2) lagen die Werte bei nur 20 – 60 mg/ha in Petrischalen im offenen Bereich und wieder höher bei 30 – 89 mg/ha im benachbarten Senf. In vertikalen Gazenetzen in 3 m Abstand zum Drillen im offenen Bereich ohne Kultur wurden bis zu etwa sieben Mal höhere Gehalte nachgewiesen als im Vergleich zu Petrischalen.

Stichwörter: Neonicotinoid, Saatgutbehandlung, Staub, Abdrift, Nachbarkultur

1. Introduction

Seeds of many crops are treated with insecticides to protect young seedlings against insect pests with the neonicotinoids imidacloprid, clothianidin and thiamethoxam being frequently used. In 2008 during sowing of maize a bee poisoning of about 12,000 hives was observed in Germany. Relevant amounts of insecticidal substances (neonicotinoids) treated to the seeds drifted into adjacent flowering crops in the form of contaminated dust. Analysis of dust abrasion from different seed batches resulted in up to several g of clothianidin/ha being abraded from seeds of maize and set free in the form of dust (Pistorius *et al.*, 2009; Heimbach *et al.*, 2010). Dust drift containing neonicotinoids such as the three mentioned above which show high effects on bees at very low dose has been thought to be relevant for bee toxification already in 2003 (Greatti *et al.*, 2003; 2006) but clear effects associated to drilling of treated maize were identified only later on in Europe (Pistorius *et al.*, 2009; Girolami *et al.*, 2012; Tapparo *et al.*, 2012) and recently in the US (Krupke *et al.*, 2012). Dust emission during drilling of treated seeds has opened a new area of research (Nuyttens *et al.*, 2013) to quantify emission and environmental concentrations in adjacent areas and to predict potential effects on organisms. Therefore in field experiments the emission of pesticides during drilling of maize, barley and oil seed rape was studied and the deposition of the active substance was measured in adjacent areas on bare soil and within adjacent crops to achieve generic drift exposure curves which may be extrapolated to other types of active substances and be used to predict environmental concentration for aquatic and soil organisms and organisms living in adjacent vegetation including crops.

2. Material and methods

Field experiments were carried out from 2008 to 2012 using different seeds of different crops as well as different batches of seeds. The abrasion potential of most seed batches was analysed using the Heubach Dustmeter (Heimbach, 2008). The content of the active substance in the dust was analysed using the filter within the Heubach-Dustmeter. Pneumatic single corn seeders were used for maize. A deflector was attached to the air outlet, resulting in a 90% drift reduction compared to unchanged systems (Anonymous, 2012). Oil seed rape and barley were sown using pneumatic pressure systems which are frequently used in Germany and only in one case for barley mechanical sowing equipment was used.

Measurements of drift were carried out on areas with bare ground adjacent in the wind direction of drift during sowing in 2008 and in experiments from 2009 onwards additionally in directly adjacent flowering crops.

Maize of different seed quality (Tab. 1) was sown in every year from 2009 to 2012 with the same pneumatic driller (suction system) with drift deflection (90% reduced drift values). For oil seed rape in 2009 and 2011 and barley (one trial 2008 and 2012) the same pneumatic driller (air pressure system), but for one trial with barley (2008) a mechanical driller was used. Seed treatments used in maize were Poncho/Poncho Pro (0.5/1.25 mg clothianidin/seed), in oilseed rape Elado (10 g clothianidin/kg seeds) and in barley 2008 Manta Plus (35 g imidacloprid/100 kg seeds) and barley 2012 Gaucho 600 FS (35 g imidacloprid/100 kg seeds). The quality of seed treatment was determined using the Heubach method (Heimbach, 2008) and the content of the active substance was analysed in dust sampled on the filter of the Heubach filter body. These quality criteria regarding dust abrasion and residue content in dust (Tab. 1) varied depending on the seed batches used. Sowing width was 40–60 m (except 100 m in 2008) and different sowing densities were used (maize usually 100,000 seeds /ha, rape about 700,000 seeds /ha, barley about 140 kg /ha) in most experiments. The soil was quite dry during experiments (between 4 and 23% soil water). Sowing took place at wind speed conditions of 2–5 m /sec. Wind direction varied between experiments and was almost to 90° into the direction of the adjacent areas used for the deposition except the maize drill experiment carried out in 2012 (where it was about 45°).

In 2008, ten Petri dishes (of 143 cm^2) per distance (with a water/acetone (1:1/v:v) solution) were used as replicates on bare ground placed in several distances from a drilled area of 100 m x 100 m in wind direction. From 2009 onwards the adjacent area of the field sown was divided into 2-3 replicated areas of about 50 m length covered either with winter oil seed rape (maize drill experiments) or mustard (oil seed rape or barley drill experiments) and at least 30 m long areas with bare soil, where the crop had been removed (Fig. 2). Before drilling the adjacent plants were wetted with glycerol/water (1:1/v:v) solution to create a worst case situation for adhesion of dust particles during harvest of plant material for neonicotinoid analysis. In the bare soil area dust drift was measured with 4-5 Petri dishes (143 cm^2 with filter paper wetted with water/acetone 1:1/v:v solution) per replicate, placed at different distances to the area sown from 0.15 m or 1 m up to 30 m. Additionally, in 3 m distance one vertical gauze net was placed (2 m height x 3.50 m length, the net wetted also with glycerol water solution) and three to five samples of 0.25 m^2 size per net at a height of 0.40 m–0.90 m were taken above ground level for residue analysis. The filter effect of the directly adjacent vegetation was calculated after measuring the a.s. deposits on all plants harvested in 0.1 m^2 large areas as well as the soil deposition in Petri dishes (143 cm^2, but only 61 cm^2 in the more dense crop *Sinapis*) within these areas (4 subreplicates for both samples per replicate and distance) and then comparing the values in Petri dishes in non crop areas to total depositions in the adjacent crop areas.

Drilling usually lasted about 1 hour. Plants, gauze nets and Petri dishes were sampled shortly after drilling and 24 hours later. Samples were deep frozen after delivery to the analytical laboratory. For the procedure of determination of insecticide contents see Fig. 1. The identification of different neonicotinoids (e.g. clothianidin and imidacloprid) in Petri dishes/filter collectors, gauze nets, and plants was carried out with a LC/MS/MS- system of Dionex UltiMate 3000 coupled to an AB SCIEX QTRAP 5500. For quantification the use of matrix-matched calibration with internal standards (acetamiprid D3 as surrogate, clothianidin D3 or imidacloprid D4) was necessary. The method was validated by conducting recovery experiments with Petri dishes, and filter inlets, gauze nets, and plants. The limits of quantification were for Petri dishes and filters 0.7 mg/ha (except in 2008 1.5 mg/ha), for gauze 4 mg/ha, and for plants parts with open flowers 1 mg/ha or the rest of the plants 0.1 µg/kg – 0.5 µg/kg per 0.1 m^2 according to the weight of harvested plants/m^2.

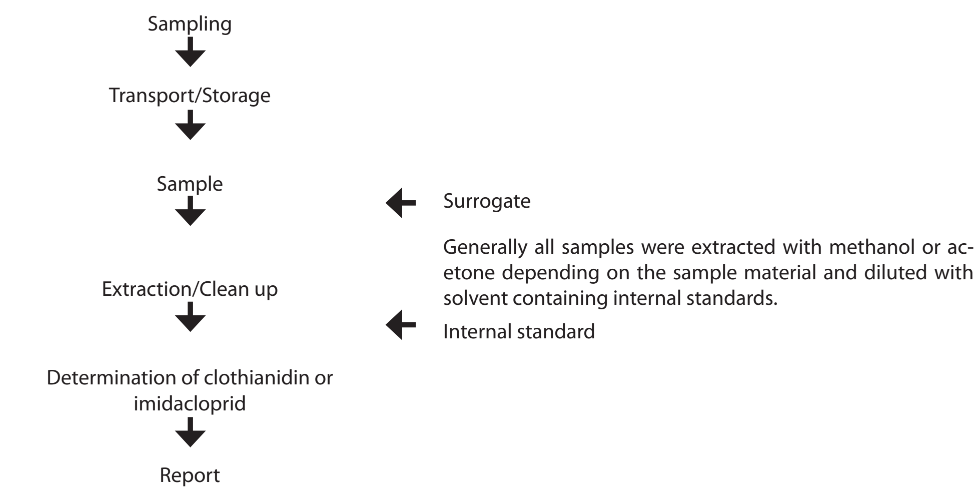

Fig. 1 Procedure for the determination of neonicotinoid concentration.

***Abb. 1** Ablaufschema für die Bestimmung der Insektizidkonzentration.*

Fig. 2 Schematic plan for drift experiments with replicated adjacent crop and non crop areas, used 2009-2012.

Abb. 2 *Schematischer Plan der JKI Abdrift-Versuche mit wiederholten Nachbarkulturen und offenen Bereichen ohne Kultur für 2009 bis 2012.*

3. Results and discussion

The release of dust into adjacent areas may generally be influenced by the amounts of dust set free during drilling (Heubach value), the percentage of the active substance in dust, dust particle size, amount of seeds drilled per ha, sowing width and type of sowing machinery (Anonymous, 2012; Biocca *et al.*, 2011) and also on wind and soil conditions. The exposure of non-target organisms depends on the imission of contaminated dust and the height and density of plants as well as on the morphology of flowers and leaves and especially on the stickiness of adjacent vegetation. Therefore all these aspects need to be considered when conducting drift experiments to derive drift values for risk assessment.

Residues in Petri dishes on bare soil only decreased slowly with increasing distance in average of all experiments as well as in single experiments (Fig. 3). The mean deposition in 1 m, 3 m and 5 m distance to the drilling area was reduced to about 70% in 20 m distance in the nine field experiments with different crops carried out since 2008. Deposition a few cm off the drilling area exceeded the 1 m value clearly with a little less than twice the value of 1 m (average of four experiments). Less but still detectable drift values were found for mechanical drilling of barley compared to pneumatic drilling in the same experiment (Heimbach and Stähler, 2010). In all experiments some samples were taken also 24 hours after drilling but residues were always distinctly lower than shortly after drilling and are not reported here.

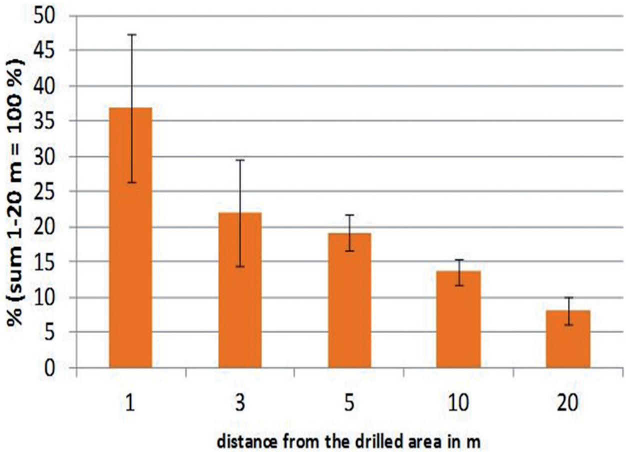

Fig. 3 Mean % and std. of neonicotinoid deposition at different distances (values of 1+3+5+10+20 m = 100%) in Petri dishes placed on bare soil in adjacent non crop areas of nine drilling experiments (4 x maize, 2 x rape, 3 x barley) in 2008–2012.

Abb. 3 *Konzentrationen unterschiedlicher Neonicotinoide in Petrischalen in verschieden Abständen auf offenem Boden auf der unbewachsenen Nachbarfläche in neun Drillversuchen (4 x Mais, 2 x Raps, 3 x Gerste) von 2008 – 2012 (Werte aus 1+3+5+10+20 m = 100%).*

Drift values in Petri dishes on bare soil varied depending mainly on the dust abrasion potential of the seed batch used for drilling. Higher Heubach values or higher amounts of the active substance in the dust resulting in higher drift values (Tab. 1). Contents of the active substance (g/ha) in adjacent flowering plants of several experiments were up to about 4.5 times higher at 1 m distance (average of all experiments in 1 m distance 2.42 times higher) compared to values of Petri dishes on bare soil in non crop areas. The ratio of the active substance values determined on areas with vegetation compared to values on Petri dishes in areas without plants at 1 m distance from the drilling area showed, that flowering oil seed rape filtered dust and resulted in 2.95 times higher amounts in four experiments whereas this value was only 1.70 times if white mustard was adjacent in three experiments (Fig. 4). In both adjacent crops with increasing distance from the drilled area, the difference between content in vegetation and Petri dishes at the same distance decreased. This emphasizes the importance of the filtering capacity of adjacent plants especially directly at the field border when carrying out any risk analysis for organisms being active in adjacent vegetation.

Tab. 1 Characteristics of seed batches concerning application rate and deposition of neonicotionoids in different samplers in wind direction in areas adjacent to drilling in field experiments 2009-2012. Pneumatic drillers were used for rape and barley (except 1 experiment with mechanical driller for barley) but pneumatic single corn drillers for maize.

***Tab. 1** Charakteristika von Saatgut-Chargen hinsichtlich Applikationsraten und Ablagerungen von Neonikotinoiden auf unterschiedlichen Sammelvorrichtungen angebracht in Windrichtung auf benachbarten Flächen in Aussaatversuchen in den Jahren 2009 bis 2012. Pneumatische Drillgeräte bei Raps und Gerste (außer 1 x Gerste mechanische Aussaat), pneumatische Einzelkornsähgeräte bei Mais.*

Crop and year of sowing	g a.s. sown/ha in the experiment	Heubach dust in g sown/ha (% a.s. in dust)	Heubach dust in g a.s. sown/ha	g a.s./ha (Petri dishes, mean of 1-5 m distance)	g a.s./ha (adjacent crop, mean of 1-5 m distance)	g a.s./ha (vertical gauze nets in 3 m distance)
Maize 2009	125	2.1/100,000 seeds (22.1%)	0.469	0.41	0.81	not used
Maize 2010	125	0.86/100,000 seeds (10.6%)	0.091	0.10	0.28	0.685
Maize 2011	50	0.45/100,000 seeds (19.1%)	0.086	0.15	0.27	0.447
Maize 2012	16.7	0.10/13,400 seeds (42%) untreated seeds added	0.041	0.022	0.051	0.026
Rape 2009	46	not determined	not determined	0.058	0.082	not used
Rape 2011	36	0.38/730,000 seeds (6.33%)	0.025	0.021	0.033	0.130
Barley 2008 mechanical driller	51	1.71/147 kg (7.98%)	0.136	0.030*	not used	not used
Barley 2008	47	1.57/135 kg (7.98%)	0.125	0.045**	not used	not used
Barley 2012	46	1.55/132 kg (5.55%)	0.086	0.024	0.030	0.097

*Corrected by the author from 0.30 **Corrected by the author from 0.45 (Nov. 2nd, 2015)

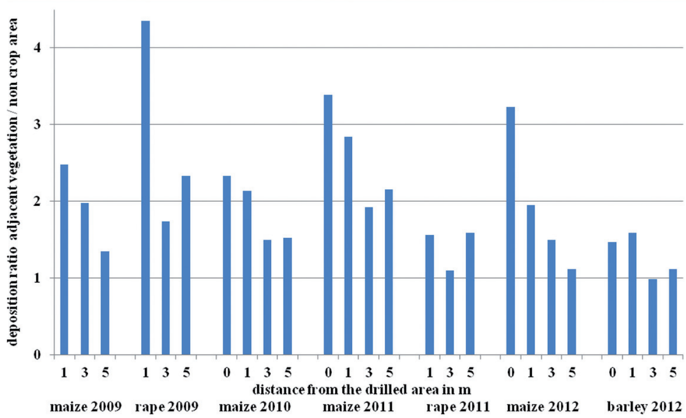

Fig. 4 Ratio of neonicotinoid deposition (at 0.1 m – 5 m distance) in adjacent crops (flowering oil seed rape after drilling of treated maize seeds, flowering white mustard after drilling of treated oil seed rape and barley seeds) and of deposition in Petri dishes placed on bare soil in adjacent non crop areas in drift experiment during sowing of maize, rape, and barley 2009-2012.

***Abb. 4** Verhältnis der Ablagerung von Neonicotinoiden (in 0,1 - 5 m Entfernung) in benachbarten, blühenden Rapsbeständen nach der Aussaat von gebeiztem Maissaatgut, in blühendem Senf nach der Aussaat von gebeiztem Raps- und Gerstesaatgut im Vergleich zur Ablagerung in Petrischalen auf offenem Boden auf unbewachsenen Flächen.*

Vertical gauze nets, representing a three-dimensional structure, are easier to handle in drift experiments than adjacent vegetation and may be used to obtain a representative worst case situation of potential drift into adjacent plants. Values of the active substance in these nets (g/m^2) were about 3 to 10 times higher compared to those in Petri dishes on bare soil, when both samplers were placed in non crop areas in 3 m distance from the drilling area in 5 experiments (Fig. 5).

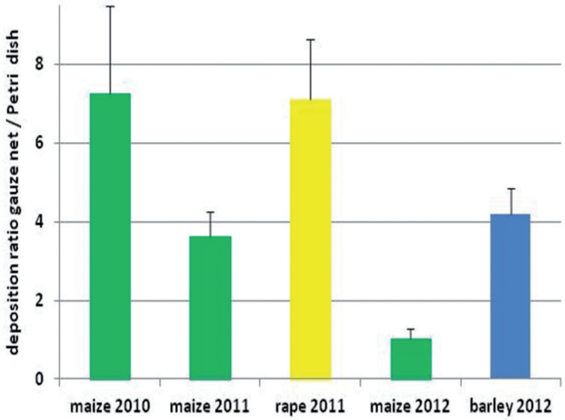

Fig. 5 Ratio of neonicotinoid deposition (mean and std. within experiments) in vertical exposed gauze nets (representing a 3-D structure) and of deposition in Petri dishes, both samplers at 3 m distance placed on bare soil in adjacent non crop areas in drift experiments during sowing of maize, oil seed rape or barley 2010-2012.

Fig. 5 *Verhältnis der Ablagerung von Neonicotinoiden (Mittelwert und Standardabweichung der a.s. in 3 m Entfernung) an vertikalen Gazenetzen (die eine 3-D-Struktur darstellen) im Vergleich zur Ablagerung in Petrischalen auf offenen Boden auf unbewachsenen Flächen nach der Aussaat von gebeiztem Mais-, Raps- oder Gerstesaatgut.*

4. Conclusions

Risk mitigation for pesticide treated seeds seems possible with an improvement of the seed coating quality regarding content of loose dust, maximum amount of active substances in dust and with improvements of sowing techniques (Forster *et al.*, 2012). There are differences between spray and dust drift (e.g. varying form and fraction size of dust particles for different crops and seed batches, evaporation of droplets but not for dust particles during drift), which need to be addressed when carrying out and interpreting dust drift experiments. For risk analysis experimental drift values need to be corrected for the maximum field rate of seeds per ha permitted and for the drilling width in an experiment if it was distinctly less than e.g. 100 m wide, because of the long travelling distance of drift.

The maize drilling in 2010 and 2011 reported here resulted in clear unacceptable effects on honey bees which were exposed in the experiments (Georgiadis *et al.*, 2012). A further improvement of the seed quality regarding abrasive or loose dust, a reduction of the content of the active substance in dust and an improvement of the sowing machinery is needed. More data are needed to predict realistic residue exposure values after dust drift in different types of adjacent crops and natural vegetation. In the experiments reported here, adjacent crops (flowering oil seed rape in spring or white mustard in summer) had up to 4.5 times higher residues compared to Petri dishes placed in non crop areas on the soil at the same distance. But data of vertically exposed gauze nets may represent a worst case situation to represent exposure in adjacent vegetation of different types including e.g. hedges.

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Dust drift during sowing of maize and oilseed rape effects on honey bees

Abdrift während der Aussaat von Mais und Raps – Auswirkungen auf Honigbienen

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In 2008 bee poisoning incidents in southern Germany revealed drift of insecticidal dusts on adjacent areas with flowering bee forage plants during sowing of maize as a considerable route of exposure. Consequently, several improvements have been proposed as possible risk mitigation measures e.g. for seed dressing quality regarding dust abrasion, taking into account Heubach values and residue content of dust. To assess potential effects on honey bee colonies following insecticidal dust drift on adjacent non-target areas, from 2009 until 2012 several large-scale drift experiments were carried out during sowing treated maize and winter oil seed rape (= WOSR) using different seed batches.

In drift experiments with maize, two different set-ups were used: 2010 the experimental area (flowering WOSR) was in the middle of two areas reserved for maize drilling depending on wind direction. In contrast, 2011 and 2012 the area reserved for drilling of maize was surrounded by flowering WOSR areas.

In 2010, on both sides directly along the edge of the WOSR 4 bee hives for the field approach as well as three gauze-covered tunnel tents (16 x 6 m) with bee hives for the semi-field approach were exposed. For "Treatment"-variant of the field and semi-field approach the hives were set up at 0 respectively 3 m and for "Control"-variant at 90 respectively 87 m from the edge of the directly exposed WOSR (= 0 m). For the field approach another 4 hives were set up in about 800 m distance. Before sowing, semi-field colonies were closed and the gauze from the tunnels removed. After sowing, the tents were covered again and the hives reopened. Bee hives in the field approach were left open during the drilling process, so forager bees were continuously exposed to contaminated dust. In 2011 and 2012, similar experiments with field and semi-field approach were performed (distances to the exposed side of WOSR of 0 m, 50 m and in the field set-up, an additional group of colonies at a distance of about 500 m).

In all experiment, maize seed was sown by a pneumatic vacuum operated precision air planter with at least 90% drift reduction due to a deflector.

In drift experiments with WOSR 2009 and 2011, the drilling area was surrounded by flowering mustard. Sowing was done by a conventional pneumatic seed drill. Experimental procedures, samplings and documentation were similar to the drift experiments with maize in 2011. Only in 2009, the design of the semi-field approach differed in tent size (4 x 4 m) and number of replicates (n=4).

The impact of dust drift on bee colonies in semi-field and field approaches were examined by assessing flight activity and mortality in dead bee traps (type "Gary"). Dead bees were documented, collected, frozen and analyzed for residues.

The results of drift experiments during maize sowing showed a clear treatment related increase of bee mortality, especially in the worst-case semi-field approach, but also in the field approach at a much lower level. Bee mortality in 2011 was slightly lower than in 2010, presumably due to a slightly lower exposure (2010: 0.091 g Clothianidin in Heubach filter dust of 100,000 seeds applied/ha; 2011: 0.086 g a.i. in Heubach filter dust of 100,000 seeds applied/ha). A very similar mortality rate compared to control was found during maize sowing in 2012 (0.041 g a.i. in Heubach filter dust per amount of seeds applied/ha). Further improvements of the seed treatment quality of maize and of the sowing technique are needed to exclude adverse effects on bees.

In contrast to sowing maize, during sowing of treated WOSR in 2009 and 2011, no treatment related increase of mortality was observed. Even in the «treated» variant of the worse-case semi-field approach, only low mortality, similar to the control was detected, barely exceeding the natural rate of mortality. The amounts of abrasion dust and its insecticidal residue content were clearly lower compared to maize (2011: 0.021 g a.i. Clothianidin in Heubach filter dust per amount of seeds applied/ha). The good seed treatment quality of WOSR and the use of a conventional pneumatic seed drill did not result in any adverse effects on bees.

The project was partly funded by the German Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Diabrotica research program.

Risk assessment and state of art on the risk for honey bees from dust drift of insecticidal dusts during sowing

Risikobewertung und Stand der Forschung zum Risiko für Honigbienen durch die Abdrift insektizider Stäube während der Aussaat

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In 2008 a large-scale honey bee poisoning in parts of southern Germany occurred during sowing of maize, caused by contamination of flowering bee forage plants with drift of dust from insecticidal seed-dressing containing the active substance Clothianidin.

Since early 2009, drift experiments during sowing insecticide treated seeds were realized to investigate the relation between abrasion potential of treated maize and oil seed rape seeds (determined by Heubach dustmeter, dust drift and resulting residues in Petri dishes on bare soil in the off crop area placed at different distances and in adjacent flowering crops as well as residues in bee matrices and the effects on honey bees. In "worst case" scenario experiments bee colonies were set up in semi-field (gauze-covered tents, 16 x 6 m) and in field trials along the edges of the drilled area to study the impacts on mortality, foraging activity and brood development of bee colonies following exposure to dust drift during sowing and exposure to contaminated pollen and nectar.

Using drift-reduced pneumatic sowing techniques, several trials with drilling of seed treated crops was conducted during bee flight activity, so foragers of the field trials were continuously exposed to dust. Control variants were set up with hives in about 50 m (control) and more than 500 m (remote) distance to the drilling area. In semi-field trials, the hive entrance of colonies were closed before daily bee flight activity and the gauze covering removed before drilling; afterwards the tents were covered with gauze again and the hives opened. Furthermore, in cooperation with a contract lab that developed new machinery that allows the application of defined amounts of dust, a field trial was conducted. A number of different experimental approaches were conducted in the last few years, some also by other research groups. Some suitable test methods were identified to assess the effects of contaminated dusts on bees for risk assessment purposes.

From the available data, conclusions on the potential exposure of bees to dust following sowing of different seed treated crops, on the potential crops of concern and the importance of different exposure routes as well as the different factors influencing the exposure can be drawn. Nevertheless, the residues which bees can be exposed to during or following a sowing operation is highly variable and depends on the quality of seed treatment, the sowing machinery used, on the size of the sown area, on the attractiveness and quantity of nectar and pollen of the vegetation in the margin of the field sown and on meteorological conditions. To allow an estimation of potential dust exposure for risk assessment purposes, it is important that quality criteria are defined and guaranteed for the seed treatment of different crops and also for sowing machinery including devices for drift reduction e.g. deflectors. With these prerequisites, reliable exposure values can be obtained.

As sowing of different crops results in different exposure, also the potential risk for bees differs. In semi-field and field trials with sowing of winter oil seed rape no adverse effects on honey bees were observed if the seed treatment and sowing technique guarantee a limited dust emission. Since 2008, clear improvements in seed dressing quality of maize and development of drift-reducing sowing technique were achieved, resulting in a significant reduction of the potential dust exposure. Still, trials with sowing of maize treated with bee toxic insecticides with improved seed treatment quality (e.g. Heubach value of less than 0.5 g/100,000 seeds and approximately 20% a.i. Clothianidin in the dusts), resulted in a high mortality of exposed bees and underline the necessity of further improvements of seed treatment quality and a reduction of potential dust emission during sowing of maize to exclude adverse effects on bees.

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Erarbeitung der fachlichen Grundlagen für ein Prüfverfahren für Sägeräte mit verminderter Abdrift von Beizstäuben

Development of a test method for sowing machines concerning the drift of dust abrasion

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Zusammenfassung

Lange Zeit galt die Saatgutbeizung als die effektivste und in Bezug auf die Beeinträchtigung des Naturhaushalts als die sicherste Form des Pflanzenschutzes. Seit den schweren, durch Beizstäube verursachten Bienenschäden im Rheintal vor 5 Jahren, steht jedoch die Zulassung von insektizidhaltigen Saatgutbehandlungsmitteln in der Diskussion.

Um das Emissionsverhalten von Sägeräten während der Aussaat auf Basis gesicherter Daten beurteilen zu können, wurden am Landwirtschaftlichen Technologiezentrum Augustenberg (LTZ) die fachlichen Grundlagen für ein Prüfverfahren zur Messung der Beizstaubabdrift bei Sägeräten erarbeitet. Es wurde ein Indoor-Prüfstand erstellt und eine standardisierte Prüfmethode entwickelt, mit der es möglich ist, das Abdriftverhalten von Sägeräten im Vergleich zu bewerten. Als Prüfsaatgut dient ein Granulat, welches mit einem fluoreszierenden Farbstoff gebeizt ist. Die „Tracertechnik" ermöglicht eine reproduzierbare, schnelle und kostengünstige Beurteilung des Abdriftverhaltens der in der Praxis eingesetzten Sägeräte.

Zur Einordnung der im Prüfstand gewonnen Abdriftwerte der Sägeräte wurden zum Vergleich Messungen im Freiland durchgeführt. Die dabei ermittelten Driftmengen lagen auf vergleichbarem Niveau wie die im Prüfstand gemessenen Werte. Die standardisierte Abdriftmessung im Prüfstand eignet sich damit auch zur Berechnung von Expositionsszenarien für die Aussaat von gebeiztem Saatgut.

Stichwörter: Prüfverfahren, Sägeräte, Beizstaubabdrift, Windkanal, Expositionsszenarien

Summary

For a long time the seed treatment was regarded as the most effective and in terms of the impact of the natural environment as the safest form of plant protection. Since the serious damage of bees, caused by dust of abraded seed treatment in the Rhine Valley five years ago, the admission of seed treatment products containing insecticide is in the discussion.

To evaluate the emission characteristics of sowing machines during sowing based on secure data, the technical basis for a test method for measuring the drift of abraded seed-dust in sowing machines were developed at the Centre for Agricultural Technology Augustenberg. An indoor test bench was created and a standardized test method by which it is possible to evaluate the drift behavior of sowing machines in comparison was developed. A granulate which is stained with a fluorescent Tracer is used as test seed. The „tracer technique" allows a reproducible, rapid and inexpensive assessment of the drift behavior of the sowing technology, which is commonly used.

To classify the obtained drift values in the test bench, measurements in the field were carried out for comparison. The determined drift volumes of those measurements were at a similar level as the measured values in the test stand. Therefore the standardized measurement of drift in the test stand is suitable for the calculation of exposure scenarios for the sowing of treated seeds.

Keywords: Test method, sowing machines, dust drift, wind tunnel, exposure scenarios

1. Einleitung

Im Frühjahr 2008 wurden in der oberen Rheinebene mehrere tausend Bienenvölker geschädigt. Ursache dafür waren offensichtlich neonicotinoidhaltige Beizmittelemissionen, die durch Abdrift bei der Aussaat von Mais auf Blüten benachbarter Kulturen gelangten und dort von den Bienen aufgenommen wurden. Abdriftmessungen während der Maissaat mit pneumatischen Einzelkornsägeräten an verschiedenen Instituten konnten eine Exposition wirkstoffhaltiger Stäube auf den Nahbereich der gesäten Fläche nachweisen. In der Folge wurden sofortige Maßnahmen zur Minimierung dieser Emissionen ergriffen, wie die Verbesserung der Beizverfahren und die Ausrüstung der Einzelkornsägeräte mit Deflektoren zur Ableitung der Gebläseabluft an die Bodenoberfläche. Die Zulassung neonicotinoidhaltiger Beizmittel wurde ausgesetzt und die Erlaubnis zur Aussaat von Saatgut, welches mit Mesurol (Wirkstoff: Methiocarb) gebeizt ist, wurde auf Sägeräte beschränkt, die eine Minderung der Abdrift um 90 % gegenüber herkömmlichen Einzelkornsägeräten ohne Umrüstsatz erzielten.

Die Einstufung der Geräte hinsichtlich ihrer Abdriftminderung erfolgt derzeit durch eine Freilandmessmethode des Julius Kühn-Instituts in Braunschweig (JKI). Da diese Messungen im Freiland auf Grund der Boden- bzw. Witterungsverhältnisse nur in engen Zeitfenstern möglich sind und die Einflussfaktoren auf die Staubexposition wie Windgeschwindigkeit, Windrichtung, Konvektion und Bodenverhältnisse dabei stark variieren, wurde die Entwicklung eines entsprechenden Indoor-Prüfverfahrens angeregt, bei dem diese Einflüsse standardisiert werden können. Um der Praxis ausreichende Möglichkeiten hinsichtlich der Sätechnik zur Verfügung zu stellen, mit der unvertretbare Auswirkungen auf den Naturhaushalt ausgeschlossen werden können, bedarf es gesicherter Prüfrichtlinien, mit denen reproduzierbare Ergebnisse erarbeitet werden können. Die Erarbeitung der fachlichen Grundlagen wie auch die Entwicklung eines Prüfverfahrens war Inhalt eines dreijährigen Forschungsprojekts am Landwirtschaftlichen Technologiezentrum Augustenberg (LTZ).

2. Material und Methoden

Indoor-Prüfverfahren zur Messung der Beizstaubabdrift bei Sägeräten

Das Prinzip der Indoor-Prüfmethode zur Messung der Beizstaubabdrift bei Sägeräten beruht auf einer Durchführung der Saat in einem Windkanal und der anschließenden Massenbestimmung abgeriebener und abgedrifteter Staubmengen sowie der darin enthaltenen Wirkstoffanteile bei einer definierten Windgeschwindigkeit. Als Nachweisstoff im Rahmen des Prüfverfahrens dient der Tracer „Pyranin 120 %“, welcher fluoreszierende Eigenschaften hat. Zusammen mit einem zur Saatgutbehandlung gebräuchlichen Kleber (Peridiam) wird das Prüfsaatgut mit diesem Tracer gebeizt.

Da sich die Abriebeigenschaften von handelsüblichem Saatgut durch Luftfeuchtigkeits- und Temperaturschwankungen verändern, ist es für ein standardisiertes Messverfahren nicht geeignet. Für Abdriftmessungen im Prüfstand wird deshalb ein aus Kunststoff und Holzstaub hergestelltes Granulat verwendet, welches vergleichbare physikalische Eigenschaften hat und sich für alle Sägerätebautypen eignet. Die poröse Oberfläche des Kunstsaatguts ermöglicht eine gute Anlagerung von Beizmitteln, welche nach der Verwendung wieder abgewaschen werden können. Damit kann das Kunstsaatgut mehrmals eingesetzt werden. Die Beizung des Kunstsaatguts erfolgt in einem Chargenbeizgerät und ist für die beschriebene Methode ebenfalls standardisiert. Durch die Ermittlung des Tausendkorngewichts und des Heubachwertes nach der Beizung wird die Einheitlichkeit des Kunstsaatguts gewährleistet. Die Saatmenge für eine Messung mit Einzelkornsägeräten beträgt 6 kg. Diese Menge entspricht einem bei der Maissaat üblichen Hektaraufwand von 100.000 Korn. Bei Getreidekulturen, deren Aussaat meist durch Universalsägeräte erfolgt, liegt der Hektaraufwand bezogen auf die Kornzahl deutlich höher (bis 4.500.000 Korn). Zur leichteren Handhabung während der Prüfung wurde hier die Saatmenge bei diesen Geräten auf 24 kg (400.000 Korn) festgelegt.

Das zu prüfende Sägerät wird auf die aus Gitterrosten bestehende Standfläche des Prüfstands gestellt. Für den Bereich direkt um die Schare werden Formteile aus 5 cm dicken Styrodur-Platten zugeschnitten, die einen Saatschlitz bilden. Der übrige Bereich der Standfläche wird mit Blechen abgedeckt. Während des Sävorganges fällt das aus der Sämaschine austretende Saatgut durch den Gitterrost auf Förderbänder. Diese führen die Körner ab und befördern sie zur anschließenden Massenbestimmung der ausgebrachten Saatgutmenge in darunter befindliche Behälter. Das Gebläse und die Säwelle des Sägeräts werden durch stufenlos regelbare Elektromotoren angetrieben. Die Einstellungen des Sägeräts werden so gewählt, dass der Saatgutdurchsatz einer Arbeitsgeschwindigkeit von 6 km/h bei üblichem Hektaraufwand entspricht. Während des Sägerätebetriebs im Prüfstand wird dieser mit einer antistatischen Folie verschlossen, um den Luftaustausch der Messzelle mit der Umgebung zu verhindern.

Ein Radialgebläse mit einer Volumenleistung von 6.500 m^3/h erzeugt den Luftstrom im Windkanal, wobei die Luft über Rohre in einem geschlossenen System zirkuliert. Die Messung der Abdrift im Prüfstand erfolgt bei einer Windgeschwindigkeit von 1 m/s, die sich durch einen Frequenzumrichter zur stufenlosen Einstellung der Gebläsedrehzahl genau einstellen lässt. Während des Saatgutdurchlaufes werden die driftenden Staubpartikel von einem Filtervlies der Filterklasse F8 abgeschieden. Zur Differenzierung der Driftmenge über die Höhe ist die Filterfläche in 5 Segmente unterteilt. Die Abmessungen der gesamten Filterfläche betragen 900 x 1500 cm (B/H). In Abb. 1 ist der Sägeräteprüfstand schematisch dargestellt.

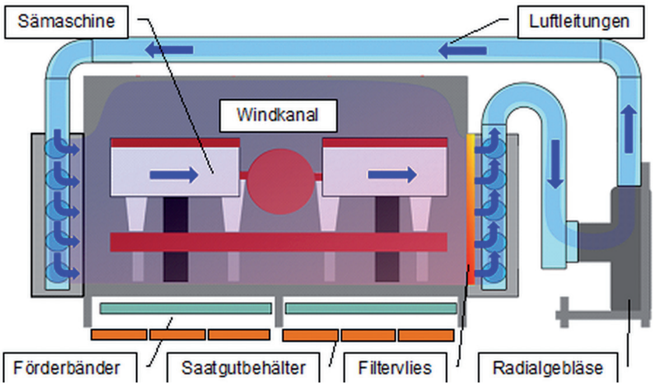

Abb.1 Schematische Darstellung des Sägeräteprüfstands.

Fig. 1 *Schematic presentation of a test stand for sowing machines.*

Die Beurteilung der Geräte erfolgt sowohl durch Wiegen der im Filter abgeschiedenen Stäube, als auch durch fluorometrische Bestimmung der darin enthaltenen Farbstoffmengen. Das Prinzip der Fluoreszenzspektrometrie ist die Anregung des in Lösung befindlichen Farbstoffs mit Licht einer bestimmten Wellenlänge. Dabei wird Licht höherer Wellenlänge zurückgestrahlt (emittiert). Diese von der Menge des Farbstoffs abhängige Strahlungsemission wird von einem Detektor gemessen. Der Vorteil dieser Tracertechnik liegt zum einen in der sehr tiefen Nachweisgrenze bis zu einer Verdünnung von 10^{-12} (Wernli, 2003), was den Einsatz geringer Tracermengen erlaubt und zum andern in einer schnellen und kostengünstigen Messung.

Die Auswertung der fluorometrisch ermittelten Pyraninkonzentration in der Abwaschflüssigkeit erfolgt mit einer modifizierten Version von APPLCALC (K. Schmidt, 2012, persönliche Mitteilung). Diese zur Ermittlung von Spritzbelagswerten im Pflanzenschutz entwickelte Excel-Tabellenkalkulation ist speziell auf die Fluoreszenzmessung abgestimmt worden. Da Fluoreszenzmessungen Konzentrationsmessungen sind, die, in einem weiten, über den bei Abdriftmessungen erforderlichen Messbereich hinaus, linear verlaufen, genügt für die Umrechnung der Messwertanzeige die Bestimmung einer „Eichgeraden" aus zwei Punkten. In die Umrechnung fließen die jeweiligen Basiswerte Saatgutmenge, Farbstoffmenge, Hektaraufwand und Fluoreszenzanzeige ein. Als Referenz für die Berechnung der Pyraninmengen in den einzelnen Proben wird der Eichwert einer bekannten Pyraninkonzentration herangezogen.

Messung der Beizstaubabdrift im Freiland

Zur Einordnung der im Prüfstand gemessenen Abdriftwerte wurden zum Vergleich Messungen im Freiland durchgeführt. Die Messmethode der Feldversuche orientierte sich an den Vorgaben des Julius Kühn-Instituts (JKI), welches in Abstimmung mit dem Umweltbundesamt und den Pflanzenschutzmittelherstellern hierzu eine „Methode zur Messung der Abdrift beim Aussäen von Maissaatgut im Freiland" definiert hat. Danach erfolgt die Messung des Bodensediments mit Petrischalen in 6 Messabständen (1, 3, 5, 10, 20, 30 m) und zehnfacher Wiederholung je Messabstand (1 m Abstand). Schwebende Stäube wurden mit einer Gaze gemessen, die in 1 m Entfernung vom Saatfeld mit einer Fläche von 4 m Breite und 2 m Höhe auf einen Bauzaun aufgespannt war. Die Probenentnahme an der Gaze erfolgte nach der Aussaat durch Herausschneiden von quadratischen Gewebestücken in einer Messhöhe zwischen 0,5 m und 1 m. Da dieses Messdesign nicht die gesamte Luftdrift erfasst, sondern auf die Messung sedimentierender und bodennah schwebender Stäube begrenzt ist, waren für einen Vergleich mit den Ergebnissen aus dem Prüfstand zusätzliche Kollektoren notwendig (Abb. 2).

Für die Messung der Luftdrift von Beizstäuben gibt es bisher kein allgemein anerkanntes Verfahren. Deshalb wurde das Staubabscheidungsvermögen folgender Kollektoren, die sich bei Abdriftmessungen im Pflanzenschutz bewährt haben, im Vergleich getestet: Nylondrähte (Ø 2 mm), die in dreifacher Wiederholung bis zu einer Höhe von 6 m aufgespannt wurden, Bälle aus Kunststoffgeflecht (Ø 5 cm), deren Anordnung in 50 cm Abständen bis zu einer Messhöhe von 4 m erfolgte (2 Wiederholungen) und tote Bienen, die in unmittelbarer Nähe zu den Bällen aus Kunststoffgeflecht angebracht waren. Die Bälle aus Kunststoffgeflecht und die Bienen blieben unbehandelt, während die Nylondrähte zur Verbesserung der Haftfähigkeit der Driftpartikel mit Silikonöl eingesprüht wurden.

Für die Anordnung der Kollektoren war die allgemeine Definition für Luftdrift („Airborne Drift"), die auch den Bereich der Beizstaubabdrift umfasst, maßgebend: "*The volume (or mass) of chemical per unit length of spray run that passes above a point at a given down-wind distance outside the field or target area*" (Online Encyclopedia, 2010). Der Messpunkt zur Abscheidung driftender Stäube hatte bei allen Kollektorarten einen Abstand zum Aussaatfeld von 1 m. Zur Untersuchung des Driftverlaufs nichtsedimentierender Stäube mit zunehmender Entfernung von der Emissionsquelle wurden Nylondrähte auch in weiteren Entfernungen (5 m, 15 m und 30 m) von der Aussaatfläche aufgespannt. Die Abmessungen der Saatfläche betrugen 45 m Länge und 27 m Breite, woraus sich bei Sägeräten mit 3 m Arbeitsbreite 9 Säbahnen ergaben.

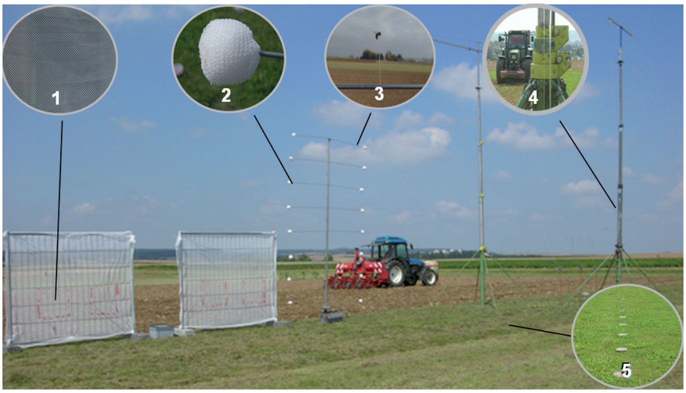

Abb. 2 Freilandmessung mit verschiedenen Kollektoren zur näheren Bestimmung der Luftdrift (1 = Gaze; 2 = Bälle aus Kunststoffgeflecht; 3 = Bienen; 4 = Nylondrähte; 5 = Petrischalen).

Fig. 2 *Outdoor measurement of various collectors to determine air drift (1=gauze; 2=balls of synthetic network; 3=bees; 4=nylon wires; 5=Petri dishes).*

3. Ergebnisse

In Abb. 3 sind exemplarisch die Abdriftmengen dargestellt, die im Prüfstand während der Aussaat von Prüfgranulat bei einem Einzelkornsägerät (ESM IV) gemessen wurden. Im linken Diagramm sind die absoluten Driftmengen abgebildet, welche in den einzelnen Höhensegmenten des Filtervlieses enthalten waren. Das rechte Diagramm zeigt die relativen Driftmengen aktiver Substanz (Pyranin) in Prozent, die das Driftpotenzial des Sägeräts darstellen. Das Driftpotenzial ist als maximal mögliche Staubemission eines Sägeräts definiert, die bei gegebener Windgeschwindigkeit während der Aussaat eines Saatguts mit vergleichbaren Abriebeigenschaften durch Drift im Nahbereich sedimentieren oder über den Nahbereich hinaus verschweben kann.

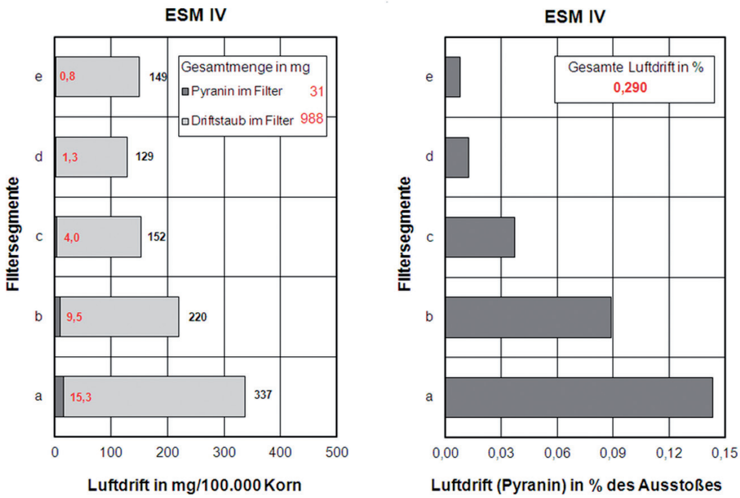

Abb. 3 Vertikal differenziertes Driftpotenzial einer Einzelkornsämaschine in % des Ausstoßes.

Fig. 3 *Vertically differentiated drift potential of a single seed sowing machine in % output.*

Nach zahlreichen Abdriftmessungen mit Sägeräten, die zur Erarbeitung der oben beschriebenen Prüfmethode durchgeführt wurden, erfolgten insgesamt 20 Geräteprüfungen, die zur Beurteilung der Reproduzierbarkeit der Prüfergebnisse dienten. Die hellen Balken der in Abb. 4 dargestellten Driftpotenziale stellen abgesehen von einer Einzelmessung („Pneum ESM III") jeweils Mittelwerte aus zwei Wiederholungsmessungen mit Granulat dar. Diese lagen bei den Einzelkornsägeräten im Bereich zwischen 0,08 und 0,45 %. Bei den beiden pneumatischen Universalsägeräten lagen die Werte zwischen 0,03 % und 0,08 %.

Zu den Messungen mit Granulat wurden bei 5 Geräten Vergleichsmessungen mit Mais durchgeführt, welcher ebenfalls mit Pyranin gebeizt war (2 g/kg). Bei diesen Abdriftmessungen mit Maissaatgut wurden im Vergleich zu den Messungen mit Granulat deutlich höhere Abdriftmengen gemessen, was offensichtlich durch den schlechteren Heubachwert des Maissaatguts begründet war. Es zeigt sich aber eine enge Korrelation zwischen den Messungen mit Granulat und Mais. Die Untersuchung mechanisch arbeitender Universalsägeräte wurde auf Grund der niederen Werte, die dabei gemessen wurden, nicht weiter verfolgt. Hierbei lagen die Driftmengen bei weniger als 0,005 %.

