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 experiments (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.

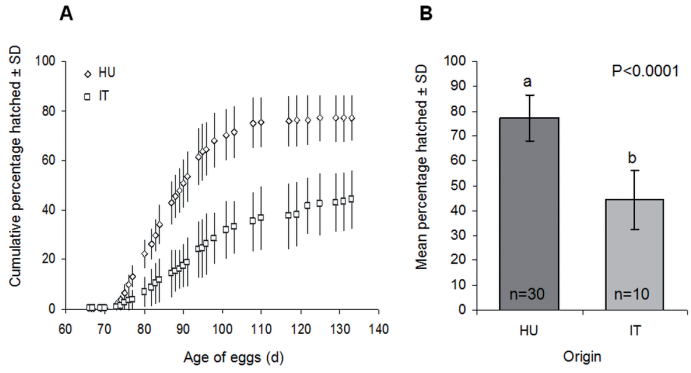

Fig. 1 Percentage of eggs of *D. virgifera virgifera* from Hungary (HU) and Italy (IT) that hatched in relation to **A** = the age of the eggs, **B** = origin of the beetles.

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 prepupae, 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.

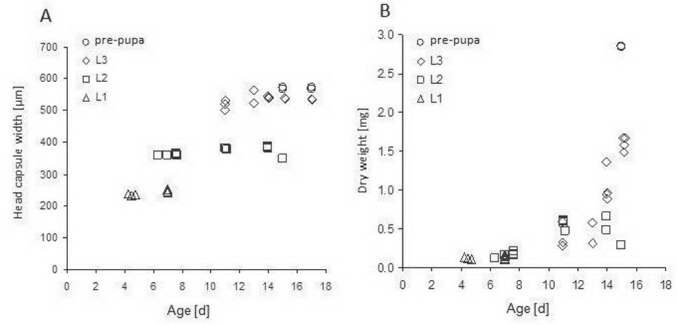

Fig. 2 A = Mean head capsule width; B = mean dry weight of different aged larvae of the non-diapausing *Diabrotica* strain reared at 20 °C.

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.

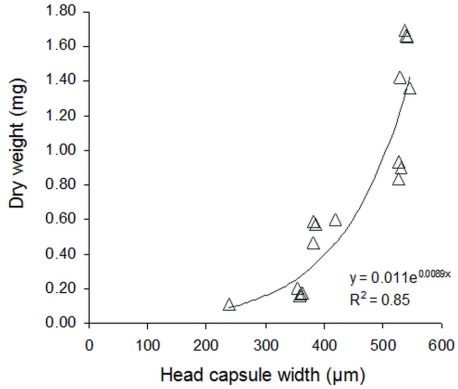

Fig. 3 The relationship between dry weight and head capsule width of the larvae of the non-diapausing strain of *Diabrotica* reared at 20 °C.

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.

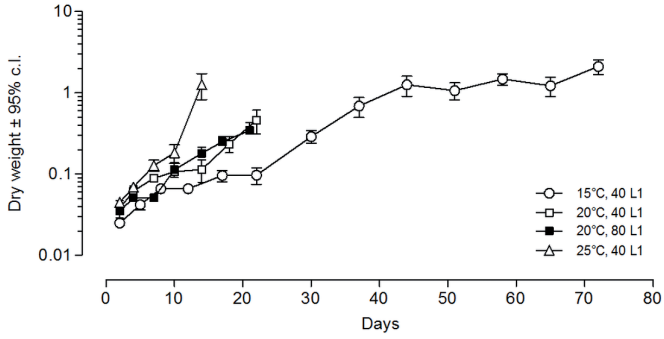

Fig. 4 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the non-diapausing strain of *Diabrotica* from USDA.

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

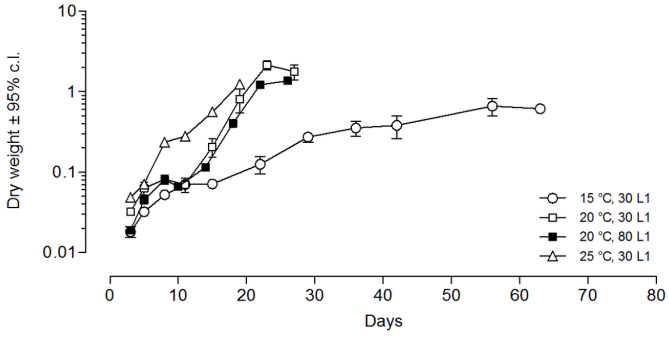

Fig. 5 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the diapausing strain of *Diabrotica* from Hungary.

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.

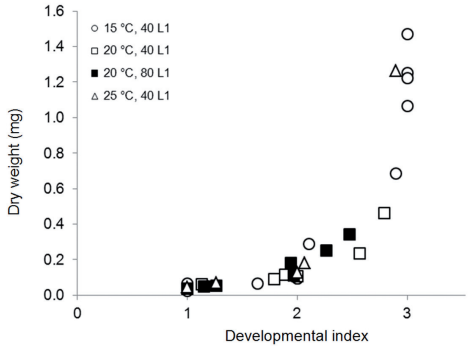

Fig. 6 Effect of temperature and density at which they were reared on the increase in dry weight of larvae of the non-diapausing strain of *Diabrotica* from the USDA in relation to the developmental index (developmental index: mean of developmental stages (L_1=1, L_2=2, L_3=3, Pre-pupa=4, Pupa=5)).

