# 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).

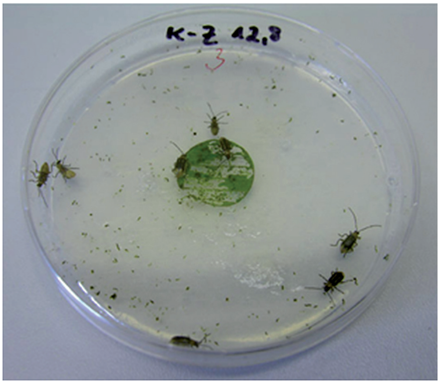


**Fig. 1** Leaf dipping bioassay.

***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.

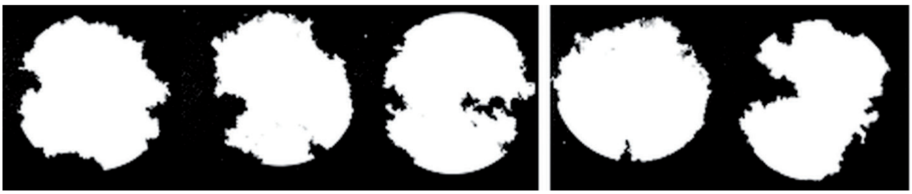

**Fig. 2** Cellulose membranes (d=25 mm) treated with Invite after 6 h of exposure to five adult *D. virgifera virgifera.*

***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.

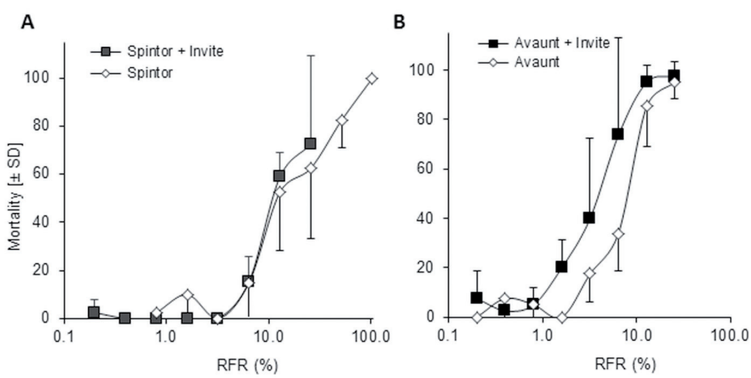


**Fig. 3** Mean percentage mortality (± SD) of adult *D. virgifera virgifera* 24 h after exposure to A – SpinTor/SpinTor + Invite; B – Avaunt/Avaunt + Invite presented on leaf discs. (RFR–recommended field rate).

***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.

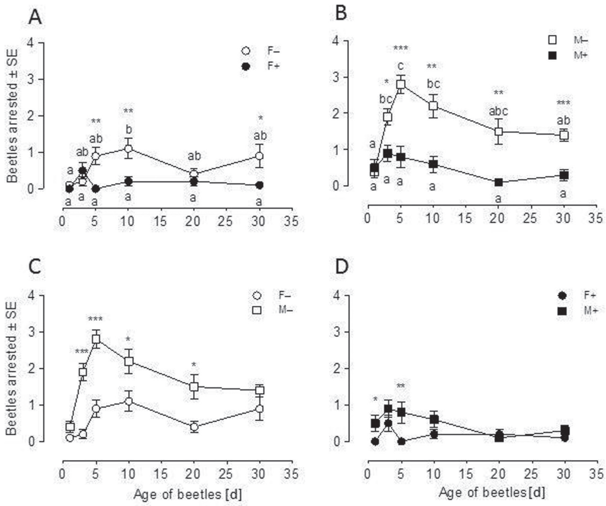


**Fig. 4 A-D** Effect of age (days), sex and pre-contact (PC) on the mean number (± SE) of *D. virgifera virgifera* present on Invite treated cellulose-membranes. (F- females without PC; F+ females with PC; M- males without PC; M+ males with PC, different letters indicate significant effects of age within 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).*

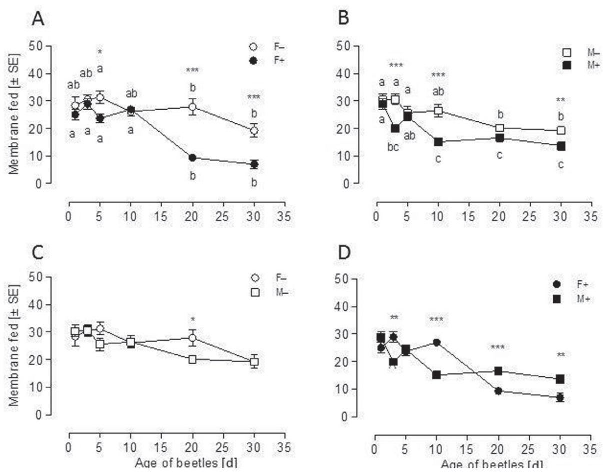


**Fig. 5 A-D** Effect of age (days), sex and pre-contact (PC) on the mean area of Invite-treated membrane consumed (± SE) by *D. virgifera virgifera.* (F- females without PC; F+ females with PC; M- males without PC; M+ males with PC, different letters indicate significant effects of age within a variant, * – 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).

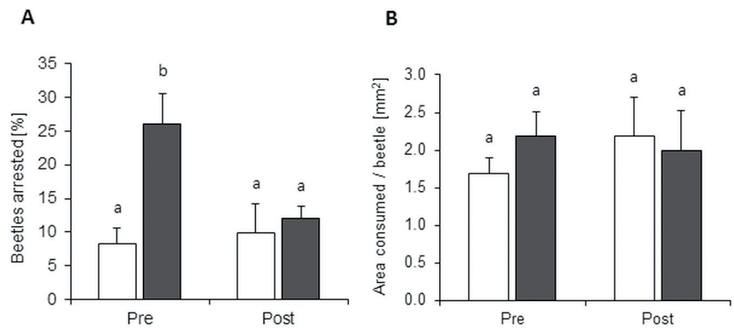


**Fig. 6** Attractiveness of repeatedly applied Invite to *Diabrotica* beetles.

***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.

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