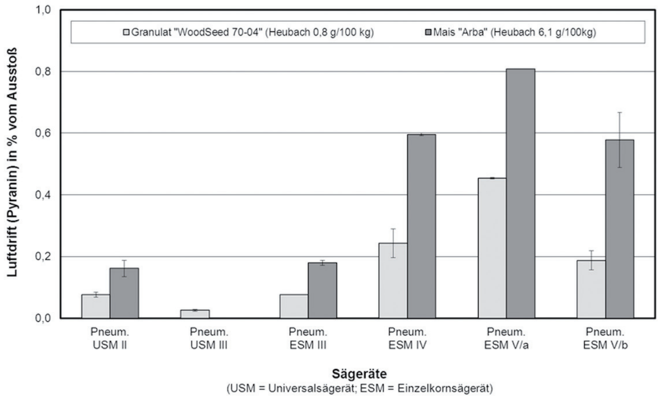

Abb. 4 Driftpotenziale verschiedener Sägeräte.

Fig. 4 *Drift potential of various sowing machines.*

Bei den Freilandmessungen zur Ermittlung der Luftdrift wurden die beschriebenen Kollektoren eingesetzt. In Abbildung 5 sind die mittleren Abdriftwerte aus vier Messungen im Vergleich dargestellt, die mit den jeweiligen Kollektorarten ermittelt wurden.

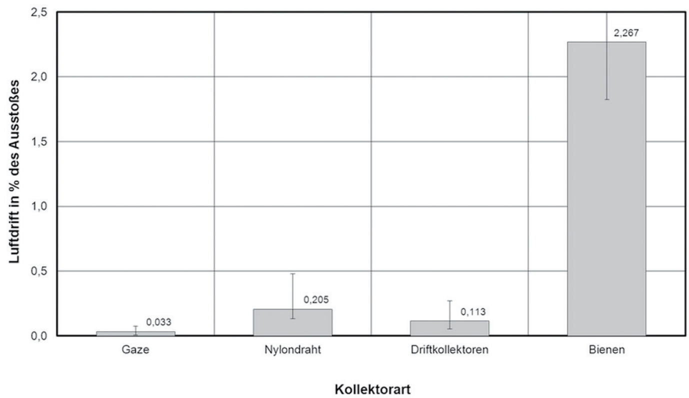

Abb. 5 Mittlere Abdriftwerte verschiedener Driftkollektoren aus vier Messungen mit Einzelkornsägeräten (gemessen von 0 bis 1 m über dem Boden bei einem Messabstand von 1 m vom Saatfeld).

Fig. 5 *Mean drift values of various drift collectors from four measurements with single seed sowing machines (measurement of 0 to 1 m above ground, measuring distance – 1 m from sown field).*

Die vergleichsweise hohen Abdriftmengen, die an Bienen gemessen wurden, konnten im Forschungszeitraum nicht ausreichend erklärt werden. Möglicherweise hängen diese hohen Werte mit der elektrostatischen Ladung zusammen, die bei Untersuchungen in den USA an Bienen nachgewiesen werden konnten (Prier *et al*, 2001). Bei allen Freilandmessungen lag das mit Gaze ermittelte Abdriftniveau unterhalb den Nachweisstoffmengen, die an den übrigen Kollektoren gemessen wurden. Wie Luftströmungsmessungen zeigten, wird das großflächig aufgespannte Gewebe von der Abdriftwolke auf Grund des hohen Luftwiderstands teilweise umströmt. Es wird also hier nur ein Teil der tatsächlichen Luftdrift erfasst. Ein ähnlicher Effekt könnte für die vergleichsweise niederen Werte der Bälle aus Kunststoffgeflecht gegenüber den Nylondrähten verantwortlich sein. Für die Beurteilung des Driftverhaltens von Sägeräten im Freiland stellen deshalb die mit Nylondraht ermittelten Driftmengen die am ehesten belastbaren Werte dar.

Die in Abb. 6 dargestellten Luftdriftwerte einer Messung mit einem Einzelkornsägerät, die hier exemplarisch für die Mehrzahl der Freilandmessungen stehen, lassen den Schluss zu, dass nur ein Teil der in den Nahbereich der Aussaatfläche hinein schwebenden Beizstäube dort auch sedimentiert. Die Messwerte in 1 m Abstand nehmen mit zunehmender Höhe über dem Boden ab. Sie zeigen eine ähnliche Verteilung wie die vertikale Verteilung der Abdrift im Prüfstand. In größerer Entfernung von der Aussaatfläche zeigt sich dagegen eine gleichmäßige Verteilung der Driftmengen bis zu einer Höhe von 6 m. Zwischen den beiden Messabständen (1 m und 5 m) sedimentiert ein Teil der Partikel, was zu einem absoluten Rückgang der Luftdrift innerhalb dieses Bereichs führt. Ein wesentlicher Anteil der bei 1 m gemessenen Luftdrift bleibt demnach in der Luft. Wie weitere Messungen zur Luftdrift zeigen, bleibt diese Nachweisstoffmenge in der Luft auch bis zu einer Entfernung von 30 m zur Aussaatfläche nahezu unverändert.

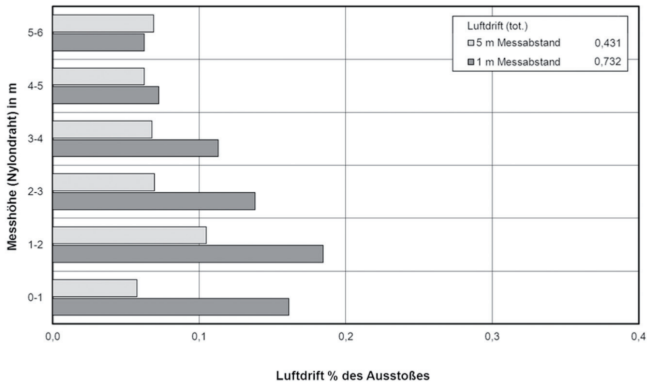

Abb. 6 Vertikale Verteilung der Abdriftmengen bei der Aussaat von Mais mit einem Einzelkornsägerät im Freiland.

Fig. 6 *Vertical distribution of the drift volume on sowing maize with a single seed sowing machine in the field.*

Die Driftkurven des Bodensediments zeigten entsprechend der physikalischen Gesetze zur Sinkgeschwindigkeit von Partikeln unterschiedlicher Größe den erwarteten Verlauf. Größere Beizstaubpartikel als Träger höherer Mengen aktiver Substanz sedimentieren schneller, weshalb im Nahbereich des Saatfeldes höhere Werte gemessen werden. Mit zunehmender Entfernung von der Nulllinie werden die sedimentierenden Partikel kleiner und somit die nachgewiesenen Abdriftmengen je Flächeneinheit geringer. Der in folgendem Diagramm dargestellte Verlauf des Bodensediments, welcher bei einer Abdriftmessung im Nahbereich der Aussaatfläche gemessen wurde, entspricht näherungsweise einer Exponentialverteilung (Abb. 7).

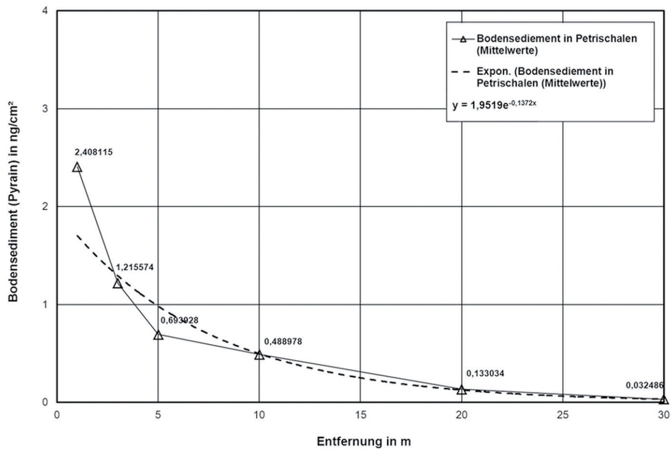

Abb. 7 Abdriftkurve zur Bilanzierung der im Nahbereich sedimentierten Beizstäube.

Fig. 7 *Drift curve to balance nearby sedimented dressing dust.*

Für die Bilanzierung der im Nahbereich sedimentierenden Beizstäube (bis 30 m) wird aus den gemessenen Abdriftwerten eine Ausgleichsfunktion ermittelt. Das Integral dieser Funktion entspricht der absoluten Abdriftmenge je Streckeneinheit (ng Pyranindrift/cm Sästrecke x Breite des Saatfelds). Bei der Aussaat verursacht jede gefahrene Säbahn eine Driftwolke. Jede dieser Driftwolken hat näherungsweise denselben Sedimentationsverlauf und kumuliert, jeweils um die Arbeitsbreite des Sägeräts versetzt, zur gemessenen Abdriftkuve. In dargestelltem Beispiel ergibt sich aus den errechneten flächenbezogenen Mittelwerten (in ng/cm²) eine Exponentialfunktion, welche den in der Legende angegebenen Verlauf hat ($y = 1{,}9519e^{-0{,}1372x}$) mit „x" für die Entfernung in m und „y" für die Driftmenge aktiver Substanz (Pyranin) in ng. Bis zu einer beliebigen Entfernung kann mit dieser Ausgleichsfunktion die absolute Driftmenge je Streckeneinheit berechnet werden. Im Beispiel ergibt sich bis 30 m Entfernung von der Nulllinie eine Driftmenge von 1.338 ng/cm Fahrstrecke.

Entsprechend der Vorgehensweise bei der Ermittlung der Luftdrift wird die so berechnete Driftmenge zur ausgebrachten Menge aktiver Substanz in Beziehung gesetzt. Im Beispiel ergibt sich aus einem Hektaraufwand von 48,2 g Pyranin bei einer Fahrstrecke von 1 cm und einer Arbeitsbreite von 3 m ein Pyraninausstoß von 144.600 ng/cm. Multipliziert mit der Anzahl gefahrener Bahnen, welche bei den durchgeführten Freilandversuchen 9 Bahnen betrug, ergibt sich eine Referenzmenge von 1.301.400 ng/cm Fahrstrecke. Daraus errechnet sich für das Bodensediment eine relative Driftmenge von 0,103 %.

Berechnung von Expositionsszenarien aus Prüfstandsmessergebnissen

Mit Hilfe dieser bei Freilandmessungen ermittelten Driftverläufe lassen sich aus den im Prüfstand ermittelten Abdriftmengen Expositionsszenarien für die Aussaat im Freiland errechnen. Für ein Worst-Case-Szenario wird dabei angenommen, dass die gesamte Driftmenge, die im Filter des Prüfstands abgeschiedenen wurde, bei einer Aussaat im Freiland im Nahbereich der Aussaatfläche sedimentiert. Mit Hilfe der bei Freilandmessungen ermittelten Driftverteilung lässt sich dann eine Driftkurve aus den im Prüfstand gemessenen Driftmengen berechnen. Diese Driftkurve entspricht dem Driftverlauf einer einzelnen Säbahn. Wird die von der Breite des Saatfeldes abhängige Anzahl von Driftverläufen, jeweils um die Arbeitsbreite des Sägeräts versetzt, aufsummiert, ergibt sich die höchstmögliche Abdrift aktiver Substanz auf den Nahbereich des entsprechenden Aussaatfelds (Abb. 8).

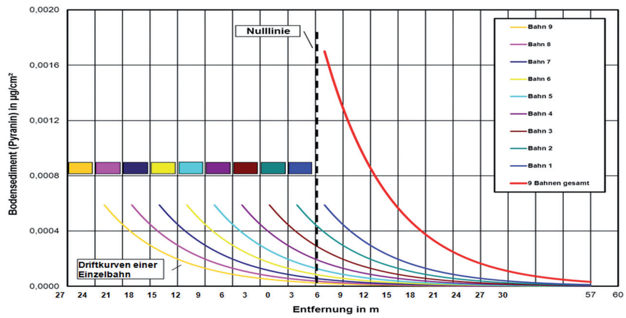

Abb. 8 Interpolierte Abdriftmengen (Bodensediment) einer Freilandmessung in % vom Ausstoß.

Fig. 8 *Interpolated drift volumes (soil sediment) of a field measurement in % output.*

Eine wesentliche Fragestellung bei der Durchführung der Freilandmessungen war, in wieweit die Ergebnisse der Prüfstandsmessungen mit den im Freiland gemessenen Driftmengen korrelieren. Aus den 21 Freilandmessungen, die mit 6 verschiedenen Sägeräten durchgeführt wurden, waren die Sägeräte-Saatgut-Kombinationen aus 3 Freilandmessungen direkt mit Ergebnissen aus den Sägeräteprüfungen vergleichbar. In Abbildung 9 sind die im Prüfstand ermittelten Driftpotenziale der drei Einzelkornsägeräte mit der jeweils im Freiland gemessenen Luftdrift und dem entsprechenden Bodensediment im Vergleich dargestellt. Wie dem Diagramm zu entnehmen ist, liegen die Werte der mit Nylondrähten gemessenen Luftdrift und des im Prüfstand ermittelten Driftpotenzials im gleichen Bereich. Das ergänzend abgebildete Bodensediment zeigt deutlich, dass für die Berechnung von Abdriftszenarien für den Nahbereich ein gewisser Anteil nicht sedimentierender Stäube vom gesamten Driftpotenzial abzuziehen ist.

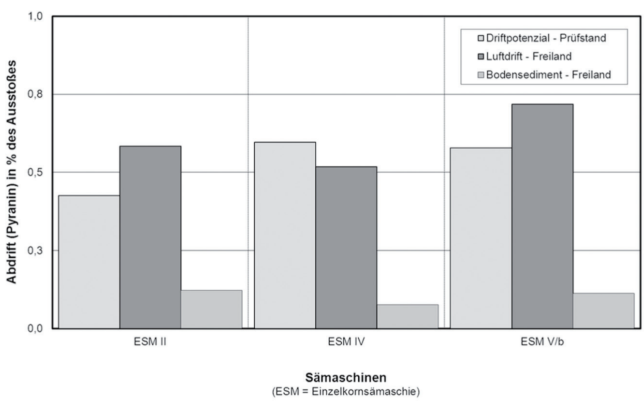

Abb. 9 Vergleich von Prüfstandsmesswerten und Driftmengen aus Freilandmessungen.

Fig. 9 *Comparison of drift values obtained from test stand and field measurements.*

4. Schlussfolgerungen

Mit dem im Forschungszeitraum entwickelten Prüfverfahren ist es möglich, das Driftpotenzial von Sägeräten unter standardisierten Bedingungen zu ermitteln. Wechselnde Witterungsverhältnisse, wie sie bei Freilandmessungen gegeben sind, können hier ausgeschlossen werden. Eine simulierte Aussaat im Windkanal ermöglicht es, sämtliche driftfähigen Staubemissionen durch einen Filter abzuscheiden und zu messen. Somit stellen die ermittelten Emissionsmengen eine durch die technischen Eigenschaften der Maschine bedingte wie auch von der Beizqualität des Saatguts abhängige „Worst-Case-Situation“ dar.

Die gewonnenen Messdaten erlauben eine Beurteilung der Geräte sowohl hinsichtlich der mechanischen Beeinträchtigung des Saatguts im Saatgutstrom als auch des Emissionsverhaltens. Mit der Verwendung eines Kunstsaatguts, welches im Rahmen des Forschungsprojekts entwickelt wurde, können mit dem Prüfverfahren reproduzierbare Ergebnisse erarbeitet werden. Es sind damit Grundlagen für die Prüfung von Sägeräten sämtlicher Bautypen geschaffen.

Wie einige methodische Ansätze im Rahmen des Forschungsprojekts zeigten, die hier nicht näher beschrieben wurden, kann auch die Effizienz technischer Verbesserungen an Maschinen durch Messungen im Prüfstand getestet werden. Eine Untersuchung der Emissionsquellen an Geräten ist möglich, wobei Lösungsansätze zur Reduzierung der Beizstaubabdrift erarbeitet werden können. Dabei ermöglicht der Prüfstand auch eine anschauliche Demonstration des Emissionsverhaltens von Sägeräten.

Das im Prüfstand ermittelte Driftpotenzial einer Sägeräte-Saatgutkombination spiegelt die maximale im Freiland zu erwartende Abdrift von Beizstäuben bei entsprechenden Windbedingungen wider. Es besteht somit die Möglichkeit, aus den im Prüfstand ermittelten Werten Expositionsszenarien zu erarbeiten. Hierfür müssen die bei Freilandmessungen ermittelten Driftkurven herangezogen werden, deren Verläufe wesentlich von der Windgeschwindigkeit abhängen. Diese Szenarien sind jedoch lediglich für den Nahbereich berechenbar. Die Driftverläufe nichtsedimentierender Stäube sind nicht ausreichend erforscht. Hierzu sind weitere Messungen im Freiland nötig.

Danksagung

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Efficacy assessment of soil insecticides and seed treatments for the control of western corn rootworm larvae

Wirksamkeit von Bodeninsektiziden und Saatgutbehandlungen zur Bekämpfung des Westlichen Maiswurzelbohrers

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Western corn rootworm larvae develop and feed below ground and cause severe damage to maize roots. The most important target of control measures against this pest are larval stages in the soil. The presented study deals with various attempts of corn rootworm larval control by insecticides. The efficacy of granular soil insecticides and insecticides as seed coating is influenced by several factors, such as the abundance of the pest insect, the formulation, the timing of the application of the control measures or soil type. These factors have been investigated in a series of experiments presented here. It was the aim to evaluate various control options and to define the most suitable and environmentally safe control method against western corn rootworm larvae.

Experiments were carried out in heavily infested corn fields and in virtually pest-free first year maize fields in Austria. Maize plants in experimental plots were artificially infested with defined numbers of *Diabrotica* eggs, to ensure a homogenous distribution of naturally occurring corn rootworm populations and to simulate different levels of infestation. Soil types were roughly differentiated into heavy, medium and light soils. Insecticides tested contained the active ingredients Clothianidin, Tefluthrin and Spinosad. Control measures were carried out during sowing of maize in mid-April. Experiments were evaluated by counting adult beetles emerging from experimental plots in emergence cages and by rating damage on the maize roots caused by corn rootworm larvae according to the "Node Injury Scale".

Insecticides showed medium efficacy in reducing the number of emerging beetles. In general, Clothianidin proved to be most efficient in protecting roots from larval damage. Therefore, the tested insecticides seem to be useful for plant protection but are of limited use for eradication measures, against the quarantine pest.

Influence of different soil types on the survival rates of the pest insect as well as on the performance of insecticides is still unclear. However, experiments have shown that rootworm larvae are susceptible to desiccation. Therefore, survival rates in sandy soils were lower than in heavier ones.

The project was funded by the German Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Diabrotica research program.

Evaluation of entomopathogenic bacteria and fungi for the control of the western corn rootworm

Untersuchungen entomopathogener Bakterien und Pilze für die Bekämpfung des Westlichen Maiswurzelbohrers

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The western corn rootworm (*Diabrotica virgifera virgifera* LeConte, WCR) is a well established maize pest in Hungary. A range of various control tools are used by farmers to keep its population under economic threshold level. These tools involve rotation of maize but in continuous cultivation, insecticide seed treatment and/or soil insecticide application against larvae are the primary tools. Foliar insecticide applications to decrease adult population and reduce egg laying are less common practices. The use of entomopathogenic bacterial and fungal products still needs research and development inputs.

We have tested the efficacy of fermented cultures of various entomopathogenic (toxin producing) strains of *Bacillus thuringiensis* (Berliner) (*Bt*), and five strains of *Metarhizium anisopliae* (Metsch.) (Sorokin) conidial fungus against the larvae of WCR.

In *in vitro* tests, newly hatched WCR larvae were fed with freshly germinated maize roots. Then, at the second larval stage, they were treated with 2 ml of the bacterial and fungal preparations. Throughout the larval development we measured the rate of surviving larvae.

In *in vivo* experiments two maize seeds were placed into pots of 15 centimeters diameter, and then the pots were grouped by six and placed into isolators. 20 WCR eggs were put directly under the seeds. The microbial preparations were applied in the same dosage as in the *in vitro* trials. Pherocon AM yellow sticky traps were put into the upper parts of the isolators to capture emerging adults. One month after the planting, the height of the plants was measured, and the damage caused by larvae was determined based on the modified IOWA 1-6 scale, along with the number of adults captured by the traps.

In every case, the efficacy of the microbial treatments was compared to untreated controls and to seed treatments with insecticide tefluthrin (Force 1,5G) and *Bacillus thuringiensis var. tenebrionis* (Novodor FC).

The efficacies of the *Bt* bacterial preparations and the *M. anisopliae* strains were significantly different from each other. Several bacterial preparations and fungal strains almost reached the efficacy of the tefluthrin treatment considering both larval mortality and the reduction of root damage. In conclusion, the cultures of the *Bt* strains and the *M. anisopliae* fungal strains tested are promising control tools and therefore need further field tests.

Research was supported by the TÁMOP-4.2.1.B-11/2/KMR-2011-0003.

A new method for efficacy testing of control measures against adult *Diabrotica* in maize

Eine neue Methode für die Prüfung der Wirksamkeit von Pflanzenschutzmaßnahmen gegen Diabrotica-*Käfer im Mais*

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Testing the efficacy of pesticides in the field is often accomplished by assessing densities of pest insects by attracting and catching them in traps. However, these methods do not show the direct effect of the control measure to be tested, e.g. the mortality caused by the insecticide. In fact, they show the abundance of alive insects, which is indirectly affected by the tested treatment together with many other factors, including mobility of the test species. This may be a challenge with highly mobile study subjects, like the western corn rootworm, *Diabrotica virgifera virgifera* (WCR).

A field method for direct determination of mortality of WCR beetles after treating the fields with insecticides is described. Monocrop maize fields with heavy *Diabrotica* infestation were treated with a neonicotinoid insecticide and compared to untreated control fields. Efficacy of the treatment was assessed with yellow sticky traps and with the newly developed method for mortality assessment: the latter consisted of cotton panels mounted between the stems of four corn plants of two neighbouring rows, in order to collect dead beetles dropping from the plants beyond the covered area.

After insecticide application, the number of dead beetles collected with panels in the treated plots was significantly higher than those in the control plots only 1 day and 3 days after application. However, no differences were found 7, 14 and 21 days after application. At the same time and in the same fields, the number of beetles caught with yellow sticky traps dropped significantly after insecticide application. Differences between treated plots and control plots were significant 1, 3 and also 7 days after treatment. Results of yellow sticky traps therefore suggested longer activity of the insecticide than proved by the mortality assessment with panels. Direct mortality assessment methods may therefore be important tools for validation of control measures in field tests.

The results of these studies have been published in detail in the Journal of Cultivated Plants, **64** (9), 2012, 342-347.

The project was funded by the German Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Diabrotica research program.

Laboratory test of the potential for using insecticide-cucurbitacin mixtures for controlling the quarantine pest *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae)

Laboruntersuchungen zur Verwendung von Insektizid-Cucurbitacin Mischungen zur Bekämpfung des Quarantäneschädlings Diabrotica virgifera virgifera LeConte (*Coleoptera*: *Chrysomelidae*)

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Summary

Due to the great ecological plasticity and adaptability of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, the unilateral use of one control option could result in it becoming less effective within a few years. Therefore, no one option alone is sufficient for resolving the problem with *D. virgifera virgifera*. In fact all possibilities have to be considered and integrated. One possibility is to control the adult beetles and so minimize egg deposition and reduce the beetle population size below the economic threshold for the following year. By applying mixtures of insecticides with bitter substances from cucurbits, which are strong feeding stimulants for *D. virgifera virgifera,* it should be possible to reduce insecticide dosages by up to 95%. This would control the beetles and minimize undesired side-effects to the agro-ecosystem and non-target organisms. Therefore, the aim of this study was to determine (i) the interactions between cucurbitacins (Invite) and five insecticides with different modes of action (indoxacarb, neonicotinoids, organophosphates, pyrethroids and spinosyns), (ii) the effects of biological factors (age, gender and pre-contact) and (iii) the possibility of selection for resistance to bitter agents.

In laboratory trials it was shown that the stimulatory effect Invite has on feeding had little or no effect on the efficacy of the five insecticides tested. The improvement in the efficacy after five hours of exposure to Avaunt (indoxacarb) and Biscaya (neonicotinoid) disappeared after 24 and 48 h, and is attributed to the slow initial effects of Avaunt and the recovery of beetles exposed to Biscaya, respectively. Although the LC-values of Biscaya and Avaunt were significantly greater than the corresponding values for mixtures with Invite, it was not possible to reduce the dosages of these active substances by up to 90%. There was no improvement in the efficacy after 48 h of exposure to any of the other insecticides analyzed. The assumption that contact (e. g. pyrethroides) and gas phase insecticides (e. g. organophosphate) are generally less suitable for mixing with Invite was only partly supported by our results. The lack of improvement in the efficacy of Spinosad when mixed with Invite is especially puzzling. Neonicotinoids and indoxacarb are suitable for mixing with Invite, especially in terms of delaying the selection for resistance. Carbamates, e. g. carbaryl (not analyzed in this study), were successfully applied in mixtures with cucurbitacins.

The results indicate that biological factors such as gender, age and pre-contact have a strong effect on the attractiveness of Invite. In the experiments, the strength of the response of young beetles to the bitter agents was greater than that of old beetles and that of females less intense than that of males, and pre-contact markedly reduced the stimulatory effect for both sexes. These results and the findings of trials using *D. virgifera virgifera* caught in Austrian maize fields before and after applications of insecticide-Invite mixtures indicate that the attractiveness of cucurbitacins varies and is subject to selection. If this control strategy is applied extensively then it is likely that this beetle will develop resistance to the bitter agents. Thus, it is important to monitor the resistance of the beetles to these substances.

Keywords: western corn rootworm, Invite, insecticides, attractiveness, feeding, resistance

Zusammenfassung

Aufgrund der großen ökologischen Vielfalt und Anpassungsfähigkeit des Westlichen Maiswurzelbohrers, *Diabrotica virgifera virgifera* LeConte, könnte die einseitige Nutzung einer Bekämpfungsmethode dazu führen, dass sie innerhalb weniger Jahre an Wirksamkeit verliert. Eine Methode allein reicht also nicht, um das Problem mit *D. virgifera virgifera* zu lösen. Tatsächlich müssen alle Möglichkeiten betrachtet und zusammengeführt werden. Eine ist die Bekämpfung adulter Käfer, um so die Eiablage zu minimieren und die Populationsgröße der Käfer im nachfolgenden Jahr unter die ökonomische Schadensschwelle zu senken. Durch die Verwendung von Insektizidmischungen mit Bitterstoffen aus Kürbisgewächsen, die starke Fraßstimulanzien für *D. virgifera virgifera* sind, sollte es möglich sein, Insektiziddosierungen um bis zu 95% zu reduzieren. Dadurch würden die Käfer bekämpft und unerwünschte Nebenwirkungen auf das Agroökosystem und Nichtzielorganismen minimiert werden. Mit Hilfe dieser Studie sollte folgendes festgestellt werden: (I) die Wechselwirkungen zwischen Cucurbitacinen (Invite) und fünf Insektiziden mit unterschiedlichem Wirkungsmechanismus (Indoxocarb, Neonicotinoide, Organophosphate, Pyrethroide und Spinosyne), (II) der Einfluss biologischer Merkmale (Alter, Geschlecht und Vorkontakt) und (III) die Möglichkeit der Resistenzbildung gegen Bitterstoffe.

Im Laborversuch wurde nachgewiesen, dass die stimulative Wirkung von Invite auf den Fraß wenig oder gar keine Wirkung auf die Wirksamkeit der fünf untersuchten Insektizide hatte. Die Wirksamkeitssteigerung nach fünf Stunden Einwirkung von Avaunt (Indoxocarb) und Biscaya (Neonicotinoid) verschwand nach 24 h bzw. 48 h und lässt sich auf die langsame Anfangswirkung von Avaunt bzw. die Erholung der Käfer von Biscaya erklären. Obwohl die LC-Werte von Biscaya und Avaunt signifikant größer waren als die entsprechenden Werte für Invite-Gemische, war es nicht möglich, die Dosierung dieser Wirkstoffe um bis zu 90 % zu verringern. Eine Erhöhung der Wirksamkeit nach 48 h Einwirkzeit konnte für keines der anderen untersuchten Insektizide festgestellt werden. Die Annahme, dass Kontakt- und gasförmige Insektizide (z. B. Pyrethroide bzw. Organphosphate) generell weniger für Gemische mit Invite geeignet sind, konnte durch unsere Ergebnisse nur teilweise gestützt werden. Die fehlende Steigerung der Wirksamkeit von Spinosad im Gemisch mit Invite ist besonders erstaunlich. Neonicotinoide und Indoxocarb eignen sich für Gemische mit Invite, insbesondere zur Verzögerung der Resistenzbildung. Carbamate, z. B. Carbaryl (in dieser Studie nicht untersucht), wurden erfolgreich in Gemischen mit Invite angewendet. Die Ergebnisse zeigen, dass biologische Merkmale wie Geschlecht, Alter und Vorkontakt eine große Wirkung auf die Attraktivität von Invite haben. In den Versuchen zeigten junge Käfer eine stärkere Reaktion auf die Bitterstoffe als alte Käfer und Weibchen eine weniger intensive als Männchen. Und Vorkontakt verringerte deutlich die stimulierende Wirkung auf beide Geschlechter. Diese Ergebnisse und die Resultate aus Versuchen mit *D. virgifera virgifera*, die in österreichischen Maisfeldern vor und nach der Anwendung von Invitegemischen gefangen wurden, zeigen, dass die Attraktivität von Cucurbitacinen unterschiedlich ist und der Selektion unterliegt. Wird diese Bekämpfungsstrategie in großem Umfang angewendet, ist anzunehmen, dass der Käfer Resistenz gegenüber Bitterstoffen entwickelt. Aus diesem Grund sollte die Resistenz der Käfer gegen diese Stoffe überwacht werden.

Stichwörter: Westlicher Maiswurzelbohrer, Invite, Insektizid, Attraktivität, fressen, Resistenz

1. Introduction

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte 1868, is one of the most serious pests of maize. Management options for controlling WCR are to target either the root feeding larvae by crop rotation, to apply soil insecticides or plant varieties of transgenic *Bt*-maize, or to treat adult beetles by aerial application of insecticides with the objective of reducing oviposition and damage by larvae in the following season. Concerns over unintended side effects, the development of resistance to organophosphates and carbamates and behavioural adaptation to crop rotation in parts of the US corn belt stimulated renewed interest in combining aerial insecticides with semiochemicals. It is known that cucurbitacins, extremely bitter secondary plant compounds of Cucurbitaceae, evoke compulsive feeding in adult WCR. It is argued that the addition of cucurbitacins to aerially applied insecticides could allow the field application rate to be reduced to about 10% of that usually recommended. The successful control of WCR using such mixtures is reported in a number of area-wide management programs in the US. Another, more worrying result of these programs is that the use of a single active ingredient (i.e. cabaryl) can result in the development of a significant level of resistance within only three to four years.

Therefore, the aim of this study was to evaluate the possibility of combining cucurbitacins with insecticides that have different modes of action (i.e. indoxacarb, neonicotinoids, organophosphates, pyrethroids and spinosyns). Further the effect that biological factors, such as age, sex and previous contact with cucurbitacins, have on the attractiveness of the bitter compounds was investigated. The third aim of this study was to determine whether the use of Invite will increase the rate at which cucurbitacins loose their attractiveness for WCR.

2. Material and Methods

Test organisms

The beetles used were reared since 2006 as a non-diapausing strain (USDA-NCARL, Brookings, USA; Branson 1976). The rearing-methods are those described by Branson *et al.* (1975) and Jackson (1986). At the beginning of experiments the beetles were 2–3 weeks old and had not previously come into contact with any food containing cucurbitacin (e.g. squash, zucchini etc.).

Experiments

Leaf-dip bio-assays (Fig. 1) were used to assess the toxicity of a mixture of cucurbitacin (Invite®EC; FFP AgroTech, Eustis, FL 32727) and insecticide compared to that of pure insecticide (i.e. oxadiazine, neonicotinoids, organophosphates, pyrethroids and spinosyns) (Tab. 1). Invite®EC consists of cucurbitacins (80%) derived from Hawkesbury watermelon, *Citrullus vulgaris* Schrad, and non-toxic edible carriers (20%) (Gerber *et al.*, 2005).

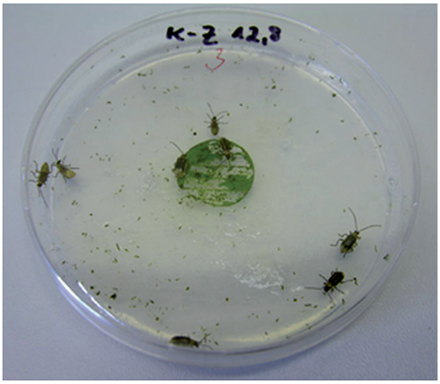

Fig. 1 Leaf dipping bioassay.

Abb. 1 *Blatt-Tauch-Bioessay.*

Tab. 1 Insecticides applied in laboratory experiments. CAI – content of active ingredient (g ai/l); RFR – recommended field rate ($ml_{Product}$/ha).

Tab. 1 *In Laborexperimenten genutzte Insektizide. CAI – Gehalt aktiver Substanz (g ai/l); RFR – registrierte Feldaufwandmenge ($ml_{Product}$/ha).*

Chemical Sub-group	Active ingredient	Trade name	CAI	RFR	Batch no
3A Pyrethroids	lambda-Cyhalothrin	Karate Zeon	100	75	3082
4A Neonicotinoids	Thiacloprid	Biscaya	240	300	PF 90156878
1B Organophosphates	Chlorpyrifos-methyl	Reldan 22	225	1500	YB 23272002
5 Spinosyns	Spinosad	SpinTor	480	50	YL 19272083
22A Indoxacarb	Indoxacarb	Avaunt	150	170	JUL09BL013

A leaf disc 24 mm in diameter was placed in each Petri dish together with 4 beetles. The leaves were taken from maize plants at BBCH 16–17 (cv. Tassilo, KWS Saat AG, Einbeck, Germany) grown in a greenhouse. To ensure a uniform wetting of the leaf discs, triton X100 (0.1% [v/v]) was added to the insecticide solutions as a wetting agent. After drying for 2 h at 22 ± 2 °C in a fume cupboard, the leaf discs were transferred to the Petri dishes (diameter 94 mm, height 16 mm) with their upper surface uppermost. Each Petri dish was filled 2 h before with 35 ml of 2% agar-solution (Kobe 1, Carl Roth GmbH & Co. K.G., product Nr: 5210.2). This agar served as a source of water for the leaf discs. Mortality and area of leaf discs consumed by the beetles (rated in 10%-steps) were recorded 5, 24 and 48 h after the beginning of the exposure. Each variant and concentration was replicated 8-times. Using this method the efficacy of insecticides ($LC_{50/90}$ with and without Invite) was assessed.

The experiments on the affect of biological factors were done using Invite treated cellulose membranes (Fig. 2). To assess the influence of age, sex and previous experience, the number of beetles that stayed on the different membranes and the area of membrane they consumed were recorded (Tallamy and Halaweish, 1993; Hollister and Mullin, 1998, 1999; Parimi *et al.*, 2003). The determination of attractiveness was done by 1st recording the number of beetles feeding on the membranes (arrested) after 15, 30 and 45 minutes and 2nd measuring the area of membrane consumed by the beetles. For this purpose a microscope (SZX 12, Olympus) was used to take pictures of each membrane at the end of the experiment and then analysed using image interpretation software cell^D (Olympus) (Fig. 1). Using the area of membrane remaining it is possible to calculate the area consumed.

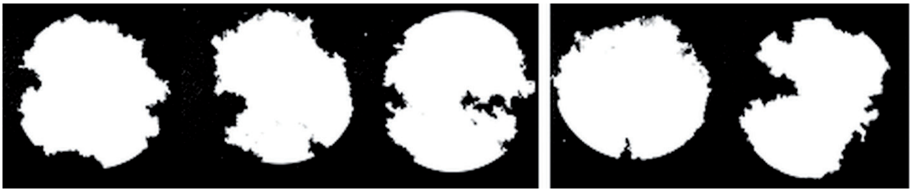

Fig. 2 Cellulose membranes (d=25 mm) treated with Invite after 6 h of exposure to five adult *D. virgifera virgifera.*

Abb. 2 *Mit Invite behandelte Zellulosemembranen (d = 25 mm) nach 6-stündiger Exposition gegen 5 adulte* D. virgifera virgifera.

The development of resistance to insecticides was determined by collecting beetles from maize fields in Austria before and after an insecticide-Invite application and assaying their resistance using Invite treated cellulose membranes. Due to quarantine regulations it was not possible to transport live beetles from Austria to Germany. Therefore these experiments were carried out by colleagues in Austria (AGES, Wien). At four locations beetles were collected in the same plots before and after the insecticide application. In the laboratory their gender was determined and response to Invite analyzed using the membrane bioassay. Preparation of materials (Petri dishes and membranes) and evaluations (planimetry and statistics) were done at BTL.

Statistical Analysis

All the basic statistical analyses were done using the computer programme SYSTAT, version 10.0. To identify the dose-response relationship the data were analysed using PoloPlus 1.0 (LeOra Software Company). In order, to determine the goodness-of-fit of the data (response of *D. virgifera virgifera* to different doses of insecticide) to the relationship predicted by the probit model the observed and predicted values were compared using chi^2 tests.

3. Results and Discussion

Cucurbitacins are oxidized tetra-cyclic triterpenes, which occur as typical secondary plant metabolites in most Cucurbitaceae and some other plant families (Metcalf and Lampman, 1989). In most of the plants containing cucurbitacins, the highest concentrations occur in roots and fruits, whereas in leaves and stems the concentrations are lower (Tallamy and Krischik, 1989). It is generally accepted that the extreme bitterness and toxicity of cucurbitacins account for why these substances are an effective defence against herbivores (e. g. Tallamy and Krischik, 1989; Metcalf, 1979; Metcalf *et al.,* 1980). A number of species of Diabroticina and Aulacophorina (Coleoptera: Chrysomelidae: Galerucinae: Luperini), to which *D. virgifera virgifera* also belongs, have become adapted to the cucurbitacins in cucurbits. These animals have overcome this defence strategy but the cucurbitacins now induce compulsive feeding behavior and are attractive for these beetles (e. g. Chambliss and Jones, 1966; Howe *et al.,* 1976; Metcalf, 1979; Fig. 1 B). This is the case for both male and female adult beetles and the larvae of *Diabrotica undecimpunctata howardi* Barber (DeHerr and Tallamy, 1991).

For adults and especially the larvae of *D. virgifera virgifera* (and most Luperini) Cucurbitaceae are not their host plants, therefore, cucurbitacins are not consumed along with their food during development (Halaweish *et al.,* 1999). The exception is the high intake of pollen by adult beetles. Most of the bitter substances in the pollen are excreted and only a small amount is metabolized (Ferguson *et al.,* 1985). The residual cucurbitacins accumulate mainly in the haemolymph, but also in the fat body, cuticula, spermatophores and developing eggs (Ferguson *et al.,* 1985; Andersen *et al.,* 1988; Tallamy *et al.,* 2000). In this way these animals sequester the anti-herbivore defence mechanism of Cucurbitaceae (Ferguson and Metcalf, 1985; Nishida and Fukami, 1990). It is reported that the ‚bitter' eggs and larvae of *D. undecimpunctata howardi* are less susceptible to the entomophagous fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, (Tallamy *et al.,*1998) and therefore these sequestered cucurbitacins not only protect them against predators but also against microbial pathogens. The way this pharmacophagy (Boppré, 1984) evolved is unknown.

Metcalf *et al.* (1987) were the first to demonstrate that a mixture of insecticides and cucurbitacins could be used to control adult *D. virgifera virgifera*. The results of this study stimulated several attempts to improve the formulation (e. g., increase the stability and persistence of mixtures in wet weather conditions) and methods of application, which resulted in a drastic reduction (up to 95–98%) in the field rate of insecticides (Chandler 2003). Commercial products registered in the USA are offered as proprietary mixtures (‚Slam' and ‚Adios' Microflow Co., Memphis, Tennessee) or as a cucurbitacin-additive in liquid (Invite) or powder form (CiteTrak) (Chandler, 2001; Tallamy *et al.,* 2005). During 1995–2002 in a number of practical trials carried out within the frame work of area wide pest management programs in the USA it was demonstrated that insecticide-cucurbitacin mixtures can be used to successfully control adult *D. virgifera virgifera* (Chandler *et al.,* 2003; Gerber *et al.,* 2005).

In our study the effectiveness of none of the insecticides tested was increased by 90% by mixing with Invite. The most suitable products for mixing with Invite were Avaunt (oxadiazine) and Biscaya (neonicotinoid). SpinTor should not be mixed with Invite as in mixture it did not show any increase in efficacy in the laboratory (Fig. 3). The biggest difference between treatments with and without Invite was recorded after five hours of exposure (Tab. 2). At later assessments (i.e. 24 and 48 h) the differences between treatments decreased. For Karate Zeon (pyrethroids), the addition of Invite caused no effect after 24 h, and for Reldan22 (organophosphate), and SpinTor (spinosyns), the addition of Invite caused no effect after 24h and 48 h. These results partly contradict those of field tests.

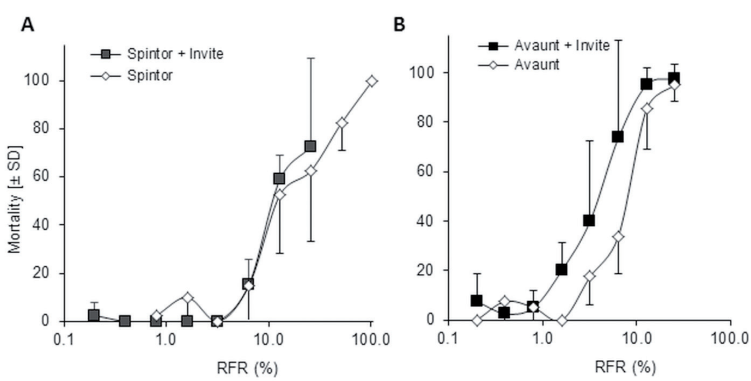

Fig. 3 Mean percentage mortality (± SD) of adult *D. virgifera virgifera* 24 h after exposure to A – SpinTor/SpinTor + Invite; B – Avaunt/Avaunt + Invite presented on leaf discs. (RFR–recommended field rate).

Abb. 3 *Mittlere prozentuale Mortalität (± SD) adulter* D. virgifera virgifera *24 h nach Exposition gegen A – SpinTor/SpinTor + Invite; B – Avaunt/Avaunt + Invite, die auf Blattscheiben ausgebracht wurden. (RFR–registrierte Feldaufwandmenge).*

Tab. 2 Mean of sum of percentage mortality (± SD) of adult *D. virgifera virgifera* after exposure to six concentrations of pure insecticides compared with mixtures of insecticides and cucurbitacin (Invite®EC) presented on leaf discs in the laboratory. (* p<0.05; ** p<0.01; *** p<0.001; ns – not significant; Mann and Whitney U-test, and t-test).

***Tab. 2** Mittlere Summe der prozentualen Mortalität (±SD) von adulten* D. virgifera virgifera *nach Exposition gegen 6 Konzentrationen eines reinen Insektizids im Vergleich mit einer Mischung aus Insektizid und Cucurbitacin (Invite®EC), die auf Blattscheiben ausgebracht wurden. (* p < 0,05; ** p < 0,01; *** p < 0,001; ns – nicht signifikant; Mann and Whitney U-test, und t-test).*

Insecticide	5 hours			24 hours			48 hours		
Avaunt	34.4	± 29.7	***	318.8	± 43.8	***	387.5	± 46.3	**
Avaunt + Invite	234.4	± 51.7		428.1	± 45.2		468.8	± 54.7	
Biscaya	115.6	± 55.0	***	237.5	± 70.7	***	290.6	± 37.7	**
Biscaya + Invite	459.4	± 37.7		371.9	± 45.2		393.8	± 62.3	
Karate Zeon	215.6	± 40.0	***	200.0	± 48.2	ns	150.0	± 50.0	*
Karate Zeon + Invite	375.0	± 35.4		218.5	± 34.0		234.4	± 66.7	
Reldan22	118.8	± 34.7	***	334.4	± 58.2	ns	393.8	± 45.8	ns
Reldan22 + Invite	225.0	± 56.7		368.8	± 39.5		393.8	± 37.2	
SpinTor	73.9	± 13.1	ns	142.5	± 27.4	ns	152.9	± 36.7	ns
SpinTor + Invite	94.3	± 38.6		146.8	± 41.7		151.8	± 25.0	

For a successful use of insecticide-cucurbitacin mixtures it is important to have a better knowledge of the biological factors that affect the interactions between beetles and bitter agents. Especially gender, age and pre-contact with cucurbitacins are important factors influencing the response of Diabroticina. There is such data for *D. undecimpunctata howardi,* published by Tallamy and Halaweish (1993), and for *Acalymma vittatum* (Fabricius), published by Smyth *et al.* (2002), but not for *D. virgifera virgifera*.

The results of our experiments indicate that the attractiveness of Invite is affected significantly by all of the three biological variables tested, i.e. Invite is most attractive to young males that have no previous experience with this substance (Fig. 4). This is also true for feeding activity (Fig. 5). Young beetles with no previous experience with this substance consumed larger areas of membrane treated with Invite than older beetles or those with previous experience. Of the beetles with previous experience of Invite young females fed more than males of the same age, but old males consumed more than old females. These results indicate that insecticide-cucurbitacin mixtures might be less effective in areas were Cucurbitaceae (e.g. melons, pumpkins) are cultivated in close proximity to maize fields. The results further indicate that beetles that survived a previous application or feed on sub-lethal residues are less likely to be attracted by subsequent applications.

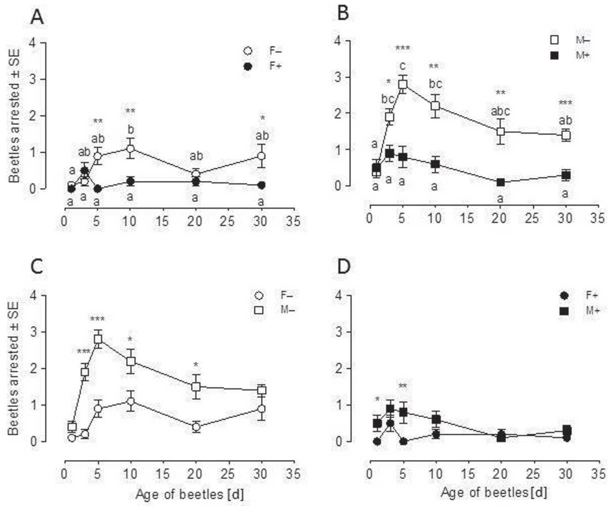

Fig. 4 A-D Effect of age (days), sex and pre-contact (PC) on the mean number (± SE) of *D. virgifera virgifera* present on Invite treated cellulose-membranes. (F- females without PC; F+ females with PC; M- males without PC; M+ males with PC, different letters indicate significant effects of age within a variant, * – significant differences between variants, * p<0.05; ** p<0.01; *** p<0.001, t-test and Mann and Whitney U test.

