Nutritional ecology of the invasive maize pest *Diabrotica virgifera virgifera* LeConte in Europe

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Für Carola und Jule!

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Summary:

The nutritional ecology of the invasive maize pest *Diabrotica virgifera virgifera* LeConte (Coleoptera, Chrysomelidae, Galerucinae) was studied with regard to larval and adult food use and performance on various host plants. The adult beetles are feeding mainly on aboveground maize tissues, while the larvae are root feeders on maize and other Poaceae species. This leaf beetle was first detected 1992 near Belgrade and has spread into 15 European countries already. Models predict infestation to occur in all but northern European countries in the following years. The results of the study aim towards a more detailed understanding of processes which determine the invasion potential and success of this pest species.

1) The use of different food resources by adult beetles in Southern Hungary were studied over a 10 week period. In order to evaluate the use of different maize tissues a gut content analyses was performed. Furthermore a detailed pollen analyses was carried out to estimate the use of alternative pollen sources.

- The adult beetles showed a high adaptability with regard to their nutritional ecology in their new range. The majority of all flowering weeds were used as alternative pollen sources.
- The use of maize tissue and pollen from alternative host plants by adult beetle was depending on maize phenology, diversity of flowering weeds in a given habitat and the sex of the beetles.

2) The performance of larvae on maize cultivars from different European countries and several alternative host plants was tested. A new method was developed that allowed to measure how efficient *D. v. virgifera* larvae performed in transferring plant biomass into own body biomass.

- The larval performance showed significant differences on different European maize varieties.
- The larvae performed well on several other grass species which are common weeds in maize fields and also on some monocot crops as winter wheat. They were unable to use roots of dicot weeds as for example *Amaranthus* sp.
- The performance of the larvae on different maize varieties and alternative host plants was related to the total nitrogen content, the C/N ratio and the phytosterol composition encountered in the host plant tissue.

Introduction:

"We must make no mistake: we are seeing one of the great historical convulsions in the world's fauna and flora"
Charles S. Elton, 1958.

Biological invasions occur when an organism is encountered beyond its previous range (Williamson, 1996). Natural invasions are usually long term events, which result in range expansions on continents or in colonization of new areas due to natural events (tectonic movement, land bridges) or historical fluctuations in climate and biota (Mooney and Cleland, 2001). In contrast to these natural invasions most invasions nowadays are results of human activities. Organisms are deliberately or accidentally transferred across natural biogeographical barriers like oceans or mountain ranges due to the worldwide transport of commercial goods and humans (Mack et al., 2000). Invasive species are considered as the second most important factor after habitat destruction responsible for biodiversity loss (Walker and Steffen, 1997). Besides their environmental impact they also pose a heavy threat to national economies (Pimentel et al., 2000). Crop pests are the most obvious invaders to cause economic damage. Invasive arthropod pests are accounting for 14.4 billion USD monetary losses per year due to decreased yield, damage, and control costs in US agriculture (Pimentel et al., 2003). Several invasions were deliberate, like the spreading of crops across the globe. However, as Elton (1958) remarked "Just as trade followed the flag, so the animals have followed the plants". Thus almost 500 years after the arrival of maize in Europe its worst insect pest finally caught up. Since its first discovery 1992 near Belgrade, Yugoslavia, *Diabrotica virgifera virgifera* (Chrysomelidae, Galerucinae) is invading European maize fields and has spread into 14 countries until 2002 (Fig. 1). This leaf beetle is most likely to colonize all European countries except for northern latitudes (Scandinavia) where climatic conditions impede larval development (Baufeld, pers. comm.). The spread may reach up to 100 km per year (Baufeld and Enzian, 2001). Models predict an economic impact of about 500 Mio. € per year in the EU member states (Baufeld, pers. comm.). *D. v. virgifera* (Western Corn Rootworm; WCR) originated in Central America, where it is thought to have coevolved with annual monocot grasses like maize (Eben et al.,

1997). In the US it is known since the beginning of the 20th century and is recognized as the most damaging insect pest in maize since the 1950s. It accounts for 1 billion USD monetary losses per year due to decreased yield and control costs (Krysan and Miller, 1986).

