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A new method for efficacy testing of control measures against adult Western Corn Rootworm in maize

Eine neue Methode zur Wirksamkeitsprüfung von Insektiziden gegen Käfer des Westlichen Maiswurzelbohrers in Mais

Abstract

Relative sampling methods, like sticky traps, are commonly used to monitor population fluctuations of flying insects, either to facilitate control decisions in integrated pest management or for evaluation of experimental insecticide treatments in efficacy testing, which is necessary before any authorisation of pesticides in Europe. Concerning the latter, however, trap results do not measure the direct effect of a treatment on a pest population. Rather, they show the activity of alive insects after treatment, which is influenced by the control measure together with many other factors.

In the present study, we describe a field method to directly assess the mortality of adult western corn rootworms (*Diabrotica virgifera virgifera* LeConte) after treatment of maize fields with an insecticide. Fields with continuous maize and heavy *D. v. virgifera* 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 a newly developed method for mortality assessment. The latter consisted of cotton panels mounted between the stems of maize plants, in order to collect dead beetles dropping from the plants beyond the covered area.

After insecticide application, the number of dead beetles collected with these panels in the treated plots was significantly higher than those in the control plots only 1 day and 3 days after application. No significant 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 persistence of the insecticide than proved by the direct mortality assessment with panels.

Key words: Sampling method, relative, absolute, direct, indirect, mortality, insecticide efficacy, *Diabrotica*

Zusammenfassung

Relative Erfassungsmethoden wie der Einsatz von Gelbtafeln sind üblich zum Monitoring von Populationsgrößen von fliegenden Insekten, entweder als Hilfe für Bekämpfungsentscheidungen oder für die Erfassung der Wirkung einer Insektizidbehandlung, wie sie bei der in Europa üblichen Zulassung von Pflanzenschutzmitteln vor einem

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Vertrieb vorgeschrieben ist. Mit diesen Methoden werden aber nur indirekte Effekte erfasst, es wird nur die Aktivität der verbliebenen lebenden Tiere gemessen, die neben der Insektizidwirkung noch von vielen anderen Faktoren beeinflusst wird.

In dieser Arbeit wird eine Methode zu Erfassung der direkten Mortalität von *Diabrotica virgifera virgifera* (LECONTE, 1858) nach einer Insektizidbehandlung in Mais vorgestellt. Dauermaisfelder mit einem starken *Diabrotica* Befall wurden mit einem neonicotinoiden Insektizid behandelt und mit unbehandelten Feldern verglichen. Die Insektizidwirkung wurde mit herkömmlichen, beleimten Gelbtafeln und mit einer neuen Methode erfasst, bei der Baumwolltücher zum Auffangen herabfallender toter Käfer zwischen Maispflanzen aufgespannt wurden.

Ein und drei Tage nach der Behandlung war die Anzahl toter Käfer in behandelten Feldern signifikant höher als in unbehandelten, nach sieben, 14 und 21 Tagen gab es keine signifikanten Unterschiede mehr. In den parallel dazu ausgewerteten beleimten Gelbtafeln fiel die Zahl der gefangenen Käfer nach der Behandlung nach ein, drei und sieben Tagen signifikant ab. Die Ergebnisse aus beiden Auswertungsmethoden attestieren der Behandlung somit eine signifikante Wirksamkeit. Die direkte Zählung toter Tiere in den Stoffbahnen zeigt jedoch, dass die Persistenz des Mittels deutlich kürzer ist, als die Ergebnisse der Gelbtafelauswertung vermuten lassen.

Stichwörter: Relative, absolute, direkte, indirekte Erfassungsmethoden, Mortalität, Wirksamkeit, Insektizide, *Diabrotica*

Introduction

Efficacy of control measures against agricultural pests is often assessed by monitoring pest population fluctuations after application of control agents such as insecticides. Assessment of these fluctuations requires an accurate and at the same time feasible sampling technique (TOLLEFSON, 1986). Highest possible accuracy is achieved with absolute sampling methods, like visual counting of pest specimens, which allows for estimation of the population density per unit of area. On the other hand, high labour costs and time consuming post-processing of huge samples are the major drawbacks of these methods. The use of a relative sampling method, like the employment of traps over a given time, is therefore often chosen as a cheaper and less labour intensive alternative to absolute sampling, albeit at the expense of accuracy.