***Abb. 4 A-D** Einfluss von Alter (Tage), Geschlecht und Vorkontakt (PC) auf die mittlere Anzahl (± SE) von* D. virgifera virgifera *auf Invite-behandelten Zellulosemembranen. (F- Weibchen ohne PC; F+ Weibchen mit PC; M- Männchen ohne PC; M+ Männchen mit PC, unterschiedliche Buchstaben bezeichnen signifikante Einflüsse des Alters innerhalb einer Variante, * – signifikante Unterschiede zwischen Varianten, * p<0.05; ** p<0,01; *** p<0,001, t-test und Mann and Whitney U-test).*

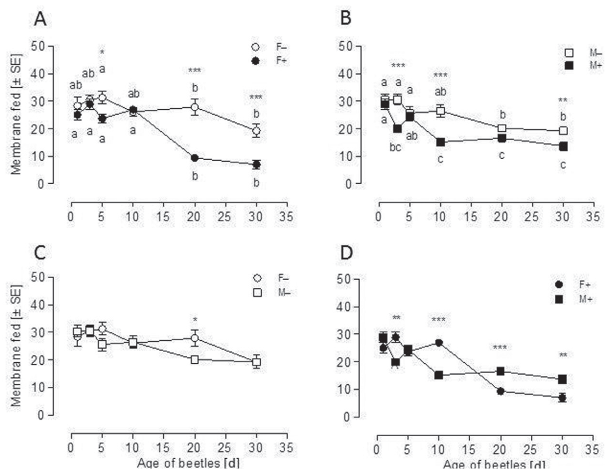

Fig. 5 A-D Effect of age (days), sex and pre-contact (PC) on the mean area of Invite-treated membrane consumed (± SE) by *D. virgifera virgifera.* (F- females without PC; F+ females with PC; M- males without PC; M+ males with PC, different letters indicate significant effects of age within a variant, * – significant differences between variants, * p<0.05; ** p<0.01; *** p<0.001, t-test and Mann and Whitney U test).

***Abb. 5 A-D** Einfluss von Alter (Tage), Geschlecht und Vorkontakt (PC) auf die durch* D. virgifera virgifera *mittlere konsumierte Fläche (± SE) von Invite-behandelten Zellulosemembranen. (F- Weibchen ohne PC; F+ Weibchen mit PC; M- Männchen ohne PC; M+ Männchen mit PC, unterschiedliche Buchstaben bezeichnen signifikante Einflüsse des Alters innerhalb einer Variante, * – signifikante Unterschiede zwischen Varianten, * p<0,05; ** p<0,01; *** p<0,001, t-test und Mann and Whitney U-test).*

A reduced susceptibility to cucurbitacins will result in beetles only ingesting small (sub-lethal) doses of insecticide, which increases the risk of this beetle becoming resistant to the active ingredient.

This risk is illustrated by the results of an experiment in which the responses of beetles collected from maize fields in Austria before and after an insecticide-Invite application were assayed using Invite treated cellulose membranes. Without regard to gender the beetles collected before and after an application of an insecticide-Invite mixture did not differ in their response to membranes treated with Invite. Both the number of beetles arrested and the area of membrane consumed did not differ. But gender-related analyses revealed that less female than male beetles caught before the application were arrested (Fig. 4). This was not the case for beetles collected after the application. There were significant differences in the number of males arrested. This revealed that Invite was more attractive to male beetles collected before than after the application (Fig. 6A). The areas of membrane consumed by both groups of beetles were the same (Fig. 6B).

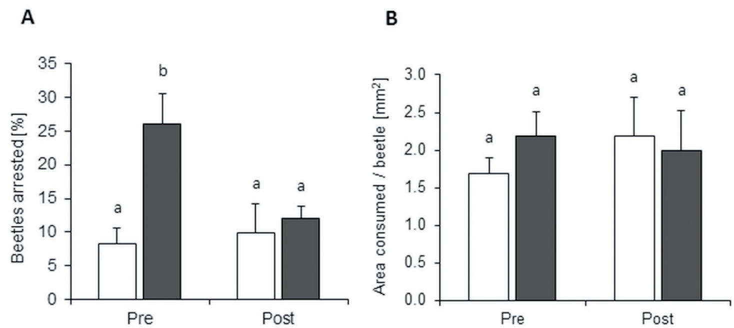

Fig. 6 Attractiveness of repeatedly applied Invite to *Diabrotica* beetles.

Abb. 6 *Attraktivität von Invite für* Diabrotica*-Käfer nach wiederholter Behandlung.*

There have been experiments carried out in the USA on the selection for both, resistance to insecticides and cucurbitacins (Siegfried *et al.* 2004). In four areas (1 Iowa, 2 Kansas, 3 Indiana/Illinois and 4 South Dakota) the susceptibility of *D. virgifera virgifera* caught in treated fields was compared with that of beetles caught in untreated fields. Using bioassays (glass residue tests) they show that in three of the areas (1–3) the susceptibility of beetles to carbamat (carbaryl) significantly declined from 1997 to 2002. Comparable results are reported by Zhu *et al.* (2001). These authors show that after only four years of applying a cucurbitacin-carbaryl-mixture (Slam) the susceptibility (LC_{90}) of the beetles was up to 20-times less. In parallel with the selection for resistance to the insecticide in these areas the response of beetles to cucurbitacins also declined. These results clearly show that large-scale and long-term application of a single active substance results in selection, which could lead to reduced efficacy and finally resistance to the pesticide.

Acknowledgements

This study was part of the German Diabrotica research program (see http://diabrotica.jki.bund.de) and funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). We thank all those colleagues from Austria who supported our experiments by supplying *Diabrotica* collected in the field.

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Manual application of insecticidal dust in semi-field experiments with honeybees

Manuelle Applikation von Insektizidstäuben in Halbfreilandversuchen mit Honigbienen

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In order to assess the risk for honey bees following exposure to different size fractions of dust containing insecticides, semi-field trials were carried out from 2010 to 2012. Aim of these experiments with manual application of insecticidal dust was to establish a "no observed effect rate" (= NOER) and a "lowest-observed-effect rate" (= LOER) for honey bees by examining adult bee mortality and development of brood (brood termination rate). In addition, using the same experimental conditions (size fraction, application rate) but different crops effects on bee mortality were investigated and compared.

Dusts of different size fractions were gained by sieving abraded dust of insecticide treated maize to different size classes, analysed for residue content and manually applied on flowering Phacelia and winter oilseed rape. To facilitate homogeneous distribution of the small amounts of active ingredient, the contaminated dust was diluted with standard soil (LUFA 2.2) of the same particle size range as the investigated dust. The effects of different rates of active substance (0.10, 0.25, 0.50 and 1.0 g a.i./ha Clothianidin; n=3) and of different particle sizes of dust ($x \leq 160$ µm, $250 < x \leq 450$ µm, $x > 500$ µm with an application rate of 2.0 g a.i./ha; n=3) on honey bees were examined.

In the experiments, 2-3 gauze-covered tents (10 x 4 m) covering the flowering crop, with one bee colony in "Hohenheimer Einfachbeuten" (Zander, 10 frames) with at least three brood combs in all developmental stages (eggs, larvae and sealed brood) were set up in each replicate. In the experiments, different mixtures of dusts and soil (seed treatment dusts and standard soil LUFA 2.2) were manually applied on the flowering crop inside the tents during full bee flight activity. Foraging intensity and mortality in dead bee traps (Type "Gary") were assessed for at least 7 days after application and samples of dead bees were taken for residue analysis.

In contrast to other dust fractions applied at the same rate of a.i. per ha, significantly increased mortality and effects on brood development were detected for fine dust particles «$x \leq 160$ µm», which is the particle size range of pollen (2–250 µm). At the same application rate and size fraction similar mortalities occurred in winter oilseed rape and Phacelia. In the trials with a target application rate of 0.1 g Clothianidin/ha, no adverse effects were observed, at 0.25 g Clothianidin/ha a slight increase of adult mortality was detected.

The project was funded by the German Ministry of Food, Agriculture and Consumer Protection (BMELV) within the German Diabrotica research program.

Investigations of application techniques for tall corn crops

Untersuchung von Applikationstechniken für hohe Maisbestände

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The treatment of adults of the corn rootworm on large areas requires high power of impact of plant protection equipment (Fig. 1). In addition to the features of the device such as reliability, tank capacity and maneuverability as well as the driving speed especially the volume rate is very important. Therefore, it should be in the lower range of the specified rate in the approval or authorization of the plant protection product. Since in most cases distance regulations to water bodies or terrestrial structures must be observed, coarse atomizing nozzles must be used, leading to a low degree of coverage on target areas. In addition, the height of the corn crop of up to 3.5 meters requires a good penetration of spray droplets to reach the target areas such as the ears of the corn.

Fig. 1 Specialized sprayer for tall crops during the drift measurements.

Abb. 1 *Spezialspritzgerät für hohe Kulturen während der Driftmessung.*

Setting the volume rate at 200 l/ha will obtain reasonable values of speed (at 7 km/h) and spraying pressure (at 3 bar) for the nozzle size 03. The testing of spray deposition distribution and drift in tall corn crops were therefore carried out with air injection nozzles of this size and three different designs. In addition to a vertical discharging standard injection nozzle a double jet injection nozzle with symmetrical spraying direction (each 30° to the front and rear) and a double jet injection nozzle with asymmetric spraying direction (10° forward, 50° to the rear) were used. The fluorometric measurements of spray deposition were carried out icluding the following parts of 10 corn plants in each case: tassel, upper 6 leaves, tips of the two ears with silks and stalk between the ears of corn. In consideration of the high mobility of the corn rootworm and its feeding sites, due to the achievable vertical distribution, experiments with additional air support to improve the penetration were dispensed. The drift measurements were carried out at distances of 1, 3, 5, 10 and 20 m from the area treated according to the guideline for measuring the direct drift of JKI.

Results of drift measurements

Despite the very high boom position of about 4 m above the ground all three nozzle types achieved a reduction of drift by more than 90% (Fig. 2) compared to standard nozzles. This applies to the entire range of 3 to 20 m from the treated area, which is relevant for standard valuation of drift reduction. The drift reduction classes according to the list of drift reducing devices of the JKI were achieved in any case.

Next to the treated area at a distance of 1 m, the measured values approximately correspond to the standard. The additional use of a rim nozzle at the end of the spraying boom could be an improvement.

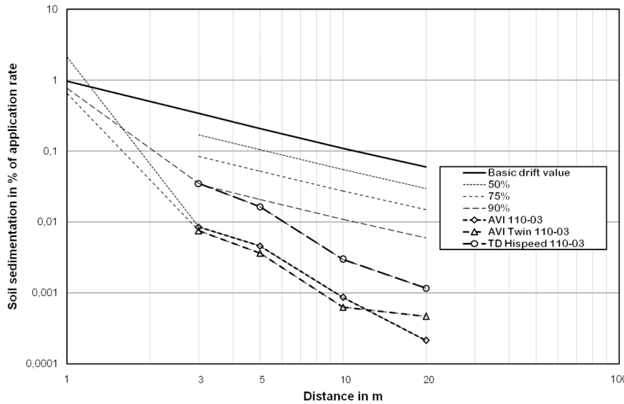

Fig. 2 Results of drift measurements.

Abb. 2 *Ergebnisse der Driftmessungen.*

Results of spray deposition measurements

With all three nozzles types the spray deposits on the leaves decrease from top (flag leaf) to bottom (sixth sheet in the ear area), at the same time showing that the symmetrical double jet injection nozzle generally scored slightly lower depositions than the other two nozzles. These are on the same level at the top, with increasing depth of penetration there are benefits on the leaves for the vertical spraying nozzle while the double flat spray nozzles converge.

At the ear tips with silk there are only small differences between the nozzles, while the asymmetrical spraying double jet injection nozzle achieves slightly higher deposition at the vertical stalk section of the plant.

Overall, Fig. 3 shows that there is no improvement both in terms of amount of spray deposition and in terms of vertical distribution of double jet injection nozzles compared to standard vertical spraying single jet injection nozzles.

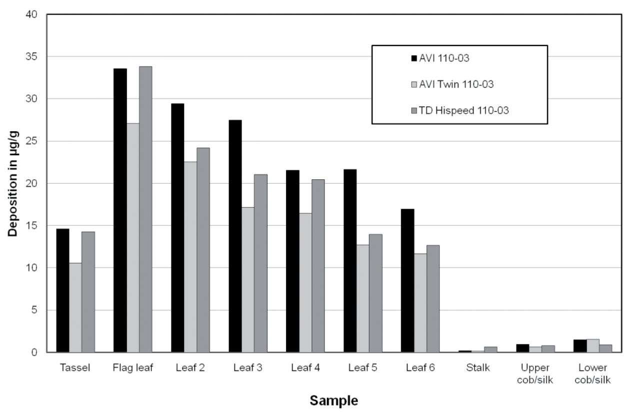

Fig. 3 Influence of nozzle type on spray deposition.

Abb. 3 *Einfluss der Düsentypen auf den Spritzbelag.*

Session 4: Agronomic measures for eradication and containment

On the influence of different soil cultivation practices in autumn and spring on the population development of the western corn rootworm *Diabrotica virgifera virgifera* LeConte (Col.: Chrysomelidae)

*Untersuchungen zum Einfluss unterschiedlicher Bodenbearbeitungsverfahren im Herbst und im Frühjahr auf die Populationsdynamik des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera *LeConte)*

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Summary

It can be expected that intensity and depth of soil cultivation have an impact on the eggs of *Diabrotica virgifera virgifera*, which are predominantly positioned 10 to 15 cm below soil surface. Main aim of this study was to investigate the relationship between different cultivation measures in autumn and spring and the hatching of adult *D. virgifera virgifera* as well as the maize root damage, caused by larvae. Gauze covered hatch-cages (2 per plot) were used to count the emerged imagines. Variants: Plough and grub, both 25 cm working depth, in autumn and superficial tillage with disc-harrow in spring. The trials were performed on fields with a significant natural *D. virgifera virgifera* infestation, in West Romania. The number of hatched adult *D. virgifera virgifera* was unsteady in the different years and did not correlate with soil-cultivation practices. This finding can be explained with the different climatic conditions in the single years. The maize-root damage under the hatch-cages was not significantly different between variants. This may on the one hand result from the limited number of samples (2 cages per plot) on the other hand also from the artificial growth circumstances for the maize under the small cages. The maize-root damage assessed in the surrounding plots was significantly lower in the plough-variant compared to those in the grubber- and disc-harrow-plots, in two of three years. A summary of all data achieved in three years, showed a significant correlation between the number of adult *D. virgifera virgifera* and the maize-root damages in the field (p=0.0001, r=0.605).

Keywords: *Diabrotica,* cultivation measures; plough; cultivator; disc-harrow; integrated control

Zusammenfassung

Es ist zu erwarten, dass Art und Tiefe der Bodenbearbeitung einen Einfluss auf die Eier von *Diabrotica virgifera virgifera* haben, die bevorzugt in einer Tiefe von 10 bis 20 cm abgelegt werden. Ziel des Projektes war es, den Zusammenhang zwischen verschiedenen Bodenbearbeitungsverfahren im Herbst und im Frühjahr und der Anzahl schlüpfender Imagines des Westlichen Maiswuzelbohrers sowie der durch die Larven verursachten Wurzelschäden am Mais, zu untersuchen. Die Versuche wurden auf Feldern mit starkem Besatz mit *D. virgifera virgifera*, in West Rumänien, durchgeführt. Versuchsvarianten waren: Pflügen und Grubbern im Herbst (jeweils 25 cm Arbeitstiefe) und oberflächliche Bodenbearbeitung mit einer Scheibenegge im Frühjahr (10 - 15 cm Arbeitstiefe). Die Anzahl geschlüpfter Käfer war in den drei Versuchsjahren sehr unterschiedlich. Grund dafür könnten die sehr unterschiedlichen Witterungsbedingungen in den einzelnen Jahren gewesen sein. Die Wurzelschäden an den Maispflanzen unter den Schlupfkäfigen unterschieden sich zwischen den Bodenbearbeitungsvarianten nicht signifikant. Das kann an der relativ geringen Probenzahl (2 Schlupfkäfige pro Parzelle) liegen, wird jedoch auch von den Wachstumsbedingungen der Maispflanze unter den relativ kleinen Gazekäfigen beeinflusst worden sein. Wurzelschäden am Mais in der umgebenden Parzelle, verursacht durch Larvenfraß, waren in der gepflügten

Variante in zwei von drei Jahren signifikant geringer als in den Vergleichsvarianten. Die Zusammenfassung aller erhobenen Daten in den drei Versuchsjahren, zeigte eine signifikante lineare Korrelation zwischen der Anzahl geschlüpfter Käfer und den Schäden an den Maiswurzeln (p = 0,0001; r = 0,605).

Stichwörter: *Diabrotica*, Bodenbearbeitung; Pflug; Grubber; Scheibenegge; integrierte Bekämpfung

1. Introduction

Since 2007 *Diabrotica virgifera virgifera* is present in Germany. As this species is classified as a quarantine pest officially required measures, following EU-Decision 2003/766/EU and EU-recommendation 2006/565/EU, have to be applied to avoid further spreading and to eradicate the population (Halm, 2007).

In order to improve and adapt control measures for *D. virgifera virgifera*, including chemical and non chemical options for German conditions two research programs were generated, funded by the German Federal Ministry of Food, Agriculture and Consumer Protection and the Bavarian State Ministry of Food, Agriculture and Forestry (Zellner *et al.*, 2009). The work presented in the following is part of this research program.

The influence of soil preparation on the population dynamics of *D. virgifera virgifera* is not well reviewed in Europe. However it could be expected that intensity and depth of soil preparation would have an impact on *Diabrotica* eggs, which are predominantly positioned 10 to 15 cm below soil surface (Schwabe *et al.*, 2010)

Main aim of this study was to investigate the relationship between different soil preparation measures in autumn and spring and the hatching of *D. virgifera virgifera* imagines and the intensity of maize-root damage, caused by larvae feeding.

The trials had to be performed on fields with a significant natural *D. virgifera virgifera* infestation were maize was grown in single-crop farming. An appropriate site was found at the boundary between Grabaţ and Lenauheim, two villages in the Banat region 45 km west of the city of Timişoara, Romania. It was a large and long field. Due to technical reasons it was decided to put both trials on this site, each at one end. The distance between the trials was about 100 m.

Soil cultivation was managed in collaboration with the owner of the field by using his farming equipment.

2. Material and methods

The project started in autumn 2009 and was completed in autumn 2012

Three cultivation measures were applied:

- plough, 25 cm working depth, in autumn (Fig. 1),
- grubber, 25 cm working depth, in autumn (Fig. 2),
- superficial cultivation by disc-harrow (10 to 15 cm working depth), in spring.

The maize was planted with a standard seed combination across all plots.

Large plots (500 m^2, 4 replications) were appropriate to work with the farmer's equipment (Fig. 3). They were also necessary to avoid adult *D. virgifera virgifera* moving across plots.

Gauze covered hatch cages (2 per plot) were used to count the emerging adult *D. virgifera virgifera*. A single plot is shown in Fig. 4; the whole set of cages in a trial can be seen in Fig. 5.

Imagines were counted weekly throughout the hatching period from mid of June till mid of August. Each year in September or October the maize root damage caused by *Diabrotica* larvae were assessed inside the hatch cages and in the surrounding plot, by using the 'Root-Node-Injury Scale' from 0 to 3 (Oleson *et al.*, 2005).

All plants (5 to 6) under the hatch-cages were assessed, whereas 5 x 5 succeeding plants in the surrounding plot were evaluated.

Fig. 1 Cultivation using a plough.

Abb. 1 *Bodenbearbeitung mit einem Pflug.*

Fig. 2 Cultivation using a grubber.

Abb. 2 *Bodenbearbeitung mit einem Grubber.*

Fig. 3 Trial site after soil cultivation in autumn, Lenauheim 2011.

Abb. 3 *Versuchsflaeche nach der Bodenbearbeitung im Herbst, Lenauheim 2011.*

Fig. 4 Hatch cage, 1.4 m height, ground surface 1 m^2, with gauze clothing.
Abb. 4 *Fangkäfig, 1,4 m hoch, Bodenfläche 1 m^2, Abdeckung mit Gaze.*

Fig. 5 Hatch cages (2 per plot), Lenauheim, 2012.
Abb. 5 *Fangkäfige (2 je Schlag), Lenauheim, 2012.*

3. Results

During the test period it became apparent, that the trial results were unsteady from year to year. It was obvious that the differences between the variants were not mono causal. As soil cultivation, sowing date and assessments were performed nearly at the same time it was decided to put the data achieved from both trials together and to regard them as one experiment with 8 replications in the statistical analysis.

- The number of hatched adult *D. virgifera virgifera* was unsteady in the different years and did not correlate with the soil cultivation practices (Fig. 6).
- The maize root damage under the hatch cages were not significantly different between variants (Fig. 7).
- The maize root damages assessed in the plots surrounding the hatch-cages were significantly lower in the plough variant compared to those in the grubber and disc harrow plots, in two of three years (Fig. 8).
- A summary of all data achieved in three years, showed a significant correlation between the number of adult *D. virgifera virgifera* and the maize root damage in the field (p=0.0001, r=0.605).

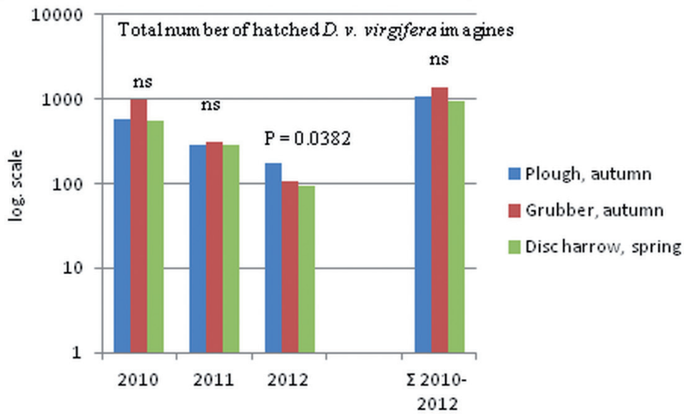

Fig. 6 Total number of adult *D. virgifera virgifera* caught in the single variants and years and mean values.

Abb. 6 *Gesamtanzahl gefangener adulter* D. virgifera virgifera *je Variante und Jahr und Durchschnittswerte.*

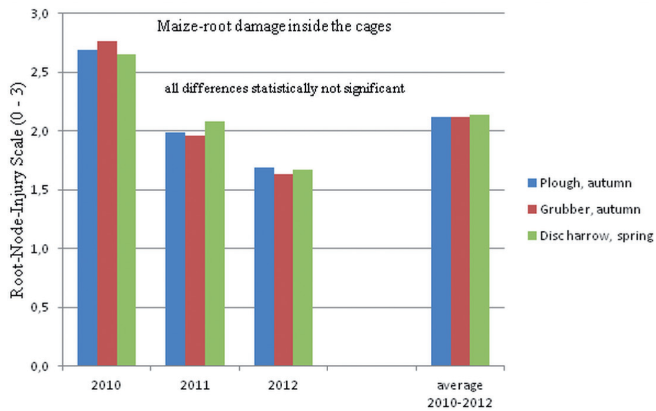

Fig. 7 Maize root damage inside the hatch cages, in the single variants and years and mean values.

Abb. 7 *Maiswurzelschaden innerhalb der Schlupfkäfige je Variante und Jahr und Durchschnittswerte.*

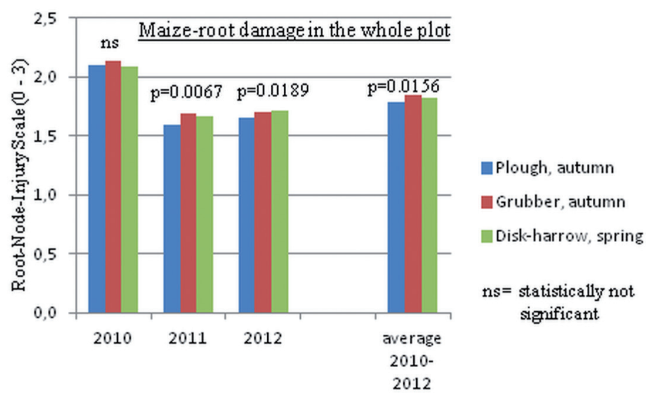

Fig. 8 Maize root damage assessed in the whole plot, single variants and years and mean values.

Abb. 8 *Schaden an Maiswurzeln im ganzen Schlag je Variante und Jahr und Durchschnittswerte.*

4. Discussion

A study on the influence of soil conditions on the survival rate of *D. virgifera virgifera* larvae in Austria (Grabenweger *et al.*, 2010) shows, that the physical soil characteristics like permeability for water and air affect the mobility of *Diabrotica* larvae. Reduced mobility increases the possibility of drying up.

On the other hand a survey performed in Kansas (Riedell and Sutter, 1995) showed, that water saturated soils during egg hatch can adversely affect western corn rootworm populations and subsequent larval feeding damage.

No ovipositional preference for a tillage system was evident in a study performed in Iowa from 1983 till 1985 (Gray and Tollefson, 1988), but egg population declined significantly from fall 1983 to spring 1984 in the plough variants whereas no significant reduction in egg population occurred in the non tillage and superficial treatments. No differences were found in the next vegetation period.

All studies confirmed, that many abiotic components have an impact on the population-dynamics of the western corn rootworm.

The results mentioned above are reflecting the situation in West Romania during the trials period (2010–2012). The first year (2010) was hallmarked by heavy rainfalls end of May and beginning of June; the trial site was partly flooded during a few days. As the *Diabrotica* egg hatching occurs in that period the population decreased significantly. In 2011 and 2012 it was very dry between June and September. This also had a negative impact on *Diabrotica* population development.

These climatic influences can explain that the number of hatched adult *D. virgifera virgifera* in the trials was unsteady in the different years and did not correlate with the soil tillage practices.

The finding that the maize-root damage under the hatch cages were not significantly different between variants may on the one hand result from the limited number of samples (2 cages per plot) on the other hand also from the artificial growth circumstances for the maize plants under the small cages.

The maize root damage assessed in the surrounding plots were significantly lower in the plough variant compared to those in the grubber and disc harrow plots, in two of three years.

In laboratory tests eggs of the western corn rootworm were placed in different soil depth; the related egg hatching rate was assessed. A negative correlation between the soil depth (40 cm) and the egg hatching rate could be observed. In a comparable field trial carried out in the USA, the laboratory finding could be confirmed in the first of two years but not in the second one (Baufeld, 2012). As these studies were performed in the laboratory and under field conditions in the USA, the results are not directly comparable with those achieved from the Romanian trials but they are confirming the tendency.

To confirm a significant correlation between the number of adult *D. virgifera virgifera* and the maize-root damages in the field seems to be natural. The various influences affecting the *Diabrotica* population development as mentioned above indicate that even this is not a matter of course.

Acknowledgements

I would like to thank all colleagues from Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara, e.g. Cristian Rancov, who strongly supported the work on this project, as well as the project leader and the project coordinator for valuable advice and active participation.

Many thanks to Mr. Dimitru Popa for his continuous support and for his friendly attendance to make his field and his farming equipment available for us.

I also would like to express my gratitude to the Bavarian State Ministry of Food, Agriculture and Forestry, who supported the project financially.

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Dispersal and oviposition behaviour of *Diabrotica virgifera virgifera* in non-maize crops to improve advice and guidelines for crop rotation

Verbreitungs- und Eiablageverhalten von Diabrotica virgifera virgifera *in Nicht-Mais-Kulturen zur Verbesserung von Beratung und Richtlinien für den Fruchtwechsel*

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Adults of the maize pest *Diabrotica virgifera virgifera* are known to perform inter-field movements to crops other than maize, mostly to access protein-rich food sources. In the USA, some populations also lay eggs into non-maize crops where maize is grown the following year which will allow larval development.

Crop rotation experiments aimed at investigating to what extent dispersing adults may also lay eggs in uninfested fields of 11 different crop habitats in Europe, and consequently may reduce the efficacy of rotation as a control measure.

Mass releases of beetles and their recaptures in crop rotation experiments at two study sites in southern Hungary in 2009, 2010, 2011 and 2012, revealed that less than 10% of the entire population in the infested maize fields disperses towards uninfested crop habitats, including maize. When not including uninfested maize in the analyses, then less than 5% of the entire population from the infested maize fields disperses towards non-maize crop habitats. Maize was the most attractive crop for the dispersing *D. virgifera virgifera*, regardless of whether males and females were considered separately or together. Second most attractive were ploughed bare soils or harvested and grubbed peas. *Sorghum* (Millet), *Sorghum* (sudan grass), potatoes, soybean, sugar beet as well as harvested and grubbed rape or winter wheat were of minor importance.

Most dispersing adults also laid eggs. Less than 20% of the entire *D. virgifera virgifera* population in the study sites emerged from maize as a result of oviposition into uninfested crop habitats the previous year. It appeared that the majority of the *D. virgifera virgifera* population from infested maize fields was ovipositing over the entire maize area, regardless of whether the maize was their natal field or not. When not including uninfested maize in the analyses, then less than 15% of the entire *D. virgifera virgifera* population in the study sites emerged from maize as a result of oviposition into uninfested crop habitats, e.g. *Sorghum* (sudan grass), sugar beet and others.

Despite of some extent of dispersal and oviposition into non-maize crops, it can be concluded that, according to the current state of knowledge, any crop can be rotated with maize to successfully manage this invasive alien maize pest in Europe.

This study was funded by the Bavarian State Ministry of Food, Agriculture and Forestry.

Extraction of *Diabrotica* eggs from soil and determining whether *Diabrotica* oviposit in fields of oil seed squash

Extraktion von Diabrotica*-Eiern aus dem Boden und Bestimmung, ob* Diabrotica *in Ölkürbisfeldern Eier ablegt*

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Summary

A soil washing apparatus was constructed and tested in collaboration with colleagues from the grant authority and project partners in Austria and Romania. It is based on a similar apparatus used in the USA but our apparatus can quickly process a large number of samples.

The eggs were extracted by sieving and then floating on $MgSO_4$-solution followed by sedimentation in water. Eggs of *D. virgifera virgifera* were separated from those of other species by studying the structure of egg surface. The efficiency of extraction of the apparatus was checked using samples spiked with blue coloured eggs. In co-operation with Austrian and Romanian colleagues a method was developed for sampling in the field. Eggs of *D. virgifera virgifera* were extracted from soil samples collected from fields in Austria and Romania in spring/ summer and autumn 2011. The beetles did not appear to prefer to lay eggs at either the edges or centres of fields. As very few eggs were found at the edges of oil seed squash fields this crop does not appear to be a suitable habitat for egg laying.

Keywords: *Diabrotica virgifera virgifera,* egg laying, extraction from soil samples, oil seed squash field

Zusammenfassung

Eine Eiauswaschanlage wurde zusammen mit Kollegen vom Zuwendungsgeber und von Projektpartnern in Österreich und Rumänien gebaut und geprüft. Sie ähnelt einer Anlage, die in den USA verwendet wird. Unsere Anlage kann jedoch eine größere Anzahl von Proben in kürzerer Zeit verarbeiten.

Die Eier wurden durch Sieben extrahiert und in einer $MgSO_4$-Lösung aufgeschwemmt, um sich dann in Wasser abzusetzen. Die Eier von *D. virgifera virgifera* wurden von denen anderer Arten anhand ihrer Oberflächenstruktur getrennt. Die Extraktionsleistung der Anlage wurde mit Hilfe von Proben, die mit blau gefärbten Eiern versetzt wurden, geprüft. Zusammen mit den österreichischen und rumänischen Kollegen wurde eine Probenahmemethode für das Freiland entwickelt. Eier von *D. virgifera virgifera* wurden aus den Bodenproben von Feldern in Österreich und Rumänien im Frühjahr/Sommer und im Herbst 2011 extrahiert. Die Käfer bevorzugten offensichtlich weder den Feldrand noch die Feldmitte für die Eiablage.

Stichwörter: *Diabrotica virgifera virgifera*, Eiablage, Extraktion aus Bodenproben, Ölkürbisfeld

1. Introduction

In order to forecast the damage caused by and control the pest *Diabrotica virgifera virgifera*, which was introduced from the US into Europe, it is important to have more detailed information on where and how many eggs it lays.

The numbers of eggs laid in the soil are directly related to the subsequent number of damaging larvae. Although it is difficult to accurately determine the number of eggs in soil because it takes a long time to process the large volumes of soil needed to do this, it is possible to forecast the expected abundance if one can measure egg density. In addition, by extracting its eggs from soil it is also possible to answer questions about the egg laying behaviour of *D. virgifera virgifera*.

Studies in the USA and Europe reveal that it is possible to obtain quantitative data on the number of eggs laid in a defined volume of soil by washing the soil through sieves of different mesh sizes and separating the eggs from the residue by a process known as floating (e.g. Foster *et al.,* 1979; Matteson, 1966; Park and Tollefson, 2006; Shaw *et al*., 1976; Ruesink, 1986; Ujvari *et al*., 2004). But whether the methods used in the USA and South-East Europe are suitable for being used elsewhere needs to be checked. For example, fields in the USA are much larger than in Europe and therefore local soil samples vary less in terms of soil types and soil structures.

Currently it is also unknown if differences in the fauna will result in the presence of eggs of other taxa that are similar to those of *D. virgifera virgifera*, which makes it difficult to identify its eggs. The objective of this project was to develop a method for extracting eggs of *D. virgifera virgifera* from soil samples suitable for determining whether *D. virgifera virgifera* oviposits in fields of oil seed squash.

2. Material and methods

To determine the number of eggs laid by females of *D. virgifera virgifera* in fields a soil washing apparatus was constructed and tested (Fig. 1-4). The apparatus had to be transportable and capable of quickly processing a large number of samples. In principal, the construction is based on equipment used for this purpose in the USA in terms of water pressure, rotation speed and position/angles (Shaw *et al*., 1976). The extraction of samples is done by washing soil trough two sieves, the first a fixed 550 µm mesh sieve followed by a rotating drum fitted with a 250 µm mesh sieve (Fig. 2). In order to determine the appropriate mesh sizes eggs of *Diabrotica* of different ages and developmental stages were measured.

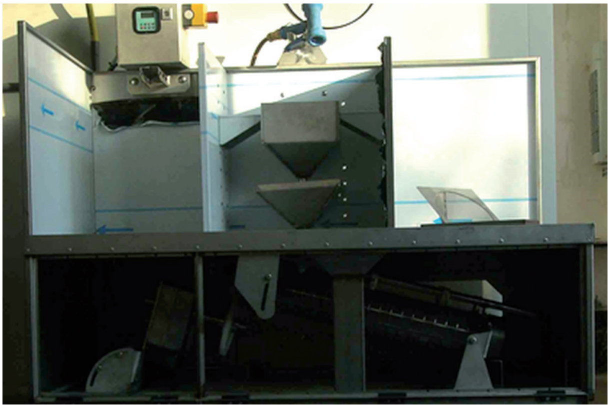

Fig. 1 Apparatus used for extracting eggs from soil.

Abb. 1 *Apparat zur Auswaschung von Eiern aus Bodenproben.*

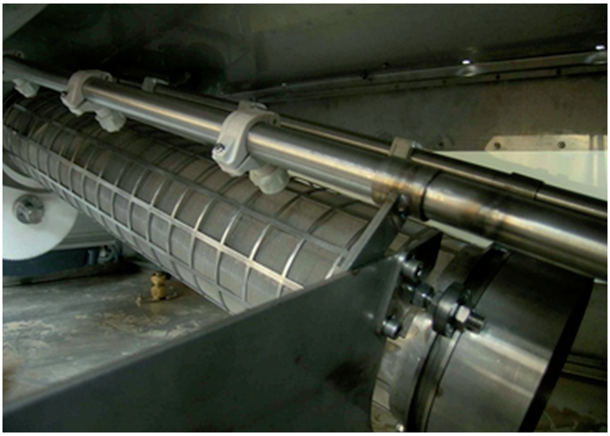

Fig. 2 Drum sieve.

Abb. *2 Trommelsieb.*

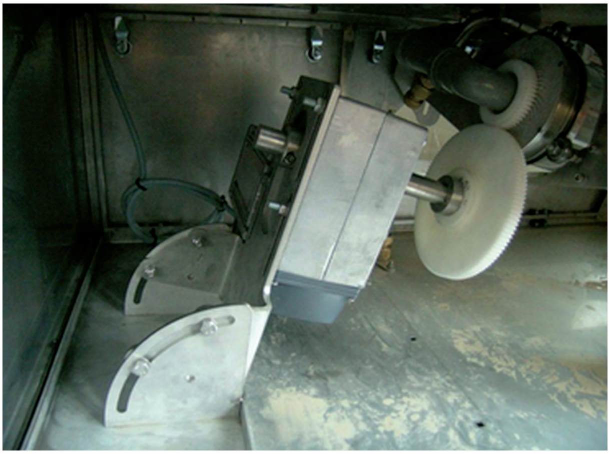

Fig. 3 Details of motor suspension.

Abb. 3 *Detailaufnahme des Motorgetriebes.*

Fig. 4 Speed control.

Abb. 4 *Geschwindigkeitskontrolle.*

The eggs were extracted by transferring the residue on the sieves to funnels filled with 1 liter $MgSO_4$-solution (2 mol/L) on which the eggs float (Fig. 5) and then replacing this solution with water (Fig. 6), which results in the sedimentation of the eggs (Palmer *et al*., 1976). Counting the eggs was done under a microscope. Eggs of other insects, which were not removed during the washing procedure because they were the same size and specific gravity as those of *D. virgifera virgifera,* were identified by checking the structure of egg surface (Fig. 7). The eggs of *D. virgifera virgifera* are characterized by sculpturing consisting of bold ridges forming polygons (Atyeo *et al*., 1964; Krysan, 1986).

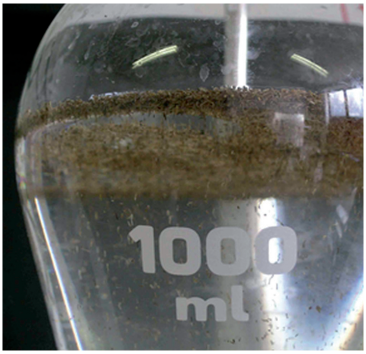

Fig. 5 Eggs floating on $MgSO_4$-solution.

Abb. 5 *Aufschwemmung der Eier in $MgSO_4$ Lösung.*

Fig. 6 Sedimentation of eggs in water.

Abb. 6 *Sedimentation der Eier in Wasser.*

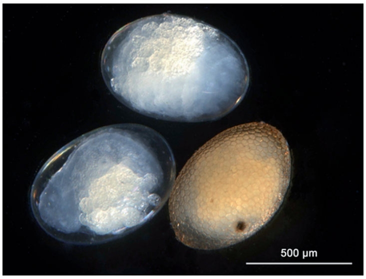

Fig. 7 Eggs of same size and density extracted from soil samples. The chorion of the egg of *D. virgifera virgifera* (*right*) has a characteristic polygonal sculpturing (Atyeo *et al*., 1964).

Abb. 7 *Aus Bodenproben extrahierte Eier gleicher Größe und Dichte. Das Chorion der Eier von* D. virgifera virgifera *(*rechts*) hat eine charakteristische polygonale Skulpturierung (*Atyeo *et al., 1964).*

To verify the efficiency of this method every fifth sample was spiked with 10 eggs coloured with Giemsa and the number coloured eggs recovered after washing and floating was determined. These eggs were produced by a *Diabrotica* culture that originated from Hungary and was reared in the laboratory.

To optimize the sampling in the field the soil samples were collected using different equipment (spade, shovel, soil core borer) and transferred in different quantities to the egg-washing apparatus.

After different durations of washing the composition of the different sized pieces of debris on the different sieves was analysed. Both published and our results were used to finalize the adopted design of the sampling method. This design was used in 2011 in field trials in Austria and Romania. These trials were done in close co-operation with colleagues, Grabenweger in Vienna and Foltin in Wulkaprodersdorf, Austria and Lauer/Gräpel in Timişoara, Romania.

3. Results

The apparatus we constructed was used to obtain qualitative and quantitative data on the abundance and distribution of *D. virgifera virgifera* eggs in the field with the aim to use this data to forecast the expected future abundance of this pest. In addition, this information will be used to address a number of questions about the egg laying behaviour and model the population dynamics of this pest.

The choice of the mesh sizes of stainless steel sieves was determined by the size of the eggs of *D. virgifera virgifera,* which originated from Hungary (length 0.63 ± 0.03 mm (min. 0.54 mm, max. 0.69 mm); diameter 0.40 ± 0.03 mm (min. 0.34 mm, max. 0.46 mm)). The fixed primary sieve has a mesh size of 550 µm and the rotating drum sieve has a mesh size of 250 µm. The efficiency of the soil washing apparatus was tested by using soil samples containing known numbers of eggs of *D. virgifera virgifera* obtained from a culture of this species reared in-house since 2003. The efficiency with which blue coloured control eggs were recovered was similar to that reported in the literature (e.g. Shaw and Hummel, 2003; Takács *et al*., 2005).

There was a negative relationship between the volume of soil sample placed in the egg-washing apparatus and number of eggs extracted (Fig. 8). This indicated that a soil sample should not be larger than 750 ml otherwise the number of eggs recovered is less than 88%.

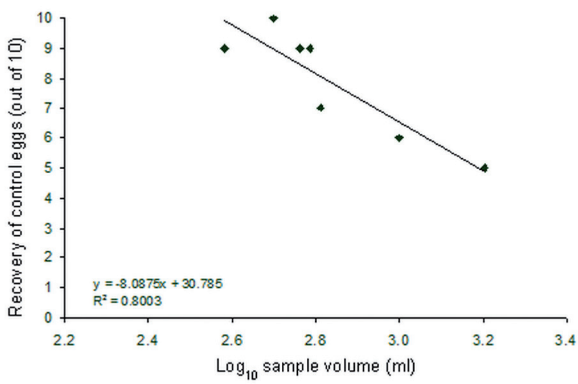

Fig. 8 Effect of soil sample size on the recovery of blue coloured eggs.

Abb. 8 *Einfluss der Bodenprobengröße auf die Wiederfindung von blau gefärbten Eiern.*

During the counting of eggs under a microscope it became clear that there were also eggs laid by other species. If these eggs were of the same size and density as those of *D. virgifera virgifera* then they would not be separated from those of the target species during the washing process. The eggs of *D. virgifera virgifera,* however, can be reliably identified as the sculpturing on the chorion is very characteristic (Atyeo *et al*., 1964; Rowley and Peters, 1972).

In collaboration with colleagues from the grant authority and project partners in Austria and Romania a method of collecting soil samples in the field was developed and used to obtain the soil samples collected in Austria and Romania, which were processed in the soil washing apparatus.

The first set of field samples was collected in spring/summer 2011. In total 36 samples were collected in Austria (locations in Styria and Burgenland) and 14 in Romania (locations in Banat).

The second set of field samples was collected in autumn 2011. In total 99 samples were collected in Austria (locations in Styria and Burgenland) and 62 in Romania (locations in Banat).

To obtain data on (i) the distribution of eggs from the edge to the centre of a field and (ii) whether eggs are laid in oil seed squash fields near a maize field, 5 sample points per area were selected (Fig. 9). Analyses of soil samples from oil seed squash fields were carried out only in Styria and only for fields close to maize fields infested with *D. virgifera virgifera*. Each sample consists of 3 subsamples of soil collected down to a depth of 20 cm using either a spade or core borer. 2 l of soil were collected from the base of plants, 2 l within a row and 2 l between rows. After carefully sieving (mesh size 1 cm) and sorting to remove stones and roots 3 subsamples 2 x 500 ml were isolated and transferred to the laboratory for extraction and the numbers of eggs of *D. virgifera virgifera* – eggs extracted are given in Table 1.

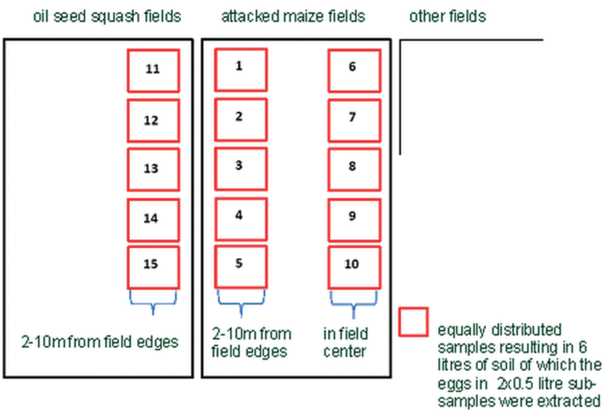

Fig. 9 Number and location of the samples collected in fields in Austria in autumn 2011. Soil samples from oil seed squash fields were collected only in Styria.

Abb. 9 *Anzahl und Platzierung der Bodenprobenahmen in Feldern in Österreich im Herbst 2011. Proben aus Ölkürbisfeldern wurden nur in der Steiermark gesammelt.*

In spring/summer 2011 the maximum number of eggs per litre of soil was 8.1 in Austria and 3.1 in Romania. The samples collected in autumn 2011 contained a higher maximum number, 69.2 eggs per litre in Austria and 45.0 eggs per litre in Romania (Tab. 1). The analyses show that in the soil samples collected during spring-summer there were more eggs recorded at the edges of 4 of the fields and more eggs from the centre in 1 field. In the former there was no significant difference in the number of eggs recorded at the edges and centres of the fields.

Tab. 1 Mean number (± SD) of *Diabrotica* eggs per litre of soil extracted from samples collected in 2011. (position: C center, E edge, M maize, OSS oil seed squash; crop rotation: B barley, M maize, OSS oil seed squash, P potato, R oilseed rape, S soya, SB sugar beet, W wheat).