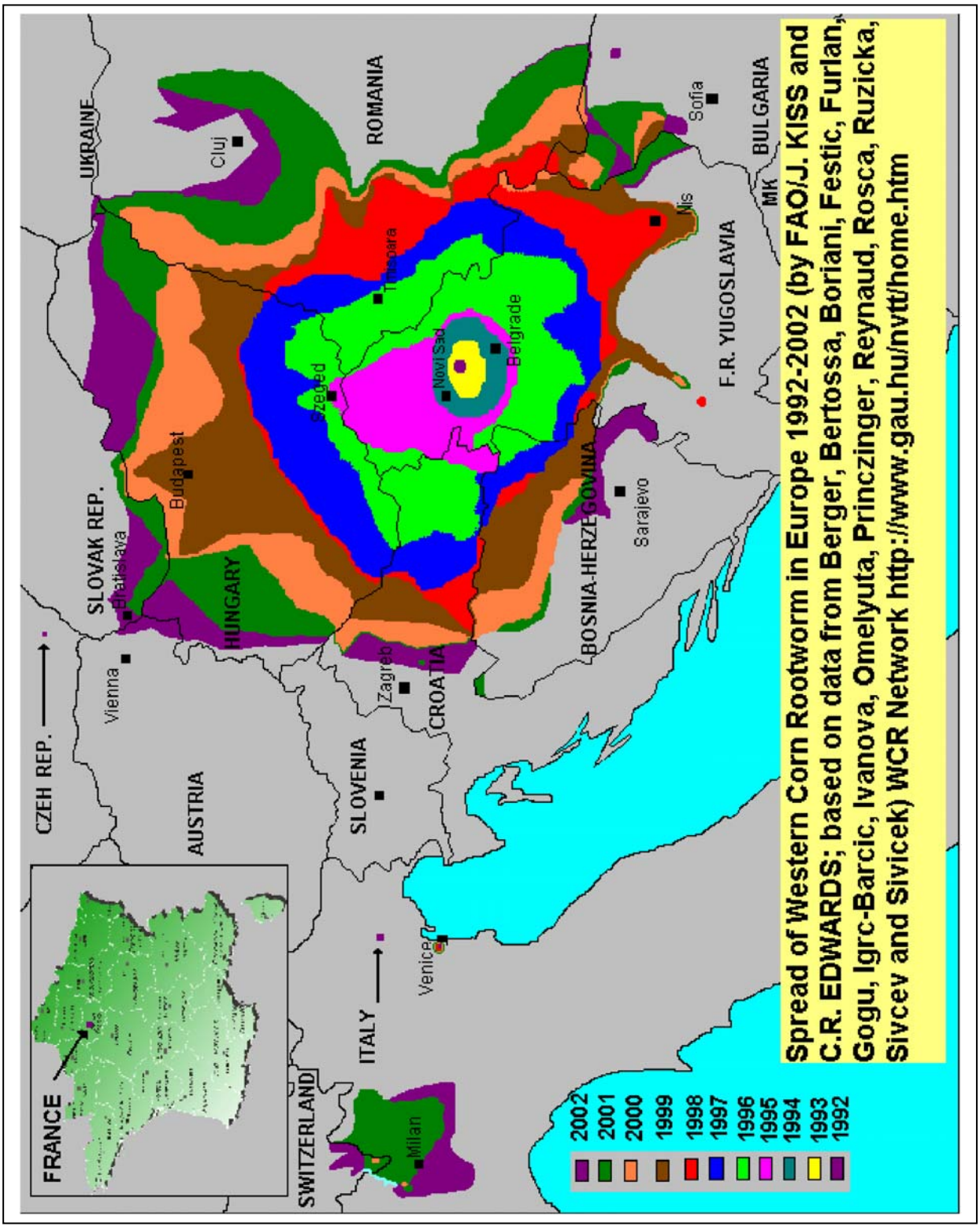

Figure 1: Spread of WCR in Europe until 2002.

The factors which determine success or failure of a given invader still remain unknown to a large extent (Williamson, 1996). Several important features of successful invaders are: the invasion (or propagule) pressure, the ability of an invader to encounter mates and suitable habitats or the invaders ability to adapt to the biotic and abiotic conditions in its new range (Williamson, 1996). Phenotypic plasticity is regarded as being another factor responsible for successful invasions (Agrawal, 2001). In case a species has successfully invaded a new area and eradication is not feasible anymore it is often called for management plans. To perform a risk assessment study and to establish a management plan, a thorough understanding of the population biology of the invasive species in its new range is indispensable (Simberloff, 2003). European maize production differs from the US with regard to production intensity and the cultural practices used. This sets up a different habitat with different resources available for WCR. Thus resource and habitat utilization of WCR in Europe may be significantly different compared to the resources used in its old range mainly in the corn belt of the USA.