In addition, population fluctuations monitored with visual counts or by catching of insects 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 activity of alive insects, which is indirectly affected by the tested treatment together with many other factors, including mobility of the test species. MORRIS (1960) determined the selection of an adequate sampling area to be one of the main factors influencing reliability of sampling results. This may be a challenge when study subjects are highly mobile, like flying insects, and plot sizes have to be large enough to minimize the influence of movement between plots and the surrounding.

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Col., Chrysomelidae) is one of the most important pests in maize worldwide and efforts for its control are huge (METCALF, 1986; RICE, 2004). *D. v. virgifera* adults are highly mobile. Flight mill experiments indicate that long distance dispersal flights of *D. v. virgifera* may cover a distance of more than 20 km. Within a series of flights, beetles may fly as far as 40 km in one day (SPENCER et al., 2009; COATS et al., 1986). Distances in every day movement of *D. v. virgifera* within a maize field or between adjacent fields are significantly shorter. SPENCER et al. (2009) estimate average intrafield movement rates of 6–17 m/day, and TOEPFER et al. (2006) have demonstrated flights up to 300 m. It is therefore no doubt that beetles have the potential to move between plots of considerable size within a few days. This may impede reliable evaluation of experiments on the control of adult *D. v. virgifera*.

In the present study, we describe a field method to determine the direct mortality of adult *D. v. virgifera* after treatment of maize fields with an insecticide. Our aim was to develop a technique to assess the direct consequences of a tested control measure on the target insect and to evade dependence on indirect methods like visual counts or employment of traps. We compared the mortality data of the new sampling technique with conventional trap catches in the same plots, in order to demonstrate the differences between direct and indirect mortality assessment and to reveal the influence of additional factors other than the treatment on the population dynamics at the experimental sites.

Material and methods

Experimental setup

Experiments were conducted in 2009 and 2010 in an area with intensive maize production in the north-eastern part of Austria. *D. v. virgifera* was detected for the first time in this region in 2002 (CATE, 2002; BERGER and CATE, 2002). Since then, high pest population densities established in fields with continuous maize, reaching levels of up to 5 beetles per plant (GRABENWEGER, unpubl.). Fields around the village of Deutsch Jahrndorf (48°00'36"N 17°06'33"E) were chosen as experimental plots. Each plot (field) had a minimum size of one hectare with a minimum of 25 m width and a minimum period of three years of continuous maize cultivation. Different maize hybrids of approximately the same maturity group (FAO 380–440) were planted in mid April in both years.

Two variants, a neonicotinoid insecticide treatment (72 g/ha Thiacloprid) and an untreated control, were tested in four randomised replicates each in 2009 and 2010, respectively. The insecticide was applied with a high clearance sprayer (working width 16 m, 24 nozzles) at a rate of 300 l/ha. Applications took place at the end of July in 2009 and at the beginning of August 2010, respectively, when flying periods of the adult beetles were about to reach their first peak. Flight monitoring was carried out with pheromone traps („PAL“ type, Csalomon, Budapest, HU). An average of about 50 to 60 beetles per day and trap is caught with these traps at peak swarming periods in continuous maize fields at the experimental site.

Data collection and analysis

Efficacy of the treatment was assessed with one conventional trapping technique and with the newly developed method for mortality assessment.

First, yellow sticky traps (unbaited „PALs“-type traps, Csalomon, Budapest, HU) were used for estimation of *D. v. virgifera* population densities within treated and untreated fields (Fig. 1). Three traps per field were wrapped around the stem of maize plants in a height of approximately 160–180 cm at distances of 50, 75 and 100 meters from the field margin. Caught beetles were counted immediately before application and on days 1, 3, 7, 14 and 21 after application. Average beetle counts (mean of three traps) were converted to beetles per trap and day and traps were replaced by new ones after counting.