Tab. 1 *Durchschnittliche Anzahl (± SD) von* Diabrotica-*Eiern je Liter Boden aus Proben gesammelt in 2011. (Lage: C Zentrum, E Rand, M Mais, OSS Ölkürbis; Fruchtfolge: B Gerste, M Mais, OSS Ölkürbis, P Kartoffel, R Raps, S Soya, SB Zuckerrübe, W Weizen).*

Country		Location	Position	Crop rotation	Sample (n)	Eggs (n)	Mean		Max/ Ltr	Min/ Ltr
Austria	May	Bruckneu-dorf	E-M	P-WW-M-M	2	0	0.0	± 0.0	0.0	0.0
			C-M	P-WW-M-M	2	0	0.0	± 0.0	0.0	0.0
	May	Dedenitz	E-M	M-M	4	0	0.0	± 0.0	0.0	0.0
			E-OSS	WW-OSS	2	0	0.0	± 0.0	0.0	0.0
	May	Deutsch Jahrndorf	E-M	M-	2	6	4.8	± 4.7	8.1	1.5
			C-M	M-	2	3	1.5	± 0.4	1.7	1.2
	June	Deutsch Jahrndorf	E-M	M-	2	1	0.7	± 0.9	1.3	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Dornau	E-M	OSS-M	4	0	0.0	± 0.0	0.0	0.0
			E-OSS	M-OSS	2	0	0.0	± 0.0	0.0	0.0
	May	Raabfeld	E-M	M-	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Wallern	E-M	M-	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
	May	Zuberbach	E-M	M-	2	3	2.0	± 1.2	2.9	1.2
			C-M	M-	2	0	0.0	± 0.0	0.0	0.0
Romania	May	Grabatz	E-M	M-M-M-WW	2	3	1.1	± 0.2	1.3	1.0
			C-M	M-M-M-WW	2	2	0.8	± 1.2	1.7	0.0
	May	Iecea Mare I	C-M	M-M-WW	2	0	0.0	± 0.0	0.0	0.0
		Iecea Mare II	E-M	M-M-WW	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-M-WW	2	5	2.0	± 1.6	3.1	0.8
	May	Lenauheim	E-M	M-M-M-WW	2	0	0.0	± 0.0	0.0	0.0
			C-M	M-M-M-WW	2	0	0.0	± 0.0	0.0	0.0
Austria	Oct.	Antau	E-M	M-W-M-R	5	74	12.9	± 12.9	26.4	0.0
			C-M	M-W-M-R	5	26	4.6	± 5.1	12.7	0.0
	Sept.	Badersdorf	E-M	M-S-WW-M-S	5	49	15.3	± 19.5	48.4	0.0
			C-M	M-S-WW-M-S	5	10	3.1	± 3.7	8.3	0.0
	Sept.	Kukmirn	E-M	M-	5	0	0.0	± 0.0	0.0	0.0
			C-M	M-	5	8	2.6	± 3.7	7.9	0.0
	Oct.	Neutal	E-M	M-WW-R-M	5	2	0.7	± 1.0	2.0	0.0
			C-M	M-WW-R-M	5	13	3.6	± 5.0	12.0	0.0

	Oct.	Siegendorf 1	E-M	M-M-Durum-WW	5	11	3.2	± 2.8	7.1	0.0
			C-M	M-M-Durum-WW	5	150	43.1	± 30.6	85.1	0.0
		Siegendorf 2	E-M	M-M-WW-SB	5	52	15.8	± 16.3	42.9	0.0
			C-M	M-M-WW-SB	5	67	20.2	± 20.9	47.7	0.0
	Oct.	Stoob	E-M	M-WG-R-M	5	1	0.3	± 0.7	1.6	0.0
			C-M	M-WG-R-M	5	9	2.8	± 2.6	5.0	0.0
	Oct.	Wallern	E-M	M-	5	17	5.2	± 7.5	17.6	0.0
			C-M	M-	5	32	10.1	± 6.7	21.7	5.7
	Oct.	Wulkapro-dersdorf 1	E-M	M-WW-SB-R	5	10	2.2	± 4.4	10.0	0.0
			C-M	M-WW-SB-R	5	28	6.7	± 7.5	18.3	0.0
		Wulkapro-dersdorf 2	E-M	M-WW-SB-R	5	4	1.3	± 2.4	5.5	0.0
			C-M	M-WW-SB-R	5	2	0.7	± 0.9	1.7	0.0
	Oct.	Halbenrain East	E-M	M-OSS-M-OSS	5	27	8.5	± 12.0	29.5	0.0
			C-M	M-OSS-M-OSS	5	15	4.8	± 5.0	11.9	0.0
			E-OSS	OSS-M-S-M	5	0	0.0	± 0.0	0.0	0.0
	Oct.	Halbenrain West	E-M	M-OSS-M-OSS	5	12	3.9	± 6.5	15.3	0.0
			C-M	M-OSS-M-OSS	5	20	6.7	± 9.5	23.3	0.0
			E-OSS	OSS-M-S-M	5	2	0.6	± 1.3	2.8	0.0
	Oct.	Poppendorf East	E-M	M-OSS-M-OSS	5	8	2.3	± 1.7	4.5	0.0
			C-M	M-OSS-M-OSS	5	26	8.7	± 7.4	20.7	1.7
			E-OSS	OSS-M-OSS-M	5	3	0.9	± 1.4	3.2	0.0
		Poppendorf West	E-M	M-OSS-M-OSS	5	1	0.3	± 0.7	1.7	0.0
			C-M	M-OSS-M-OSS	5	25	8.1	± 6.5	18.3	1.9
			E-OSS	OSS-M-OSS-M	5	2	0.6	± 0.9	1.6	0.0
	Oct.	Zelting 1	E-M	M-M-WG	5	163	48.3	± 25.4	69.2	4.2
			E-OSS	OSS-M-M	5	7	2.0	± 1.9	4.3	0.0
		Zelting 2	E-M	M-M-M-M-M	9	37	9.1	± 11.8	33.3	0.0
			E-OSS	OSS	10	1	0.2	± 0.7	2.3	0.0
Romania		Grabatz I	E-M	M-M-M	8	17	3.5	± 6.1	13.3	0.0
			C-M	M-M-M	8	1	0.2	± 0.6	1.7	0.0
		Grabatz II	E-M	M-M-M	8	0	0.0	± 0.0	0.0	0.0
		Grabatz III	E-M	M-M-M	2	0	0.0	± 0.0	0.0	0.0
		Iecea Mare II	C-M	M-M-M-WW	10	9	1.5	± 3.7	11.7	0.0
		Lenauheim I	E-M	M-M-M	8	69	15.0	± 18.9	45.0	0.0
			C-M	M-M-M	8	0	0.0	± 0.0	0.0	0.0
		Lenauheim II	C-M	M-M-M	10	13	2.1	± 2.1	6.1	0.0

For the soil samples collected in autumn there were more eggs at the edges of 5 fields and more eggs in the centre of 9 fields. If the results for the samples collected in both periods are combined, then more eggs were recorded at the edges of 10 fields and more eggs at the centres of 11 fields. That is, we did not find a preference for laying eggs either at the edges or centres of fields.

Only very few eggs were laid in oil seed squash fields close to maize fields. For the 8 oil seed squash fields sampled the number of eggs/litre of soil varied between 0.0 (± 0.0) - 0.9 (± 1.4). In only one field (located near Zelting, Austria) were 2.0 (± 1.9) eggs/litre and that was close to a maize field that was heavily infested with *Diabrotica* and in the soil of which there were 48.3 ± 25.4 eggs /litre.

4. Conclusions

In order to determine the number of eggs laid by females of *D. virgifera virgifera* in fields a soil washing apparatus was constructed and tested in collaboration with colleagues from the grant authority and project partners in Austria and Romania. This apparatus is transportable and can be used to quickly process a large number of samples. It is based on a similar apparatus used in the USA. The soil is first washed through a 550 µm sieve and then in a rotating sieve drum with a mesh size of 250 µm.

The eggs were extracted from the residue by first floating them in $MgSO_4$-solution followed by sedimentation in water. The numbers of eggs were counted under a microscope. Eggs of *D. virgifera virgifera* can be easily identified as the surface of its eggs has a characteristic reticulated structure. Thus it was possible to separate its eggs from those of other insects, which were not removed during the washing procedure because they are the same size and specific gravity as those of *D. virgifera virgifera*. The extraction efficiency of this apparatus was checked by extracting soil samples spiked with blue coloured eggs. In co-operation with Austrian and Romanian scientists a method was developed for sampling in the field that could be quickly used to determine both the horizontal and vertical distribution of eggs of *Diabrotica* in the soil.

The beetles did not appear to prefer to lay their eggs at the edges or centres of fields and only very few eggs were recorded at the edges of oil seed squash fields.

Acknowledgements

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The effect of flooding on the mortality of larvae of *Diabrotica virgifera virgifera* under Bavarian conditions

Der Einfluss von Überschwemmungen auf die Mortalität von Diabrotica*-Larven unter Bedingungen in Bayern*

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Bavaria has a lot of areas threatened by flooding in continuous maize. According to Zellner (oral communication, 2008) 20,000 ha (57%) out of 35,000 ha in continuous corn are grown in such areas. There was no information available on the effect of flooding, which usually occurs in Bavaria in June, on larvae. The flood from 19 to 21 June 2009 in lower Bavaria was simulated in a laboratory trial. Water temperature was 13 °C. It has a detrimental impact on the mortality rate of the larvae in case of flooding. The higher the temperature, the higher the mortality (Wyatt Hoback *et al*., 2002). In 2010, first laboratory experiments on the effect of flooding on population development gave surprising results. Although 2nd and 3rd larval stages (L2/L3) were exposed to 24-h or 96-h flooding at 13 °C, the numbers of hatched beetle did not differ significantly. 2011 showed a tendency to reduction with increasing duration of flooding, although non-significant. This may result from various reasons. The larval stages L2 and L3 are more robust, and living in the roots (survival reservoir for oxygen and nutrition) makes them less sensitive to flooding. This conclusion must not be applied to the first larval stage, which is assumed to be very sensitive and shows high natural mortality already. Furthermore, the first larval stage occurring in the soil and feeding on root hairs is directly exposed to flooding. The experiments on flooding were continued in 2012 to be able to provide three years results.

Fig. 1 The 3rd larval stage of the western corn rootworm is boring into the roots of maize which presented a refuge to survive (survival reservoir for oxygen and nutrition) thus making it less sensitive to flooding in this stage (photo taken in quarantine laboratory, 2011).

Abb. 1 *Larven des 3. Larvenstadium des Westlichen Maiswurzelbohrers bohren sich in Maiswurzeln, die den Larven Schutz für das Überleben bei Überschwemmung boten (Sauerstoff- und Futterreservoir). Foto aufgenommen im Quarantänelabor, 2011.*

This project was part of the German Diabrotica Research Program. It was partly funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

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The effect of *Diabrotica*-resistant corn cultivars on the larval development in lab-based studies

Der Einfluss Diabrotica*-resistenter Maissorten auf die Larvenentwicklung in Laboruntersuchungen*

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The aim of the project was to investigate the influence of *Diabrotica*-resistant corn cultivars on larval development and hatching rate of *Diabrotica virgifera virgifera*. Three laboratory trials under quarantine conditions were conducted. Resistant (Sunrise Group, variety No. 4, 6 and 7) and non-resistant (No. 1, 2, 3 and 5) corn cultivars were planted. Beside two uninfected control pots up to 15 replicates of each cultivar were used. After the plants had reached the BBCH 13 stage freshly hatched larvae of *Diabrotica* were transferred into the pots. After a period of 21 days, the trials were evaluated, in order to prevent the development of adult beetles: plants were measured, weighed, and the length of their roots was determined. Surviving larvae of *Diabrotica* were collected from the soil and roots, counted, and also weighed. The number and weight of the larvae found was compared between resistant and non-resistant corn cultivars. During the first two trials mainly the plants of the Sunrise cultivar showed clear symptoms of a heavy infection with *Fusarium* (Fig. 1). Plants of other cultivars were less infected.

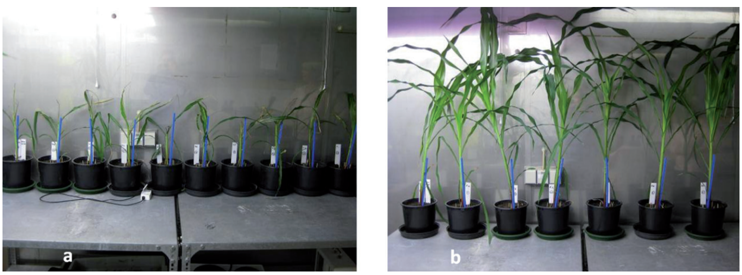

Fig. 1 Different plant length and weight of the *Fusarium* infected sunrise variety No. 4 (a) in comparison to the non resistant variety No. 1 (b) at the same time.

Abb. 1 *Unterschiede in Pflanzenlänge und –gewicht zwischen der mit* Fusarium *infizierten Sunrise-Sorte Nr. 4 (a) und der nicht resistenten Sorte Nr. 1 (b) im gleichen Versuchszeitraum.*

In the first experiment the *Fusarium*-infected varieties No. 4, 6 and 7 showed a reduced plant growth and plant weight. Nevertheless the highest number and weight of larvae were found for cultivar No. 1 and 7. The lowest number of larvae was found for cultivar No. 4. For the non-resistant cultivar No. 2, 3 and 5, no significant differences in larval size and weight were found. The second trial showed comparable results: The larvae of cultivars No. 4 and 6 were smaller and lighter than larvae from other cultivars. The survival rate of larvae of the second trial was lower than in the first trial. The highest weight of larvae was found for cultivar No. 3, 5 and 7. Resistance of the Sunrise cultivars was not confirmed by these results. With the last experiment the larval development at different plant stages of a non-resistant cultivar (RONALDINIO) was tested. Unfortunately, these plants were also infected with *Fusarium*. This trial showed contrasting result: more and larger larvae were detected on the youngest and smallest plants. This illustrates, that a valid interpretation of these results is still difficult. All laboratory trials were more or less influenced by the infection with *Fusarium* which caused a reduced plant growth depending on the cultivar used. It is not clear which influence the fungal infection and the developmental stage of the maize had on the larval development.

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Dispersion behavior and oviposition of adult western corn rootworm after an early maize harvest

Ausbreitungs- und Eiablageverhalten von Maiswurzelbohrerkäfern nach früher Maisernte

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An early harvest of maize, at a time when flight period of the pest is still in progress, may trigger migratory flights of adults out of the harvested plots into adjacent maize fields. If the migrating beetles are females, they may lay a considerable portion of their egg load into the invaded fields. This harvest-driven migration may therefore be disadvantageous in eradication or containment programmes against the quarantine pest insect.

In the presented experiments, migratory flights of western corn rootworm adults in relation to different dates of maize harvest were investigated. We monitored fluctuations of the corn rootworm adult populations with yellow sticky traps in several maize fields in close vicinity to each other. Traps were installed on maize plants before harvest and on pegs on the stubble fields after harvest. Captured beetles were counted weekly. In addition, alive females were caught and brought to the lab where they were held in cages with oviposition substrate and nutritional supply. After death of these females, eggs were sieved out of samples of the substrate and counted to calculate oviposition rates. In addition, separate samples of the substrate were hibernated and used for analysis of the emergence rates of *Diabrotica* larvae after diapause in the following spring.

The abundance of active adults in the maize fields decreased steadily at the end of the flight period of the pest. Population levels dropped significantly after harvest in the stubble field. However, we were not able to prove an increase of the population levels in maize fields close to the harvested plots. Consequently, evidence for migratory flights of adults in the course of the harvest was not found. Reasons may be that the majority of the beetles die during the harvesting process. In addition, beetles were found to be active on the stubble fields for weeks. This shows that a small fraction of the population remains on their native plots despite harvest or moves back into the stubble fields after harvest. Moreover, the fraction of the pest population which was forced to migrate by the harvest of single plots may be very small in comparison to the total population in an infested region. Population fluctuations as a consequence of harvesting small parts of the total maize area may therefore be superimposed by other factors with stronger impact on overall population dynamics in infested regions.

Results show that an early start of the maize harvest has negligible impact on the fluctuations of western corn rootworm populations in regions where the pest is established since years. Consequently, it is unlikely that early harvesting of maize impairs the efficacy of containment programmes in infested zones.

However, *Diabrotica* females caught at the end of the flight period of the pest were still able to lay a considerable amount of eggs. In addition, these eggs proved to be viable and larvae emerged after diapause in the following spring. It is therefore clear that females are able to found a population in recently invaded areas right to the end of the flight period, independent from their age. These results have to be taken in consideration in eradication programmes of isolated outbreaks of western corn rootworm.

This study was carried out under the German *Diabrotica* research programme (for more information see http://diabrotica.jki.bund.de) by the Austrian Agency for Health and Food Safety (AGES), Vienna. We are grateful to our colleges Helmut Klapal, Otto Wurm and Vera Andrejic for help with field and lab work. The study was partly funded by the Bavarian State Ministry for Nutrition, Agriculture and Forestry (StELF), Munich.

Halting the western corn rootworm by crop rotation – yes, but! Effects of oil pumpkin in Styria

Aufhalten des Westlichen Maiswurzelbohrers durch Fruchtwechsel – ja, aber!

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1. Introduction

The western corn rootworm - *Diabrotica virgifera virgifera* has invaded large parts of the maize growing areas of Central Europe since it was first detected in 1992 near Belgrade, Serbia (Edwards *et al.*, 2011). In 2002 the beetle arrived in the eastern parts of Austria and extended in 2012 into the southern and western parts. Only a few cases with observed economic damages have been recorded since in those areas. Official control measures according to EU Decision 2003/766/EG are in place to prevent the further spread of the western corn rootworm. Crop rotation as the most efficient strategy to suppress the western corn rootworm should be applied either alone or in combination with insecticide treatments. In our study we aimed to test the effects of Styrian oil pumpkin rotated with maize on the reproduction of *Diabrotica* in isolation cages in the field.

2. Material and methods

Several experiments in isolation cages have been set up to identify potential host plants of the western corn rootworm and those plants which would interfere with the development of the corn rootworm. These experiments were carried out on a maize site in Austria near Graz in the federal-state of Styria from 2009 to 2012. In specially designed well isolated cages (1.4 m x 1.4 m x 2.5 m) each planted with 20 maize plants a defined number of female and male beetles were released in 2009 (see this issue Foltin and Robier, 2013a, Population dynamics and host plant specificity of *Diabrotica virgifera virgifera*). Descendant generations i.e. hatching of beetles were recorded regularly. These studies resulted in beetles hatching with larval damage only when maize after maize was grown (variants 1 to 3) whereas in all other variants no descendants appeared. Beside cereals in these studies also oil pumpkin turned out to be definitely no host plant.

Learning from observations in commercially grown oil pumpkins („Styrian oil pumpkin" Cucurbita pepo var. styriaca), its flowers - unlike soybeans - are highly attractive to *Diabrotica virgifera virgifera* beetles. In this context female beetles leave maize to feed in pumpkin flowers in autumn of adjacent plots. A significant proportion of them do not return into maize but lay their eggs into the soil of pumpkin fields.

Various flowering broadleaved weeds in soybeans, sunflowers and sugar beet are also attractive to *Diabrotica* (Moeser and Vidal, 2004). Our own observation cornfirm this for:

- common thornapple, *Datura stramonium*
- ragweed, *Ambrosia artemisiifolia*
- lambsquaters, *Chenopodium* sp.
- cocklebur, *Xanthium strumarium*
- bindweeds, *Convolvulus arvensis*, *Callystegia sepium*, etc.

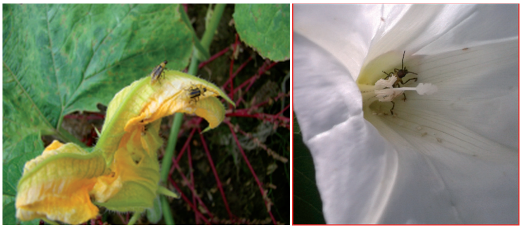

Fig. 1 *Diabrotica* adults visiting pumpkin and bindweed flowers.

Abb. 1 *Adulte* Diabrotica *zu Besuch bei Kürbis- und Ackerwindeblüten.*

Field experiments in Bad Radkersburg district

Apart from the a.m. cage tests in Graz new experiments have been conducted 2011 and 2012 on 3 sites in southeast Styrian maize fields near Bad Radkersburg where maize and oil pumpkin is grown in alternating crop rotations (see scheme in Figure 3 below).

This second experimental series was designed to study oviposition behavior of the western corn rootworm.

Similarly isolation cages as in Graz were used and yellow sticky traps with pheromone and floral baits were placed in the cages.

In fall of 2011 soil was sampled from maize and adjacent oil pumpkins (Fig. 1). The number of eggs of *Diabrotica* was estimated within the soil samples to indicate the potential risk of the corn rootworm infestation in the following year.

Figure 1 shows the experimental design for oil pumpkin and maize. Maize was planted in spring 2012 all over both fields while in 2011 maize was planted adjacent to oil pumpkins. Experiments were conducted on two field sites near Bad Radkersburg.

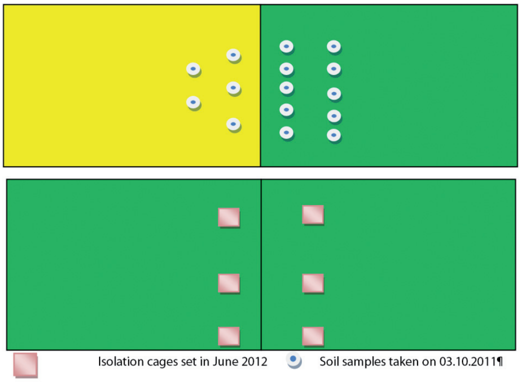

Fig. 2 Experimental design - isolation cages (pink squares) with yellow sticky traps and baits in maize 2012 planted after oil pumpkin (yellow plot in 2011 above) and after maize (green plot in 2011 above). White-blue spots indicate soil samples taken on 3rd October 2011. Field plots ca. 1 hectare each in 2011 maize (green) adjacent to oil pumpkin (yellow); same field plots planted with mais all over in 2012 (below); oviposition in oil pumpkin in autumn 2011 observed, soil sampled in October 2011; maize planted all over in 2012, number of hatching beetles recorded in cages with a ground area 2 m^2 and 2.5 m height.

Abb. 2 *Versuchsaufbau – Isolationskäfige (rosa Vierecke) mit Gelbtafeln und Ködern in Mais im Jahr 2012, der nach Ölkürbis (gelbes Feld im Jahr 2011 s. oben) und nach Mais (grünes Feld im Jahr 2011 s. oben) angepflanzt wurde. Die weiß-blauen Kreise stehen für Bodenproben, die am 3. Oktober 2011 genommen wurden. Die Schläge waren im Jahr 2011 jeweils ca. 1 ha groß für Mais (grün) und Ölkürbis (gelb); dieselben Schläge waren in 2012 vollständig unter Mais (unten); Untersuchung der Eiablage im Herbst 2011, Bodenbeprobung im Oktober 2011; Flächen vollständig unter Mais in 2012, Anzahl geschlüpfter Käfer in Käfigen mit einer Grundfläche von 2 m^2 und 2,5 m Höhe.*

3. Results and discussion

The results of the soil samples taken in 2011 (see Table 1) showed that the number of eggs detected was tenfold higher in maize fields compared to the adjacent oil pumpkin fields. A small proportion of eggs were laid into oil pumpkin fields, since flowering pumpkin is highly attractive to beetles (unlike soybean) although oil pumpkin is not a host plant of *Diabrotica*.

The experiments in the isolation cages resulted in ten times more beetles hatched in fields with maize after maize compared to those on field with maize after oil pumpkin (Tab. 1). In Styria (Southern Austria) there are regions with very small field units where an alternating crop rotation of oil seed pumpkin followed by maize is common practice. Particularly after the end of the maize flowering period in July mainly female *Diabrotica* beetles search for pollen in neighboring fields. Since oil pumpkin flowers from May until the end of September it is therefore strongly attracting *Diabrotica* adults. Oil pumpkin female flowers responsible for the yield appear long time before *Diabrotica* hatching. However, in autumn dozens of mostly female beetles invade male pumpkin flowers (Fig. 2) to collect their pollen. It is not known if all the females return from oil pumpkins to lay their eggs into maize fields. It is rather likely that oviposition partly occurs also in oil pumpkin fields (see Fig. 3).

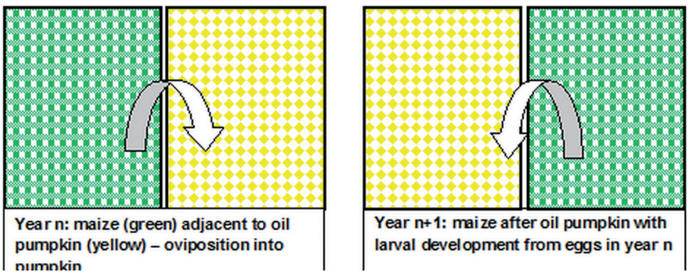

Fig. 3 Schematic *Diabrotica* dispersal and oviposition in maize and adjacent oil pumpkin fields.

Abb. 3 *Schematische Ausbreitung von* Diabrotica *und Eiablage in Mais- und benachbarten Ölkürbisfeldern.*

Tab. 1 Eggs dedected from soil samples in autumn 2011 near Bad Radkersburg (BTL Bio-Test Labor Sagerheide/D, LfL Freising, Versuchsreferat Stmk, AGES, Agro DS Österreich).

Tab. 1 *Eier, die in Bodenproben aus der Nähe von Bad Radkersburg vom Herbst 2011 gefunden wurden (BTL Bio-Test Labor Sagerheide/D, LfL Freising, Versuchsreferat Stmk, AGES, Agro DS Österreich).*

Location	Date of sampling	Position of sample in the fields	No. of samples	No of eggs extracted	Ø No of eggs/ L	Sd Standard deviations	Max eggs L	Min eggs L
Halbenrain*	03.10. 2011	edge maize	5	12	3.9	± 6.5	15.3	0.0
		centre maize	5	20	6.7	± 9.5	23.3	0.0
		edge pumkin	5	2	0.6	± 1.3	2.8	0.0
Zelting*	03.10. 2011	edge maize	5	163	48.3	± 25.4	69.2	4.2
		edge pumkin	5	7	2.0	± 1.9	4.3	0.0
Zelting	05.11. 2011	edge maize	9	37	9.1	± 11.8	33.3	0.0
		centre maize	10	19	4.2	± 3.5	11.1	0.0
		edge pumkin	10	1	0.2	± 0.7	2.3	0.0

* Sites in further tests with isolation cages in 2012

Tab. 2 Numbers of recorded adult western corn rootworm beetles hatched in maize after maize fields compared to maize after oil pumpkin fields.

Tab. 2 *Anzahl gefundener adulter* Diabrotica*-Käfer, die auf Flächen mit Mais-nach-Mais-Anbau geschlüpft sind, im Vergleich zu Flächen mit Mais-nach-Ölkürbis-Anbau.*

	Crop	
Year 2011	oil pumpkin	maize
Year 2012	maize	
	Beetles trapped/isolation cage	
Site 1 Halbenrain Ost	28	96
	122	155
	54	121
Site 2 Zelting	46	198
	39	309
	45	271
Mean	55.67	191.67
Ratio	3.44	
Risk-index for oil pumpkin	29.04	

* Risk-index for *Diabrotica* reproduction: Wheat =< 1%, maize=100%
Kurt Foltin and Johann Robier, 2014

Alternating crop rotation of oil pumpkin and maize

Oviposition in oil pumpkin is less than 10% compared to maize (see Tab. 1).

On the same plots counts of hatching adults in isolation cages with yellow sticky traps + pheromone and floral baits in Southeast Styrian maize fields indicate however higher numbers:

- the average ratio between the pre-crops maize vs. oil pumpkin was 3.4 : 1,
- reproduction rates maize fb cereals (wheat) were <1%,
- reproduction rates maize fb maize were 100%,
- reproduction rates oil pumpkin fb maize were 29.04%.

Cereals are therfore:

- ca. 30x safer than alternating crop rotations with oil pumpkin,
- ca. 100x safer than mono maize crop rotations.

4. Conclusions

From our experiments we conclude that there is a risk of *Diabrotica* reproduction in oil pumpkin fields rotated with maize in alternation (15-30% of the rates compared to maize after maize). Compared to cereals on the other hand the risk is 15 to 30 times higher. Larval damage on maize roots could only be observed in fields with maize after pumpkin. Various other flowering broadleaved plant species can serve with their pollen as a food resource and female beetles may lay eggs to a limited extend into such fields of alternative crops.

Acknowledgements

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Do undersowings in maize influence the development of larvae of the western corn rootworm?

Beeinflussen Untersaaten in Mais die Entwicklung der Larven des Westlichen Maiswurzelbohrers?

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Larvae of the western corn rootworm (*Diabrotica virgifera virgifera*) have to find suitable roots for feeding after hatching from overwintering eggs. We hypothesized that the roots of undersown plants, when mixed with the target roots of maize, would reduce the ability of larvae to localize the roots of their host plant, thus reducing the survival of larvae, resulting in less damage on maize roots.

We used perennial rye grass, Italian ryegrass, a mixture of Italian ryegrass and white clover, white clover, yellow mustard, and sunflowers as undersowings; the standard maize cultivar used in the experiments was Ronaldinho. For the experiments we used semi field plots simulating field conditions, filled with a silt loam and peat soil mixture. The containers were placed in a greenhouse and the larvae were extracted from the soil about 3 weeks after application of the eggs. Due to the quarantine status of the pest, the experiments needed to be terminated after this time span to avoid hatching of adults. Kempson extraction was used to extract and count the larvae developing in the different treatments.

Contrary to our hypothesis, most of the undersowings tested did not result in significantly lower number of larvae. When undersowing maize with clover even significantly more larvae than in the control were extracted. In case of dicots (yellow mustard or sunflower) lower larval numbers were extracted; however, only underdowings with sunflower caused a significant reduction of larval numbers recovered. All treatments with undersowings had no significant effect on larval dry weight nor did the undersowings significantly enhance or delay larval development.

In conclusion, undersowings do not provide an additional or alternative control measure against western corn rootworm larvae. Even in the case of sunflowers mixed with maize plants, it needs to be tested, whether the effects found in the greenhouse can be translated into field conditions.

Oviposition preferences of *Diabrotica virgifera virgifera*: Multiple-choice field cage trials

Eiablagepräferenzen von Diabrotica virgifera virgifera*: Multiple-choice-Käfigversuche im Feld*

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Adults of the maize pest *Diabrotica virgifera virgifera* are known to primarily lay their eggs into the soil of maize fields. This is, because the larvae of this pest are largely restricted in their feeding to maize. In the USA, some populations, however, also lay eggs in non-maize crops where maize is grown the following year which will allow larval development.

Therefore, the oviposition behaviour of *D. virgifera virgifera* adults was studied in large multiple choice field cages at two field sites under European conditions between 2009 and 2011. Between 8 and 22 large gauze cages (ca. 4.5 x 2 x 2 m) were placed into each of the two study fields, each covering three different crops. Totally 10 different crops were used in different combinations. About 50 newly emerged female and 50 male adults were released in each cage (usually in mid-July) of each year. As a result of oviposition of *D. virgifera virgifera* in the different crop habitats within the multiple choice field cages, a new generation of beetles emerged the following year when maize was planted over the entire experimental area. These beetles were captured in separated smaller cages placed over the area of the previous large multiple choice cage. The number of emerging adults largely differed between the previous crops before maize, indicating that oviposition was influenced by the crop habitat. As expected, the largest proportion of oviposition appeared to have happened in maize. To some extent also *Sorghum* (Millet) was more used for oviposition than other crops. Final quantitative analyses are on-going.

This study was funded by the Bavarian State Ministry of Food, Agriculture and Forestry.

Winter wheat and volunteer cereals as host plants for the western corn rootworm in Europe

Winterweizen und Ausfallgetreide als Wirtspflanzen für den Westlichen Maiswurzelbohrer in Europa

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Several cereals – including wheat - have already been proved to be suitable as host plants of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in laboratory experiments. Under field conditions, however, additional factors such as crop phenology may impede successful juvenile development of the pest insect. Early winter wheat varieties, for example, may mature - and consequently lose root vigour – at a time when larvae are still foraging in the soil. Late varieties, on the other hand, may be more suitable for pest development. Last but not least, corn rootworm larvae may not be able to complete development before maturation of the regular crop, but some may survive until volunteer wheat plants appear after harvest. This small portion of the larval population may then be able to complete development in the already harvested winter wheat fields.

The aim of the presented experiments was to investigate the possibility of corn rootworm development on winter wheat under field conditions. An early and a late winter wheat variety were sown in autumn 2009 and 2010, respectively. Small plots within these experimental fields were infested with defined amounts of *Diabrotica* eggs. Small maize plots in the same fields, similarly infested with corn rootworm eggs, served as control. Germination of volunteer winter wheat plants after harvest was suppressed in one part and augmented in the second part of the early winter wheat plots. Emergence of any western corn rootworm adults was monitored with emergence cages.

Results were similar in both years. A small number of *D. virgifera virgifera* adults emerged in control plots from maize roots, while no beetles were caught in any cages erected above winter wheat plots, independent from variety and the presence of volunteer wheat plants. A comparison of crop phenology and juvenile developmental periods of the corn rootworms showed a lack of synchrony. Both winter wheat varieties started to mature before the main part of the pest population was estimated to reach the pupal stage. Therefore, older larval stages of *D. virgifera virgifera* suffer from severe loss of food supply, making survival of the pest population unlikely. Germination of volunteer wheat plants, on the other hand, starts only after the majority of the larval population has presumably died from starvation.

Our two years results do not allow to completely exclude the possibility of a successful development of small parts of a *D. virgifera virgifera* population in winter wheat under central European field conditions. Nevertheless, results show clearly that an epidemic population development as known from continuous corn is impossible. One reason for this is a lack of synchrony between the phenological development of the juvenile pest stages and the host plants.

Crop rotation therefore remains to be a very effective pest management tool for containment measures in already infested regions, even if winter wheat follows maize in the crop rotation system. It may, however, not bee effective enough as a standalone method in isolated outbreak zones. In these cases, it is recommendable to plant only dicotyledone crops after maize and to employ additional eradication measures.

This study was carried out under the German *Diabrotica* research programme (for more information see http://diabrotica.jki.bund.de) by the Austrian Agency for Health and Food Safety (AGES), Vienna. We are grateful to our colleges Helmut Klapal, Otto Wurm and Vera Andrejic for help with field and lab work. The study was partly funded by the Bavarian State Ministry for Nutrition, Agriculture and Forestry (StELF), Munich.

Sorghum, Miscanthus & Co: Energy crops as potential host plants of western corn rootworm larvae

Sorghum, Miscanthus *& Co: Energiepflanzen als potenzielle Wirtspflanzen des Westlichen Maiswurzelbohrers*

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Summary

In a series of greenhouse experiments the host status and quality of 49 biofuel plants for the larvae of WCR were evaluated. The plants tested (18 species and varieties of *Sorghum*, 16 forage grasses, 6 *Miscanthus* genotypes, 6 *Panicum* varieties and 3 broadleaf species) were grown for at least three weeks before they were used in the bioassays.

The insects used in the experiments were obtained from a non diapausing laboratory strain originally from the US and maintained by BTL since 2006. Only neonate larvae (not older than 24 hours) were used in the bioassays.

In each experiment up to six species or varieties of plants were tested each with 10 replicates (containers). A susceptible maize variety was used as a positive control in each experiment. Each plant container was infested with ten neonate WCR larvae using a fine art brush. After inoculation the plants were not watered for at least 24 hours to facilitate the establishment of the larvae. The experiments were terminated after 18 days. To extract surviving larvae the soil and roots of test plants were carefully examined by hand and then transferred to a modified MacFadyen heat extractor with an extraction temperature of 45 °C.

To assess the host quality the number of larvae recovered, the widths of their head capsules and dry weights were recorded. The larvae were dried at 40 °C for at least 72 hours and then weighed on an electronic micro balance

Of the 21 forage and switch grasses examined 16 hosted WCR larvae. However, the percentage of larvae that survived for 18 days, their dry weights and head capsule widths were significantly less than that recorded for larvae that developed on maize roots. The roots of most (i.e. 15) of the 18 *Sorghum* species or varieties tested were unsuitable for the development of WCR larvae. For the remaining three *Sorghum* species a maximum of only two larvae (of 100 inoculated) were recovered. These results indicate that species of *Sorghum* are very poor quality hosts for WCR as previously reported in other studies. The opposite was true for the *Miscanthus* species tested. The number of larvae recovered from *Miscanthus* x *giganteus* roots, their dry weight and head capsule widths were the same as those recorded for larvae reared on the maize control. The other *Miscanthus* genotypes were less suitable than Mxg, but still more acceptable than all the forage and switch grasses tested in this study. In accordance with all previous studies, which used host plants other than maize, no larvae developed on the three broad leaf species.

Keywords: *Diabrotica virgifera virgifera*, field grasses, energy-crops, recovery, dry weight, head capsule width

Zusammenfassung

In Gewächshausversuchen wurde die Wirtspflanzeneignung von 49 Pflanzenarten und –sorten für Larven des Westlichen Maiswurzelbohrers einer nicht diapausierenden Linie des USDA untersucht. Getestet wurden Pflanzen, die interessante Optionen für die Biomasse-Produktion darstellen. Es wurden 18 *Sorghum*-Hirsen, 16 Ackergräser, jeweils sechs Rutenhirsen und Chinagräser, sowie drei zweikeimblättrige Pflanzenarten untersucht. Die Einschätzung der Wirtsqualität der überprüften Pflanzen erfolgte anhand der Wiederfunde der eingesetzten Larven, deren Kopfkapselbreite und des erreichten Gewichts.

Alle getesteten *Sorghum*-Arten und -Sorten wiesen keine, oder nur eine minimale Wirtseignung für *Diabrotica*-Larven auf. Die getesteten Hirsen können auf Basis dieser Ergebnisse uneingeschränkt als Mais-Alternative im Energiepflanzenanbau empfohlen werden. In einer wechselnden Fruchtfolge mit Mais bieten sie die Chance, hohe Biomasseerträge mit einer effektiven Reduktion der Populationsdichte zu verbinden.

Die Wirtspflanzeneignung der geprüften Ackergräser ist dagegen arten- und sortenabhängig. Fünf der 16 getesteten Gräser stellten sich für die Entwicklung der Larven als ungeeignet heraus. Die Wirtspflanzenqualität der elf Testpflanzen, von denen Tiere extrahiert wurden, muss auf Basis der erhobenen Versuchsparameter als reduziert bis minimal eingestuft werden. Im Vergleich zur Maiskontrolle waren insbesondere die Wiederfunde, aber auch die Kopfkapselbreiten und das erreichte Gewicht signifikant kleiner. Gleiches gilt auch für die geprüften *Panicum*-Sorten. Eine der sechs überprüften Sorten eignete sich nicht als Wirtspflanze für die Larven und die anderen fünf in nur sehr reduziertem Maße. Diese Ergebnisse zeigen, dass diese Gräser in der Fruchtfolge nicht als Ersatz für Mais bei Eradikationsbemühungen punktuell auftretender Käfer-Populationen zu empfehlen sind. Da die Entwicklungsbedingungen für die Larven aber stark reduziert sind, können in *Diabrotica*-Eingrenzungsgebieten sowohl Ackergräser als auch Rutenhirsen erfolgreich in wechselnden Fruchtfolgen mit Mais eingesetzt werden.

Die einzige Testpflanze an der sich *Diabrotica*-Larven genau so gut wie an Mais entwickelten, ist das Chinagras (*Miscanthus* x *giganteus*). Die anderen fünf getesteten *Miscanthus*-Genotypen ermöglichten ebenfalls eine Entwicklung der Larven. Die Wirtspflanzenqualität dieser Genotypen ist jedoch geringer als von *M.* x *giganteus*.

Stichwörter: *Diabrotica virgifera virgifera*, Ackergräser, Energiepflanzen, Wiederfunde, Trockengewicht, Kopfkapselbreite

1. Introduction

One of the most important biofuel crops is maize. To reduce the negative effects of large scale and continuous cultivation of maize a number of other species of potential biofuel plants have been suggested. Although there are a considerable number of studies on the economic, environmental and social aspects of their cultivation, the direct or indirect effects of these plants on agricultural pests are rarely investigated (Spencer and Raghu, 2009). In particular, whether these plants are suitable hosts for the larvae of the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, is unknown and will be analyzed in this study.

Reports from the USA indicate that *Diabrotica* larvae not only feed on maize, but can reach the adult stage also on other monocotyledonous plants (Branson and Ortman, 1967a, b, 1970; Oyediran et al., 2004; Wilson and Hibbard, 2004). Some of the plants tested in the USA, other than weeds and wheat, also occur in Germany. Moeser (2003) reports that *Diabrotica* larvae develop as well on wheat and some other species of grass (*Setaria* spp. and *Panicum milliaceum*) as on maize. Unfortunately these experiments were only done using second instar larvae and over a period of only six days. Therefore, it is uncertain whether they can complete their development and reproduce feeding on these grasses.

Field experiments in Romania have confirmed that this beetle will feed on three species of *Setaria* in the field (Breitenbach *et al*., 2005). In contrast to the findings of Moeser and Vidal (2004) no adults were found on wheat in Romania. Cabrera Walsh (2007) report that a species of *Diabrotica* (*D. speciosa* [Germar]) from South America can also complete its development on *Sorghum halepense*. This is of special interest as *Sorghum* spp. are thought to be no suitable host plants for WCR because the roots contain high levels of hydrocyanic acid (Branson *et al*., 1969). As in previous studies only a small number of *Sorghum* plants were analyzed but *Sorghum spp.* could be used for biofuel production instead of maize as a consequence these plants were the main focus of this project.

2. Methods

In a series of greenhouse experiments the host status and quality of 49 biofuel plants and a cultivar of maize for the larvae of WCR were compared. The biofuel plants tested (16 forage grasses, 6 *Panicum* varieties, 18 species and varieties of *Sorghum*, 6 *Miscanthus* genotypes, and 3 broadleaved species) were potted in a 1:1 (w/w) mixture of a commercial compost and sandy field soil previously sieved and heat steamed for four hours. The containers used were 1 liter plastic pots. The drainage holes in the containers were closed with a fine stainless steel mesh (mesh size 100 µm) to prevent the escape of *Diabrotica* larvae. Plants were grown for at least three weeks before they were used in the bioassays. The plants were cultivated and experiments done in a greenhouse kept at 22 ± 2 °C, 65 ± 15% RH and a 16 hour photoperiod. Additional light was provided by 400 W Philips Son-T Agro high pressure sodium vapour lamps if the ambient light intensity fell below 10,000 lux. The plants were watered as necessary, no additional fertiliser was added.

Insects – The insects used in the experiments were obtained from a non-diapausing laboratory strain originally from the US and maintained by BTL since 2006. The non-diapausing strain was selected in the USA at the end of the 1960s over a period of 9 generations and since then kept at the Northern Grain Insect Research Laboratory (USDA-NGIRL), Brookings, USA, and produces approximately 6 generations per year (Branson, 1976). The rearing methods applied by us are those described by Branson *et al*. (1975, 1988). Only neonate larvae not older than 24 hours (Fig. 1) were used in the bioassays (Fig. 2).

Fig. 1 *D. virgifera virgifera*: **A** – developmental stages of larvae (L1–L3) and **B** – typically shortened and bent pre-pupa in the earthen cell in which it pupates.

Abb. 1 D. virgifera virgifera*: **A** –Entwicklungsstadien der Larven (L1–L3) und **B** – Typisch verkürzte und gebogene Vor-Puppe in ihrer Puppenhöhle.*

Fig. 2 Design of trial for testing host quality using *Sorghum bicolor* (Arlys, Biomass 150), *S. bicolor* x *sudanense* (Green Grazer), *S. sudanense* (Akklimat) and maize (Tassilo) as the control.

Abb. 2 *Versuchsdesign zur Testung der Wirtsqualität von* Sorghum bicolor *(Arlys, Biomass 150),* S. bicolor *x* sudanense *(Green Grazer),* S. sudanense *(Akklimat) und Mais (Tassilo) als Kontrolle.*

Bioassays – The proof of acceptability of plant species and cultivars was done in a series of successive experiments. In each experiment up to six species or varieties of plant were tested each with 10 replicates (containers). A susceptible maize variety (Tassilo, kindly provided by KWS) was used as a positive control in each experiment. The experiments were set up in a randomized block design. As according to Chege *et al*. (2005) and Hibbard *et al*. (2008), the quality of host plants depends on their age the test plants were at a susceptible vegetative developmental stage (e. g. maize and *Sorghum* spp. at BBCH 14–15, field grasses at the tillering stage, BBCH macro stadium 2; BBCH working group, [2001]). Each plant container was infested with ten neonate WCR larvae using a fine art brush (size 000). After inoculation the plants were not watered for at least 24 hours to facilitate the establishment of the larvae. According to the quarantine rules the experiments were terminated after 18 days, before the animals reached the pre-pupal stage.

To extract surviving larvae the soil and roots of test plants were carefully examined by hand and then transferred to a modified MacFadyen heat extractor (MacFadyen, 1961). Heat extraction was continued for up to four days with an extraction temperature of 45 °C provided by red light bulbs (Fig. 3).

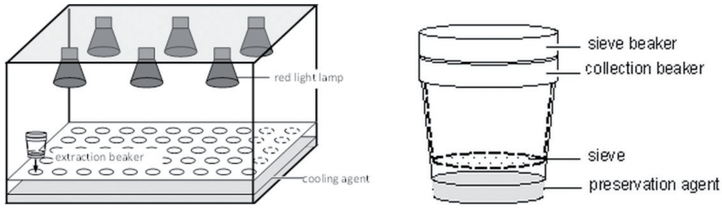

Fig. 3 A – Design of the modified MacFadyen apparatus; B – Extraction beaker.

Abb. 3 *A – Design des modifizierten MacFadyen Apparates; B – Extraktionsbecher.*

To assess the host quality the number of larvae recovered, the widths of their head capsules and dry weights were recorded. Head capsule measurements were obtained using a stereo microscope (Olympus, SZX 12) fitted with a CCD camera (Olympus, colour view III) and cell^D software was used to analyze the pictures (Olympus Deutschland GmbH, Hamburg, Germany and Olympus Soft Imaging Solutions GmbH, Münster, Germany). The larvae were dried at 40 °C for at least 72 hours and then weighed on an electronic micro balance (XP 26, d=0.1 µg; Mettler-Toledo GmbH Giessen, Germany).

Statistics – The number of larvae recovered was expressed as percentage recovered per container (n_{ex}*100/n_{ino}; n_{ex}=number larvae extracted; n_{ino} number of larvae inoculated). For the calculation of the mean head capsule width or dry weight the value for each larva was treated as a replicate. Comparisons of experimental treatments were done using a t-Test or Mann-Whitney Test. To compare more than two treatments a one-way ANOVA and Tukey post hoc test were performed. To test if the preconditions for parametric statistics (normality and equal variances) were met the D'Agostino and Pearson omnibus normality- and Bartlett-test were used. Non parametric comparisons were done using the Kruskall-Wallis test. For all calculations Microsoft Excel® 2002 (10.6871.6870; SP 3) and GraphPad Prism 5.03 for Windows (GraphPad Software Inc., San Diego, USA) were used.

3. Results

A comparison of the results for all the maize controls in the successive experiments shows that the development of larvae was dependent, but not exclusively so, on food quality (Fig. 4). There are significant differences between recovery (ANOVA, Tukey post-hoc test, F=6.572, df=13; p< 0.0001), dry weight (Kruskal-Wallis- and Dunn post-hoc-test, H=286.3, df=13, p< 0.0001) and head capsule width (Kruskal-Wallis- and Dunn post-hoc-test, H=109.0, df=13, p< 0.0001). It is difficult to explain these differences as in each experiment the relevant abiotic and biotic factors (e. g. temperature, soil composition, air- and soil-temperature, duration of experiment, plant age, number of larvae per container) were identical. It cannot be excluded that there are rhythms in the development of beetles and plants. These variations document the accurateness and thresholds of the executed experiments and should be accounted for in the interpretation of the results. The data for each experiment were calculated relative to those for the maize control (=100%) in order to compare the results of the different experiments.

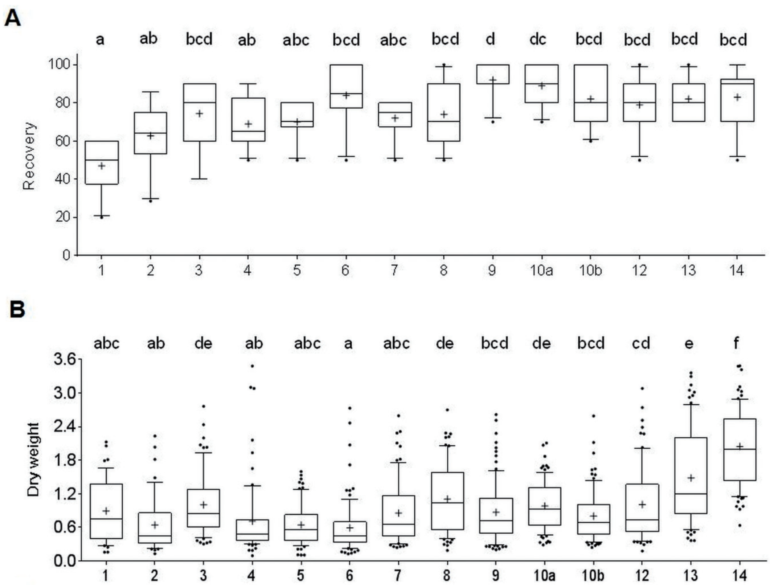

Fig. 4 Results of the individual successive tests in which was analysed (A) percentage recovery, (B) dry weight (mg) and (C) head capsule width (µm) of *D. virgifera virgifera* larvae reared on maize**.** (boxplots with median, mean (‚+') and 10–90 percentiles; different letters indicate significant differences (**A**: one-way ANOVA, Tukey-test; **B** and **C**: Kruskal-Wallis Test, Dunn's-Test; $p<0.05$).