WCR is an univoltine species, where the eggs diapause during the winter. The larvae hatch in spring and reach pupation within 40 to 50 days of larval development depending on environmental conditions. All three larval stages feed on maize roots, while the first stage feeds externally and the later two stages mine inside the primary roots (Chiang, 1973). The main host is maize. However larval development is also possible on other monocot host plants (Branson and Ortman, 1970). When reared on alternative hosts, the adult fertility is not significantly decreasing (Branson and Ortman, 1967). Larval mobility is limited and the larvae are not able to discriminate between host and non-host plants by the means of olfaction (Krysan and Miller, 1986). Therefore larval survival is determined by the females ability to find an appropriate host plant for oviposition (Branson and Krysan, 1981). After hatching the adult beetles feed on all aboveground tissue of maize especially pollen and silk (Ludwig and Hill, 1975). The females are able to lay up to 1000 eggs and oviposit during their entire life span from mid summer to late autumn (Chiang, 1973). Preferred sites for oviposition are the base of maize plants. If maize is not available or already harvested, females prefer clumps of monocot weeds over bare soil or maize stalks (Johnson and Turpin, 1985).

The encounter of suitable hosts is essential for all life stages of an invasive species. Which nutritional resources can be used by insect depends on their suitability for insect metabolism. Host suitability is determined by several factors like the content of nutrients and of secondary compounds such as phenols and alkaloids (Scriber and Slansky, 1981). For females it is especially important to find nitrogen rich food for egg maturation (Wheeler,1996). Because root feeding larvae have to cope with very nitrogen poor food (Slansky and Sriber, 1985), their performance is especially influenced by the content of nitrogen.

The carbon-nutrient balance hypothesis has long been used as a tool to predict resource allocation of secondary compounds in plants (Hamilton et al., 2001). This hypothesis predicts that the plant should invest excess carbon or nitrogen into defense metabolites, depending on environmental conditions (Lerdau and Coley, 2002). Besides C- or N-based plant defenses, carbon and nitrogen ratios may also allow to interpret the insects ability to use a given food item, because C and N may be incorporated in nutritive substances like sugars or amino acids as well. Nitrogen may be found as protein- or non-protein-nitrogen (secondary compounds like alkaloids) in plant tissues (Slansky and Scriber, 1985). The influence of nitrogen on insect performance has been well documented by more than 200 studies revised by Scriber (1984), investigating the influence of fertilizers on herbivores. In the majority of these studies a surplus of nitrogen led to increased larval growth and feeding damage. Thus the C/N ratio may be considered a valuable parameter to explain the performance of herbivore insects on different host plants.

Besides the nitrogen content other essential compounds, which are present at much lower quantity in plant tissues play an important role as well. One group of these essential compounds for insects are phytosterols (Svoboda and Thompson, 1985). Sterols have numerous functions in insect biochemistry. They are essential components of cell membranes and serve as precursors of molting hormones (ecdysteroids) in many insects (Svoboda, 1984). Insects as many other invertebrates are unable to synthesize the steroid nucleus. Thus they rely on exogenous sources of sterols for regular development (Svoboda and Thompson, 1985). Metabolic constraints may limit which sterols could be used to support normal growth and development (Behmer and Elias, 2000). The phytosterol content of food items has

been shown to influence herbivore behavior (Behmer and Elias, 1999) and performance (Behmer and Grebenok, 1998). Thus a phytosterol analysis of the host plant tissue may be used to interpret insect performance on different host plants (Nes et al., 1997).

Objectives:

To determine the factors which influence the success of the invasion of WCR in Europe the nutritional ecology of adults and larvae was investigated.

1) A gut content and pollen analyses of field caught adults was performed to show the plasticity in food resource utilization in European maize production systems. More specifically we addressed the questions:
    - ❖ Does maize phenology has an impact on the nutritional ecology of adult WCR?
    - ❖ Is resource utilization of adult WCR habitat dependent?
    - ❖ Do female and male WCR use different components or proportions of the available resources?
2) Furthermore food conversion efficiency studies were conducted to determine the suitability of different European maize varieties and alternative host plants for WCR larval development.
    - ❖ How do WCR larvae perform on different European maize varieties?
    - ❖ Are WCR larvae able to use alternative host plants for their development?
    - ❖ Is the performance on alternative hosts comparable to the performance on maize?
    - ❖ Is the larval performance correlated to the C/N ratio and the phytosterol content we determined in the host plant tissue?

The following chapters have been submitted under the following titles:

- **Chapter 1**: Moeser, J and S. Vidal, 2003b. Does phenotypic plasticity in the nutritional ecology of adults facilitate the invasion of Europe by the maize pest *Diabrotica virgifera virgifera*? Submitted to Agriculture, Ecosystems and Environment.