Second, mortality of *D. v. virgifera* adults caused by the treatment was assessed by collecting dead beetles per defined unit of area. Development of an adequate collection advice was inspired by total fauna studies in orchards (BROWN, 1998) and side effect testing of honeybees („water permeable sheets“ in ANONYMOUS, 2010). Cotton panels covering one square meter of soil surface (approx. 75 cm width and 134 cm length) were mounted between the stems of four maize plants of two neighbouring rows in a height of 30 to 40 cm above ground, in order to collect all dead beetles dropping from the plants beyond the covered area (Fig. 2 and 3). Panels did not touch the ground to avoid predation by ground-dwelling insects or rodents. They were made of undyed and unbleached cotton (IKEA® Bomull) which provides a contrastive background to facilitate detection of dead beetles without being visually attractive to live *D. v. virgifera* adults or other insects. A stone (approx. 150–200 g) was placed as a weight in the centre of each panel to avoid loss of beetles due to agitation by wind. In addition, the slope towards the centre of the panel should reduce the loss of sublethally affected beetles trying to leave the panel before they die. One panel was positioned in a distance of about two metres to each of the yellow sticky traps (three per plot). Dead beetles on the upper surface of the panels were counted and removed at the same sampling intervals as shown above. Average beetle counts (mean of three panels) were converted in dead beetles per panel (= square meter) and day.

Data collected with both methods were analysed graphically with box plots and numbers of beetles in treated and control plots were compared at each collection date with Mann-Whitney-U tests, using SPSS.

Fig. 2. Cotton panels mounted between maize plants to collect dead *Diabrotica virgifera virgifera* after an insecticide application (Photo: EGARTNER, AGES Wien).

Fig. 1. Yellow sticky trap to estimate the population density of *Diabrotica virgifera virgifera* (Photo: EGARTNER, AGES Wien).

Fig. 3. Dead beetles of *Diabrotica virgifera virgifera* on cotton panels after insecticide application (72 g Thiacloprid/ha). (Photo: EGARTNER, AGES Wien).

Prior to the experiment, reliability of the panel method was tested by examining recovery rates of marked dead beetles. A total of 60 dead beetles were marked by cutting off the head and one leg and five beetles each were placed into twelve randomly selected cotton panels. After one week of exposure, marked beetles were recovered and counted.

Results

Flight of adult *D. v. virgifera* started at the end of June in 2009 and at the beginning of July in 2010, respectively. The number of caught beetles in the sticky traps before application of the insecticide varied between the experimental plots in both years, showing a heterogeneous distribution of the *D. v. virgifera* population at the experimental sites. On average, however, daily trap catches in control plots and plots assigned to insecticide treatment did not differ in daily trap catches before application (Fig. 4; Mann-Whitney-U, $p > 0.05$).

At the same time, almost no dead beetles were collected with panels in any of the plots, indicating a very low natural mortality of the beetles at the beginning of the flight period (Fig. 5). From five marked dead beetles exposed prior to the experiment, an average of 3.8 ± 1.19 marked beetles per panel was recovered after one week of exposure, resulting in an average recovery rate of 77%.

After insecticide application, the number of dead beetles collected with panels in the treated plots was significantly higher than those in the control plots one day and three days after application in both years (Fig. 5; Mann-Whitney-U, $p < 0.05$). With one exception on day 14 in 2009, beetle collections with panels did not show significant differences between control plots and treated plots seven, 14 and 21 days after application, although there was a general tendency to higher beetle mortality in treated plots.

The number of beetles caught with sticky traps dropped significantly after insecticide application in the treated plots. At the same time, numbers of beetles remained on the same level or even increased in control plots. Differences between treated plots and control plots were significant one, three and seven days after treatment (Fig. 4; Mann-Whitney-U, $p < 0.05$). After that period, numbers of caught beetles in treated plots increased slowly and reached about the same level as before the insecticide application approximately two weeks after the treatment. Hence, no significant differences were found with sticky traps after 14 and 21 days, respectively, in both years (Mann-Whitney-U, $p > 0.05$).