Abb. 4 *Ergebnisse der einzelnen aufeinander folgenden Versuche, in denen analysiert wurde (A) prozentualer Anteil an Wiederfunden, (B) Trockengewicht (mg) und (C) Kopfkapselbreite (µm)* von D. virgifera virgifera *Larven die an Mais gehalten wurden (boxplots mit Median, Mittelwert (‚+') und 10–90 Perzentile; unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede (**A**: one-way ANOVA, Tukey-Test; **B** and **C**: Kruskal-Wallis Test, Dunn's-Test; p<0,05).*

The survival and development of WCR larvae feeding on 49 plant species/varieties, including 16 forage grasses (e.g. a number of *Lolium* and *Festuca* varieties), six switch grass varieties (*Panicum virgatum*), 18 *Sorghum* species/varieties, *Miscanthus* x *giganteus* (Mxg) and five other *Miscanthus* genotypes and three broadleaved species were monitored. To assess the quality of these plants as hosts for WCR the number of larvae that survived and the widths of their head capsules and dry weights were recorded.

Of the 21 forage and switch grasses examined 16 hosted WCR larvae (Tab. 1 and 2). However, the percentage of larvae that survived for 18 days, their dry weights and head capsule widths were significantly less than that recorded for larvae that developed on maize roots. The recovery of larvae from the field grasses tested was only 12.1 ± 22.1% of that for the maize control (78.7 ± 13.6%).

Tab. 1 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on field grasses ([1] LfL – Bavarian State Research Center for Agriculture, Freising, Germany; S – seeds).

Tab. 1 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven, die an Ackergräsern gehalten wurden ([1] LfL – Bayerische Landesanstalt für Landwirtschaft, Freising, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
Alopecurus pratensis	Alko	LfL	S	0.0		
Arrhenatherum elatius	Arone	LfL	S	0.0		
*Bromus mollis**	wild population	LfL	S	0.0		
*Dactylis glomerata**	Husar	LfL	S	59.3	33.9	81.5
Festuca arundinacea	Lipalma	LfL	S	2.4	11.8	61.3
F. pratensis	Cosmolit	LfL	S	29.8	31.8	69.6
F. rubra	Condor	LfL	S	0.0	0.0	0.0
Lolium multiflorum	Fastyl	LfL	S	8.3	15.4	64.2
L. multiflorum	Liberta	LfL	S	11.9	17.6	66.4
L. perenne	Ivana	LfL	S	0.0		
L. perenne	Niata	LfL	S	5.7	31.2	71.7
L. perenne	Pionero	LfL	S	15.7	20.1	65.9
L. perenne	Pomerol	LfL	S	1.4	26.0	93.2
Phalaris arundinacea	wild population	LfL	S	36.5	13.0	64.7
Phleum pratense	Phlewiola	LfL	S	9.7	15.7	63.0
Poa pratensis	Nixe	LfL	S	0.0		

* several times tested

Tab. 2 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Panicum virgatum* ([1] NRS – Natur-Rostoff-Service, Kanzem, Germany; S – seeds).

Tab. 2 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* Panicum virgatum *gehalten wurden ([1] NRS – Natur-Rostoff-Service, Kanzem, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
Panicum virgatum	Alamo	NRS	S	6.1	10.2	59.1
P. virgatum	Carthage	NRS	S	7.3	6.4	60.0
P. virgatum	Cave in Rock	NRS	S	0.0		
P. virgatum	Forestburg	NRS	S	7.3	13.8	71.4
P. virgatum	Kanlow	NRS	S	15.9	10.3	63.0
P. virgatum	Sunburst	NRS	S	1.2	4.6	66.3

Also the head capsule width of larvae feeding on field grasses was smaller than for the maize control. The largest head capsule width was recorded for larvae that developed on the forage grass var. Husar (432.7 ± 79.8 µm; n=140) and both ryegrass varieties Niata and Cosmolit (371.2 ± 65.9 µm and 351.2 ± 60.5 µm). The head capsule width of larvae in the corresponding maize control was 521.8 ± 37.3 µm. In comparison to the larvae on maize those on ryegrasses var. Pomerol stand out, as at 93.2% they are only slightly smaller than those on maize. But as there was only a single larva extracted from this plant its food quality will be rated very low. The reduced head capsule width of larvae that developed on field grasses can be accounted for in terms of their slow development. While on maize roots most larvae (96.4%) reached the third larval stage (L3), only 38.6% reached this stage on field grasses with 60.6% still only at the second stage (L2) and a smaller percentage (0.8%) at the first stage.

The roots of most (i.e. 15) of the 18 *Sorghum* species and varieties tested were unsuitable for the development of WCR larvae. For the remaining three species of *Sorghum* a maximum of only two larvae (of 100 inoculated) were recovered (Tab. 3). These species are not seen as alternative biofuel plants to maize. Only one larva was extracted from the roots of *S. caffrorum* and *S. dochna* and two from *S. durra*. Compared with maize the other characters used for rating host quality were also strongly reduced (Tab. 3). This supports the results of other authors that analyzed a smaller number of species/varieties (e.g. Branson *et al*., 1969; Clark and Hibbard, 2004; Oyediran *et al*., 2004). Due to the low number of recoveries the results are not presented graphically.

Tab. 3 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Sorghum* ([1] TFZ – Technologie und Förderzentrum, Straubing, Germany; KWS – KWS Saat AG, Einbeck, Germany; JKI – Julius Kühn-Institut, Braunschweig, Germany; S – seeds).

Tab. 3 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven, die an* Sorghum *gehalten wurden ([1] TFZ – Technologie und Förderzentrum, Straubing, Deutschland; KWS – KWS Saat AG, Einbeck, Deutschland; JKI – Julius Kühn-Institut, Braunschweig, Deutschland; S – Samen).*

Taxon	Variety	Source[1]		R	DW	HC
S. bicolor	Arlys	TFZ	S	0.0		
	Biomass 150	TFZ	S	0.0		
	Branco	KWS	S	0.0		
	Bulldozer	KWS	S	0.0		
	Goliath	TFZ	S	0.0		
	Maja	TFZ	S	0.0		
	Sucrosorgo 405	TFZ	S	0.0		
	Zerberus	TFZ	S	0.0		
	wild population	JKI	S	0.0		
S. bicolor x sudanense	Green Grazer	TFZ	S	0.0		
	Lussi	TFZ	S	0.0		
	Inka	TFZ	S	0.0		
S. caffrorum	wild population	JKI	S	1.1	27.3	66.8
S. dochna	wild population	JKI	S	1.1	16.7	68.0
S. durra	wild population	JKI	S	2.2	55.7	83.5
S. nervosum	wild population	JKI	S	0.0		
S. saccaracum	wild population	JKI	S	0.0		
S. sudanense	Akklimat	TFZ	S	0.0		

These results indicate that species of *Sorghum* are very poor quality hosts for WCR as previously reported in other studies. The opposite was true for the *Miscanthus* species tested. The number of larvae recovered from *Miscanthus* x *giganteus* roots, their dry weight and head capsule widths were the same as those recorded for larvae reared on the maize control (Tab. 4). The other *Miscanthus* genotypes were less suitable than *Miscanthus* x *giganteus*, but still more acceptable than all the forage and switch grasses tested in this study. In relation to the number of larvae inoculated at the start of the experiments the number extracted varied between 14.0 ± 12.6% for *M. sacchariflorus* (Robustus) and 70.0 ± 13.3% for *M.* x *giganteus* (rhizome-plants). In comparison with the maize control this indicates 17.7% for *M. sacchariflorus* (Robustus) and 95.7% for *M.* x *giganteus* (Tab. 4). Omitting the results for the genotype Robustus the recovery of larvae from the *Miscanthus* tested was 52.3 ± 13.5%. This value is equivalent to 74.7% of larvae extracted from maize controls.

Tab. 4 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *Miscanthus* ([1] IVT – *in-vitro*-tec Gesellschaft zur Pflanzenvermehrung für den Umweltschutz mbH; Berlin, Germany; MBT – Mendel Biotechnology, Hayward, CA, USA; RH – rhizome-plants, IV – *in vitro*-plants).

Tab. 4 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* Miscanthus *gehalten wurden. ([1] IVT –* in-vitro-*tec Gesellschaft zur Pflanzenvermehrung für den Umweltschutz mbH; Berlin, Deutschland; MBT – Mendel Biotechnology, Hayward, CA, USA; RH – Rhizome-Pflanzen, IV –* in vitro-*Pflanzen).*

Taxon	Variety	Source[1]		R	DW	HC
Miscanthus x giganteus	Mxg	IVT	RH, IV	95.7	146.3	100.0
M. sacchariflorus	Robustus	MBT	IV	17.7	25.3	69.1
M. sacchariflorus x sinensis	Amuri 1	MBT	IV	78.5	23.5	72.6
M. sacchariflorus x sinensis	Amuri 2	MBT	IV	53.2	32.5	82.2
M. sacchariflorus x sinensis	Nagara	MBT	IV	50.6	60.5	84.2
M. sinensis	Hybrid	MBT	IV	50.6	29.0	81.7

The dry weight of the larvae that developed on *Miscanthus* roots varied by a factor of 11.4. The larvae that developed on *M. sacchariflorus* x *sinensis,* Amuri 1 (0.243 ± 0.168 mg) and the genotype Robustus (0.261 ± 0.134 mg) were mostly very light in weight. The heaviest larvae were extracted from *in vitro*-plants of *Miscanthus* x *giganteus* (experiment 14) and were 2.126 ± 0.932 mg, which is the largest dry weight recorded during this study. In comparison to the dry weight of larvae reared on the maize control these values correspond to 23.5% for larvae reared on Amuri 1 (Tab. 4).

The comparison of the host quality of *Miscanthus* x *giganteus* revealed only small differences between *in vitro*- and rhizome-plants (Tab. 5). Although the percentage recovery and head capsule width of larvae that developed on these plants did not differ the dry weight of those reared on *in vitro*-plants was larger (Fig. 5).

Tab. 5 Percentage (compared to the maize control within each experiment) of recovery (R), dry weight (DW) and head capsule width (HC) of *D. virgifera virgifera* larvae reared on *in vitro*- and rhizome plants of *Miscanthus* x *giganteus*.

Tab. 5 *Prozentualer Anteil (im Vergleich zur Maiskontrolle in jedem Versuch) an Wiederfunden (R), Trockengewicht (DW) und Kopfkapselbreite (HC) von* D. virgifera virgifera *Larven die an* in-vitro- *und Rhizome-Pflanzen von* Miscanthus x giganteus *gehalten wurden.*

Taxon		R	DW	HC
M. x giganteus	*in vitro*-plants	80.7	103.7	97.3
M. x giganteus	rhizome-plants	84.3	68.3	96.6

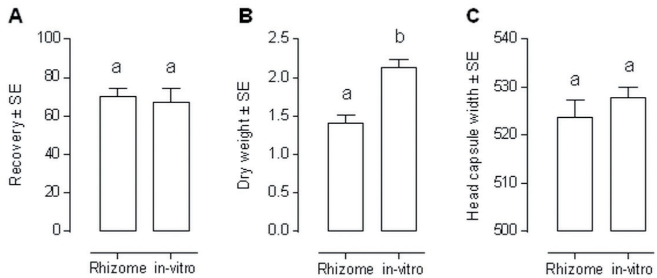

Fig. 5 Results of tests in which was analysed (A) percentage recovery, (B) dry weight (mg) and (C) head capsule width (µm) of *D. virgifera virgifera* larvae reared on *Miscanthus* x *giganteus* grown from *in vitro*- and rhizome-plants. (Different letters indicate significant differences between variants, t-test, p<0.05).

Abb. 5 *Ergebnisse der Versuche, in denen analysiert wurde (A) prozentualer Anteil an Wiederfunden, (B) Trockengewicht (mg) und (C) Kopfkapselbreite (µm) von* D. virgifera virgifera *Larven die an* Miscanthus x giganteus *gehalten wurden, welche sich aus* in vitro- *und Rhizome-Pflanzen entwickelten. (unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede zwischen den Varianten, t-Test, p<0,05).*

No larvae developed on the three broad leaved species of dicotyledonous plants tested. This is in accordance with all previous studies, which used hosts other than maize (e. g. Branson and Ortman, 1967a, 1970; Behle *et al.,* 2008).

4. Conclusion

It is reported that larvae of the western corn rootworm are able to develop on plant species other than maize. Therefore, it is not surprising we recorded something similar for the biofuel plants used in this study. However, in accordance with previous studies the quality of most of these 'alternative hosts' for WCR is considerably less than that of maize, which resulted in fewer larvae becoming established and surviving and a prolonged larval development, as indicated by smaller head capsule widths and reduced dry weights. Based on the results presented the majority of the forage and switch grasses tested are not suitable for eradicating small and recently established populations. Despite this they can be used as an alternative to maize as part of a crop rotation strategy when *D. virgifera virgifera* is established in an area. The low host quality of these grasses will result in a decrease in the population size of *D. virgifera virgifera* and a diversification of the crops that can be used as biofuels.

All *Sorghum* varieties tested were not suitable hosts for WCR larvae, but are promising biofuel crops. Therefore, if grown in rotation with maize, *Sorghum* provides a highly effective and ecological friendly way of controlling WCR, and is highly productive.

That species of *Miscanthus*, especially *M.* x *giganteus,* are good hosts for WCR is alarming. The long crop cycle of *Miscanthus* rhizomes of up to 20 years would appear to provide an excellent and long lasting source of high numbers of WCR adults, as continuous maize production does. However, this depends on the egg laying behaviour of the females. Only if they lay their eggs in established *Miscanthus* stands they will complete their life cycle there. Despite some early findings of studies carried out in the US this question is still unanswered and should be evaluated at a large spatial scale. Based on what is known about the oviposition behaviour of WCR they lay very few eggs in crops other than maize. As the oviposition sites are located very close to adult feeding sites, e. g. plants that produce large amounts of pollen, it is very unlikely that WCR will lay eggs in crops of *Miscanthus*.

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Host plant specificity studies of the western corn rootworm - experiments in isolation cages

Untersuchungen zur Wirtspflanzenspezifität des Westlichen Maiswurzelbohrers – Versuche in Isolationskäfigen

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1. Introduction

Maize is a profitable crop for central European growers. Many farmers prefer maize for feeding cows or pigs and for the maintenance of biogas plants. The western corn rootworm has invaded large parts of the maize growing areas of Central Europe (Edwards and Kiss, 2011). In 2002 the beetle arrived in the eastern parts of Austria and extended in 2012 as well into the southern and western parts. Only a few cases with observed economic damages have been recorded since in invaded areas. Official control measures according to EU Decision 2003/766/EG is in place to prevent the further spread of the western corn rootworm. Crop rotation is known to be the most efficient strategy to suppress the western corn rootworm. The required crop rotation may however lead to economic impacts for growers. In the experiments presented here different plant species to be rotated with maize were studied with respect to their effects on the reproduction of *Diabrotica* in the field. The population dynamics of *Diabrotica* was studied in isolation cages in the field (Figure 1).

Fig. 1 Isolation cages for recording *Diabrotica* populations (photo: K. Foltin).

***Abb. 1** Isolationskäfige zur Untersuchung von Diabrotica-Populationen (Foto: K. Foltin).*

2. Material and methods

Since crop rotation is the most efficient measure to suppress *Diabrotica* reproduction and spread, several experiments have been set up to identify those plant species that could either interfere the development of western corn rootworm or are potential host plants. Experiments were carried out on a maize site in Austria near Graz in cages especially designed for an isolated development of *Diabrotica* beetles in the field. Cages were of about 2 m^2 ground size and 2.5 m height (1.4 m x 1.4 m x 2.5 m, see Fig. 1). Maize was planted with 20 plants per cage. Maize plants were infested artificially by defined numbers of female and male beetles released into the cages (Foltin and Robier, 2014). Descendant generations i.e. hatching beetles were recorded regularly.

The most common arable crops and plant species cultivated in Austria were planted after maize into these isolation cages in 2010 and subsequent years:

1. Maize
2. Green rye – hibernal green cover between maize followed by maize
3. Field peas undersown in maize
4. Winter and spring cereals
5. Oil Pumpkin („Styrian oil pumpkin" *Cucurbita pepo var. styriaca*)
6. *Miscanthus*
7. Warm season grasses: barnyard grass - *Echinochloa crus-galli*, yellow foxtail - *Setaria glauca*

3. Results and conclusions

The experiments resulted in beetles hatching only when maize after maize was grown (plant species/variant numbers 1 to 3). Larval damage as well was only observed in continuous maize fields. All other variants led to the hatching of no or only small and negligible numbers of beetles (Figure 2).

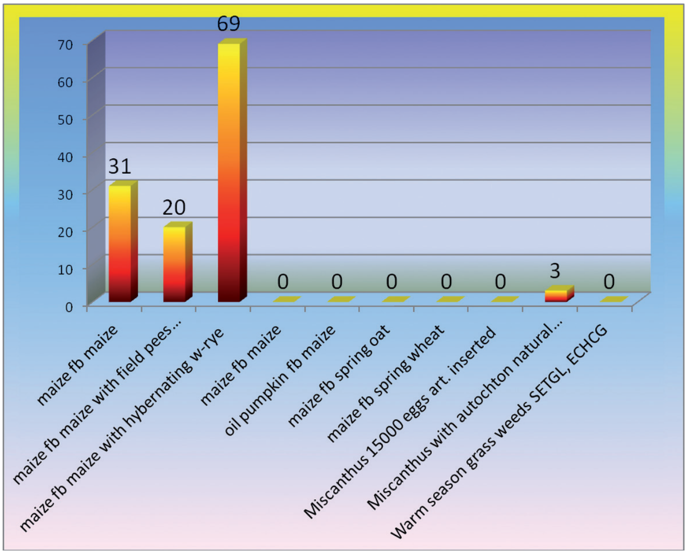

Fig. 2 CROP rotational experiments in isolation cages in Graz (Austria). No. of specimen of *Diabrotica virgifera virgifera* hatched and caught in yellow sticky traps within isolation cages from July to October 2012 within 10 different variants of crop rotation in four replications.

Abb. 2 *Versuche zur Fruchtfolge in Isolationskäfigen in Graz (Österreich). Anzahl* Diabrotica*-Käfer, die in der Zeit von Juli bis Oktober 2012 geschlüpft waren und mit Gelbtafeln gefangen wurden, in 10 unterschiedlichen Fruchtfolgevarianten mit jeweils vier Wiederholungen.*

Table 1 shows a summary of results derived from the isolation cage experiments from 2010 to 2012. Spring cereals and oil pumpkin plants were not suitable as host plants for larval development, because reproduction of western corn rootworm was not possible. Autumn planted hibernating winter rye had no suppressive effect on western corn rootworm as maize follows maize in this variant. Undersown fodder peas as well did not have any suppressive effect on *Diabrotica* due to the continuous maize in this rotation. Although elephant grass (*Miscanthus*) was described as a host plant of *Diabrotica* only very few beetles hatched in these experiments (Gloyna *et al.*, 2011).

Despite artificial infestation of 15,000 eggs per cage in spring only very small numbers of beetles could be detected. In warm season grass weeds (*Echinochloa crus-galli*, *Setaria glauca* and *Setaria viridis*) *Diabrotica* could only reproduce at high population densities the year before in maize and only very few hatched beetles were found. In 2012 no hatched adults could be observed at all until August.

Table 1 reflects scenarios of 100% and 50% maize in crop rotation. When maize is even less than 50% in crop rotation the risk of reproduction and spread of *Diabrotica* is almost zero. But the risk also depends very much on the current infestation level and the population densities of western corn rootworm.

Tab. 1 Summary of host suitability and estimated risk for the reproduction of *Diabrotica* in crop rotations of maize with various plant species.

Tab. 1 *Zusammenfassung der Wirtspflanzeneignung und geschätztes Reproduktionsrisiko von* Diabrotica *in Maisfruchtfolgen mit unterschiedlichen Pflanzenarten.*

Common arable crops tested in isolation cages after maize	**Suitability as host plant**	**Estimated risk (%) in alternating crop rotation with maize (mono maize=100%)**
Spring cereals – spring oat, spring wheat	-no-	**< 1**
Maize	+ + +	**100**
Oil pumpkin*	-no-	**15–30***
Fodder peas undersown in maize	+ + + (no for peas) (no suppression by undersown fodder peas in maize)	**100**
Winter rye/maize	+ + + (no for rye) (no suppression when maize drilled into hibernating rye)	**100**
Miscanthus – Elephant grass	(+)	**<10**
Warm season grass weeds *Echinochloa crus-galli, Setaria glauca, Setaria viridis*	(+)	**<10**

*Oil pumpkin is due to its long period of flowering a strong attractant for *Diabrotica* which might lead to oviposition there. Pumpkin however is not a host plant for larvae.

Acknowledgements

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Session 5: Alternatives to maize in arable feed cropping

Ermittlung regionalspezifischer Ertrags- und Qualitätsdaten von Alternativen zu Mais im Futterbau – Feldversuche zu Futtergräsern und deren Gemengen, Hirsen sowie Getreide–Ganzpflanzensilage

Determination of region-specific data of yield and quality of alternatives to silage maize in fodder crops – field trails with forage gras and clover grass mixtures, Sorghum *as well as whole plant silage of grain*

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Zusammenfassung

In diesem Projekt wurden aktuelle regionalspezifische Daten zu den Parametern Ertrag und Qualität von alternativen Futterpflanzen zu Silomais über einen Versuchszeitraum von drei Jahren untersucht. Dabei wurden regional adaptierte Silomaissorten mit Futtergräsern und Kleegrasgemengen, Sudangräser und Hirsen sowie Getreide-Ganzpflanzensilagen miteinander verglichen. Die getesteten Silomaisvarianten erzielten über den Versuchszeitraum mit ca. 230 dt/ha die höchsten und konstantesten Trockenmasse-Erträge (TM) bei gleichzeitig geringer Streuung der Einzelwerte. Die TM-Erträge der *Sorghum*-Arten lagen ca. 30 – 50 dt/ha unter den Erträgen von Silomais. Zudem schwankten die Sudangräser und Hirsen sehr stark in den Erträgen und es konnten bei fast allen Varianten mit Werten weit unter 28 % keine silierfähigen Trockensubstanz-Gehalte bis zur Ernte erreicht werden. Die Kleegräser erzielten hohe und stabile TM-Erträge, die an das Niveau von *Sorghum* heranreichten. Mit den ausgezeichneten Trockensubstanz-Gehalten dienen sie als hochqualitative und etablierte Alternativen zu Mais für die Silage. Die Getreide-Ganzpflanzensilagen erzielten Erträge bis zu 78 % verglichen zu Silomais. Im Versuchszeitraum erreichte keine der getesteten Kulturen das Niveau von Silomais in dieser Region, allerdings wäre eine Kombination zweier Kulturen bei Bedarf zu empfehlen. Einzelne angepasste Sorten oder Mischungen der Alternativkulturen reichten bis ca. 80 % an den Ertrag des Silomais heran. Die Werte der Netto-Energie-Laktation (NEL) zeigten, dass Silomais von allen getesteten Kulturen die höchsten NEL-Werte hatte, gefolgt von den Futtergräsern und deren Gemengen und den Getreide-GPS-Varianten, wobei es hierzu nur einjährige Ergebnisse gab. Die niedrigsten NEL-Werte zeigten die *Sorghum*-Arten. Bei dem Rohproteingehalt waren die Futtergräser den anderen Kulturen weit überlegen. Silomais, die *Sorghum*-Arten und die Getreide-GPS-Varianten hatten Rohproteingehalte, die etwa im Bereich der Hälfte der Werte der Futtergräser lagen.

Stichwörter: Silomais, Futtergräser, *Sorghum*, Getreide-Ganzpflanzensilage, Trockenmasseerträge, Trockensubstanzgehalte, Futterqualität.

Summary

This project should generate current regional results over a period of three years about the parameter yield and quality of alternative fodder crops to maize; this includes grass and clover grass mixtures, silage maize, varieties of *Sorghum/*millets and whole plant silages of wheat, rye and triticale. The tested silage maize showed the highest and most reliable average dry matter yield with 23 tons per hectare, with a very low variance. The *Sorghum* and millet varieties had dry matter yields of 3 to 5 tons per hectare below the silage maize yield but with individual values fluctuating in a broad range within years and locations. With values far below 28% the dry matter contents were not suitable for ensiling. The grass and clover grass mixtures are good, stable and established alternatives to maize for silage. They achieved high yields comparable with these of *Sorghum* but stable and with a highly suitable dry matter content for ensiling. The yield of the whole plant silages was up to 22% lower compared with maize. So none of the alternative crops can compete with the high level yield of silage maize in its favoured region, therefore would be a combination of two crops recommended. But some individual locally adapted mixtures or varieties of the alternative crops reached nearly 80% of the maize yield. Silage maize showed the highest level of the net energy content for lactation (NEL), followed by the values of the fodder crops and the whole plant silages. The *Sorghum* varieties showed the lowest NEL value of all tested cultures. The highest crude protein showed the fodder crops contents. Silage maize, *Sorghum* and the whole plant silages had values lying nearly around the 50% mark of the fodder crops.

Keywords: Silage maize, fodder crops, *Sorghum*, millets, grain whole plant silage, dry matter yield, fodder quality.

1. Einleitung

Silomais ist in Bayern die meist angebaute Kultur (InVeKos-Daten, 2010). Aufgrund der im niederbayerischen Landkreis Passau vorherrschenden günstigen Böden ist sie auch dort die meist favorisierte Feldfrucht. Da Mais selbstverträglich ist wird er in dieser Region in sehr engen Fruchtfolgen aber auch als Monokultur angebaut. Das erweist sich als problematisch, da sich der Landkreis Passau im Einwanderungsgebiet des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera* LeConte) befindet. Dieser Quarantäneschädling hat sich seit seinem Erstauftreten 1992 am Belgrader Flughafen in den europäischen Ländern mit ausgedehnten Maisanbauregionen etabliert, wobei die Hauptausbreitungsgebiete in den osteuropäischen Balkanländern, in den östlichen Regionen von Österreich, aber auch in Norditalien liegen (Wudkte, 2005; Baufeld, 2009, Edwards *et al.*, 2012). Der Schädling verursacht dort durch massives Auftreten enorme Ertragsausfälle an Mais (Deuker *et al.*, 2012). In den letzten Jahren belegen Käferfunde das Vorkommen des Westlichen Maiswurzelbohrers auch im Süden von Deutschland (Boegel, 2012). Der Käfer wurde zum einen passiv eingeschleppt, wie entlang der Ostwest-Transitstrecken oder an den Flughäfen, zum anderen erfolgt eine aktive Einwanderung des Käfers durch Zuflug aus befallenen Maisfeldern der Nachbarländer.

Durch seinen Quarantänestatus hat der Westliche Maiswurzelbohrer in Deutschland neben ertraglichen Einbußen die Einleitung von besonderen betrieblichen Maßnahmen, wie Ausrottungs- und Eingrenzungsmaßnahmen zur Folge, und macht die Anwendung von integrierten Behandlungsmitteln notwendig (JKI, 2013). Dazu gehören vorbeugende und ackerbauliche Maßnahmen wie eine erweiterte Fruchtfolge, der Einsatz von Insektiziden und die Ausbringung natürlicher Feinde und soweit vorhanden, der Anbau von Sorten, die einen Züchtungsfortschritten gegen den Schädling vorweisen können. Je nach Produktionsausrichtung kann eine Einschränkung des Maisanbaues für die betroffenen Betriebe enorme ökonomische Schäden und Benachteiligungen nach sich ziehen.

Ziel dieses Projektes war die Erhebung regionalspezifischer Daten zur Ertragsleistung und den Qualitätsparametern von Futterpflanzen, die in dieser Region Alternativen zu Silomais in der Tierfütterung darstellen können. Dazu zählen Pflanzen des Feldfutterbaus wie Futtergräser im Rein- und Gemengeanbau, Kleegrasmischungen, *Sorghum*-Arten (Sudangräser/Hirsen) und Getreide-Ganzpflanzensilage (GPS).

Für die genannten Kulturen lagen aus den bayerischen Befallsgebieten nicht genügend Daten vor, so dass weder exakte pflanzenbauliche Empfehlungen abgeleitet werden, noch Berechnungen zur Wirtschaftlichkeit in dieser Region zu den alternativen Nutzpflanzen angestellt werden konnten. Für den Getreide-GPS-Versuch wurden zwei Versuchsjahre als ausreichend erachtet, da aus Versuchen der letzten Jahre auf Datenmaterial zurückgegriffen werden kann.

2. Material und Methoden

In den Feldversuchen wurden die möglichen Alternativen zu Mais in direktem Vergleichsanbau geprüft. Dazu wurden in dem Befallsgebiet drei Standorte für eine dreijährige Laufzeit des Versuches ausgewählt.

2.1 Standorte und Standortbedingungen

Der Versuch wurde 2009 im Befallsgebiet des Westlichen Maiswurzelbohrers an den Standorten Rotthalmünster, Kirchham und Egglfing in Niederbayern, Landkreis Passau, im Bodenklimaraum 116 („Gäu, Donau- und Inntal“, Fig.1) angelegt. Die einzelnen Standorte wurden so ausgewählt, dass sich ihre Böden geologisch unterschieden um damit die Bodenvariation im Befallsgebiet hinreichend abzudecken, zugleich sollten durch möglichst geringe Rüst- und Wegezeiten eine immer zeitgerechte und kosteneffiziente Bearbeitung von der Höheren Landbauschule Rotthalmünster (HLS) aus sichergestellt sein.

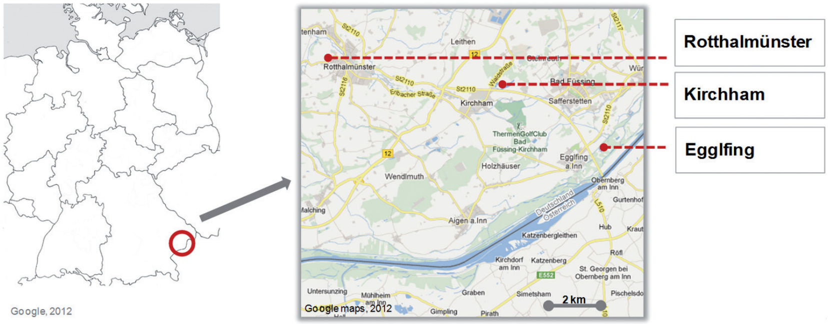

Abb. 1 Darstellung der Standorte 2009 – 2011.

Fig. 1 *Map of the field sites 2009-2011.*

Die Böden der Versuchsflächen in Rotthalmünster liegen im direkten Umgriff der HLS, bestehend aus Parabraunerde aus diluvialem Löß und befindet sich im tertiären Hügelland im Flusstal des Inns. Die Versuchsfläche liegt exponiert im freien Feld.

Die Böden des Standortes Kirchham bestehen aus Braunerde mit einer Krumenauflage von ca. 20 cm. Darunter folgt Schotter, was eine gute Wasserführung an diesem Standort gewährleistet, allerdings auch schnell zu Trockenheit führt. Der Standort zeichnet sich durch eine niedrige Bodenfruchtbarkeit aus und liegt am Waldrand.

Der Standort Egglfing besitzt einen Aueboden, der reich an organischer Substanz mit mineralischen Anteilen ist und unmittelbar im Überschwemmungsbereich des Inns liegt. Durch den Bau eines Dammes ist der Standort vor den negativen Auswirkungen eines Inn-Hochwassers geschützt. Das Feldstück liegt inmitten eines Waldstücks, was dem Standort ein eigenes Kleinklima verleiht, d.h. hohe Luftfeuchte und Wärme halten sich dort im Sommer länger als in frei exponierten Lagen, im Winter aber auch die Kälte. In Tab. 1 sind die Standortparameter aller Versuchsorte aufgeführt.

Tab. 1 Standortparameter.

Tab. 1 *Field site parameters.*

Standort	**Rotthalmünster**	**Kirchham**	**Egglfing**
Höhe (über NN)	360 m	335 m	322 m
langj. Mittel Niederschlag	800-850 mm	800-900 mm	800-900 mm
Mittl. Tagestemperatur	7-8 °C	8 °C	8 °C
Bodenart	sL4D	SL6Alg	sL4Al
Bodenzahl	70	36	60
Ackerzahl	68	36	59
Geologie	Tertiäres Hügelland	Innterrassenschotter	Flusssandsedimente
Klassenbeschrieb	Parabraunerde aus diluvialem Löß	Braunerde	Aueboden
pH-Wert (Okt.,09)	6,7	6,7	7,2
P_2O_5 (mg/100g Boden)	14	54	23
K_2O (mg/100g Boden)	12	27	7

2.2 Kulturen

Die verschiedenen Kulturen wurden in direktem Vergleichsanbau geprüft, um vergleichbare Daten zu erhalten. Die Sorten und Mischungen wurden so ausgewählt, dass sie für die Region angepasst waren (Bayerische Qualitätssaatgutmischungen bei den Futtergräsern) bzw. es sich um regional bewährte Sorten handelte (Silomais, *Sorghum*, GPS).

2.2.1 Gräser- und Kleegräsermischungen

Das Sortiment umfasste 15 verschiedene Gräser- und Kleegrasmischungen, die z.T. in zwei Schnitt-Intensitäten geprüft wurden (Tab. 2 und 3). Die Intensität „intensiv" umfasste fünf Schnitte pro Jahr, „extensiv" beinhaltete vier Schnitte pro Jahr. Die fünfschnittigen Varianten sollten ihren Einsatz in der Tierfütterung finden. Durch die häufig angesetzten Schnitte sollte qualitativ hochwertiges Futter, wie es bei Wiesennutzung und Weidehaltung in der Praxis durchgeführt wird, bereitgestellt werden. Die vierschnittigen Varianten waren nicht auf Qualität, sondern auf Quantität ausgerichtet, um die Aufwüchse energetisch in einer Biogasanlage verwerten zu können.

Beide Schnittintensitäten wurden nur an den Versuchsgliedern 1 - 5 durchgeführt, da sich genau diese Mischungen auch in einem anderen bundesweit durchgeführten Projekt wiederfinden (Projekt „EVA-II, Fachagentur für Nachwachsende Rohstoffe), das die Energiegewinnung aus Gras für die Biogasproduktion untersucht. Somit kann in späteren projektübergreifenden Auswertungen auf Daten von sechs anstelle von drei bayerischen Standorten zurückgegriffen werden. Daher resultiert auch der Einsatz der Versuchsglieder 4 und 5 (Mischungen „A 3" und „A 3 mit Rotklee"), die empfohlene Gräsermischungen für Norddeutschland sind und in Bayern normalerweise keine Anwendung finden. Der Grund hierfür ist, dass sie aufgrund der ausgewählten Grasarten, -mischung und der ausgewählten hochertragreichen aber wenig ausdauernden Sorten unter den härteren bayerischen Witterungsbedingungen nicht über drei Jahre beständig sind. Sie decken damit die Spanne nicht-empfohlener, aber noch im Qualitätssegment stehender Alternativen zur staatlichen Empfehlung dar. Die Versuchsglieder 6 bis 15 wurden generell nur „intensiv" geschnitten.

Die Versuchsglieder 6, 7 und 8 sind sogenannte „überjährige Mischungen", d.h. nach der Saat werden sie im Ansaatjahr geerntet, überwintern und erreichen im zweiten Jahr ihre volle Ernteleistung. Für eine Kultur im dritten Jahr sind diese Grasmischungen nicht ausgerichtet, die Bestände werden gepflügt und neu angelegt. Die Mehrheit der Versuchsglieder stellten „mehrjährige Mischungen" dar, d.h. sie werden angesät, was häufig unter einer Deckfrucht geschieht, in diesem Fall unter die Deckfrucht Hafer, der mit 70 kg/ha ausgesät wurde. Damit kann im Ansaatjahr ein Ertrag von ca. 80 % im Vergleich zum Vollertrag erreicht werden (Keller *et al*., 1997, Hartmann *et al*., 2006). Die in den Mischungen verwendeten Sorten sind auf Ertrag und Ausdauer ausgewählt und bringen den Vollertrag in dem Ansaatjahr folgenden sog. 1. Hauptnutzungsjahr und dem darauffolgenden 2. Hauptnutzungsjahr. Danach erfolgt auch für diese Mischungen ein Umbruch der Fläche. Die Tabelle 2 zeigt die verschiedenen Artenanteile der getesteten Mischungen, in Tabelle 3 sind die Prozentanteile der einzelnen Sorten der Artenmischungen aufgeführt.

Tab. 2 Übersicht der angebauten Gräser- und Kleegrasmischungen und deren Artenanteile.

Tab. 2 *Cultivated grass and clover grass mixtures and their species composition.*

	Mischungs-bezeichnung	Bezug	Artenzusammensetzung und %-Anteile													Saat-stärke
Vgl			WD	WB	WV	WL	WSC	FEL	WRP	KL	GL	ROT	RKL	WKL	LUZ	kg/ha
1	**FM 3-K**	BQSM				22	41				15		7	4	11	27,0
2	**FM 4-K**	BQSM	30			15	37						11	7		27,0
3	**FM 4**	BQSM	19			15	33						22	11		27,0
4	**A3**	EVA II Ref	43	26	31											35,0
5	**A3 mit Klee**	EVA II Ref	29	20	20								31			35,0
6	**FE 1**	BQSM				19	43						38			21,0
7	**FE 3-K**	BQSM		46	51								3			36,0
8	**FM 2**	BQSM				10	20								70	30,0
9	**Agravit 040R**	Advanta	75										15	10		32,5
10	**MG 8 Standard**	Freuden-berger				17	50		10			10	7	7		30,0
11	**Country 2052**	DSV	30	10	10	10	20	20								35,0
12	**Landgreen KG 550**	BSV	43			15	14		6	6			4	7	5	30,0
13	**Intensiv-mi-schung Kleegras**	Andreae	30			23	20						20	8		30,0
14	**Tetrafix intensiv m. Klee**	Stroet-mann	60					30					5	5		40,0
15	**Mehrjähr. Klee-gras m. Luzerne High Quality**	Dehner	10			15	10	45			5		5		10	30,0

Abkürzungen:

WD Deutsches Weidelgras; **WB** Bastardweidelgras; **WV** Welsches Weidelgras; **WSC** Wiesenschwingel; **FEL** Festulolium; **WRP** Wiesenrispe; **WL** Wiesenlieschgras; **GL** Glatthafer; **KL** Knaulgras; **ROT** Rotschwingel; **RKL** Rotklee; **WKL** Weißklee; **LUZ** Luzerne; **BQSM** Bayerische Qualitätssaatgutmischung; **EVA II REF** Saatgutreferenzgras FNR-Projekt EVA II; **Wdh** Wiederholung; **Vgl** Versuchsglied

Tab. 3 Darstellung der verwendeten Sorten in den Gräser- und Kleegrasmischungen.

***Tab. 3** Variety composition of grass and clover grass mixtures.*

Art	**Gräser**														
	WB								**WD**						
Reifegruppe1)	3	3	1	8	7	5	4	3	8	5	4	3	9	7	7
Ploidie (T = Tetraploid)	T				T	T	T		T	T	T		T		
Sorte	Ibex	Pirol	Ivana	Herbie	Navarra	Twins	Aubisque	Indiana	Castle	Missouri	Alligator	Lilora	Zocalo	Feeder	Aberavon
Züchter	EGB	STEI	BPZ	DLF	DLF	DLF	DLF	DLF	DLF	DLF	EGB	EGB	EGB	INSE	STEI
Mischung															
FM 3-K															
FM 4-K										9,9	9,9			9,9	
FM 4										9,3				9,3	
A 3	25,7					42,9									
A 3 + Klee	20,0					28,6									
FE 1															
FE 3-K	22,9	22,9													
FM 2															
Advanta Agra-vit 040R							45,0	30,0							
Freudenberger MG8 Standard															
DSV Country 2052	10,0											30,0			
BSV Landgreen KG 550				13,0											30,0
Andrae In-tensivmisch. Kleegras			5,0						10,0						
Stroetmann Tetrafix int. mit Klee					25,0	35,0									
Dehner Mehrj. Kleegras m. Luzerne high Quality													10,0		

1) Ähren-/Rispenschieben bzw. Blühbeginn: 1 = sehr früh, 3 = früh, 4 = früh bis mittel, 5 = mittel, 6 = mittel – spät, 7 = spät, 8 = spät – sehr spät, 9 = spät

Fortsetzung Tabelle 3

	Art	**Gräser**								
			WV				**WL**			**WRP**
	Reifegruppe[1)]	4	5	5	4	4	3	2	3	7
	Ploidie (T = Tetraploid)	T	T							
	Sorte	Mondora	Tarandus	Tigris	Oryx	Corner	Lischka	Licora	Phlewiola	Oxford
	Züchter	DLF	EGB	EGB	FREU	DLF	EGB	EGB	RZG	DLF
Mischung	**FM 3-K**					22,2				
	FM 4-K					14,8				
	FM 4					14,8				
	A 3	31,4								
	A 3 + Klee	20,0								
	FE 1					19,0				
	FE 3-K			25,7	25,7					
	FM 2					10,0				
	Advanta Agra-vit 040R									
	Freudenberger MG8 Standard								17,0	10,0
	DSV Country 2052		10,0				10,0			
	BSV Landgreen KG 550					15,0				6,0
	Andrae Intensiv-misch. Kleegras						12,5	10,0		
	Stroetmann Tetrafix int. mit Klee									
	Dehner Mehrj. Kleegras m. Luzerne high Quality					15,0				

[1)] Ähren-/Rispenschieben bzw. Blühbeginn: 1 = sehr früh, 3 = früh, 4 = früh bis mittel, 5 = mittel, 6 = mittel - spät, 7 = spät, 8 = spät - sehr spät, 9 = sehr spät

Fortsetzung Tabelle 3

	Art	Gräser									
			FEL		GL	KL	ROT				
	Reifegruppe[1)]	7	6	4	4	o.A.	6	5	5	3	4
	Ploidie (T = Tetraploid)	T	T	T							
	Sorte	Paulita	Lifema	Achilles[2)]	Arone	Lidaglo	Reverent	Pradel	Preval	Liherold	Cosmolit
	Züchter	DLF	EGB	DLF	STEI	EGB	FREU	BAHO	EGB	EUR	STEI
Mischung	**FM 3-K**				14,8				20,4		20,4
	FM 4-K								18,5		18,5
	FM 4								16,7		16,7
	A 3										
	A 3 + Klee										
	FE 1								21,5		21,5
	FE 3-K										
	FM 2							10,0	10,0		
	Advanta Agra-vit 040R										
	Freudenberger MG8 Standard						10,0				50,0
	DSV Country 2052		20,0						8,0	12,0	
	BSV Landgreen KG 550					6,0					14,0
	Andrae Intensiv-misch. Kleegras										20,0
	Stroetmann Tetrafix int. mit Klee	30,0									
	Dehner Mehrj. Kleegras m. Luzerne high Quality			45,0	5,0						10,0

[1)] Ähren-/Rispenschieben bzw. Blühbeginn: 1 = sehr früh, 3 = früh, 4 = früh bis mittel, 5 = mittel, 6 = mittel - spät, 7 = spät, 8 = spät - sehr spät, 9 = sehr spät
[2)] in der Zulassung

Fortsetzung Tabelle 3

	Art						Leguminosen							
			LUZ								WKL			
	Reifegruppe[1)]	4		2	3	4	5	4	5	5	o.A.	6	5	6
	Ploidie (T = Tetrapoid)				T	T	T	T						
	Sorte	Plato	Franken neu	Sanditi	Temara	Tempus	Maro	Titus	Riesling	Milkanova	Seminole	Klondike	Jura	Vysocan
	Züchter	FREU	SCHM	BAHO	EGB	FREU	NPZ	STEI	INSE	DLF	CAL	DLF	FREU	NPZ
Mischung	**FM 3-K**			11,1				7,4						3,7
	FM 4-K							11,1						7,4
	FM 4				11,1			11,1						11,1
	A 3													
	A 3 + Klee				31,4									
	FE 1							38,1						
	FE 3-K							2,8						
	FM 2			70,0										
	Advanta Agravit 040R						5,0	10,0		10,0				
	Freudenberger MG8 Standard							6,5					6,5	
	DSV Country 2052													
	BSV Landgreen KG 550	5,0						4,0	7,0					
	Andrae Intensiv-misch. Kleegras					10,0		10,0		5,0	2,5			
	Stroetmann Tetrafix int. mit Klee					5,0						5,0		
	Dehner Mehrj. Kleegras m. Luzerne high Quality		10,0					5,0						

[1)] Ähren-/Rispenschieben bzw. Blühbeginn: 1 = sehr früh, 3 = früh, 4 = früh bis mittel, 5 = mittel, 6 = mittel - spät, 7 = spät, 8 = spät - sehr spät, 9 = sehr spät
[2)] in der Zulassung

2.2.2 Silomais

Die Referenz für den Vergleich der Kulturen war Silomais. Damit sollten Ertragsfähigkeit und Qualitätsparameter verglichen werden. Drei Silomaissorten wurden so ausgewählt, dass sie über eine hohe regionale Anbauakzeptanz verfügten und zugleich sollten unterschiedliche Reifegruppen mit einbezogen werden. Die Anbauparameter orientierten sich an der pflanzenbaulichen Praxis (Tab. 4).

Die Sorten Torres und ES Bombastic gehören der Reifegruppe „mittelfrüh“ (S 230-S 250), die Sorte PR 39 F 58 „mittelspät“ (S 260 – S 290) an.

Tab. 4 Übersicht der ausgewählten Silomaissorten und deren Anbauparameter.

Tab. 4 *Chosen silage maize varieties and their cultivation parameters.*

Vgl	Sorte	Züchter/ Sorteninhaber	Reifegruppe	Pfl./m²	Reihenentfernung (m)	Einheiten/ha
1	Torres	KWS	S250	10	0,75	ca. 2,4
2	ES Bombastic	ARLS	S240	10	0,75	ca. 2,4
3	PR 39 F 58	PIOS	S260	10	0,75	ca. 2,4

2.2.3 *Sorghum*-Arten (Sudangräser und Hirsen)

Einige *Sorghum*-Arten werden aufgrund ihres vergleichbar hohen Ertragspotentials als mögliche Alternative zu Mais angesehen. Im Anbau befanden sich zwei Arten und deren Hybriden: Die Sudangräser, *Sorghum sudanense,* dünnstängelige, bestockungsfreudige und rispenbildende *Sorghum*-Arten und die Hirsen, *Sorghum bicolor,* kolbenbildende *Sorghum*-Arten, wozu auch die Zuckerhirsen zählen, sowie Hybriden aus beiden Arten. Es wurden vier verschiedene *Sorghum*-Sorten und zwei Artenmischungen verwendet, die zum Zeitpunkt des Projektbeginns für den Anbau im süddeutschen Raum empfohlen wurden bzw. in Vorversuchen vielversprechende Ergebnisse gezeigt hatten (Tab. 5).

Tab. 5 Übersicht der ausgewählten Sudangräser und Hirsen und deren Anbauparameter.