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)).*

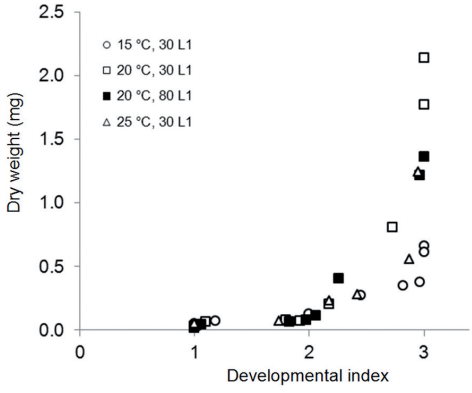

Fig. 7 Effect of temperature and density at which larvae were reared on the increase in dry weight of larvae of the diapausing strain of *Diabrotica* from Hungary in relation to the developmental index (developmental index: Mean of developmental stages (L_1=1, L_2=2, L_3=3, Pre-pupa=4, Pupa=5)).

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:

$$MRGR = (\ln W2 - \ln W1)/D$$

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.

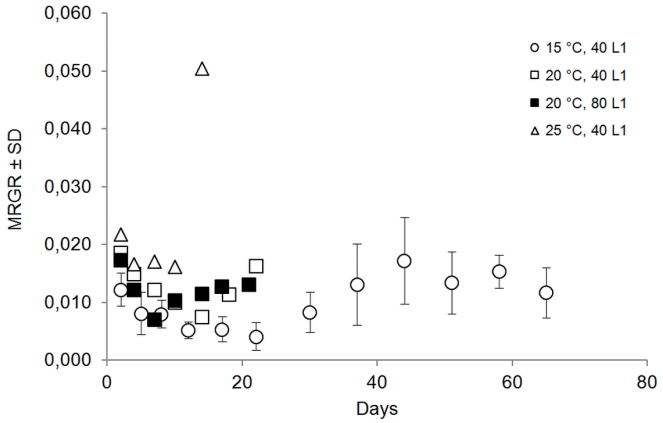

Fig. 8 Effect of temperature and density on the mean relative growth rate (MRGR) of *Diabrotica* larvae of the non-diapausing strain from USDA.

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

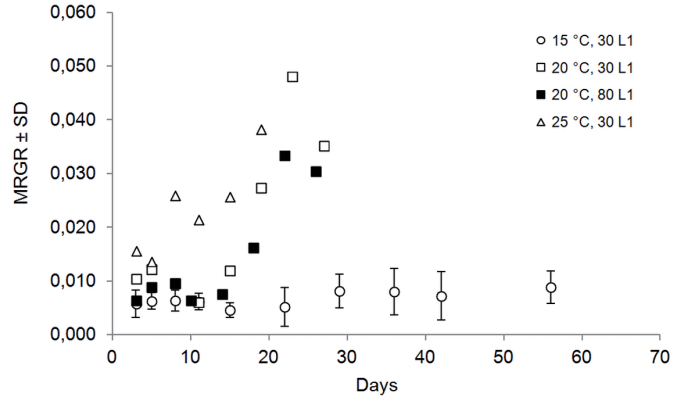

Fig. 9 Effect of temperature and density on the mean relative growth rate (MRGR) of *Diabrotica* larvae of a diapausing strain from Hungary.

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

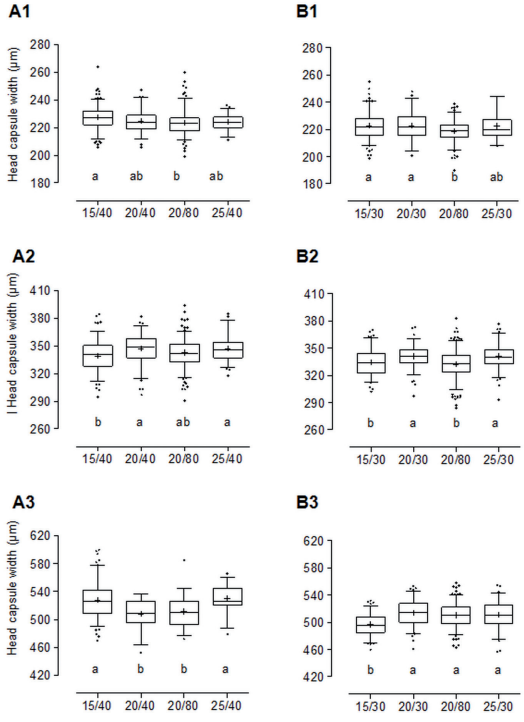

***Fig. 10** Head capsule width of different larval stages (1 L1, 2 L2, 3 L3) reared at different temperatures and larval densities (e.g. 15/40=15 °C, 40 L1). **A** = non-diapausing laboratory strain. **B** = diapausing strain collected in the field in Hungary (different letters indicate significant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