Discussion

The significant differences in beetle numbers between treated and untreated plots showed a high impact of the thiacloprid treatment on *D. v. virgifera* adults. Yellow sticky trap catches as well as beetle counts from cotton panels proved the efficacy of the control measure. Despite this basic concurrence, however, both methods differed in their results on the effective period of the treatment. Results obtained from cotton panels show that the insecticide remains active against adult *D. v. virgifera* for a few days only. After seven days, numbers of dead beetles collected with panels were similar in treated and untreated plots and remained on a similar low level until three weeks after insecticide application. Contrary, yellow sticky trap results indicated significantly increased *D. v. virgifera* mortality (by decreased activity of the beetles) for at least

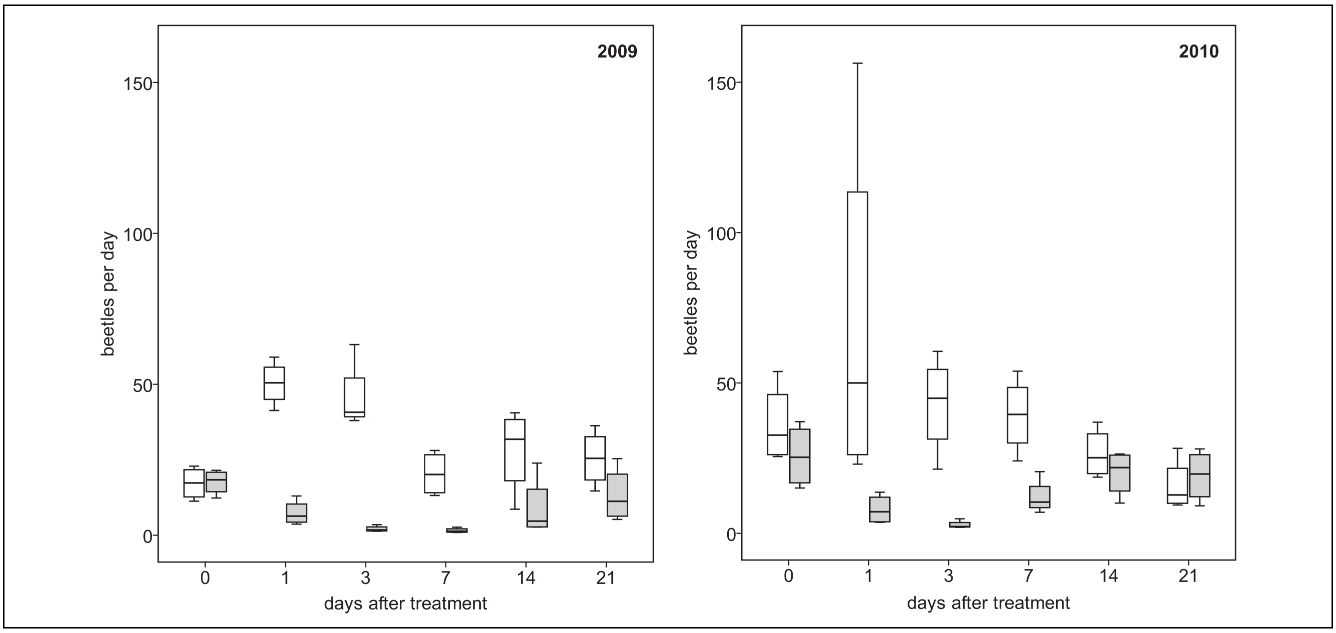

Fig. 4. Boxplots showing the average number of *D. v. virgifera* beetles per day and trap caught with yellow sticky traps in untreated (white) and treated (grey, 72 g Thiacloprid/ha) plots.