Tab. 5 *Chosen sudan and* Sorghum *grasses and their cultivation parameters.*

Vgl	Sorte	Züchter/ Sorteninhaber	Art/Kreuzung	Saatstärke (kg/ha)	Reihenabstand (cm)
4	Mithril	Andreae-Saaten	*Sorghum sudanense* x *S. bicolor*	20,0	37,5
5	Sucrosorgo 506	Syngenta	*Sorghum bicolor*	8,0	37,5
6	Energiemischung	Andreae-Saaten	*S. bicolor* x *S. sud.* + *S. bicolor*	18,0	37,5
7	Sorghum spezial	Andreae-Saaten	diverse *Sorghum*-Arten	17,0	37,5
8	Branco	KWS	*Sorghum bicolor*	8,0	37,5
9	Inka	KWS	*Sorghum sudanense* x *S. bicolor*	20,0	37,5

2.2.4 Getreide-Ganzpflanzensilage (GPS)

Für die GPS wurden drei Arten ausgewählt, die sich in vorangegangenen Versuchen als besonders massewüchsig herausgestellt haben. Dazu zählten Winterweizen (WW), Wintertriticale (WTRI) und Winterroggen (WR). Es wurden von jeder Art zwei Sorten getestet (Tab. 6). Die GPS-Erträge wurden nur zweijährig getestet, da schon ausreichend regionales Datenmaterial von dieser Kultur vorhanden war. In 2010 wurde die Wintertriticale-Sorte „Trisol“ als Versuchsglied 4 getestet, da die ursprünglich geplante Sorte kurzfristig als Saatgut nicht zur Verfügung stand. In 2011 wurde „Trisol“ gegen die bewährte Sorte „Massimo“ ausgetauscht.

Tab. 6 Übersicht der verschiedenen Getreide-GPS-Sorten und –Arten.

Tab. 6 *Cereal GPS varieties and species.*

Vgl	Sorte	Züchter/Sorteninhaber	Saatmenge Körner/m²
Winterroggen			
1	Visello	LOCH	270
2	Balistic	LOCH	270
Triticale			
3	Benetto	DNKO	330
4	Trisol (2010)	LOCH	330
4	Massimo (2011)	HEGE	330
Winterweizen			
5	Akratos	STRU	330
6	Inspiration	BRGD	330

2.3 Aussaat und Ernte

Die Aussaat- und Erntetermine der verschiedenen Kulturen sind in den Tab. 7 und Tab. 8 aufgelistet. Die mehrjährigen Kleegräser wurden zu Projektbeginn Anfang Mai 2009 gesät. Die überjährigen Varianten 6-8 wurden nach guter fachlicher Praxis in 2011 umgebrochen und neu angesät.

Für den Zeitpunkt des ersten Schnittes der Gräser-Mischungen gab es zwei Vorgaben: Die vielschnittigen Varianten sollten zu Beginn Ähren- bzw. Rispenschieben (BBCH 51), die extensiv gehaltenen Varianten zum Ende Ähren- bzw. Rispenschieben (BBCH 59) geerntet werden. Die Folgeschnitte wurden diesem Schnittregime angeglichen.

Die Aussaat des Getreide-GPS fand 2009 Ende Oktober statt, in 2010 Anfang Oktober. Der Zeitpunkt der Ernte wurde für die GPS-Varianten zur Mitte der Milchreife (BBCH 73-75) festgesetzt.

Die Saat des Silomais fand in den Versuchsjahren immer um den 20. April statt. Der geeignete Trockensubstanzgehalt (TS-Gehalt) war für die Ernte bestimmend und daher fand diese gegen Ende September oder Anfang Oktober statt.

Als wärmeliebende Kultur wurden die *Sorghum*-Arten mit Anfang Juni spät gesät. Dieser Saatzeitpunkt sollte auch auf eine mögliche Kombination mit der Vorkultur Getreide-GPS abgestimmt sein. Für *Sorghum* wird eine Vegetationsdauer von 120-135 Tagen vorgegeben, bis ein akzeptabler TS-Gehalt erreicht wird (LfULG, 22.01.2013). Je nach Witterung fand die Ernte am Anfang oder Ende Oktober statt, wobei der genaue Zeitpunkt durch die Witterung und die Verfügbarkeit der Erntemaschinen festgelegt wurde.

Tab. 7 Aussaattermine der verschiedenen Kulturen.

Tab. 7 *Sowing dates for the crops.*

Kultur	Datum Aussaat		
	2009	**2010**	**2011**
Kleegräser	08.05.09	-	14.03.11*
Silomais	21.04.09	19.04.10	19.04.11
Sudangräser/Hirsen	03.06.09	10.06.10	31.05.11 03.06.11
GPS	-	22.10.09	04.10.10

* Neuansaat überjährige Mischungen

Tab. 8 Erntetermine der verschiedenen Kulturen.

***Tab. 8** Harvest dates for the crops.*

		Datum Ernte Kleegräser														
		2009					2010					2011				
	Inten-sität	Schnitt					Schnitt					Schnitt				
Kultur		I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Klee-gräser	intensiv	01.07.	19.08.	29.10.	-	-	17.05.	23.06.	19.07.	07.09.	28.10.	09.05.	14.06.	13.07.	23.08.	12.10.
	extensiv	01.07.	19.08.	05.11.	-	-	28.05.	21.07.	07.09.	28.10.	-	30.05.	13.07.	23.08.	12.10.	-
Silomais		27.09.09					15.10.10					21.09.11				
Sudan-gräser Hirsen		09.10.09					20.10.10					03.10.11				
GPS		kein Anbau					01.06.10					27.06.11				

2.4 Versuchsbeschreibung

Bei den Versuchsanlagen handelte es sich um randomisierte Blockanlagen mit vierfacher Wiederholung. Die Parzellengröße betrug für alle Kulturen 10 m², die Anlagen wurden 1,5 m breit und 6,7 m lang ausgesät und waren von den Abmessungen auf die Sä- und Erntetechnik abgestimmt. Als Besonderheit galten innerhalb der Gräser-Mischungen die extensiv geführten Varianten, die als Extrablock an die Versuchsanlage angehängt werden mussten, da sie ein anderes Erntemanagement erforderten (Abb. 2). Mit den „überjährigen Mischungen" wurde ebenso verfahren.

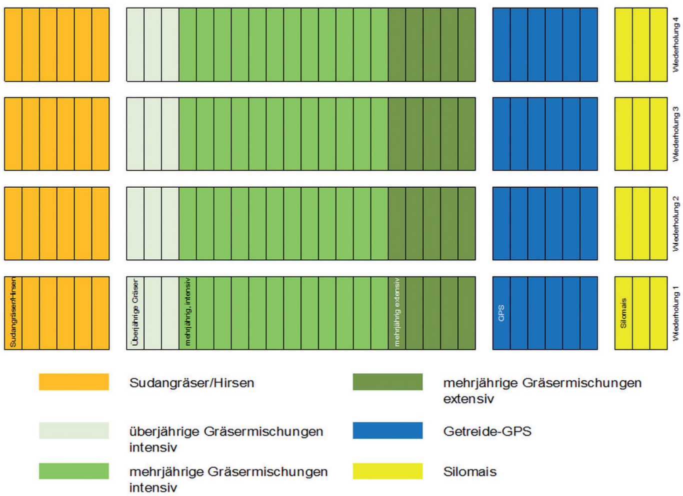

Abb. 2 Skizze des Anlageplans: Randomisierte Blockanlage am Beispiel des Standortes Egglfing, 2010.

***Fig. 2** Layout of the field design: randomized block design of the field site Egglefing, 2010.*

Bis auf die mehrjährigen Kleegräser rotierten alle anderen Kulturen im Anbau in den einzelnen Versuchsjahren.

2.5 Sä- und Erntetechnik

Das Saatgut der einzelnen Kulturen wurde mit den HLS-eigenen Parzellensämaschinen ausgebracht.

Die Gräser- und Kleegrasmischungen wurden mit einem Grüngut-Parzellenvollernter Haldrup GPE 100 geerntet. Der Silomais, die *Sorghum*-Arten und die GPS-Varianten wurden mit einem Reihenunabhängigen frontgetriebenen Mähvorsatz (Firma Kemper) geerntet und das Gut gleichzeitig mit einem integrierten Parzellenhäcksler Modell Hege 214 auf eine Länge von ca. 1 cm zerkleinert.

2.6 Witterung

Die Temperaturen der Versuchsjahre 2009 und 2011 waren im Jahresdurchschnitt 18 % bzw. 19 % höher als das vieljährige Mittel des Deutschen Wetterdienstes von 1961-1990. In 2010 lagen die Temperaturen mit ca. 3 % nur knapp über dem langjährigen Mittelwert (Tab. 9).

Die Jahresniederschläge waren aber in allen drei Versuchsjahren geringer als im vieljährigen Mittel. In 2009 wurden ca. 11 % weniger Niederschläge gemessen als im Durchschnitt. Die Jahre 2010 und 2011 fielen mit 22 % und 30 % weniger Niederschlägen trocken aus.

Nach der Kleegrassaat im Mai 2009 kam es immer wieder zu Regenereignissen (Abb. 3), was die frisch bearbeitete Bodenoberfläche am Standort Rotthalmünster verschlämmen ließ. Das führte dazu, dass anfangs kein Auflaufen der Mischungspartner Leguminosen zu erkennen war und für diese Arten eine Nachsaat drohte. Die Samen liefen letztendlich verzögert auf und etablierten sich an allen drei Standorten sehr zufriedenstellend.

Die hohen Niederschlagsmengen und niedrigeren Temperaturen Anfang Juni bedeuteten anfangs für das Auflaufen des wärmeliebenden *Sorghum*-Saatgutes eine Herausforderung, es gab aber im weiteren Verlauf keine nachteiligen Auswirkungen auf das Wachstum und die Entwicklung bis zur Ernte. Die Ernte fand unter widrigen Bedingungen statt und musste in den längeren Regenpausen durchgeführt werden. Flurschäden konnten aber durch vorsichtige Handhabung der Erntemaschine vermieden werden.

Der Silomais konnte wegen der günstigen Witterung zum ortsüblichen Termin Ende April gesät werden und entwickelte sich ohne Probleme. Die Getreide-GPS-Varianten wurden Ende Oktober gedrillt.

2010 gab es im Februar bis April eine ausgeprägte Frühjahrstrockenheit, die eine leichte Wachstumsverzögerung der Kleegräser zur Folge hatte. Das wurde nach der Mais-Saat Ende April durch reichliche Niederschläge im Mai wieder wettgemacht und die Vegetation holte dann auf, was vorher infolge von Wasserknappheit an Biomassenzuwachs fehlte.

Reichliche Niederschläge gab es im Juni und besonders im Juli, wobei es sich oft um Starkregenereignisse handelte.

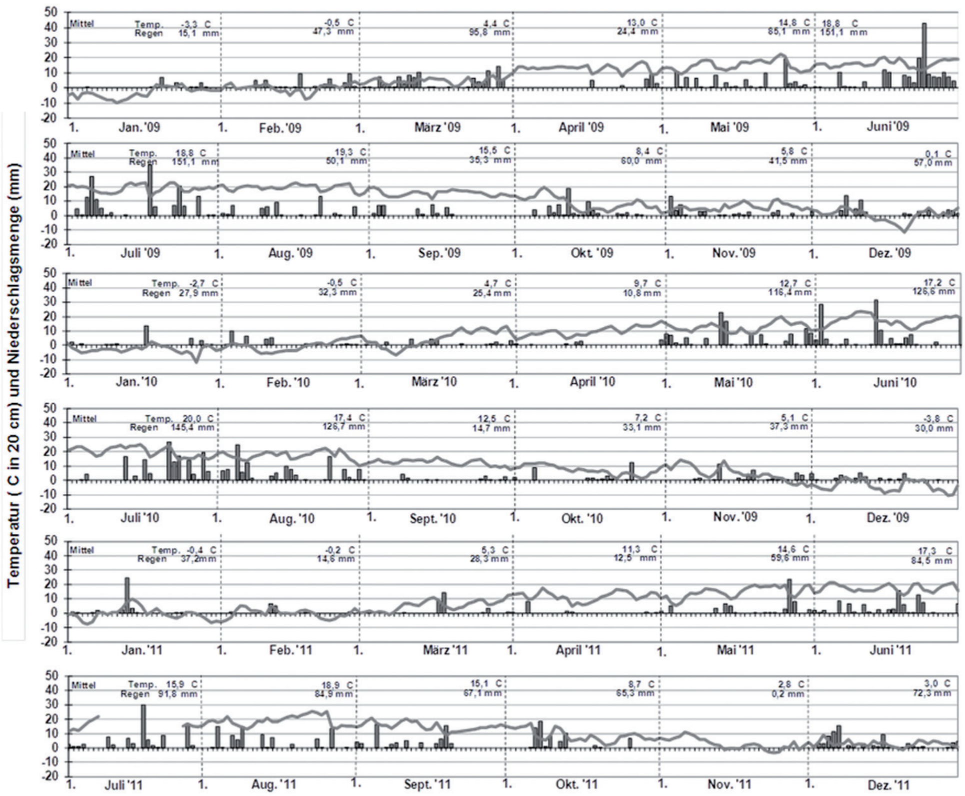

Abb. 3 Darstellung der Temperatur und Niederschläge über den Versuchszeitraum 2009 bis 2011, Aufzeichnungen der Wetterstation 128 in Rotthalmünster.

Fig. 3 *Temperature and rainfall over the experimental period from 2009 to 2011, recordings of the weather station 128 in Rotthalmünster.*

Der August 2010 war ebenfalls verregnet. In diesen feuchten Sommermonaten stagnierten die Kulturen von *Sorghum* und Silomais im Wachstum etwas im Vergleich zu den vorangegangenen Jahren, konnten aber im regenarmen September noch stark an Biomasse zulegen. Die Zeit von Oktober bis Dezember fiel trockener aus als im Vorjahr.

Das Versuchsjahr 2011 startete wie 2010 mit einer ungewöhnlich ausgeprägten Frühjahrstrockenheit, nur dass diese bis Juni anhielt, es fielen bis dahin nur ca. die Hälfte der Niederschläge wie z.B. in 2009. Die frisch angesäten überjährigen Kleegrasmischungen litten besonders unter der Trockenheit. Erst ab Juni gab es immer wieder Niederschläge und die Kulturen konnten sich erholen. Die Sommer- und Herbstmonate waren gekennzeichnet durch Regenfälle, die aber unterdurchschnittliche Niederschlagsmengen im Vergleich zu den Vorjahren brachten. Insgesamt hatte 2011 ein Drittel weniger Niederschläge als das vieljährige Mittel (Tab. 9) und diese verteilten sich hauptsächlich auf die Monate Juni bis Oktober. Eine Darstellung der Monatsmittelwerte von Temperatur und Niederschlägen über die drei Versuchsjahre ist in Abb. 4 dargestellt.

Tab. 9 Darstellung der langjährigen Temperatur- und Niederschlagsmittelwerte der Wetterstation Passau.

***Tab. 9** Long-term mean temperature and rainfall of the weather station Passau.*

Jahr	Temp (2m) Ø	Langj. Temp. (8,0 °C) (Abweichung abs.)	Langj. Temp. (8,0 °C) (Abweichung rel.)	Niederschlag Σ	Langj. Niederschlag (936,6 mm[1)]) (Abweichung abs.)	Langj. Niederschlag (936,6 mm[1)]) (Abweichung rel.)
	[°C]	[K]	[%]	[mm]	[mm]	[%]
2009	9,5	1,4	17,5	829,7	-106,6	-11,4
2010	8,3	0,3	3,8	730,0	-206,6	-22,1
2011	9,5	1,5	18,8	658,2	-278,4	-29,7
Ø	**9,1**	**1,1**	**13,4**	**739,3**	**-197,2**	**-21,1**

[1)] Vieljähriges Mittel DWD (von 1961 bis 1990): Passau-Oberhaus (WST)

Quelle: http://www.am.rlp.de/Internet/AM/NotesBAM.nsf/bamweb/71560a9bf1629eb8c1257393002e83a7? OpenDocument&TableRow=3.1.2%2C3.5#3.1. aus Agrarmeteorologie LfL vom 23.01.13

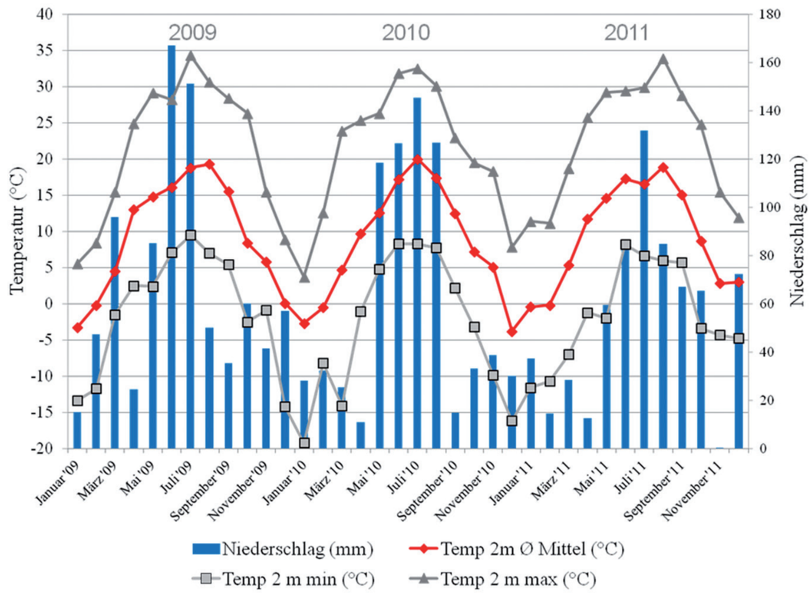

Abb. 4 Übersicht der Monatsmittelwerte von Temperatur und Niederschlag 2009-2011.

***Fig. 4** Monthly mean temperature and rainfall, 2009 to 2011.*

2.7 Pflanzenschutzmaßnahmen

Die Kleegrasmischungen wurden keinem chemischen Pflanzenschutz unterzogen. Damit die Versuchsparzellen keinen Schaden durch Wildverbiss erlitten, wurde um die Versuchsanlage die Bayerische Qualitätssaatgut-Wiesenmischung „D 2" gesät, um den Umgriff attraktiv für das Wild zu gestalten. Zur olfaktorischen Abschreckung des Wildes wurden in 2010 zusätzlich WC-Steine in Kirchham und Egglfing um die Versuchsanlagen auf Pflöcken montiert.

In Egglfing bestand ein massives Problem durch große Wühlmaus- oder Maulwurfhügel in der Gräser-Versuchsanlage. Diesen war nur durch mechanische Beseitigung und vorsichtiges Abtragen beizukommen. Allerdings waren die Hügel so zahlreich in der Versuchsanlage vorhanden, dass diese schon im ersten Jahr stark in Mitleidenschaft gezogen wurde. Grabaktivitäten durch Füchse oder Hunde schädigten zudem einzelne Parzellen. Ein umsichtiges Führen des Parzellenvollernters war notwendig, um das Erntegut so wenig wie möglich durch mineralische Bestandteile des Bodens zu verunreinigen. Einer massiven Verunkrautung durch das kleinblütige Franzosenkraut (*Galinsoga parviflora)* konnte durch Schröpfschnitte erfolgreich entgegen getreten werden.

Eine große Attraktivität schien die junge GPS-Kultur auf das Wild an den Waldstandorten Kirchham und Egglfing auszuüben. Dort wurde im Frühjahr 2010 starker Verbiss festgestellt, dem nur durch Umzäunung mit Hühnerschutznetzen entgegenzutreten war. Diese Maßnahme wurde auch 2011 beibehalten. Die Kulturen wurden jeweils im Frühjahr mit Halmverkürzern behandelt. Bei Winterweizen trat 2011 massiv *Fusarium* spp. an den Ähren auf.

Bei Silomais und *Sorghum*-Arten fanden handelsübliche Fungizide ihre Anwendung, wobei der Krankheitsdruck gering ausfiel. Auch hielt sich der Unkrautdruck bei beiden Kulturen in Grenzen, so dass keine Behandlung erfolgen musste. Als Hauptunkraut waren Hühner- und Borstenhirse zu verzeichnen.

2.8 Düngung

Zu Versuchsbeginn wurden an den Standorten Bodenproben gezogen und auf die Inhaltstoffe P_2O_5, K_2O und dem pH-Wert untersucht. Die Düngung wurde dementsprechend auf den Nährstoffentzug der einzelnen Kulturen angepasst und ausgebracht (Tab 10).

Tab. 10 Übersicht über die Bodenverhältnisse zu Versuchsbeginn und die Düngung an den Standorten.

***Tab. 10** Soil parameters at the beginning of the experiment and fertilization at the field sites.*

Versuchsort Landkreis	**Boden**		**Acker**	**Bodenuntersuchung ,09 (mg/100g Boden)**		**Vorfrucht**	**Bemerkung**	**Düngung kg/ha (rein)**				
	Art	Zahl	Zahl	P_2O_5 K_2O MgO	pH-Wert			N	P_2O_5	K_2O	MgO	S
										Kleegräser		
Rotthalmünster	sL4D	70	68	14	6,7	Mais	Versuchsbeginn		70	140		
				12								
PA				o.A.		ZF Senf	währ. Versuchszeit		70	140		
							nach jedem Schnitt	40				
		Parabraunerde aus diluvialem Löss								Mais		
							Versuchsbeginn		25	50		
							währ. Versuchszeit	150	25			55
										Sudangräser		
							währ. Versuchszeit	130	70	140		
										Kleegräser		
Kirchham	SL6Alg	36	36	54	6,7	Mais	Versuchsbeginn		120	240		
				27								
PA		Braunerde		o.A			währ. Versuchszeit		120	240		
							nach jedem Schnitt	40				
										Mais		
							Versuchsbeginn					
							währ. Versuchszeit	140				
										Sudangräser		
							währ. Versuchszeit	130	70	140		
										Kleegräser		
Egglfing	sL4Al	60	59	23	7,2	Luzerne-	Versuchsbeginn		120	240		
				7								
PA		Aueboden		o.A.		Kleegras	währ. Versuchszeit		120	240		
							nach jedem Schnitt	40				
										Mais		
							Versuchsbeginn					
							währ. Versuchszeit	140				
										Sudangräser		
							währ. Versuchszeit	130	70	140		

2.9 Statistische Auswertung

Für die statistischen Analysen wurde das Programm SAS 9.2 (SAS Institute, Cary, NC, USA) genutzt. Die Varianzanalysen erfolgten unter Zuhilfenahme der GLM Procedure. Als Post-Hoc-Test wurde der Student-Newman-Keuls-Test (SNK) angewandt.

2.10 Berechnung der Energiedichte

Die Energieberechnungen des Silomais konnten mit Hilfe der Ergebnisse der Nah-Infrarot-Spektroskopie-Verfahrens (NIRS) durchgeführt werden.

Alle anderen Kulturen wurden im Labor der LfL auf Inhaltstoffe untersucht und die Berechnungen zu der Energiedichte wie unten beschrieben durchgeführt.

Zur Berechnung der Umsetzbaren Energie (ME) wurde die Formel von Hoffmann *et al.* (1971) herangezogen und für die Nettoenergie Laktation (NEL) die Formel von van Es, (1978) (Kirchgeßner, 1992, DLG-Futterwerttabellen, 1997), da aufgrund fehlender Parameter in den Laborwerten wie z.B. der Gasbildungswerte eine andere Formel nicht angewandt werden konnte:

$$ME\ (MJ) = 0{,}0312 * DXL + 0{,}0136 * DXF + 0{,}0147 * NfE + 0{,}00234 * DXP$$

$$NEL\ (MJ) = 0{,}6 * [1 + 0{,}004 * (q - 57)] * ME;\ q\ (\%) = ME / GE * 100$$

DXL = verdauliches Rohfett (g)

DXF = verdauliche Rohfaser (g)

NfE = N-freie Extraktstoffe (g)

DXP = verdauliches Rohprotein (g)

GE = Bruttoenergie

Die Berechnung der Energiedichte der Futtergräser und von deren Gemenge stellte sich als Herausforderung dar, da die verschiedenen Mischungen zum einen unterschiedliche Arten enthielten und zum anderen der Klee- und Luzerneanteil innerhalb der Mischungen stark variierte. Ein weiterer zu beachtender Punkt war die Veränderung des Pflanzenbestandes über die Jahre bei dieser mehrjährigen Kultur, wie z.B. durch Auswinterung, tierische Schädlinge und Bodeneinflüsse. Bei der Anwendung *einer* Formel zur Berechnung für „Kleegras" wäre der Energiegehalt der Mischungen homogen berechnet worden, ungeachtet der unterschiedlich vorhandenen Prozentanteile von Leguminosen und Gräserarten. Die Ergebnisse der Einzelmischungen wären nicht auf die tatsächlichen Bestände angepasst und würden eine verfälschte Energiedichte widergeben. Die mehrjährigen Kleegraskulturen zeigten über die drei Versuchsjahre eine starke Veränderung in der Pflanzenzusammensetzung durch die Bodeneinflüsse (Verringern des Leguminosenanteils), die an einzelnen Standorte so gravierend war, dass sie bei der Berechnung zu berücksichtigen war, um das Ergebnis der Energiedichte nicht falsch abzuschätzen.

Zur Berechnung der NEL-Werte wurden daher die Ertragsanteilschätzungen der einzelnen Arten herangezogen, die ab 2010 jeweils zum ersten, dritten und letzten Schnitt für die Kleegraskulturen durchgeführt wurden. Damit konnten die aktuellen Anteile der Mischungspartner mit einbezogen werden und die Veränderung des Pflanzenbestandes innerhalb der Mischungen über die Jahre und an den einzelnen Standorten in der Berechnung berücksichtigt werden. Für die Ergebnisse von 2009 wurde die *erste* Ertragsanteilschätzung von 2010 verwendet.

Für die Futterwertberechnungen gibt es angepasste Formeln, wie z.B. die Kleegrasformel zur Berechnung der NEL. Da stellte sich das Problem der Definition, ab wie viel Prozentanteile Klee wird eine Mischung als „Kleegras" bezeichnet. Als „Kleegras" wurde dann eine Futtergrasmischung angesehen, die 10 % und mehr Kleeanteil enthielt (persönliche Mitteilung, Hartmann). Lagen die Prozentanteile darunter, wurde die Berechnung, wie im folgenden Beispiel beschrieben, durchgeführt:

Kleegras-Mischung mit einem Artenanteil von 8 % RKL und 95 % WSC

8 % RKL + 8 % WSC (entspricht 16 %) Anwendung Kleegrasformel

84 % WSC Anwendung WSC-Formel

Zur Berechnung der Gesamt-NEL wurde das *gewogene Mittel* verwendet, d.h.

Summe NEL-Endergebnis = Kleegrasergebniss * 0,16 + WSC-Ergebnis * 0,84

3. Ergebnisse und Diskussion

3.1 Trockenmasse-Erträge der Futtergräser und deren Gemenge

Die Aussaat der Futtergräser und deren Gemenge wurde Anfang Mai 2009 durchgeführt. Die Witterungsbedingungen nach der Saat waren durch reichliche Niederschläge gekennzeichnet und führten auf den frisch bestellten Versuchsflächen zu einer Oberflächenverschlämmung. Das hatte zur Folge, dass zwar die Grasmischungspartner sehr gut, die Leguminosen aber erst sehr verspätet und zögerlich aufliefen, so dass eine Nachsaat derselben drohte. In der darauffolgenden Zeit aber konnten sich die Leguminosen an allen Standorten sehr gut etablieren. Die Kleegrasmischungen wurden unter die Deckfrucht Hafer gesät, um der jungen Kultur zum einen Schutz vor Hitze und Austrocknung zu bieten (Anonymus, 2010), und um zum anderen im ertragsschwächeren Ansaatjahr dennoch einen passablen Ertrag zu erzielen (Gujer, 1997).

Tabelle 10 zeigt die Trockenmasse-Erträge der drei Standorte und Jahre. Farblich gekennzeichnet ist die Eingruppierung der Erträge in sogenannte Quartile, die die Verteilung der Stichprobe in vier Viertel teilen (Lohninger, 2012). Das erste und niedrigste Quartil ist weiß gekennzeichnet, das zweite hellgrau mit schwarzer Schrift, das dritte mittelgrau mit weißer Schrift und das vierte mit den höchsten Erträgen dunkelgrau.

Am ertragstärksten zeigten sich in 2009 bei den *intensiv* geschnittenen Varianten die Weidelgras-betonten Mischungen. Von den drei Standorten lieferte Kirchham den höchsten durchschnittlichen Trockenmasseertrag mit 103,4 dt/ha (Tab. 11), gefolgt von Rotthalmünster (88,5 dt/ha) und schließlich Egglfing (87,3 dt/ha). Die Weidelgras-betonten Mischungen „A 3", „A 3 mit Rotklee" und „FE 3-K" waren über alle Standorte am ertragreichsten. Ebenso stark waren die Mischungen „Mehrjähriges Kleegras" in Egglfing, „Country 2052" in Kirchham und „Agravit 040R" in Rotthalmünster. Die Mischungen „FM 3-K", „FM 4" und „MG8 Standard" waren an den meisten Standorten vom Ertrag schwach. „FM 4-K", „FE 1" und „Landgreen kg 550" lagen an den einzelnen Versuchsorten in verschiedenen Quartilen und zeigten eine Spreizung von gering über mittel bis zum hohen Ertragsniveau. Die restlichen Mischungen zeigten mittlere Erträge.

Der Standort mit dem höchsten Ertrag war bei den *extensiven* Schnittvarianten Kirchham (100,2 dt/ha), gefolgt von Rotthalmünster (87,1 dt/ha) und Egglfing (57,4 dt/ha). Auch bei diesem extensiven Schnittregime zeigten sich die „A 3" und „A 3 mit Rotklee" am ertragreichsten. Schwach fiel dagegen „FM 4" in Egglfing und Kirchham aus, diese Mischung war aber in Rotthalmünster sehr stark. Die Werte der restlichen Mischungen bewegten sich im Mittelfeld.

Im zweiten Versuchsjahr 2010, dem sogenannten ersten Hauptnutzungsjahr, zeigte sich vom Durchschnittsertrag ein gänzlich anderes Bild. Obwohl die Futtergräser nach der Ansaat an allen Standorten von den Mischungspartnern ein einheitliches Bild erkennen ließen, differenzierte sich die Zusammensetzung der Bestände schon stark bis Ende 2010 durch Bodeneinflüsse, wie z.B. stark mineralisierende Böden. Am Standort Egglfing verminderte sich bis zum vierten Schnitt der Leguminosenanteil auf nahezu Null Prozent, dort zeigte sich aber ein unglaublicher Massewuchs der Gräser, der Durchschnittsertrag der *intensiv* geschnittenen Varianten war mit 155,2 dt/ha am höchsten.

Rotthalmünster wies den zweithöchsten Durchschnittsertrag mit 154,3 dt/ha auf. In Kirchham war durch den leichten Boden ein Rückgang der Leguminosen zu erkennen, die Bestände waren nicht so dicht und hoch wie an den anderen beide Standorten und das wirkte sich negativ im Ertrag aus (147,8 dt/ha).

Auch in 2010 waren bei den *intensiv* geschnittenen Varianten die „A 3" und „A 3 mit Rotklee" Varianten über alle Standorte die ertragreichsten. Die Mischung „Country 2052" steigerte sich an zwei Standorten im Vergleich zu 2009. Auffällig war die Zunahme der Erträge von „FM 3-K" und „FM 4" über alle Versuchsorte im Vergleich zum Vorjahr. Der starke Rückgang des Leguminosenanteils in Egglfing machte sich bei den kleebetonten Mischungen wie Mehrjähriges Kleegras oder Intensivmischung Kleegras im Ertrag bemerkbar. Die übrigen Mischungen wiesen eine starke Spreizung im Ertrag auf, d.h. an einem Standort waren sie ertragsreich, am anderen –schwach.

Die extensiven Varianten lagen vom Ertrag leicht unter dem Niveau der intensiven. Egglfing führte die Rangfolge mit einem Durchschnittsertrag von 141,9 dt/ha an, gefolgt von Rotthalmünster mit 136,8 dt/ha und schließlich Kirchham mit 128,4 dt/ha. Auch in der Quantität erzeugenden Variante waren die „A 3 mit Rotklee" an allen Standorten am ertragreichsten. Die Mischung „A 3" war in Egglfing und Kirchham ertragstark, in Rotthalmünster aber –schwach. Verglichen zum Vorjahr hatte die Mischung „FM 3-K" an allen Standorten stark an Ertrag zugelegt.

Das letzte Versuchsjahr 2011 war für den Großteil der Mischungen das zweite Hauptnutzungsjahr. Drei Varianten („FE 1", „FE 3-K" und „FM 2") wurden neu angesät, da es sich um überjährige Mischungen handelt. Die ausgeprägte Frühjahrstrockenheit machte den Beständen und vor allem den Neuansaaten stark zu schaffen, zum ersten Schnitt der mehrjährigen Kulturen waren die Überjährigen noch nicht schnittreif. Daher ergab sich ein Ausfall von einem Schnitt, was sich auch im Ertrag auswirkte und in Tabelle 10 grau dargestellt ist. Die Durchschnittserträge wurden nur von den mehrjährigen Kulturen miteinander verglichen, die Überjährigen sind extra erfasst und dargestellt.

Auch im zweiten Hauptnutzungsjahr war das Verhältnis Leguminosen/Gräser in Rotthalmünster sehr ausgewogen und fast ebenso hoch wie zu Versuchsbeginn. Die Bestände waren sehr dicht, hoch und kaum durch Unkräuter beeinflusst. Egglfing war bis zum Ende der Vegetation nur noch ein reiner Grasbestand mit enormer Wüchsigkeit, und in Kirchham waren die Bestände vom Wuchs sehr niedrig und dünn, was sich letztendlich in einem niedrigen Ertrag widerspiegelte.

Für den Großteil der Mischungen, war bei den *intensiv* geschnittenen Varianten Egglfing der ertragsstärkste Standort mit 156,6 dt/ha, gefolgt von Rotthalmünster (152,9 dt/ha) und Kirchham (111,6 dt/ha). Die Neuansaaten wurden 2011 nicht unter die Deckfrucht Hafer gesät, erreichten aber dennoch ein höheres Ertragsniveau als zu Versuchsbeginn in 2009, mit Ausnahme von Kirchham. Aufgrund der geringen Krumendicke und des wasserdurchlässigen Bodens war 2011 besonders Kirchham vom Frühjahrstrockenstress betroffen. Selbst trockenheitstolerante Arten wie das Knaulgras zeigten eingerollte Blätter oder vertrocknete Blattspitzen.

Am ertragsstärksten war die Mischung „FM 3-K" an allen drei Standorten. Diese etablierte sich über die Jahre sehr gut und steigerte kontinuierlich von Versuchsbeginn bis –ende zunehmend den Ertrag. „FM 4K" und „Landgreen kg 550" zeigten sich über alle Standorte ertragsstabil. Stark abnehmend und variierend waren zu Versuchsende „A 3" und „A 3 mit Rotklee" und die restlichen Mischungen.

Die *extensiv* geschnittenen Varianten lagen in Rotthalmünster mit 160,5 dt/ha an erster Stelle, gefolgt von Egglfing (130,8 dt/ha) und Kirchham (99,5 dt/ha). Auch in diesen Varianten war die „FM 3-K" die ertragsstärkste Mischung an allen drei Standorten, gefolgt von „FM 4" und „FM 4-K". Auch hier zeigten „A 3" und „A 3 mit Rotklee" den schwächsten Ertrag.

Tab. 11 Darstellung der mittleren Trockenmasse-Erträge (dt/ha) und die Eingruppierung in die erste bis vierte Quartile über die Einzelstandorte und Jahre.

Tab. 11 *Mean dry matter yield (dt/ha) and grouping into the first to fourth quartile over the individual sites and years.*

Variante	Mischung	Gesamt-TM (dt/ha)							
		2009						alle Standorte 2009	
		Egglfing		Kirchham		Rotthalmünster		Ø	Ø
		ext.	int.	ext.	int.	ext.	int.	ext.	int.
1	FM 3-K	52,9	70,5	95,2	94,1	80,5	81,0	76,2	81,9
2	FM 4-K	49,5	82,7	93,1	96,4	83,8	82,9	75,5	87,3
3	FM 4	49,4	76,2	78,1	93,2	84,4	88,3	70,6	85,9
4	A3	70,3	110,4	117,2	112,2	90,5	92,8	92,7	105,1
5	A3+Klee	64,9	99,6	117,5	122,7	96,1	96,1	92,8	106,1
6	FE 1 (überjährig)	-	71,1	-	98,4	-	90,6	-	86,7
7	FE 3-K (überjährig)	-	104,4	-	119,9	-	97,0	-	107,1
8	FM 2 (überjährig)	-	78,6	-	92,5	-	88,2	-	86,4
9	Agravit 040R	-	93,7	-	103,0	-	93,3	-	96,7
10	MG8 Standard	-	76,1	-	96,3	-	84,6	-	85,7
11	Country 2052	-	93,3	-	112,1	-	90,2	-	98,6
12	Landgreen kg550	-	79,2	-	99,2	-	81,3	-	86,6
13	Intensivmischung Kleegras	-	79,2	-	98,6	-	85,2	-	87,7
14	Tetrafix intensiv m. Klee	-	95,9	-	104,4	-	90,8	-	97,1
15	Mehrj. Kleegr.m. Luz. HQ	-	99,0	-	108,3	-	85,9	-	97,7
Ø TM (dt/ha) alle		57,4	87,3	100,2	103,4	87,1	88,5	81,6	93,1
Ø TM (dt/ha) nur Überjährige			84,7		103,6		91,9		93,4

1. Quartil	2. Quartil	3. Quartil	4. Quartil

Variante	Mischung	Gesamt-TM (dt/ha)							
		2010						alle Standorte 2010	
		Egglfing		Kirchham		Rotthalmünster		Ø	Ø
		ext.	int.	ext.	int.	ext.	int.	ext.	int.
1	FM 3-K	140,0	151,4	129,0	151,1	142,2	157,5	137,1	153,3
2	FM 4-K	125,0	146,6	127,0	143,7	127,8	161,0	126,6	150,4
3	FM 4	129,1	145,1	115,7	147,9	133,5	163,6	126,1	152,2
4	A3	156,2	174,3	129,2	138,6	104,3	118,7	129,9	143,9
5	A3+Klee	159,4	179,8	140,9	152,6	176,2	191,9	158,8	174,8
6	FE 1 (überjährig)	-	144,1	-	135,1	-	172,6	-	150,6
7	FE 3-K (überjährig)	-	153,3	-	151,9	-	152,4	-	152,5
8	FM 2 (überjährig)	-	169,6	-	143,3	-	119,4	-	144,1
9	Agravit 040R	-	147,0	-	141,1	-	156,1	-	148,1
10	MG8 Standard	-	148,5	-	148,6	-	170,6	-	155,9
11	Country 2052	-	162,1	-	152,5	-	182,0	-	165,5
12	Landgreen kg550	-	168,0	-	148,2	-	145,8	-	154,0
13	Intensivmischung Kleegras	-	140,1	-	154,4	-	148,4	-	147,6
14	Tetrafix intensiv m. Klee	-	158,2	-	151,1	-	117,1	-	142,2
15	Mehrj. Kleegr.m. Luz. HQ	-	139,3	-	156,8	-	157,8	-	151,3
Ø TM (dt/ha) alle		141,9	155,2	128,4	147,8	136,8	154,3	135,7	152,4
Ø TM (dt/ha) nur Überjährige			155,7		143,4		148,1		149,1

1. Quartil	2. Quartil	3. Quartil	4. Quartil

Variante	Mischung	Gesamt-TM (dt/ha)							
		2011						alle Standorte 2011	
		Egglfing		Kirchham		Rotthalmünster		Ø	Ø
		ext.	int.	ext.	int.	ext.	int.	ext.	int.
1	FM 3-K	136,3	161,1	121,9	132,7	175,5	165,1	144,5	152,9
2	FM 4-K	133,6	156,2	117,6	115,6	153,2	153,9	134,8	141,9
3	FM 4	135,2	156,2	119,3	113,5	157,0	153,7	137,2	141,1
4	A3	125,0	156,6	71,8	109,8	146,7	133,3	114,5	133,3
5	A3+Klee	123,8	150,9	67,0	94,8	170,1	166,7	120,3	137,5
6	FE 1 (überjährig)	-	89,8	-	60,6	-	94,4	-	81,6
7	FE 3-K (überjährig)	-	114,3	-	84,6	-	117,0	-	105,3
8	FM 2 (überjährig)	-	85,3	-	64,1	-	121,0	-	90,1
9	Agravit 040R	-	136,2	-	90,3	-	152,3	-	126,3
10	MG8 Standard	-	159,7	-	122,7	-	152,6	-	145,0
11	Country 2052	-	149,2	-	109,9	-	133,1	-	130,8
12	Land-green kg550	-	184,6	-	136,5	-	153,8	-	158,3
13	Intensivmischung Kleegras	-	146,0	-	95,1	-	156,2	-	132,4
14	Tetrafix intensiv m. Klee	-	148,8	-	100,8	-	162,6	-	137,4
15	Mehrj. Kleegr.m. Luz. HQ	-	173,7	-	117,8	-	150,9	-	147,5
Ø TM (dt/ha) Mehrjährige		130,8	156,6	99,5	111,6	160,5	152,9	130,3	130,8
Ø TM (dt/ha) Überjährige			96,5		69,8		110,8		92,4

1. Quartil	2. Quartil	3. Quartil	4. Quartil

Tab. 12 Ergebnisse des Post-Hoc-Tests (SNK, α = 0,05) der Futtergräser/Gemenge beim Vergleich der Gesamt-TM-Erträge.

Tab. 12 *Results of the Post Hoc tests (SNK, α=0.05) for fodder grasses/mixtures from the comparison of the total dry matter yields.*

Vari-ante	Mischung	2009													
		Egglfing int.					Kirchham int.				Rotthalmünster int.			
1	FM 3-K				d	e	a			d	a			d
2	FM 4-K			c	d	e			c	d			c	d
3	FM 4				d	e				d	a	b	c	d
4	A3	a					a	b			a	b	c	d
5	A3+Klee	a	b				a				a	b		
6	FE 1 (überjährig)			c	d	e			c	d	a	b	c	d
7	FE 3-K (überjährig)	a												
8	FM 2 (überjährig)					e				d	a	b	c	d
9	Agravit 040R		b	c	d			b	c	d	a	b	c	
10	MG8 Standard			c	d	e			c	d		b	c	d
11	Country 2052	a	b	c			a	b	c		a	b	c	d
12	Landgreen kg550		b	c	d	e			c	d				d
13	Intensiv-mischung Kleegras			c	d	e			c	d		b	c	d
14	Tetrafix intensiv m. Klee	a	b					b	c	d	a	b	c	d
15	Mehrj. Kleegr.m. Luz. HQ	a	b				a	b	c	d	a	b	c	d

SNK (α = 0,05)

Variante	Mischung	2010 Egglfing int.	2010 Kirchham int.	2010 Rotthalmünster int.
1	FM 3-K	d e	a	d
2	FM 4-K	d e	a	d
3	FM 4	d e	a	d
4	A3	a b	a	e
5	A3+Klee	a	a	a
6	FE 1 (überjährig)	d e	a	d
7	FE 3-K (überjährig)	c d e	a	e
8	FM 2 (überjährig)	d e	a	d
9	Agravit 040R	e	a	c
10	MG8 Standard	c d e	a	d
11	Country 2052	a b c	a	e
12	Landgreen kg550	c d e	a	d
13	Intensiv-mischung Kleegras	d e	a	c
14	Tetrafix intensiv m. Klee	b c d	a	b
15	Mehrj. Kleegr.m. Luz. HQ	b c d	a	d

Variante	Mischung	2011 Egglfing int.	2011 Kirchham int.	2011 Rotthalmünster int.
1	FM 3-K	a b c	a	a
2	FM 4-K	a b c	a b	a b
3	FM 4	a b c	a b	a b
4	A3	b c	c e	b
5	A3+Klee	b c	d e	a
6	FE 1 (überjährig)	d e	f	e
7	FE 3-K (überjährig)		e	d
8	FM 2 (überjährig)	e	f	c d
9	Agravit 040R	c	d	b
10	MG8 Standard	a b c	a b	b
11	Country 2052	b c	b c d	c
12	Landgreen kg550	a	a	b
13	Intensiv-mischung Kleegras	b c	d e	b
14	Tetrafix intensiv m. Klee	b c	c d e	a b
15	Mehrj. Kleegr.m. Luz. HQ	a b	a b c d	b

SNK (α = 0,05)

Die statistische Auswertung mittels SNK-Test zeigt, dass bis zu sechs homogene Untergruppen der TM-Gesamt-Erträge aller Standorte und Versuchsjahre der Futtergräser und –gemenge existieren (Tab. 12). Die meisten Varianten sind in mehreren Untergruppen zu finden. In Rotthalmünster war 2010 und 2011 eine schärfere Untergruppierung der TM-Erträge festzustellen. Auffällig war der Standort Kirchham in 2010. Dort gab es eine so geringe Spanne der TM-Erträge, dass kein signifikanter Unterschied zwischen den einzelnen Varianten zu berechnen war und somit nur eine homogene Untergruppe existierte.

3.2 Trockenmasse-Erträge der Silomais-Varianten

Da in dieser Region Silomais die meistangebaute Kultur ist, war sie für den Vergleich der Trockenmasse-Erträge die Referenzkultur zu den anderen getesteten Arten. Zu Versuchsbeginn in 2009 war Rotthalmünster der Standort mit dem höchsten Ertrag (241,3 dt/ha, Tab. 13), gefolgt von Egglfing (232,0 dt/ha) und Kirchham (226,4 dt/ha). In 2010 zeigte Egglfing den höchsten Ertrag (241,3 dt/ha), Rotthalmünster lag mit 235,0 dt/ha etwas darunter, Kirchham hatte 219,0 dt/ha. Im letzten Versuchsjahr in 2011 konnten in Rotthalmünster 231,6 dt/ha, in Kirchham 231,2 dt/ha und in Egglfing 224,0 dt/ha erzielt werden. Im letzten Versuchsjahr hatten sehr viele Maisstandorte in Bayern mit ausgeprägter Frühjahrstrockenheit zu kämpfen. Da die Ablage der Maiskörner bei der Saat an allen Standorten verhältnismäßig tief erfolgte (Geisberger, HLS, mündl. Mitteilung), gab es für die Keimlinge einen guten Bodenschluss und genügend Feuchtigkeit um zu überleben und sich optimal zu entwickeln. Die Trockensubstanzgehalte (TS-Gehalte) des Silomais lagen innerhalb der optimalen Silierfähigkeit zwischen 28-35 % TS und sind in diesem Bericht nicht eigens aufgeführt.

Die statistische Auswertung zeigt lediglich für Egglfing 2009 einen signifikanten Unterschied der Sorte „PR 39 F 58" zu den anderen an, die durch den niedrigeren Ertrag zustande kam (Tab. 13). Sonst ergaben sich für die Silomaisvarianten über die Standorte und Jahre keine signifikanten Unterschiede.

Tab. 13 Übersicht der Trockenmasse-Erträge der Silomais-Varianten von 2009 bis 2011 und der dazugehörigen statistischen Signifikanzen (Student-Newman-Keuls-Test, α = 0,05).

Tab. 13 *Dry matter yield of the silage maize variants from 2009 to 2011 and their statistic significances (Student Newman Keuls test, **α**=0.05).*

Trockenmasse-Ertrag (dt/ha)										
Sorte		**Egglfing**			**Kirchham**			**Rotthalmünster**		
	Reifegruppe	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**
Torres	S 250	243,2	249,5	211,9	227,9	221,4	232,2	240,2	229,5	243,7
ES Bombastic	S 240	232,7	234,1	229,6	222,3	228,1	231,3	234,1	233,3	222,2
PR39F58	S 260	220,1	240,4	230,7	229,1	207,4	230,1	249,5	242,3	228,8
Durchschnitt		232,0	241,3	224,0	226,4	219,0	231,2	241,3	235,0	231,6

SNK (α = 0,05)

Sorte		**Egglfing**			**Kirchham**			**Rotthalmünster**		
	Reifegruppe	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**
Torres	S 250	a	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ES Bombastic	S 240	ab	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PR39F58	S 260	b	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = nicht signifikant

3.3 Trockenmasse- und Trockensubstanz-Erträge der *Sorghum*-Arten

Die Kulturen Sudangräser und Hirsen wurden Anfang Juni 2009 in ein sehr fein bereitetes Saatbett gesät. Der Aufgang war zufriedenstellend. Da es in unseren Breitengraden für die *Sorghum*-Arten ein Problem darstellt, silierfähiges Erntegut zu erzeugen, wurde die Ernte während der Versuchszeit Anfang bis Mitte Oktober durchgeführt, also gut eine Woche später als die des Silomais, um die maximalen TS-Gehalte für die Witterung unserer Region zu erreichen. Tab. 14 gibt die Trockenmasse- und Trockensubstanzgehalte der *Sorghum*-Arten wider.

In 2009 hatten die *Sorghum*-Varianten in Rotthalmünster mit 134,6 dt/ha den höchsten, in Egglfing mit 129,4 dt/ha den mittleren und in Kirchham mit 114,4 dt/ha den niedrigsten Ertrag. Verglichen mit dem TM-Ertrag des Silomais entspricht das 55,8 %, 50,5 % bzw. 55,8 % (Tab. 18).

In 2010 waren starke Niederschläge von Juni bis August gefallen, die Bestände entwickelten sich anfangs zögerlich, konnten aber ab Ende August noch viel an Biomasse zulegen. Wahrscheinlich war Kirchham wegen der guten Wasserführung in der Zeit der hohen Niederschläge in diesem Jahr der Standort mit den höchsten Erträgen (163,9 dt/ha), da es zu keiner Staunässe kam. In Egglfing betrug der Trockenmasse-Ertrag 153,6 dt/ha und in Rotthalmünster 123,0 dt/ha. Das entspricht einem prozentualen TM-Ertrag im Vergleich zum des Silomais von 74,8 %, 63,7 % und 55,3 %.

In 2011 wurde zu optimalen Bedingungen im Juni gesät, nach der ausgeprägten Frühjahrstrockenheit gab es in diesem Monat immer wieder Niederschläge. Die durchschnittlichen Erträge lagen in Egglfing bei 153,6 dt/ha, in Kirchham und in Rotthalmünster bei 149,6 dt/ha. Das entspricht einem prozentualen TM-Ertrag verglichen zu Silomais von 68,6 %, 64,7 % und 64,6 %.

In Prozent ausgedrückt lagen die Durchschnitts-TM-Erträge der getesteten *Sorghum*-Arten zwischen 50,5 % und 74,8 % im Vergleich zum Silomais (Tab. 18). Allerdings gab es Sorten und Mischungen, die in den Einzeljahren und –standorten an den Silomaistrockenmasse-Ertrag heranreichten: In 2010 war das „Energiemischung II" mit 197,0 dt/ha in Egglfing und 221,4 dt/ha in Kirchham, sowie die Sorte „Inka" mit 212,4 dt/ha in Kirchham. Die Problematik lag an der Stabilität und Verlässlichkeit der Erträge über die Jahre. Hohe Erträge einzelner Varianten lagen im Folgejahr wieder weit unter dem vertretbaren Maß.

Werden die Durchschnittserträge über die Versuchsjahre verglichen, so zeigt sich, dass „Energiemischung II" und „Inka" die ertragsstärksten *Sorghum*-Varianten waren (159,6 dt/ha und 155,1 dt/ha). Am schwächsten zeigte sich „Branco" mit 123,6 dt/ha.

Tab. 14 Übersicht der Trockenmasse-Erträge und Trockensubstanzgehalte der *Sorghum*-Varianten von 2009 bis 2011.

Tab. 14 *Dry matter yields and content of the* Sorghum *variants from 2009 to 2011.*

Sorte/Mischung	Trockenmasse-Ertrag (dt/ha)									
	Egglfing			Kirchham			Rotthalmünster			
	2009	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	Ø
Mithril	132,0	108,5	165,3	91,0	129,3	163,0	124,7	138,9	154,3	**134,1**
Sucrosorgo 506	146,4	134,2	161,6	100,6	130,3	122,4	151,3	123,8	163,9	**137,2**
Energiemischung II	129,2	197,0	175,2	112,2	221,2	180,3	123,3	124,6	164,0	**158,6**
Sorghum spezial	146,4	154,2	157,2	127,6	169,4	158,4	127,7	121,6	148,8	**145,7**
Branco	82,9	140,2	89,8	110,7	120,8	96,4	136,7	142,2	92,4	**112,5**
Inka	139,5	187,7	172,6	144,5	212,4	177,0	143,8	86,8	174,5	**159,9**
Durchschnitt	**129,4**	**153,6**	**153,6**	**114,4**	**163,9**	**149,6**	**134,6**	**123,0**	**149,6**	**141,3**

Fortsetzung Tabelle 14

Trockensubstanz-Gehalt (%)

Sorte/Mischung	Egglfing			Kirchham			Rotthalmünster			
	2009	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**	Ø
Mithril	22,0	25,3	23,1	21,8	23,9	24,8	24,3	23,9	23,0	**23,6**
Sucrosorgo 506	20,8	22,2	22,0	19,7	20,9	22,7	23,5	25,7	22,2	**22,2**
Energiemischung II	21,2	29,3	24,7	20,9	28,7	25,8	23,2	24,0	24,8	**24,7**
Sorghum spezial	22,3	26,2	23,7	22,6	24,3	24,8	24,0	22,7	22,9	**23,7**
Branco	20,8	22,7	21,0	19,7	19,4	21,3	21,7	24,4	19,7	**21,2**
Inka	21,6	24,7	22,8	22,5	23,4	24,1	22,4	23,9	23,0	**23,1**
Durchschnitt	**21,5**	**25,0**	**22,9**	**21,2**	**23,4**	**23,9**	**23,2**	**24,1**	**22,6**	**23,1**

Ein ebenso großes Problem des *Sorghum*-Anbaus war, dass über die Jahre keine silierfähigen Durchschnitts-Trockensubstanzgehalte (TS) erreicht werden konnten (Tab. 14). „Silierfähig" ist Pflanzenmaterial, das TS-Gehalte in einem Bereich zwischen 28 % und 35 % aufweist (Zeise *et al.*, 2011). Lediglich die Variante „Energiemischung II" hätte in 2010 mit TS-Gehalten von 29,3 % in Egglfing und 28,7 % in Kirchham siliert werden können. Alle anderen Sorten und Mischungen aller Jahre und Standorte lagen z.T. weit unterhalb dieser Spanne.

3.4 Statistische Auswertung auf Signifikante Unterschiede der *Sorghum*-Arten

Die statistische Auswertung der *Sorghum*-Varianten zeigt, dass es in Egglfing 2009 keine signifikanten Unterschiede gab, lediglich die Sorte „Branco" unterschied sich aufgrund ihres niedrigen TM-Ertrages zu allen andern (Tab. 14 und Tab. 15). In Kirchham zeigten sich deutlichere Unterschiede, die *Sorghum*-Arten konnten in drei Signifikanzgruppen dargestellt werden. Die *Sorghum*-Hybrid-Varianten „Mithril" und „Inka" differenzierten. Die *Sorghum bicolor*-Varianten „Sucrosorgo 506" und „Branco" zeigten keinen Unterschied, die Artenmischungen „Energiemischung II" und „Sorghum spezial" ebenfalls. Signifikant unterschiedlich waren „Sorghum spezial" und „Inka" zu „Energiemischung II" und „Branco" und diese differenzierten zu „Mithril" und „Sucrosorgo 506". In Rotthalmünster konnten in 2009 keinerlei Unterschiede festgestellt werden.

In 2010 gab es in Egglfing auch drei Signifikanzgruppen. Unterschiede gab es bei „Energiemischung II" zu „Sucrosorgo 506" und „Mithril". Alle anderen waren in mehreren, übergreifenden Signifikanzgruppen vertreten. Die Varianten in Kirchham zeigten nur zwei Gruppen. Dort unterschieden sich signifikant „Energiemischung II" und „Inka" durch ihre sehr hohen Erträge von den restlichen. Rotthalmünster hatte keine Unterschiede im TM-Ertrag aufzuweisen.

In 2011 war in Egglfing dieselbe Situation wie 2009, nur „Branco" unterschied sich durch den niedrigen TM-Ertrag vom restlichen Sortiment. In Kirchham war ähnliches zu beobachten, dort hoben sich „Sucrosorgo 506" und „Branco" durch den niedrigen Ertrag von den restlichen Versuchsgliedern ab. Und in Rotthalmünster war ebenfalls „Branco" die einzige Variante, die sich signifikant von den anderen unterschied.

Tab. 15 Übersicht statistischer Signifikanzen zum TM-Ertrag der *Sorghum*-Arten (Student-Newman-Keuls-Test, α = 0,05).

***Tab. 15** Statistic significances of the dry matter yields of the* Sorghum *species (Student Newman Keuls test, α=0.05).*

Sorte/ Mischung	SNK (α = 0,05)								
	Egglfing			**Kirchham**			**Rotthalmünster**		
	2009	**2010**	**2011**	**2009**	**2010**	**2011**	**2009**	**2010**	**2011**
Mithril	a	c	a	c	b	a	a	a	a
Sucrosorgo 506	a	b c	a	c	b	a b	a	a	a
Energie-mischung II	a	a	a	b c	a	a	a	a	a
Sorghum spezial	a	a b c	a	a b	b	a	a	a	a
Branco	b	a b c	b	b c	b	b	a	a	b
Inka	a	a b	a	a	a	a	a	a	a

3.5 Trockenmasse-Erträge der Getreide-GPS-Varianten

Die Getreide-GPS-Varianten wurden nur zweijährig geprüft. In 2010 lief die Kultur Winterroggen außer Konkurrenz zu Wintertriticale und Winterweizen. Der Roggen litt stark unter dem Winter 2009/2010. Obwohl mit Halmverkürzern behandelt worden war, gingen die Winterroggensorten Anfang Mai stark ins Lager, und mussten einen Monat früher als die anderen GPS-Varianten geerntet werden. Das spiegelte sich sehr stark im Ertrag wider, der durch den einmonatigen früheren Schnitt sehr gering ausfiel. In Egglfing wurden 47,4 dt/ha, in Kirchham 40,2 dt/ha und in Rotthalmünster 35,3 dt/ha geerntet (Tab. 16). Bei Wintertriticale waren es 125 dt/ha in Rotthalmünster, 119,2 dt/ha in Kirchham und 101,2 dt/in Egglfing. Winterweizen erreichte in Kirchham 120,9 dt/ha, in Egglfing 109,5 dt/ha und bis zu 95,9 dt/ha in Rotthalmünster.

In 2011 konnte von Winterroggen ein Höchstertrag von 180,8 dt/ha in Rotthalmünster geerntet werden. In Egglfing wurden 139,5 dt/ha und in Kirchham 132,3 dt/ha erzielt. Wintertriticale lag auf ähnlich hohem Niveau mit 175,1 dt/ha in Rotthalmünster, 151,4 dt/ha in Egglfing und 133,4 dt/ha in Kirchham. Winterweizen war vom Ertrag etwas niedriger als die anderen Arten. In Rotthalmünster wurden 152,4 dt/ha erreicht, in Egglfing 139,9 dt/ha und in Kirchham 118,5 dt/ha.

Tab. 16 Übersicht Trockenmasse-Erträge der Getreide-GPS Varianten in 2010 und 2011.
Tab. 16 *Dry matter yields of the cereal GPS variants in 2010 and 2011.*

Art/Sorte	Trockenmasse-Ertrag (dt/ha)					
	Egglfing		Kirchham		Rotthalmünster	
	2010	2011	2010	2011	2010	2011
Winterroggen						
Visello	48,0	130,6	40,8	134,3	36,0	183,1
Balistic	46,9	148,3	39,6	130,2	34,6	178,6
Durchschnitt WR	47,4	139,5	40,2	132,3	35,3	180,8
Wintertriticale						
Benetto	106,4	153,4	129,9	132,2	123,0	177,3
Trisol	95,9	-	108,4	-	126,9	-
Massimo	-	149,4	-	134,6	-	173,0
Durchschnitt WTri	101,2	151,4	119,2	133,4	125,0	175,1
Winterweizen						
Akratos	113,1	140,9	139,6	117,3	97,0	155,2
Inspiration	105,9	138,8	102,3	119,7	94,9	149,6
Durchschnitt WW	109,5	139,9	120,9	118,5	95,9	152,4

3.6 Statistische Auswertung auf signifikante Unterschiede der GPS-Varianten

Aufgrund der viel zu frühen Ernte des Winterroggens, bedingt durch Lager, wird ein signifikanter Unterschied in Egglfing, Kirchham und Rotthalmünster zu den anderen Getreidearten errechnet, der in Wahrheit nicht vorhanden sein dürfte. Wintertriticale „Trisol" unterscheidet sich in Kirchham signifikant von „Benetto", der Winterweizen „Inspiration" ebenso von „Akratos". In Rotthalmünster gibt es keine signifikanten Unterschiede innerhalb der Kulturen, diese bestehen aber zwischen Wintertriticale und Winterweizen (Tab. 17).

Tab. 17 Übersicht statistischer Signifikanzen zum TM-Ertrag der GPS-Varianten (Student-Newman-Keuls-Test, α = 0,05).

***Tab. 17** Statistic significances of the dry matter yields of the GPS variants (Student Newman Keuls test, α=0.05).*

	SNK (α = 0,05)																	
Sorte/Mischung	**Egglfing**						**Kirchham**						**Rotthalmünster**					
	2010			**2011**			**2010**			**2011**			**2010**			**2011**		
Winterroggen																		
Visello		b		a					c	a					c	a		
Balistic		b		a					c	a					c	a	b	
Wintertriticale																		
Benetto	a			a			a			a			a			a	b	
Trisol	a			-	-	-		b		-	-	-	a			-	-	-
Massimo	-	-	-	a			-	-	-	a			-	-	-		b	
Winterweizen																		
Akratos	a			a			a			a				b				c
Inspiration	a			a				b		a				b				c

In 2011 waren für alle Kulturen vergleichbar hohe TM-Erträge festzustellen. In Egglfing und Kirchham konnten keine signifikanten Unterschiede über alle Getreidearten und Sorten festgestellt werden. In Rotthalmünster deuteten sich Unterschiede an. Beide Sorten des Winterweizens waren signifikant unterschiedlich zu allen anderen Arten. Winterroggen und Wintertriticale ließen eine Signifikanz zwischen „Visello“ und „Massimo“ erkennen. Die TM-Erträge der anderen Sorten waren relativ ähnlich.

3.7 Prozentualer Vergleich des Durchschnitt-TM-Ertrages der Kulturen mit Silomais

Um einen Vergleich der Alternativen mit der Referenz Silomais darstellen zu können, wurden die Standort-TM-Erträge der einzelnen Jahre und Standorte mit dieser Kultur ins Verhältnis gesetzt und ein prozentualer Wert gewonnen (Tab. 18).

Bei den Futtergräsern und Gemengen wurde dabei in extensive, intensive und überjährige Varianten unterschieden. Die extensiv geführten Futtergräser und deren Gemenge erreichten einen relativen TM-Ertrag von 24,7 % im Ansaatjahr bis hin zu 69,3 % in 2011 im Vergleich zu Silomais. Die intensiv geführten Varianten zeigten eine Spannweite von 36,4 % im Ansaatjahr bis zu einem prozentualen Maximalertrag von 69,9 %. Die drei überjährigen Mischungen sind eigens aufgeführt, da sie in 2011 neu angesät wurden und dadurch einen geringen Ertrag aufweisen als die mehrjährig intensiven Varianten. Ihre Spanne reichte von 30,2 % zu 65,5 %.

Die *Sorghum*-Varianten konnten TM-Ertragswerte von 50,5 % bis zu 74,8 % erreichen.

Der Durchschnittsertrag der Getreide-GPS-Variante Winterroggen im Versuchsjahr 2010 war durch frühes Lager nicht zufriedenstellend. Die Kultur erreichte nur einen Anteil von 18 % des Silomaisertrages. In 2011 reichten die Werte von 57,2 % bis 78,1 %. Wintertriticale zeigte eine Spannweite von 41,9 % bis 75,6 %. Die Kultur Winterweizen erzielte prozentuale Werte von 40,8 % bis zu 65,8 %.

Bei diesem Vergleich sei darauf hingewiesen, dass es sich um den Vergleich des *Durchschnitts*-Ertrages handelt, der die Leistung von Einzelmischungen bzw. Sorten nicht erkennen lässt. Trotz niedriger Werte können Einzelsorten bzw. –mischungen sehr wohl nahe an den Ertrag des Silomais heranreichen. Bei den Futtergräsern können im Hauptnutzungsjahr durch eine standortangepasste Wahl der Mischung und der darin befindlichen Sorten prozentuale Werte über 80 % im Vergleich zu

Silomais erreicht werden. Das sieht man z. B. in Rotthalmünster in 2010 mit den Mischungen „A3 mit Rotklee“ und 2011 mit „FM 3K“ (Tab. 11). Auch die überjährigen Varianten „FE 1“ und „FM 2“erzielten hohe Werte in den Einzeljahren und an den Standorten.

Tab. 18 Darstellung der prozentualen Trockenmasse-Erträge der Alternativen bezogen auf den Orts- und Jahresdurchschnitt von Silomais (= 100 %).

Tab. 18 *Percentage dry matter yields of the alternatives in relation to the local and annual mean of silage maize (=100%).*

Kultur	TM-Ertrag (%) im Vergleich zu Silomais																	
	Egglfing						Kirchham						Rotthalmünster					
	2009		2010		2011		2009		2010		2011		2009		2010		2011	
	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.
Ø Futtergräser Gemenge	24,7	37,9	44,3	45,7	36,1	36,4*)	58,8	64,2	58,6	68,0	58,2	66,3*)	58,4	69,9	43,1	48,3	69,3	66*)
Ø Futtergräser überjährige	-	36,5	-	45,7	-	38,1	-	64,5	-	65,5	-	63,0	-	43,1	-	30,2	-	47,9
Ø Sorghum	55,8		63,7		68,6		50,5		74,8		64,7		55,8		55,3		64,6	
Ø GPS/WR	-		19,6		62,2		-		18,4		57,2		-		15,0		78,1	
Ø GPS/WTRI	-		41,9		67,6		-		54,4		57,7		-		53,2		75,6	
Ø GPS/WW	-		45,4		62,4		-		55,2		51,3		-		40,8		65,8	

*) Durchschnitt aller mehrjährigen Futtergräser, ohne überjährige Mischungen

3.8 Vergleich der Alternativen über die drei Jahre und drei Standorte

Ein Vergleich des TM-Ertrages der getesteten Kulturarten über die drei Versuchsjahre und Standorte ist in Fig. 5 dargestellt. Silomais zeigt relativ konstante TM-Erträge mit gleichzeitig geringer Streuung der Werte über die Jahre und Orte. Die restlichen Kulturen liegen von den Ergebnissen unter den Werten von Silomais, und zeigen eine mehr oder weniger starke Streuung innerhalb der einzelnen Standorte oder Jahre.

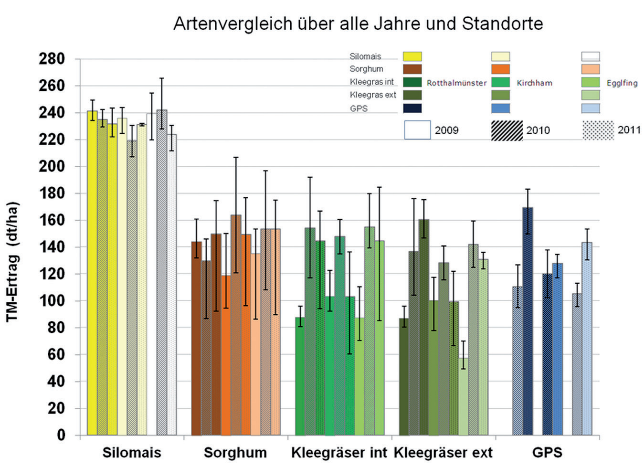

Abb. 5 Graphische Darstellung des TM-Ertrages der getesteten Kulturen über alle Versuchsstandorte und Jahre.

Fig. 5 *Graphic chart of the dry matter yields of the tested crops over all experimental sites and years.*

3.9 Nettoenergie-Laktation (NEL)

Silomais

Die getesteten Silomaissorten erreichten von allen getesteten Kulturen die höchsten NEL-Werte, die zwar innerhalb der einzelnen Standorte variierten aber über die Versuchsjahre auf ähnlich hohem Niveau blieben (6,86 bis 7,33 MJ NEL/kg TM, Fig. 6).

Sorghum-Arten und Mischungen

Ein ähnlicher Verlauf war bei den *Sorghum*-Arten festzustellen. Die NEL-Werte variierten sehr wenig an den einzelnen Standorten und zeigten ein Beibehalten des Niveaus über alle Versuchsjahre. Die Werte reichten von 5,32 bis 5,55 MJ NEL/kg TM.

Getreide-GPS

Das Getreide-GPS wurde zweijährig im Vergleichsanbau geprüft. Leider stehen für 2010 keine Laboruntersuchungen zur Verfügung, da das Pflanzenmaterial nach der TS-Bestimmung versehentlich entsorgt wurde. In 2011 waren höhere NEL-Werte erreicht worden als die der *Sorghum*-Arten. Die Werte lagen mit 5,53 bis 5,85 MJ NEL/kg TM aber niedriger als die des Silomais.

Futtergräser und deren Gemenge

Bei der Betrachtung der Energiewerte der Futtergräser muss zwischen den intensiven, fünffach geschnittenen und qualitativ hochwertigen Varianten und den extensiven, vierfach geschnittenen und quantitativen Varianten unterschieden werden.

Die Futtergräser und deren Gemenge erreichten die zweithöchsten NEL-Werte von allen getesteten Kulturen. Sie lagen in der Höhe zwischen den Werten des Silomais und der Getreide-GPS-Varianten.

Die NEL-Werte der Futtergräser waren im Ansaatjahr 2009 allgemein niedriger als in den Folgejahren, wobei sie auch innerhalb der Standorte starke Unterschiede aufwiesen. In Rotthalmünster und Kirchham konnten nur Werte bis 5,67 MJ NEL/kg TM erreicht werden, in Egglfing dagegen lagen sie mit 6,58 MJ/kg TM sogar um 1 MJ/kg TM höher. Die niedrigeren NEL-Werte lassen sich durch die unterschiedliche Pflanzenzusammensetzung an den Standorten erklären. In Rotthalmünster und Kirchham waren die Bestände sehr reich an Leguminosen, in Egglfing sank dieser Anteil bis Jahresende leicht ab, was durch den dort stark nachmineralisierenden Boden zu erklären ist. Die Spanne der Werte aller Standorte in 2009 reichten für die intensiv geschnittenen Varianten von 5,63 bis 6,58 MJ NEL/kg TM und für die extensiv geschnittenen Varianten von 5,51 bis 6,53 MJ NEL/kg TM (Fig. 6).

Im ersten Hauptnutzungsjahr 2010 waren die Schwankungen der Energiewerte dagegen nicht sehr ausgeprägt und sie lagen allgemein auf höherem Niveau als im Vorjahr. Die NEL-Werte bewegten sich in den intensiven Varianten von 6,36 bis 6,66 MJ NEL/kg TM, in den extensiven Varianten von 5,93 bis 6,45 MJ NEL/kg TM.

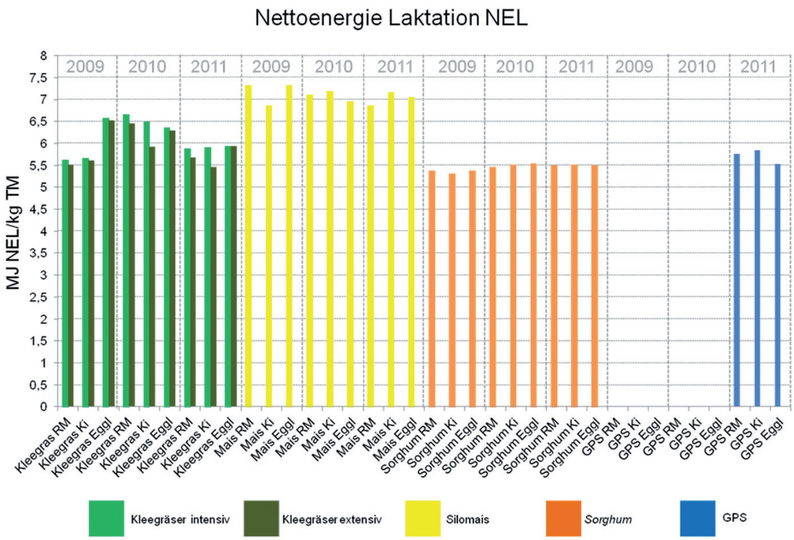

Abb. 6 Darstellung der Nettoenergie Laktation (MJ/kg TM) der getesteten Kulturen über alle Versuchsstandorte und –jahre.

Fig. 6 *Net energy lactation (MJ/kg dry matter) of the tested crops over all experimental sites and years.*

Im zweiten Hauptnutzungsjahr 2011 sanken die Werte der NEL leicht ab. Da es sich bei den Futtergräsern um eine mehrjährige Kultur handelt, zeigen Witterungseinflüsse (z.B. Auswinterung durch Kahlfröste), Bodeneinflüsse, Schädigungen durch Tiere und Unkrautdruck bei diesen Kulturen einen nicht zu vernachlässigenden Einfluss und spiegeln sich in einer Veränderung der Pflanzenzusammensetzung wider, was wiederum Auswirkungen auf die Energiedichte zur Folge hatte. In 2011 gab es nur leichte Schwankungen der NEL-Werte zwischen den Orten in den intensiven Varianten, es wurden Werte von 5,89 bis 5,93 MJ NEL/kg TM erreicht, die extensiven Varianten erzielten Werte von 5,46 bis 5,94 MJ NEL/kg TM.

3.10 Rohproteingehalte

Futtergräser und deren Gemenge

Die Rohproteingehalte der Futtergräser und deren Gemenge waren im Vergleich zu den anderen Kulturen am höchsten. In 2009 konnten bei den intensiven Varianten sehr hohe Werte erreicht werden, sie beschrieben eine Spanne von 16,75 % bis 21,56 % bezogen auf die TM. Die extensiven Varianten lagen mit 14,06 % bis 19,57 % in TM unter diesen Werten (Abb. 7).

In 2010 konnten die hohen Rohproteingehalte vom Vorjahr nicht mehr erreicht werden. Die intensiven Varianten hatten Gehalte von 15,10 % bis 16,71 %, die extensiven von 12,15% bis 14,84 %. Am Standort Rotthalmünster blieb der hohe Anteil der Leguminosen im Vergleich zum Vorjahr konstant, wobei an den Standorten Kirchham und Egglfing ein langsamer Rückgang dieser Pflanzen zu erkennen war, der sich auch in niedrigeren Proteingehalten erkennen ließ.

In 2011 konnte ein ähnlicher Verlauf der Kurven wie 2010 festgestellt werden, allerdings mit leicht erhöhten Werten im Vergleich zum Vorjahr. Diese reichten bei den intensiven Varianten von 16,39 % bis 17,51 % und von 13,41 % bis 14,51 % bei den extensiven. Egglfing, inzwischen ein reiner Grasbestand, lag an letzter Stelle.

Silomais

Der Silomais erreichte nur ca. ein Drittel des Proteingehaltes, den die Futtergräser enthielten. In 2009 betrug die Spanne Proteingehalte 6,12 % bis 6,46 %, in 2010 reichte sie von 7,06 % bis 7,23 % und in 2011 lag sie zwischen 6,37 % und 6,82 %. Die Werte variieren nur schwach, es wurden nur drei Sorten untersucht. Vom Standort Rotthalmünster sind aus dem letzten Versuchsjahr keine Laborwerte zu den Rohproteingehalten vorhanden.

Sorghum-Arten

Die *Sorghum*-Arten erzielten ähnlich hohe Proteingehalte wie der Silomais. Die Werte lagen 2009 zwischen 5,19 % und 6,47 %, in 2010 erreichten sie 6,42 % bis zu 7,96 % und in 2011 konnten Proteingehalte von 7,66 % bis 8,93 % erzielt werden. Die Streuung der Werte ist allerdings stärker als beim Silomais.

Getreide-GPS

Wie auch schon bei den NEL-Ergebnissen erwähnt, liegen für die GPS-Varianten nur die Werte von 2011 vor. Diese bewegten sich in dem Wertebereich von Silomais und den *Sorghum*-Arten. Es wurden Proteingehalte von 6,32 % bis 9,53 % der TM erreicht.

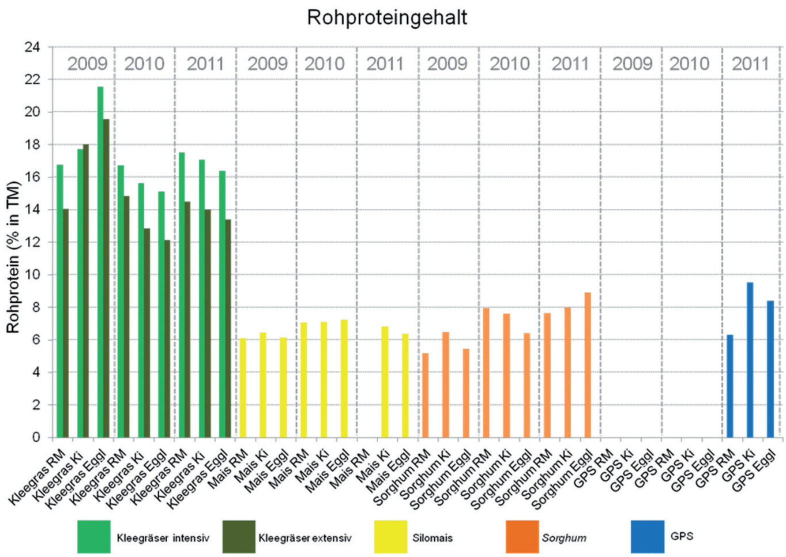

Abb. 7 Darstellung des Rohproteingehaltes (% in TM) der getesteten Kulturen über alle Versuchsstandorte und –jahre.

Fig. 7 *Raw protein content (% in dry matter) of the tested crops over all experimental sites and years.*

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Investigations on the replacement of maize products in rations for dairy cows and fattening bulls

Untersuchungen zum Ersatz von Maisprodukten in Rationen für Milchkühe und Mastbullen

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Summary

For different reasons as for example the occurrence of pests like *Diabrotica virgifera* there may be a shortage in availability of maize products for ruminant feeding. Therefore, different feeding studies on replacement of maize products in rations for dairy cows and fattening bulls were conducted as a part of the "Diabrotica research programme" set up by the federal government of Germany and the federal state of Bavaria. The main focus was set to on suitability of alfalfa as well as grass silage as a roughage source for dairy cows and fattening bulls. It is shown that partial replacement of maize silage by these products allows producing at high production level in Simmental cows and fattening bulls. A decisive advantage is the possibility to reduce the portion of protein concentrates like soybean or rape meal in the diets. Moreover, other beneficial aspects for ruminant feeding such as the high structural value of alfalfa silage are discussed. In ruminant feeding, corn is used as an energy rich dietary component. As a part of the programme a dairy cow feeding trial was conducted to evaluate whether replacement of corn by wheat is a promising possibility to reduce portion of maize products in ruminant diets. Results of the study demonstrate the limits of these feeding strategies, especially in maize silage based diets high in energy concentration and in the concentration of soluble carbohydrates. In these diets, concentrations of degradable starch and sugar are often near or above the maximum recommended amounts. For these reasons use of corn as a source of undegradable starch is preferable to the use of other cereals to prevent conditions that promote rumen acidosis.

Key words: dairy cows, fattening bulls, roughage source, starch source

Zusammenfassung

Aus unterschiedlichen Gründen, wie zum Beispiel dem Auftreten von Schadorganismen wie *Diabrotica virgifera*, könnte Mais als Rinderfutter knapp werden. Deshalb wurden verschiedene Fütterungsversuche zum Ersatz von Maiserzeugnissen in Futterrationen für Milchkühe und Mastbullen im Rahmen des „*Diabrotica*-Forschungsprogramms" des Bundes und des Bundeslandes Bayern durchgeführt. Das Hauptaugenmerk lag auf Luzerne- und Grassilage als Raufutter für Milchkühe und Mastbullen. Es wurde festgestellt, dass ein teilweiser Ersatz von Silomais durch diese Erzeugnisse ein hohes Produktionsniveau bei Simmentalkühen und Mastbullen ermöglicht. Ein entscheidender Vorteil besteht darin, dass der Anteil an Proteinkonzentraten wie Soja- oder Rapsextraktionsschrot am Futter verringert werden kann. Außerdem werden weitere positive Aspekte bei der Rinderfütterung hohe Strukturwert von Luzernesilage diskutiert. Bei der Rinderfütterung wird Mais als energiereiche Nahrungsquelle verwendet. Im Rahmen eines Milchkuhfütterungsversuchs sollte festgestellt werden, ob der Ersatz von Mais durch Weizen eine aussichtsreiche Möglichkeit für die Verringerung des Maisanteils am Rinderfutter ist. Die Ergebnisse der Studie zeigen die Grenzen solcher Fütterungsstrategien, insbesondere bei maissilagebasierten Rationen mit hohem Energiegehalt und hohen Gehalten an löslichen Kohlenhydraten. In solchen Futtermitteln liegt die Konzentration abbaubarer Stärke und Zucker oft nahe an oder über den maximal empfohlenen Mengen. Aus diesem Grund wird Mais als Quelle nicht abbaubarer Stärke anderen Getreiden vorgezogen werden, um die Förderung von Azidose zu vermeiden.

Stichwörter: Milchvieh, Mastbulle, Grobfutter, Stärkequellen

1. Introduction

For several reasons maize silage became a more and more important roughage source for ruminant feeding over the past years. Maize normally shows high dry matter (DM) and energy yields. Preservation of maize is quite low in risk for deterioration of silage and easy to handle for the farmer. Moreover, compared to other roughage sources there is a large time frame for optimum harvest date in maize. Fibre concentration increases with time comparable to other field crops, resulting in a decrease of digestibility and energy concentration, mainly in stalks. In maize, however, this decrease is widely compensated by the increase of portion of the maize cob and the increase in starch concentration of the corn (Schwarz and Ettle, 2000). Thus, among the most common field crops, maize shows not only the highest energy yields per ha but is also suitable to give roughage with a very high energy concentration, which is more important from the perspective of animal nutrition. High milk yield in dairy cows and high daily gains in fattening bulls can only be obtained at a high level of feed intake. This, in turn, is largely determined by the energy concentration of the basal diet (Dlg, 2006). Another reason for the high value of maize or corn in ruminant nutrition is the low ruminal degradability of corn starch. Starch of wheat or barley has a degradability of about 85% whereas degradability of corn starch is assumed to be only 58% (Dlg, 2001, 2008). Ensiling, however, seems to modify structure of starch and therefore starch degradability of good maize silages seems to be comparable to that of wheat. A high proportion of ruminally undegradable starch is important in ruminant feeding for different reasons. Firstly, starch directly delivered and digested in the duodenum is used energetically more efficiently than starch fermented in the rumen. Thereby, maximum amount of starch used efficiently in the duodenum of dairy cows seems to be about 1.0 to 1.5 kg/day (Flachowsky *et al.*, 2000). A second reason for the need of undegradable starch in ruminant diets is that high amounts of ruminally fermentable starch increase the risk for ruminal acidosis (Steingass and Zebeli, 2008). As shown above, current diets for high yielding ruminants need to have a high energy density and this is mostly linked to low fibre concentrations of total rations as well as high starch concentration. Risk for rumen acidosis, however, is not only determined by the level of intake of (rumen degradable) starch but also by the intake of fibre compounds (Steingass and Zebeli, 2008) and therefore by the relation of starch to fibre of the diet. This high starch and low fibre concentration, however, is typical for current high energy maize based ruminant diets and therefore a well directed substitution with concentrates is needed, one option is corn starch of low degradability.

Despite of the high feeding value of maize silage and corn for ruminants, increased need of maize silage for biogas production or occurrence of pests such as *Diabrotica virgifera virgifera* may result in a shortage of availability of maize (silage) for animal feeding. Therefore, feeding studies in dairy cows and fattening bulls evaluating alternative roughage and concentrate sources were conducted as a part of the "Diabrotica research programme" set up by the federal government of Germany and the federal state of Bavaria. The results of these trials are presented below.

2. Feeding studies on partial replacement of maize silage by grass or alfalfa silage in fattening bulls

Studies on the use of grass silage, which may be considered as an alternative roughage source in fattening bulls, mainly resulted in depressed performance due to inclusion of grass silage in diets. In these studies, diets were corrected for low protein concentration of maize silage, but not for lower energy concentration of grass silage (e.g. Juniper *et al.*, 2005). For this reason, two feeding studies were conducted to evaluate the effects of partial replacement of maize silage by grass (trial 1) or alfalfa (trial 2) silage in isoenergetic diets for fattening bulls. Each trial involved a total of 72 Simmental bulls (trial 1: 250 ± 18 kg, 197±7 days old; trial 2: 222 ± 19 kg, 180±5 days old). Within each trial, animals were equally assigned to three feeding groups according to body weight and ancestry. Group 1 (group 0% grass and group 0% alfalfa silage) was fed a Total Mixed Ration (TMR) based on maize silage, straw and concentrates. In the diets for groups 2 and 3, about 30% and 60% of maize silage and straw (based on DM) were substituted by grass silage (trial 1; groups 30% and 60% grass silage) or alfalfa silage (trial 2; groups 30% and 60% alfalfa silage). Crude protein concentration was partially equalized by reducing portion of protein concentrates (extracted soybean meal or rape meal, respectively) in groups fed grass or alfalfa silage. To obtain comparable energy concentration between feeding groups within each trial, portion of corn and rape cake was increased in these feeding groups. In trial 2, portion of concentrates in the TMR was also slightly increased. Animals were housed in group boxes (12 bulls/box) equipped with a slatted floor. In each box, four animals of each feeding group were kept. TMR and water were provided *ad libitum*. Individual feed intake was automatically recorded daily using automatic feeding troughs with sensors for animal identification. Live weight was recorded every 4 weeks and back fat depth every 12 weeks. More details on diet composition and materials and methods are given in Ettle *et al.* (2010, 2012a).

Tab. 1 Feed intake, nutrient and energy intake, growth and slaughter performance and selected meat characteristics (mean±SD) in fattening bulls fed with varying amounts of grass silage.

Tab. 1 *Futter-, Nährstoff- und Energieaufnahme, Wachstum und Schlachtleistung und ausgewählte Fleischmerkmale (Durchschnitt±SD) von Mastbullen bei unterschiedlichen Anteilen an Grassilage in der Ration.*

	Group 1, 0% grass silage			**Group 2, 30% grass silage**			**Group 3, 60% grass silage**		
Feed intake (kg DM/day)	9.3	±	1.1	9.6	±	0.8	9.3	±	1.1
CP intake, g/day	1215	±	151	1322	±	125	1285	±	153
ME intake, MJ/day	110	±	13	113	±	10	109	±	13
Initial weight, kg	248	±	19	252	±	15	252	±	17
End weight, kg	748	±	13	747	±	12	744	±	28
Average daily gain, g	1595	±	158	1615	±	122	1550	±	196
Dressing proportion, %	58.7	±	1.1	58.8	±	1.4	59.0	±	1.1
Carcass classification (EUROP)*	2.48	±	0.51	2.63	±	0.50	2.57	±	0.51
Fat classification**	2.57	±	0.51	2.84	±	0.50	2.76	±	0.54
Back fat depth at slaughter (cm)	1.74	±	0.26[c]	1.90	±	0.37[b]	2.12	±	0.31[a]
Intramuscular fat (%)	2.49	±	0.72	2.59	±	0.5	2.54	±	0.72
Fat colour (b*; haunch)	5.29	±	1.81[b]	7.07	±	2.16[a]	8.12	±	2.59[a]
ω-6/ω-3 – ratio of m. l. dorsi	11.96	±	1.39[a]	6.81	±	0.74[b]	5.14	±	0.84[c]

[a,b] Values differ at P< 0.05; *E=1,…, P=5;** 1=lean, …, 5=fat

In trial 1, feed, CP and energy intake in group 30% grass silage was slightly higher than in other groups (Tab. 1). Time to reach end weight of 750 kg was 316, 308, and 321 days for groups 0%, 30% and 60% grass silage, respectively. Average daily gain was not affected by treatment. Moreover, there was no influence on hot carcass weight, dressing or carcass conformation. Carcass fatness classification was slightly higher in animals fed diets with grass silage, and back fat depth in the middle of the fattening period and at slaughter increased significantly (P<0.05). Intramuscular fat content and pH value, share force, drip losses and grilling time of meat were not influenced by treatment. ω-6/ω-3 – ratio of meat of m. l. dorsi decreased (P<0.05) when higher proportions of grass silage were fed. In a comparable manner, pasture feeding increased ω-3 fatty acids in meat of German Holstein steers compared to concentrate feeding (Ender *et al*., 2000). Lower ω-6 and higher ω-3 fatty acid intake is discussed to positively affect consumer's health (Hollo *et al*., 2005). There was no influence on meat colour, but subcutaneous fat was more yellow (P<0.05) in grass silage fed bulls. No effect on serum GLDH-activity and concentration of urea, total protein and glucose could be observed. In summary, inclusion of grass silage in diets for fattening bulls had only minor influence on growth performance or carcass characteristics of fattening bulls. Therefore, partial replacement of maize silage by grass silage may be a valid alternative in situations where availability of maize products is limited, but lower energy concentration of grass silage has to be accounted for in diet formulation.

In the second feeding trial with fattening bulls, feed intake in group 0% alfalfa silage was slightly lower than in other groups (Tab. 2). Despite of low energy concentration of alfalfa silage (9.0 MJ ME/kg DM) and some differences in energy concentration of TMR (calculated from diet composition and energy concentration of different components: 11.6, 11.5, and 11.3 MJ ME/kg DM for groups 0%, 30%, and 60% alfalfa silage, respectively), mean daily ME intake was comparable between groups. CP intake in group 0% alfalfa silage was lower (p<0.05) compared to other groups. Time to reach end weight of 750 kg was 335, 321, and 338 days for groups 0%, 30%, and 60% alfalfa silage, respectively. There were no major differences in growth and slaughter performance and meat characteristics between groups. On the contrary to the grass silage trial, there were no differences in colour of adipose fat. Comparable to trial 1, ω-6/ω-3 – ratio of meat of m. l. dorsi decreased ($P<0.05$) when higher proportions of alfalfa silage were fed. Serum GLDH activity was higher ($p<0.05$) but serum urea concentration was lower ($P<0.05$) in group 0% alfalfa silage compared to other groups. GLDH is a liver specific enzyme that is only released when some liver cells are damaged. Therefore, one may speculate that the alfalfa silage with its high fibre content and structural value had positive effects on animal health. These specific properties of alfalfa may be of particular importance in fattening bulls fed maize silage based diets with high concentrations of soluble carbohydrates. Blood urea concentration is correlated to dietary crude protein concentration and to ruminal N-balance. Thus, higher values in groups fed alfalfa silage may be discussed in the context of an inefficient use of dietary N.

Tab. 2 Feed intake, nutrient and energy intake, growth and slaughter performance and selected meat characteristics (mean±SD) in fattening bulls fed varying amounts of alfalfa silage.

Tab. 2 *Futter-, Nährstoff- und Energieaufnahme, Wachstum und Schlachtleistung und ausgewählte Fleischmerkmale (Durchschnitt±SD) von Mastbullen bei unterschiedlichen Anteilen an Luzernesilage in der Ration.*

	Group 1, 0% alfalfa silage			**Group 2, 30% alfalfa silage**			**Group 3, 60% alfalfa silage**		
Feed intake (kg DM/day)	9.1	±	1.3	9.4	±	0.8	9.3	±	0.6
CP intake, g/day	1266	±	176[b]	1454	±	118[a]	1461	±	84[a]
ME intake, MJ/day	105	±	15	108	±	9	105	±	6
Initial weight, kg	223	±	23	224	±	18	223	±	19
End weight, kg	755	±	35	751	±	30	755	±	28
Average daily gain, g	1599	±	168	1652	±	197	1580	±	144
Dressing proportion, %	59.1	±	1.3	59.6	±	1.5	59.2	±	1.4
Carcass classification (EUROP)*	2.67	±	0.48	2.45	±	0,51	2.41	±	0.5
Fat classification**	2.86	±	0.57	3.00	±	0.44	2.73	±	0.63
Back fat depth at slaughter (cm)	2.00	±	0.45	2.03	±	0,37	1.93	±	0.43
Intramuscular fat (%)	2.96	±	0.79	3.32	±	1.11	3.23	±	1.03
Fat colour (b*; haunch)	6.31	±	1.65	7.43	±	2.3	6.39	±	2.57
ω-6/ω-3 – ratio of m. l. dorsi	9.2	±	1.2[a]	5.9	±	1.3[b]	4.6	±	0.7[c]

[a,b] Values differ at $P< 0.05$; *E=1,…, P=5;** 1=lean, …, 5=fat

In conclusion, inclusion of alfalfa silage in diets for fattening bulls tended to positively affect feed intake and had no negative effects on growth performance, which was at a high level in all groups. Therefore, inclusion of alfalfa silage in rations for fattening bulls allows reducing consumption of maize silage where availability is limited, and also helps to reduce portion of protein rich concentrates in the rations. However, as in grass silage based diets, sufficient energy supply has to be considered.

3. Feeding studies in dairy cows

In a first feeding study in dairy cows, effects of replacement of maize and grass silage by alfalfa silage were investigated. The feeding trial involved a total of 40 Simmental cows and lasted 10 weeks. Cows were divided into two groups (treatment (treat) 1: maize silage; treat 2: alfalfa silage) according to milk yield, stage of lactation, and feed intake. The cows had *ad libitum* access to partial mixed rations (PMR). PMR of treat 1 contained maize silage (47% of DM), grass silage (16% of DM) and hay/straw (4.8/4.3% of DM). In treat 2, grass silage and hay/straw were completely replaced by alfalfa silage, and maize silage by about 50%. Considering an intake of 18 kg DM of PMR/day that is equal to an amount of 7.5 kg DM alfalfa silage daily. Lower energy concentration of alfalfa silage was partly compensated by variation of type of concentrates as well as a slightly higher portion of concentrates in the PMR, respectively. However, as determined in digestibility trials in rams there was still a difference in energy concentration of PMR of 0.4 MJ NEL/kg DM. The utilizable crude protein concentration was partly equalized by a reduced portion of rape products in PMR of treat 2. In the present example, the sparing effect for protein concentrates was 1.2 kg DM/cow and day. Calculated concentration of utilizable crude protein (uCP), starch and sugar, and ruminally undegradable starch were comparable between feeding groups. More details on composition of diets are given in Ettle *et al*. (2012b).

Tab. 3 Feed intake, milk yield, milk composition, and net acid-base excretion (NABE) of cows fed maize or alfalfa silage based diets (mean±SD).