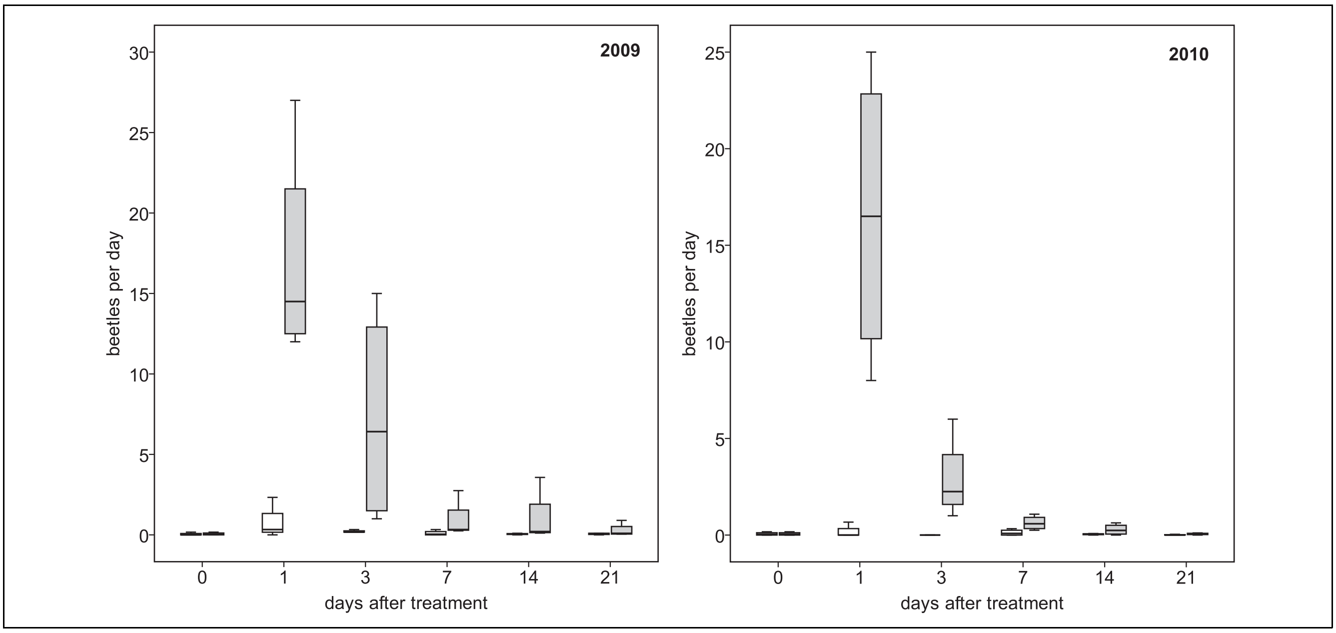

Fig. 5. **Boxplots showing the average number of dead *D. v. virgifera* beetles per day and panel (surface 1 m²) collected from cotton panels in untreated (white) and treated (grey, 72 g Thiacloprid/ha) plots.**

one week. Beetle catches in treated plots caught up to those in control plots only after two weeks. Results were similar in both years.

Apparently, the exclusive employment of sticky traps would have led to an overestimation of the effective period of the insecticide. This demonstrates a weak point of indirect methods for mortality assessment in general. Yellow sticky traps attract and catch alive, flying insects and do not collect specimens killed by an experimental treatment. The trap catches in the presented experiments are a relative measure for the activity of *D. v. virgifera* adults in the traps' capture zones. Low captures in the treated plots therefore showed that few beetles are actively flying around. However, the impact of the insecticide treatment is only one reason for low beetle activity in the treated plots. Besides that, sticky trap results probably also show that there were only few beetles immigrating from adjacent fields. This may be due to plot size, or may even indicate a repellent effect of the insecticide treatment. In addition, phenological effects may be important, like the number of newly hatched beetles emerging from the soil within the plots after the application date. Variation in the sticky trap data is therefore not necessarily linked to beetle mortality but results from many different biotic and abiotic factors influencing beetle abundance and activity.