Tab. 3 *Futteraufnahme, Milchleistung, Milchzusammensetzung und Netto-Säuren-Basen-Ausscheidung (NSBA) bei mit mais- oder luzernesilagebasierten Rationen gefütterten Kühen (Durchschnitt±SD).*

	Feeding group					
	Maize silage			**Alfalfa silage**		
DM intake, kg/day	21.8	±	2.5	21.8	±	2.7
CP intake, g/day	3503	±	412	3791	±	517
uCP intake, g/day	3502	±	401	3438	±	448
Energy intake, MJ NEL/day	156	±	18	150	±	19
Milk yield, kg/day	34.0	±	5.7	32.7	±	6.4
Milk fat, %	3.78	±	0.39	3.98	±	0.55
Milk protein, %	3.45	±	0.22	3.44	±	0.19
Milk urea, mg/l	236	±	28[b]	311	±	35[a]
ECM, kg/day	33.2	±	5.3	32.5	±	5.2
NABE 1 (4. week), mmol/l	111	±	49[b]	151	±	22[a]
NABE 2 (8. week), mmol/l	155	±	48[b]	202	±	23[a]

Mean daily feed intake was 21.8 kg DM in both groups (Tab. 3). Given that alfalfa is characterized by a high DM degradability per time unit (Flachowsky *et al*., 1992) and a high ruminal transition rate (Hoffmann *et al*., 1998) one can generally expect positive effects of alfalfa silage on feed intake. Such positive effects have been demonstrated in dairy cow trials comparing grass silage based diets and alfalfa based diets (Bulang *et al*., 2006; Ettle *et al*., 2011). Positive effects of alfalfa silage on feed intake compared to maize silage were demonstrated in dairy cows which were more than 60 days in milk (Bulang *et al*., 2006). Such additional effects on feed intake were not seen in the present study, but as feed intake is largely determined by energy concentration of basal diet (Dlg, 2006) it is of interest that feed intake in the alfalfa group was not decreased. Daily milk yield in the alfalfa group was numerically lower than in the maize silage group and that is in accordance with differences in energy intake. Nevertheless, the present data demonstrate that also diets very high in portion of alfalfa silage allow a high production level in Simmental cows and the role of alfalfa as a stabilizing diet component is underlined. There were only minor differences in milk fat concentration between groups. Because of the high fiber concentration in diets of both groups a milk fat depression may not be expected. The increased ($P<0.05$) milk urea concentration in the alfalfa silage group is a consequence of the higher ruminal N-balance.

In week 4 and week 8 of the trial urine samples were taken to determine net acid-base excretion (NABE). Higher values ($p<0.05$) were obtained for group alfalfa silage at both sampling dates. Moreover, values below reference values were seen more often in cows of the maize silage group than in other groups. There is a correlation between NABE and the fibre intake in dairy cows (Scholz *et al*., 2010) and therefore the differences may be discussed as a benefit of supply of dietary structure due to feeding of alfalfa. However, at given high dietary fibre concentrations differences may also be a consequence of differences in dietary cation-anion balance, which is also known to influence NABE (Scholz *et al*., 2010). From the result of the present trial it can be concluded that alfafa silage is suitable as a roughage source in high yielding dairy cows, even if proportion of alfalfa in the total diet is high. Benefits of supply of structural fibre may be seen in diets with a high starch concentration. Characterisation of influence of alfalfa on feed intake in relation to its energy concentration should be further investigated.

A second dairy cow feeding trial within the Diabrotica research programme was conducted to determine the effects of replacement of corn by wheat. The study involved 34 dairy cows each having more than 34 days in milk. Conditions of the study were comparable to the first dairy cow feeding trial and are described in more detail in Ettle *et al.* (2012c). PMR was based on maize silage (29% of DM), alfalfa silage (31% of DM), hay and straw (2.4% of DM each), and concentrates. In the concentrates portion, corn was replaced by wheat in a 1:1 ratio. Considering a daily intake of 19 kg DM of PMR this corresponds to 4.6 kg DM corn or wheat, respectively. According to diet calculation, PMR of the two feeding groups were comparable with energy and concentration of most nutrients, but there were considerable differences in concentration of rumen undegradable starch. Digestibility trials with rams, however, resulted in a slightly increased energy concentration (0.2 MJ NEL/kg DM) in PMR of cows fed corn. DM intake in corn fed animals was about 1 kg/day higher than in the other group (Tab. 4). These differences were not significant but resulted in a slightly increased intake of energy and utilizable crude protein in the corn fed group. In a study of Gozho and Mutsvangwa (2008) feed intake in corn fed cows was increased by 2 kg DM/day compared to wheat fed cows, whereas in a comparable study of Daenicke (2000) there was no effect on feed intake. Reasons for different effects on feed intake in studies on different starch sources fed to dairy cows may be the composition of total diet, dietary concentration of starch and total soluble carbohydrates, as well as other factors like conditioning of starch sources. Concentration of readily available carbohydrates in PMR wheat was 253 g/kg DM and therefore slightly above the critical concentration of 250 g/kg DM given by Dlg (2001). The additionally given concentrates were also based on wheat and barley and therefore a further source of soluble carbohydrates, thus supporting conditions for rumen acidosis. On the other hand one can speculate on some beneficial effects of structural fibre of the alfalfa silage preventing cows of the wheat group from a significant decrease of feed intake.

Mean milk yield was 28.4 and 26.9 kg/day for corn and wheat fed cows, respectively. A literature overview on several comparable studies (Daenicke, 2000) resulted in a mean increase of milk yield of about 1.1 kg/day when barley or wheat was replaced by corn. The present results are fairly within this range. Former tables on feed composition for ruminants (Dlg, 1997) indicated a slightly lower energy concentration of maize (8.39 MJ NEL/kg DM) than of wheat (8.51 MJ NEL/kg DM). On the other hand, respiration trials indicated higher energy concentration of corn compared to wheat (Gädeken *et al.*, 1995; Daenicke, 2000), and this is in accordance with digestibility studies conducted with the PMR, wheat and corn used in the present study. Therefore, energetic value of wheat and corn needs to be further investigated.

In accordance with other studies (Daenicke, 2000) there were no differences in milk fat or protein concentration. Milk fat concentrations of 4.1% in both feeding groups do not support any incidence acidosis. Milk urea concentration in the wheat group was significantly increased which may be a result of differences of ruminal N-balance. Blood urea concentration, which is closely correlated to milk urea concentration (Burgos *et al.*, 2007) was, however, not influenced in the present study. Serum GLDH activity, total protein, and glucose concentration were not influenced by treatment and within reference limits in both groups. Therefore, these data do not support differences in health status or energy supply between the feeding groups.

In summary, replacement of corn by wheat resulted in a slightly depressed feed intake, a decreased energy intake and a reduction of milk yield of about 1.5 kg/day. These data are in good agreement with data from literature. There is no evidence that in alfalfa and maize silage based diets corn, a provider of undegradable starch, should be replaced by wheat or other cereals. Combination of maize silage and wheat results in high dietary levels of soluble carbohydrates. Therefore there is an increased risk to exceed the current reference value of 250 g starch and sugar/kg DM (Dlg, 2001) and in consequence an increased risk for rumen acidosis.

Tab. 4 Feed intake, milk yield, and milk composition of cows fed corn or wheat (mean±SD).

Tab. 4 *Futteraufnahme, Milchleistung und Milchzusammensetzung bei mit Körnermais oder Weizen gefütterten Milchkühen (Durchschnitt±SD).*

	Feeding group					
	Corn			Wheat		
DM intake, kg/day	22.2	±	2.6	21.1	±	2.2
CP intake, g/day	3565	±	496	3431	±	418
uCP intake, g/day	3458	±	443	3249	±	367
Energy intake, MJ NEL/day	157	±	19	146	±	16
Milk yield, kg/day	28.4	±	9.8	26.9	±	7.4
Milk fat, %	4.09	±	0.47	4.06	±	0.40
Milk protein, %	3.60	±	0.23	3.71	±	0.23
Milk urea, mg/l	249	±	25[b]	298	±	38[a]
ECM, kg/day	28.8	±	9.0	27.4	±	6.9

4. Conclusions

Feeding studies in fattening bulls and dairy cows were conducted to investigate the potential of replacement of maize silage and corn by grass or alfalfa silage and wheat, respectively. In both, bulls and dairy cows, partial replacement of maize silage by alfalfa silage had only minor influence on performance, which was at a high level in all trials. Moreover, there are some indications that alfalfa silage may positively affect feed intake and health, especially when diets high in energy and soluble carbohydrates and low in fibre concentration are fed. The use of alfalfa and grass silage moreover allows reducing the portion of protein concentrates as soybean or rape meal in the rations, but there is the risk of a surplus of protein which is not effectively used by the animal. The relatively low energy concentration grass and alfalfa silage has to be considered and corrected for in diet formulation. Besides maize silage, corn is used in ruminant nutrition. The main advantage over other cereals is its high concentration of ruminally undegradable starch. The present results do not indicate any advantage for the animal, when corn in diets for dairy cows is replaced by wheat.

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Session 6: Economy

Die Bedeutung des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera LeConte)* in Bayern – regionale Relevanz und einzelbetriebliche Auswirkungen

*The importance of the western corn rootworm (*Diabrotica virgifera virgifera *LeConte) in Bavaria – local relevance and economic impact on single farms*

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Zusammenfassung

Der Westliche Maiswurzelbohrer (*Diabrotica virgifera virgifera LeConte*), der in der Europäischen Union einen Quarantänestatus innehat, wurde zum ersten Mal 2007 in Südbayern entdeckt. Im Zuge des *Diabrotica*-Forschungsprogramms des Bundes und der Länder Bayern und Baden-Württemberg, wird in Bayern eine ökonomische Begleitforschung auf einzelbetrieblicher Ebene durchgeführt. Diese zielt darauf ab, unterschiedliche betriebliche Anpassungsmaßnahmen bezüglich der Ausrottungs- und Eindämmungsstrategien ökonomisch zu taxieren. Im Vordergrund der Betrachtung steht hier vor allem die Fruchtfolgegestaltung bzw. die Reduzierung des betrieblichen Maisanteils.

Um die ökonomische Relevanz des Schädlings in Bayern besser beurteilen zu können, werden InVeKoS-Daten der bayerischen Landwirtschaftsverwaltung analysiert. Diese ermöglichen eine detaillierte Auskunft über die Entwicklung des Maisanbaus sowie der Anbauintensität. Es werden Untersuchungsregionen festgelegt, in denen auf Grund einer hohen Maisintensität verstärkt mit Schäden, bedingt durch den Käfer, zu rechnen ist. In diesen eruierten Gebieten werden für die weiteren Analysen Betriebe herausgegriffen.

Für die einzelbetrieblichen Betrachtungen werden Betriebe mit unterschiedlichen Betriebsschwerpunkten gewählt, anhand derer Anpassungsalternativen bewertet und realistische Folgenabschätzung getätigt werden können. Der erste Schritt ist hierbei die Durchführung von verfahrensökonomischen Kalkulationen unterschiedlicher Anpassungsstrategien für den Gesamtbetrieb. In einem zweiten Schritt folgt, um die gewonnenen Ergebnisse zu untermauern und zu vertiefen, eine qualitative Erhebung in Form von Leitfadeninterviews. Eine vergleichende Betrachtung von möglichen, durch den Käfer verursachten Schäden und Kosten für die Eindämmung schließen die Studie ab.

Stichwörter: *Diabrotica virgifera virgifera*, Anpassungsmaßnahmen, ökonomische Auswirkungen, ökonomische Bewertung, einzelbetriebliche Ebene

Summary

The western corn rootworm (*Diabrotica virgifera virgifera LeConte*), holding a quarantine status in the European Union, was first discovered in 2007 in southern Bavaria. In the course of Diabrotica Research Program of Germany and the states of Bavaria and Baden-Württemberg, an economic accompanying research is conducted at single farm level in Bavaria. This aims to evaluate different adaptation measures concerning the eradication and containment strategies economically.

The focus of the analysis is mainly the crop rotation and the reduction of the proportion of maize at farm level. InVeKoS-data are analyzed, in order to assess the economic relevance of the pest in Bavaria in a better way. These enable detailed information on the development of maize production and cropping intensity. Subsequently research regions are defined with a high maize density, expecting enhanced damage from the beetle. In these selected areas single farms are selected.

For considerations on single farm level, farms with different farm types are chosen by which adaptation measures are evaluated and realistic impact assessment could be made.

For the evaluation of the economic impacts a whole-farm simulation has been used to include indirect effects of different adoption strategies. Additional, semi-structured interviews were conducted at selected farms in order to prove the results of the case study, obtain more information about the consequences on farm level and to evaluate the proposed cultivation alternatives for maize. A benefit-cost analysis and the comparative analysis of possible damage caused by the beetle and cost adjustments complete the study.

Keywords: *Diabrotica virgifera virgifera*, adoption measures, economic impacts, economic evaluation, single farm level

1. Einleitung

Der Westliche Maiswurzelbohrer ist einer der bedeutendsten Maisschädlinge weltweit. Die Hauptschäden werden durch die Larven an den Wurzeln verursacht, was zu einer Beeinträchtigung der Wasser- und Nährstoffaufnahme der Pflanze sowie ihrer Standfestigkeit führt. Der Käfer gilt als sehr mobil, in intensiven Maisanbaugebieten liegt die jährliche Ausbreitungsdistanz der Population zwischen 60 und 80 Kilometern (Albert *et al.*, 2004; Vidal *et al.*, 2004; Baufeld *et al.*, 2006).

Seit dem Auftreten des Käfers in Europa wurde von der Europäischen Union ein Maßnahmenpaket für die Ausrottung und Eindämmung des Maisschädlings verabschiedet. In Bayern werden die geforderten Quarantänemaßnahmen durch eine Allgemeinverfügung der Bayerischen Landesanstalt für Landwirtschaft (LfL) umgesetzt.

Ziel der Studie ist es, die ökonomischen Konsequenzen für landwirtschaftliche Betriebe aus der Umsetzung von Eindämmungsmaßnahmen zu ermitteln. Dabei werden geeignete Maßnahmen berücksichtigt, unabhängig davon, ob sie in der bestehenden Allgemeinverfügung vorgesehen sind.

Ebenfalls soll die regionale Relevanz des Schädlings für Bayern ermittelt werden. Hintergrund hierfür ist die Annahme, dass die ökonomischen Kosten der Anpassungsmaßnahmen sowie die möglichen eintretenden Schäden, die *Diabrotica* verursachen kann, in Abhängigkeit mit der Maisanbauintensität stehen.

2. Material und Methode

Ausgehend von der Annahme, dass die ökonomische Bedeutung des Käfers mit der Maisanbaudichte korreliert, werden im ersten Schritt aus den Daten des Integrierten Verwaltungs- und Kontrollsystems (InVeKoS) und ergänzenden Expertenbefragungen die Untersuchungsgebiete ermittelt. Auswahlkriterien sind die Lage in Bayern, der prozentuale Maisanteil an der Ackerfläche (> 50 % Maisanteil a. d. Ackerfläche) sowie die flächenbezogene Entwicklung des Maisanbaus in den vergangenen Jahren (2005 - 2009). Außerdem wird die Einschätzung von lokalen Beratern berücksichtigt, inwieweit ein Auftreten des Schädlings Anpassungsmaßnahmen in den landwirtschaftlichen Betrieben erforderlich macht.

In den Untersuchungsgebieten erfolgt mit Hilfe von InVeKoS-Daten sowie von Experteninterviews die Auswahl typischer Betriebe. Dabei spielt neben dem regionalen (> 50 % a. d. AF) und dem einzelbetrieblichen Maisanteil (> 66 % a. d. AF) sowie den unterschiedlichen betrieblichen Schwerpunkten (Marktfruchtbau-, Milchvieh-, Biogas-, Bullenmast- und Schweinemastbetrieb) auch die Bereitschaft der Betriebsleiter, an der Untersuchung teilzunehmen, eine Rolle. Insgesamt werden 59 Betriebe in die Untersuchungen einbezogen.

In neun Betrieben werden detaillierte verfahrensökonomische Untersuchungen durchgeführt. Dazu werden die Betriebsdaten jeweils mittels Betriebszweiganalyse (BZA) auf Vollkostenbasis erhoben, ergänzt durch eine mündliche Befragung der Landwirte. Verstärkt eingegangen wird dabei auf Informationen über den Maisanbau in den untersuchten Gebieten, auf Einschätzungen bezüglich Schadpotentials des Maiswurzelbohrers sowie insbesondere auf einzelbetriebliche (Anbau-) Alternativen zum Mais.

Aus den Betriebszweiganalysen, den Befragungsergebnissen sowie weiteren Datengrundlagen werden für die untersuchten Betriebe Deckungsbeiträge der wichtigsten Betriebszweige im fünfjährigen Mittel abgeleitet. Auf dieser Grundlage erfolgt die ökonomische Bewertung verschiedener Anpassungsmaßnahmen auf Betriebsebene. Neben einer Veränderung des Deckungsbeitrags bzw. der variablen Kosten wird für Mehrarbeit ein Lohnansatz (15 €/AKh) berücksichtigt.

Mittels einer Break-Even-Analyse wird sich der Frage genähert, ab welchen Zeitpunkt ein Betrieb freiwillig Anpassungsmaßnahmen wie eine Fruchtfolge vornimmt. Hierfür werden die Anpassungskosten der untersuchten Betriebe den möglichen auftretenden Schäden durch einen *Diabrotica*-Befall gegenübergestellt.

Ergänzend zu den verfahrensökonomischen Untersuchungen wird in 50 weiteren Betrieben aus den Untersuchungsgebieten eine qualitative Befragung in Form eines Leitfadeninterviews durchgeführt. Die qualitative Befragung ermöglicht es, das Vorwissen aus den vorangegangenen einzelbetrieblichen Betrachtungen zu vertiefen und abzusichern. Zusätzlich wird mit dieser Methode ein qualitativer Wissenszuwachs bezüglich der einzelbetrieblichen Konsequenzen, der Bewertung von Alternativen sowie deren Akzeptanz bei den Landwirten gewonnen.

Sowohl in den verfahrensökonomischen Kalkulationen als auch in den qualitativen Befragungen werden unterschiedliche Maisanbauverhältnisse bewertet. Die Varianten umfassen die Betrachtung der Ausgangssituation inklusive Anwendung von Insektizidmaßnahmen, die Reduzierung der Maisanbaufläche auf 67 % und 50 % an der Ackerfläche sowie ein komplettes Maisanbauverbot.

3. Ergebnisse

Mittels Analyse der InVeKoS-Daten und der Experteninterviews werden fünf Untersuchungsgebiete in Bayern eruiert, in denen sowohl die Durchführung von Anpassungsmaßnahmen als auch das vermehrte Auftreten des Schädlings zu bedeutenden ökonomischen Konsequenzen führen kann.

Die betrachteten Regionen liegen im südöstlichen Niederbayern, im südöstlichen Oberbayern, im bayerischen Alpenvorland, in Schwaben und in Mittelfranken. Diese Gebiete definieren sich alle gemeinsam über eine hohe Maisanbaudichte. Hierfür sind zum Teil unterschiedliche Faktoren wie z. B. eine hohe Viehdichte, günstige Standortverhältnisse, ein geringer Anteil Ackerfläche an der landwirtschaftlich genutzten Fläche oder eine hohe Biogasanlagendichte verantwortlich.

Jedoch zeigt sich bereits bei der Auswahl der Einzelbetriebe die Schwierigkeit, in den Untersuchungsregionen, trotz hoher Maisanbaudichte, Betriebe mit mehr als 67 % Maisanteil zu finden.

Die für die einzelbetrieblichen Auswertungen ausgewählten Betriebe, wirtschaften in den Produktionsrichtungen Milchviehhaltung, Schweinemast, Marktfruchtbau, Biogas und Bullenmast. Sie bestellen zwischen 3,7 ha und 240 ha Ackerfläche. Der Maisanteil an der Ackerfläche beträgt zwischen 40 % und 100 %.

Auf die Verknappung der Maisverfügbarkeit müssen die Betriebe individuell reagieren. Je nach Produktionsschwerpunkt entstehen dem Betrieb aus den Anpassungsmaßnahmen Nachteile im mehrjährigen Mittel zwischen 100 und 800 € pro Hektar ersetzte Maisfläche (siehe Tabelle 1).

Tab. 1 Anpassungskosten pro Hektar ersetzte Maisfläche.

Tab. 1 *Adaptation costs per hectare replaced maize fields.*

Produktionsrichtung	Anpassungsmaßnahme	Kosten [€/ha]
Marktfruchtbau	Anbau von Alternativfrüchten (hier: Winterweizen)	100-200
Schweinemast	Zukauf Feuchtmais, Anbau Winterweizen als Marktfrucht, Reduzierung des Maisanteils in der Futterration	100
Milchvieh	Änderung Futterration (grasbetonter), Anbau Klee-/ Weidelgras, Zukauf Futtergetreide, Reduzierung Sojaextraktionschrot	550-650
Bullenmast	Änderung Futterration (grasbetonter), Anbau Klee-/ Weidelgras, Zukauf von Futtergetreide, Reduzierung Sojaextraktionschrot	100-350
Biogas	Substratzukauf (Silomais), Anbau Kleegras	650-800

Marktfruchtbetriebe weisen vergleichsweise geringe Anpassungskosten auf. Dies beruht vor allem auf den hohen Deckungsbeiträgen der Alternativfrüchte Winterweizen.

Da Schweinemastbetriebe in der Regel nur ein Drittel ihrer Körnermaisfläche für die Futterproduktion nutzen, werden sie überwiegend im Marktfruchtbaubereich von den Einschränkungen tangiert und haben daher ebenfalls niedrige Aufwendungen zu leisten.

Futterbaubetriebe treffen dem hingegen relativ beträchtliche ökonomische Auswirkungen. Dafür verantwortlich sind insbesondere der große Wettbewerb um Silomais, hohe Futterzukaufskosten sowie ein erheblicher Anstieg des Arbeitszeitbedarfs in Folge der Substitution von Mais- durch Kleegrasanbau. Auf die Kosten wirkt sich zusätzlich der Pachtzins für zuzupachtende Fläche aus, um gegebenenfalls ein Defizit beim Grundfutter decken zu können. Die Berechnung der Anpassungskosten ist hier von vielen Faktoren abhängig. Der Zukauf von Silomais als Futter ist für die Futterbaubetriebe die einfachste Alternative, wenn die Möglichkeit besteht den Mais zu angemessenen Preisen zuzukaufen. Da aber allgemein wenig Silomais zur Verfügung steht und die Zukaufskosten dementsprechend hoch sind, muss der Maisverbrauch in der Futterration reduziert werden. Hierbei ist zu beachten, dass für einen Hektar Silomais knapp 1,8 ha Kleegras kultiviert werden müssen, um den Grundfutterbedarf decken zu können. Steht dem Betrieb die Fläche zur Verfügung, werden die Anpassungskosten in der Regel niedriger ausfallen, als wenn er Fläche zupachten muss.

Im Biogasbereich sind insbesondere die Zukaufskosten für Silomais für die hohen Anpassungskosten verantwortlich.

Ein Vergleich der gesamtbetrieblichen Auswirkungen weist große Unterschiede bezüglich der Maßnahmen auf. Das Spektrum der jährlichen Anpassungskosten liegt zwischen 760 € für einen Milchviehbetrieb bei Durchführung der Eindämmungsvariante mit 67 % Mais an der Ackerfläche und knapp 130.000 € für einen Gemischtbetrieb, im Falle eines kompletten Maisanbauverbotes. Allerdings ist gegenwärtig nicht damit zu rechnen, dass ein vollständiger Verzicht auf Maisanbau notwendig wird. Die Kosten sind von der Größe der betroffenen Fläche, der Höhe der Maiseinschränkung sowie den betriebsspezifischen Anpassungskosten abhängig.

Bei einem Zukauf von Futtergetreide, Silomais und Feuchtmais wird in dieser Betrachtung davon ausgegangen, dass die Transportwege gleich bleiben. Man muss aber berücksichtigen, dass die Entfernung und die dadurch entstehenden Transportkosten entscheidend dafür sind, ob sich der Zukauf lohnt oder ob andere, günstigere Alternativen gesucht werden müssen. Die Transportkosten liegen bei gut 14 € für einen zugekauften Hektar Silomais und jeden zusätzlich gefahrenen Kilometer und beziehen sich auf einen Zukaufsradius von 5 - 15 Kilometer.

Die benötigte Mehrarbeit wird monetär bewertet und ist in die oben aufgeführten Anpassungskosten mit eingerechnet. Die Spanne dieser zusätzlichen Arbeitszeit ist, ebenso wie die Kosten für die Anpassungsmaßnahmen, sehr weit. Sie liegt zwischen 0 AKh/ha und 10 AKh/ha pro Hektar ersetzten Mais. Bei den Marktfruchtbetrieben liegt der Mehraufwand im unteren Bereich, wohingegen bei den Milchvieh- und Biogasbetrieben mindestens ein Drittel der ursprünglichen Arbeitszeit zusätzlich für die Anpassungen benötigt wird. Grund hierfür ist der arbeitsintensive Ersatz von Silomais- durch Kleegrasanbau. Zum einen muss aufgrund der niedrigeren Erträge mehr Futterfläche bestellt werden, zum anderen steigt der Arbeitszeitbedarf je Hektar wegen der mehrmaligen Ernte von Kleegras.

Die Ergebnisse der Break-Even-Analyse zeigen einen deutlichen Unterschied zwischen Marktfrucht- und Futterbaubetrieben. Sie werden in der folgenden Abbildung 1 graphisch dargestellt.

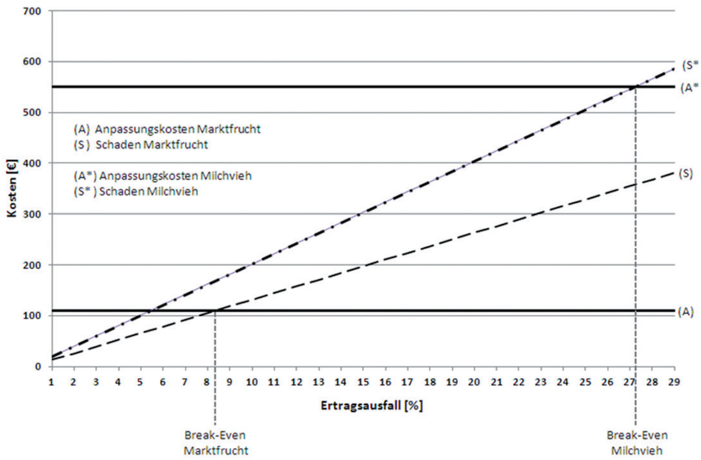

Abb. 1 Ökonomische Schadschwelle für einen Marktfrucht- und einen Milchviehbetrieb in Bezug auf Anpassungskosten und den durch Ertragsausfall bedingten Schaden (nach Musshoff und Hirschauer, 2010, S. 42).

Fig. 1 *Economic threshold for a crop farm and a dairy cattle farm in relation to the adaptation costs and the damage due to yield loss (according to Musshoff and Hirschauer, 2010, p. 42).*

Marktfruchtbetriebe, die vergleichsweise niedrige Anpassungskosten (~ 100 €/ha) zu tragen haben, weisen gegenüber dem möglichen Schaden eine geringe Toleranz auf. Schon ab einem Ertragsausfall von gut 8 % ist der Break-Even Punkt erreicht, der monetäre Schaden ist höher als die Anpassungskosten. Man kann davon ausgehen, dass solche Betriebe bereits relativ schnell mit freiwilligen Anpassungen reagieren, wenn der Käfer in größeren Mengen auftritt.

Anders wiederum ist es bei Betrieben die mit hohen Anpassungskosten (~ 550 €/ha) zu rechnen haben. Für sie ist ein schnelles Handeln nicht unbedingt nötig, da erst bei einem relativ hohen Ertragsausfall (~ 27 %) die Rentabilitätsschwelle erreicht ist. Betriebe mit hohen Anpassungskosten können länger abwarten bzw. ein Jahr mit moderaten Ertragsausfällen verkraften bevor sie handeln müssen.

Im Rahmen der qualitativen Befragung wird auf einer größeren Basis die Thematik diskutiert und die Ergebnisse der vorrausgegangenen einzelbetrieblichen Untersuchungen abgesichert.

Die Entwicklung des Maisanbaus in Bayern wird von den befragten Landwirten ambivalent betrachtet. Zum einen werden die stetige Zunahmen des Maisanbaus und die stärker werdende Konkurrenz um den verfügbaren Mais wahrgenommen. Beides wird insbesondere dem kontinuierlichen Zuwachs sowie der regionalen Kumulation von Biogasanlagen zugeschrieben und zum Teil kritisch bewertet. Zum anderen ist die Tatsache nicht außer Acht zu lassen, dass sich viele der Landwirte in den vergangenen Jahren selbst für den Bau einer Biogasanlage entschieden haben.

Die durchgeführten Interviews machen deutlich, dass die betriebliche Betroffenheit in den ermittelten Untersuchungsgebieten verhältnismäßig gering ist. So ist es schwierig in den Untersuchungsregionen Betriebe mit deutlich höheren Maisanteilen als 67 % an der Ackerfläche zu finden. Ebenso zeigte sich, dass Schweinemastbetriebe eine relativ niedrige Betroffenheit aufzeigen, da sie in der Fütterung nur einen Teil ihrer Körnermaisflächen benötigen. Die notwendigen Anpassungsmaßnahmen bei Auftreten des Westlichen Maiswurzelbohrers stellen nur in einzelnen Betrieben bzw. sehr begrenzten Regionen ein größeres Problem dar. Die Akzeptanzanalyse zeigt, dass die Einstellung der Betriebsleiter gegenüber den Eindämmungsmaßnahmen überwiegend positiv ist. Die Notwendigkeit für die Beschränkung des Maisanbaus auf 2/3 der Fruchtfolge wird erkannt. Sie ist in den Augen der meisten Landwirte Teil der „guten Fachlichen Praxis“ und für eine nachhaltige Landwirtschaft unabdingbar.

Die Frage nach betrieblichen Konsequenzen aufgrund von Anpassungsmaßnahmen wird je nach Betriebstyp und Lage des Betriebes unterschiedlich beurteilt.

Die Hälfte der Befragten gibt an, dass bei einer Situation wie es die 2/3 Regelung vorgibt, es zu keinen größeren Auswirkungen auf ihrem Betrieb kommen wird. Zu erklären ist dies mit der relativ geringen Betroffenheit ihrer Betriebe. In den Betrieben, die angeben von der der Reduzierung des Maisanteils auf 2/3 tangiert zu werden, sind die am häufigsten genannten Konsequenzen die anfallende Mehrarbeit, höhere Kostenaufwendungen, der nötige Maiszukauf sowie Ertrags- und Gewinnausfälle. Der zusätzliche Arbeitsaufwand durch die Substitution von Mais wird als Konsequenz besonders hervorgehoben. In Abbildung 2 werden die erwarteten Konsequenzen und ihre Gewichtung dargestellt.

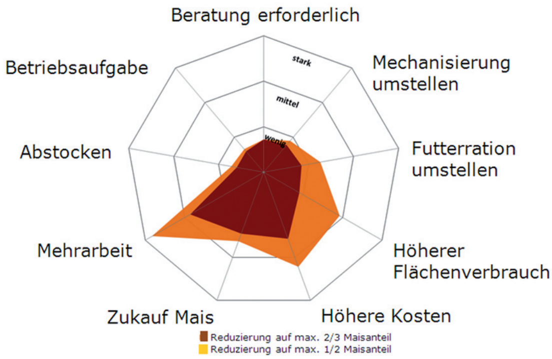

Abb. 2 Gewichtung der betrieblichen Konsequenzen im Fall einer Reduzierung der Maisfläche.

Fig. 2 *Weighting of the consequences resulting for a farm from the reduction of the maize growing area.*

Bei einer Reduzierung der Maisfläche auf 50 % wird von knapp 80 % der Landwirte angegeben, dass es zu betrieblichen Auswirkungen kommt. Besonders häufig werden die Konsequenzen Mehrarbeit, anfallende Zusatzkosten und erhöhter Flächenbedarf von den Befragten genannt (vgl. Abbildung 2).

Als hemmende Faktoren, die Maisflächen zu reduzieren, werden genannt: Standort, Umweltprogramme und die bestehende Futterration.

Die Lage der Ackerflächen spielt besonders in hochwassergefährdeten Regionen, wie z. B. den „Inn- und Rottauen", eine ausschlaggebende Rolle. Das Überschwemmungspotential und das feuchte Klima stellt für viele dort ansässige Betriebe ein Risiko dar, auf den betroffenen Schlägen Getreide anstatt des hochwassererprobten Mais anzubauen. Aber auch in anderen Regionen werden ungünstige Standorte als hemmende Faktoren für den Anbau von Alternativfrüchten angeführt.

Durch die Teilnahme an Umweltprogrammen sind besonders in den Voralpen die Betriebe in ihrem Handeln sehr eingeschränkt. Im KULAP-Programm ist der Grünlandumbruch verboten. Somit ist es nicht möglich, zusätzliche Ackerflächen für den Maisanbau in einer Fruchtfolge zugewinnen.

Grünlandbetriebe mit einer geringen Ackerfläche benötigen diese meistens komplett, um die Maisration ihres Viehbestandes decken zu können. Eine Flächeneinschränkung macht für viele Betriebe das Aufrechterhalten der bestehenden Futterration ohne Zukauf unmöglich.

Bedingt durch die Reduzierung der Maisfläche müssen Biogasbetriebe ihre Ration umstellen und auf weniger energiereiche Energiepflanzen umstellen. Dies hat für sie zur Folge, dass die Energieausbeute nicht optimal aufrechterhalten werden kann.

4. Diskussion

Aus den Kalkulationen und Befragungen zeigt sich deutlich, dass notwendige Anpassungsmaßnahmen bei Auftreten des Westlichen Maiswurzelbohrers nur in einzelnen Betrieben bzw. sehr begrenzten Regionen ein größeres Problem darstellen. Grund dafür ist, dass in vier von fünf Untersuchungsregionen, trotz hohen regionalen Maisanteils, Betriebe mit einem Maisanteil über 67 % relativ selten sind. Zudem sind die Anpassungskosten für Marktfruchtbetriebe vergleichsweise gering. Daher sind die genannten ökonomischen Nachteile nur für einen vergleichsweise kleinen Anteil aller Betriebe zu erwarten. Die erforderlichen Anpassungsmaßnahmen werden von den Landwirten in der Regel als verhältnismäßig geringes Problem gesehen.

Nur in der untersuchten Region in Niederbayern (Unterer Inn und Untere Rott) stößt die Umsetzung der Maßnahmen zum Teil auf größeren Widerstand. Dies liegt vor allem daran, dass das Gebiet, begünstigt durch Klima und Infrastruktur, sehr gut für den Körnermaisanbau geeignet ist. Der Prozentsatz an Betrieben mit einem sehr hohen betrieblichen Maisanteil ist in diesem Gebiet überdurchschnittlich hoch. Der Anbau von alternativen Feldfrüchten wird in Erwartung von möglichen Mehrkosten und -arbeit, bedingt durch erhöhte Überflutungsgefahr, verstärkte Verpilzung durch feuchtes Klima und zum Teil ungünstige Bodenverhältnisse, vermieden. Da für die feuchten Anbaulagen dieses Gebietes bisher nur wenig Erfahrung mit der Kultivierung von Alternativen zu Mais gibt, können bestehende Vorbehalte nicht endgültig bewertet werden. Es liegt aber die Vermutung nahe, dass konkrete andere Gründe für das Ablehnen der Fruchtfolgeregelungen (z. B. mangelnde Erfahrung, Infrastruktur, Mechanisierung, Tradition) gibt.

Allgemein lässt sich sagen, dass es bei einer festen Etablierung des Schädlings immer zu Mehrkosten für die Betriebe kommen wird entweder durch Anpassungsmaßnahmen oder durch auftretende Schäden bei Nichthandeln. Insgesamt kann man jedoch davon ausgehen, dass der Schädling in Bayern weit weniger Probleme macht als beim ersten Auftreten vor wenigen Jahren befürchtet wurde.

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Assessment of economic impacts of the western corn rootworm (*Diabrotica virgifera virgifera*) in Germany

*Bewertung der ökonomischen Auswirkungen des Westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera*) in Deutschland*

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1. Introduction

The western corn rootworm *Diabrotica virgifera virgifera* is known to cause enormous damage to maize crops especially in regions with high maize densities and continuous maize production where the pest can build up high abundances (reported yield losses of 10-30% in south eastern Europe and 20-30% in Italy in 2009 to up to 50% under very favorable conditions for the beetle). Since 2007 *Diabrotica* has been observed in Germany and mandatory quarantine measures according to EC directives 2003/766/EC and 2006/564/EC have been implemented and are in place since that time. Small outbreaks could be eradicated successfully where measures had been taken immediately and consequently like in the federal states of Hesse and Northrhine-Westfalia (Dicke *et al*., 2014). In the federal states of Bavaria and Baden-Württemberg, eradication measures have in contrast not been successful and containment zones have been established in 2010. These zones where suppression measures should be applied now include rather big areas especially in Bavaria (Baufeld, 2014 and Kehlenbeck and Krügener, 2012). In 2012 the complete containment zone reached 1.8 M ha with an estimated maize production area of about 280,000 to 370,000 ha (15-20% maize). Direct yield losses by damage of the beetle, however, have so far not been observed in Germany.

The mandatory measures cause high costs for the Regional Plant Protection Organizations (RPPO) of the Federal States and as well for the maize growing farmers in regions with *Diabrotica* infestation. While RPPOs can claim a part of their expenditures for eradication measures from the EU solidarity funds, economic impacts due to yield losses of maize growers are not compensated. The aim was to evaluate the appropriateness of the plant health measures in comparison to other control strategies of the western corn rootworm in Germany.

2. Methods

Costs of different control strategies and plant health measures were compared to a "no control scenario" without any measures and a "no official control scenario" where maize growers start to apply soil insecticide applications five years after the infestation started and damage becomes obvious. The control strategies consisted of (1) an eradication strategy based on crop rotation with additional insecticide treatments, (2) a containment strategy with two insecticide applications against adults per year or a crop rotation (two times maize within three years) and (3) an integrated approach with measures applied according to injury levels with chemical and non-chemical control options.

Data analysis was based on observed experiences in Germany concerning costs of RPPOs and the range expansion of *Diabrotica* from 2007 to 2012 as well as on estimated data for the further spread of the beetle and expected yield losses. Simulations were performed for the different scenarios with respect to their effect on dispersal parameters according to the spatial explicit spread model developed by Krügener *et al*. (2013) for a period of up to 30 years.

3. Results

Spread, establishment and subsequent economic impact of *Diabrotica* depend on the availability of maize and are favored by non-rotated maize production. Maize production is an important sector of agriculture in Germany and has increased during the past 10 years by about 70% (Fig. 1). This increase could mainly be attributed to an enormous increase in silage maize production while grain maize production did not change significantly. The increase in silage maize runs parallel to an increase in biogas production. In 2012 about 800,000 ha of silage maize were grown for biogas production which represents 30% of the total maize acreage and 40% of the silage maize acreage (FNR, 2013). The main areas for maize production in southern Germany coincide with the regions where *Diabrotica* has established. In addition, *Diabrotica* may find favorable climatic conditions almost all over Germany.

The potential economic impact of the western corn rootworm and the measures to be applied in Germany were expected to be very high since maize growing areas still increase (about 40% from 2007 to 2012) and especially the high percentage of non-rotated cultivation of maize for biogas production supports the further establishment and spread of the beetle.

The costs of the official control measures in Germany are summarized in Table 1. Overall, costs for monitoring, insecticide treatments, and administration sum up to 2.8 M € for the period from 2007 to 2012. Costs of farmers depend very much from their production. Costs per ha can be very high in case of biogas or animal production and moderate in case of grain maize production.

The application of the spread model over a period of 30 years showed that both the "no control" and the "no official control" scenarios lead to the spread of *Diabrotica* all over Germany with high abundances of the beetle and with high potential economic impact (all German maize producing areas are infested and suffer from yield losses or control costs, Tab. 2). The official measures (1) and (2) are successful in avoiding or slowing down spread and therefore the potential impact will never or hardly be reached. Control costs depend on the percentage of maize in crop rotation. For the integrated approach (3) with an efficacy of 90% it took more than 30 years until the potential impact was reached.

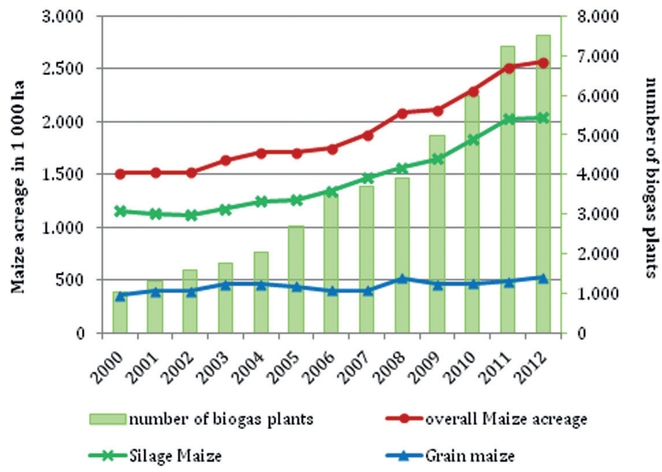

Fig. 1 Maize production and development of the number of biogas plants in Germany (sources: Deutsches Maiskomitee DMK, Fachagentur für nachwachsende Rochstoffe (FNR), Fachverband Biogas 2013).

Abb. 1 *Maisanbau und Entwicklung der Anzahl von Biogasanlagen in Deutschland (Quellen: Deutsches Maiskomitee DMK, Fachagentur für nachwachsende Rochstoffe FNR, Fachverband Biogas 2013).*

Tab. 1 Costs of official control measures incurred in Germany since 2007.

Tab. 1 *Kosten der Bekämpfungsmaßnahmen in Deutschland seit 2007.*

Year	Costs of RPPOs[1] (monitoring, insecticide treatment, administration, etc.)	Costs of farmers
2007	680,000 €	- no documented data available
2008	1,025,000 €	- up to now no yield losses reported (very low *Dvv* abundances) - If crop rotation is applied:
2009	345,000 €	in case of >50% continous maize:
2010	141,000 €	• grain maize about 150€/ha
2011	380,000 €	• silage/green maize about 250€/ha
2012	185,000 €	• maize for biogas 150– 350 €/ha (Bavaria: 650-800 €/ha, Köhler and Schätzl, 2014)
Total	**2,756,000 €**	• animal production – 100– 650 €/ha (Köhler and Schätzl, 2014)

[1] based on data from solidarity claims

Tab. 2 Estimation of spread and impact of *Diabrotica* with respect to different control options based on simulation results according to a spread model (Krügener et al., 2014) with a time horizon of 30 years and a reproduction rate of 10 for *Diabrotica*.

Tab. 2 *Abschätzung der Ausbreitung und der ökonomischen Auswirkungen von* Diabrotica *unter der Annahme unterschiedlicher Bekämpfungsmaßnahmen auf der Grundlage von Simulationen mit einem Ausbreitungsmodell (*Krügener *et al., 2014) über 30 Jahre bei einem Reproduktionsfaktor von 10 für* Diabrotica.

Control option	Expected efficacy	Direct costs of measures per ha and year	Yield loss	Indirect costs[1]	Number of years until potential impact[2] reached
Baseline: "no control"	0 %	0 €	high	----	30
"no official control" (maize growers start to apply control measures - soil insecticide application - after 5 years and if beetle numbers exceed EIL[3])	40%	70 €	high	depend on measure	30
(1) Crop rotation	100%	150– 650 (800) €[4]	very low	depend on measure	Never
(2) Maize growers start to apply control (e.g. two insecticide applications against adults or crop rotation) options immediately irrespective of the EIL	90% 60 %	90 € or >80 €/150 €	very low	depend on measure	------- (no beetles after 10 years) ------- (after 30 years area comparable to start area)
(3) Maize growers start to apply control options (e.g. two insecticide applications against adults or crop rotation) after 5 years and and if beetle numbers exceed EIL[3])	90 %	90 € or >80 €/150 €	low low	depend on measure	takes longer than 30 years

[1] Indirect effects are e.g. effects on bees, problems, with contracts for biogas production in case of crop rotation

[2] Potential impact = all German maize producing areas are infested and suffer from yield losses or control costs

[3] EIL= economic injury level; difficult to estimate; in literature: 1 beetle per plant (about 80,000 beetles per ha) -

[4] 10% maize in crop rotation: 8.64-13.56 M€, 50% maize in crop rotation: 11.61 - 19.74 M€, 70% maize in crop rotation: 13.10-22.83 M € (focus zone (1-2 km radius = 514 ha), safety zone (radius 5 km = 11,000 ha); measures: crop rotation with maize once within 3 years)

4. Conclusions

Eradication by crop rotation showed to be the most cost-effective strategy for single outbreaks and a low maize density in the safety zone (see as well Kehlenbeck and Krügener, 2012). The containment and the integrated approach depend on the efficacy of the measures and the consequent application of the control strategy. Control costs and yield losses are expected to be higher for the integrated approach (due to the later start of the measures and the further spread of the beetle). Crop rotation, however, still is expected to be the more sustainable strategy with less environmental impacts and resistance problems in the long term.

Acknowledgements

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Internationale Fachtagung zum Forschungsprogramm über den Westlichen Maiswurzelbohrer, 14.-16. November 2012, Berlin

Der westliche Maiswurzelbohrer Diabrotica virgifera virgifera, einer der bedeutendsten Maisschädlinge weltweit, wurde 1992 nach Europa eingeschleppt und hat sich inzwischen in vielen wichtigen Maisanbaugebieten Europas ausgebreitet. In Deutschland sind besonders die Bundesländer Bayern, Baden-Württemberg und Rheinland-Pfalz betroffen. In Hessen, Nordrhein-Westfalen, und Sachsen wurden bisher nur einzelne Käfer entdeckt, aber der Maiswurzelbohrer hat sich dort noch nicht etabliert. Um die weitere Ausbreitung des Käfers zu verzögern und die deutschen Maisanbaugebiete langfristig zu schützen, werden effektive Bekämpfungsmaßnahmen benötigt. Daher wurde 2008 vom Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) und dem Bayerischen Landesministerium für Ernährung, Landwirtschaft und Forsten (StMELF) unter Beteiligung des Landes Baden-Württemberg ein Forschungsprogramm aufgelegt. Der vorliegende Tagungsband fasst die Ergebnisse der Projekte zusammen, die während einer internationalen Konferenz im November 2012 in Berlin vorgestellt wurden. Die Projekte umfassten sehr unterschiedliche Aspekte – von der Biologie des Käfer, der Wirksamkeit und Umweltverträglichkeit unterschiedlicher Bekämpfungsmaßnahmen gegen Larven und Käfer bis hin zu den ökonomischen Auswirkungen. Die umfangreichen Kenntnisse, die durch das Forschungsprogramm gewonnen wurden, tragen wesentlich zur Ableitung wissenschaftlicher Empfehlungen für die Eingrenzung und Bekämpfung des Maiswurzelbohrers bei.

Proceedings International Conference on the German Diabrotica Research Program, November 14-16, 2012, Berlin, Germany

The Western Corn Rootworm Diabrotica virgifera virgifera, one of the most significant corn pests, was introduced into Europe in 1992 and has spread and invaded many important corn growing areas in Europe. In Germany, especially the federal states of Bavaria, Baden-Württemberg and Rhineland-Palatinate are affected. Hesse, North Rhine-Westphalia, and Saxony have detected individual specimens but the beetle has not yet established there. Effective control measures are required to slow down further spread of the beetle in Germany to protect corn production in the areas concerned in the long term. To achieve these aims, an extensive research programme was established in 2008 by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) and the Bavarian State Ministry of Food, Agriculture and Forestry (StMELF) with the participation of the federal state of Baden-Württemberg. The proceedings summarize the research results of the projects presented at an International Conference in November 2012 in Berlin. They considered the most varied aspects, from the biology of the beetle, efficacy and environmental compatibility of different control measures for beetles and larvae to economic impacts. The comprehensive knowledge gained within the research program provides scientific recommendations regarding necessary measures for containing and controlling the Western Corn Rootworm.

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