Panels, on the other hand, collected dead beetles only. Consequently, variation in these data was directly linked to *D. v. virgifera* mortality. Using these beetle counts as a relative measure of mortality in treated versus untreated plots is therefore much more reliable. One might argue that it is impossible to prove that beetles collected from the panels actually died from the experimental treatment. Collections before the treatments and in control plots, however, only occasionally yielded any beetles and resulted in nearly zero *D. v. virgifera* mortality in both years of the experiment. The risk to overestimate mortality with panel collections is therefore considered to be negligible.

Another advantage of the cotton panels is that beetle counts may be assigned to a defined unit of area (one square meter in our experiments). This makes the assessment of absolute mortality rates possible, when combined with an adequate estimation of the size of a *D. v. virgifera* field population (see Tollefson, 1986 for examples). Contrary, the range of yellow sticky traps is not clearly defined, although recommendations on the number of traps to be used per field for monitoring purposes exist (Hein and Tollefson, 1984). Hence, absolute population estimates are not possible with capture results from yellow sticky traps.

The use of panels has additional advantages over the employment of sticky traps, which are not directly linked to efficacy evaluation of insecticide treatments. For example, beetles may be collected easily from panels and remain in good condition for further examination on morphological and physiological parameters, e.g. sex, fertilization status or fecundity. Contrary, solvents need to be applied in order to remove specimens from the surface of yellow sticky traps. Nevertheless, beetles are often too severely damaged for any post-processing in the laboratory. Last but not least, panels are not attractive to non-target organisms, while yellow colour is a widespread stimulus among insects and sticky traps therefore catch masses of specimens from many different taxonomic groups, including many pollinators. Cotton panels may even be considered to monitor non-target effects in control experiments with insecticides, although they are probably only suitable for the collection of adult, flying insects. Last but not least, initial costs for panels are higher than those for yellow sticky traps. The latter, however, need to be replaced after every rating, while panels remain in place during the whole experimental period and may even be used for several experiments/seasons. In the long run, the use of panels is therefore sustainable and cost-efficient.

We demonstrated that the use of cotton panels allows a more realistic estimation of the effective period of an experimental treatment against *D. v. virgifera* adults in maize. It should, however, be mentioned that they are prone to underestimate total efficacy of an insecticide for two reasons. Firstly, insects getting in contact with sublethal doses of an insecticide show protective behaviour in order to avoid contact with the toxicant (HAYNES, 1988; PLUTHERO and SINGH, 1984). In our case, beetles may try to hide and remain inactive for a while in their shelter, e.g. in the axillae of maize leaves. Although they eventually die later, they will probably not drop onto the panels. In addition, sublethally affected beetles which dropped on the panel will try to escape and leave the open arena. These effects may increase with time, as the insecticide degrades, and therefore lead to an underestimation of its persistence.

Secondly, pretesting of our cotton panels has shown that about 23% of the marked beetles put into the panels were lost during one week of exposure. Reasons may be that some beetles get lost during heavy rainfall or when panels are agitated by strong gusts of wind. Experiments on side effects of insecticides have shown that predators reduce the numbers of dead insects to be collected from the ground, thus leading to an underestimation of the mortality (HEIMBACH, 1991). Therefore, panels were mounted on the stems of maize plants in order to avoid contact to the ground and minimize the impact of epigeic predators. However, it cannot be excluded that highly mobile predators, like birds, may also have contributed to these losses.

In conclusion, the employment of cotton panels contributed significantly to the evaluation of the experimental insecticide treatment. While sticky trap results were suitable to demonstrate the general efficacy of the insecticide, they probably overestimated its effective period and varied depending on additional factors other than treatment-driven mortality. The persistence of the insecticide was shown to be considerably shorter when estimated from collection results with cotton panels. These may, however, underestimate the effective period of an insecticide due to the reasons discussed above. Hence, a combination of both methods is advisable to enable a realistic interpretation of test results. This recommendation was recently integrated in a specific standard for efficacy testing of insecticides against adult *D. v. virgifera* by the European and Mediterranean Plant Protection Organization (ANONYMOUS, 2011). In future, dead beetle counts from cotton panels may also be assigned to a defined unit of area. This may facilitate the calculation of mortality rates when combined with absolute field population estimates before the treatment.

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