- **Chapter 2**: Moeser, J. and S. Vidal, 2003a. How to measure the food utilization of subterranean insects: a case study with the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Submitted to Journal of Applied Entomology.

- **Chapter 3**: Moeser, J. and S. Vidal, 2003c. Highly variable response of larvae of the invasive maize pest *Diabrotica virgifera virgifera* to European maize varieties. Submitted to Journal of Economic Entomology.

- **Chapter 4**: Moeser, J. and S. Vidal, 2003d. Do alternative host plants enhance the invasion of the maize pest *Diabrotica virgifera virgifera* in Europe? Submitted to Environmental Entomology.

Does plasticity in adult feeding behaviour facilitate the invasion of Europe by the maize pest *Diabrotica virgifera virgifera*?

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ABSTRACT - The food utilization of adults of the invasive maize pest western corn rootworm (WCR; *Diabrotica virgifera virgifera*) was studied in its newly colonized range in Southern Europe. During a period of ten weeks we collected ten beetles per field per week from six fields with a high abundance of flowering weeds and six fields with a low abundance with the aim of understanding adult feeding behaviour in Europe. Gut content analysis was performed to determine the use of maize tissue and weed pollen with regard to maize phenology. Furthermore, all pollen found within the gut was identified and quantified to plant species level. The use of maize tissue by adult WCR changed with time according to maize phenology. Furthermore, pollen resources other than maize were used more frequently as the maize matured. A more detailed pollen analysis of the beetles revealed that adults fed on a high diversity of pollen, comprising 73% of all weed species (19 different plant species from 25 in total) found within maize fields. The use of different pollen resources was not dependent on their abundance but was determined by the preference of adult WCR for specific weed pollen. Pollen other than maize was found more frequently in beetles from fields with a high abundance of weeds compared to beetles from fields with a low abundance of weeds. Female and male beetles differed significantly in their use of alternative pollen resources; total numbers of pollen were higher in females, whereas males fed on a higher diversity of host plants. The pollen resources used by adult WCR in Southern Hungary are more diverse in comparison to data from the USA. Adaptation of their feeding behaviour to more heterogeneous environmental conditions may contribute to the invasion success of WCR in Europe.

KEY WORDS - Corn rootworm, pollen feeding, nutritional ecology, invasive species, C:N ratio, phytosterols

INTRODUCTION

Invasive species are regarded as the second most important factor responsible for biodiversity loss (Walker and Steffen, 1997) and pose heavy monetary losses on national economies (Pimentel et al., 2000). However, it is as yet a matter of discussion (debate) why invasive species are successful in the areas they invade. One factor responsible for the success of invading animal species is their capability to adapt to the biotic and abiotic settings of their new habitat (Williamson, 1996). To anticipate possible threats to the environment it is mandatory to know the ecological requirements of an invasive organism in its newly colonized habitat in order to carry out a risk assessment analysis and finally consider countermeasures, if eradication is no longer feasible (Simberloff, 2003). However, Simberloff's "first shoot then ask" paradigm is no longer applicable to the invasion of Europe by the Western Corn Rootworm.

Since the first discovery of *Diabrotica virgifera virgifera* LeConte (western corn rootworm; WCR) in 1992 near Belgrade, Yugoslavia, the insect has spread considerably and is now encountered in more than 15 European countries (EPPO, 2003). The numbers of beetles and countries infested is rising each year. WCR has been known in the USA since the beginning of the 20th century. Since the 1950s, it has become the most important pest of maize, causing economic losses of about 1 billion U.S. dollars per year (Krysan and Miller, 1986). WCR is an univoltine species where the females are able to oviposit during their entire adult life span. The adult beetles feed on all above ground parts of maize plants, especially maize pollen and silk (Chiang, 1973; Ludwig and Hill, 1975). However, little is known about the use of plants other than maize as food for adult WCR.

The North American and European maize production systems differ with regard to size of the farms and intensity of maize production per unit area. Farms are more than 10 times larger in the corn belt of the USA compared to the average size in the EU (233:19 ha). Moreover, in the corn belt region maize is grown on more than 23 % of the utilized agricultural area (USDA, 2003), whereas in Europe this is just 3 % (EU

Commission, 2003). Therefore, food resources used by WCR in North America may differ from those used under European conditions.

We hypothesized, that the invasion potential of WCR might be enhanced by more diverse habitats, providing alternative and/or additional food resources. We therefore compared populations of adult WCR from maize fields differing in their abundance of weeds. We used gut content and pollen analyses to identify parameters determining the nutritional behaviour of females and males WCR in southern Hungary both in time and space.

MATERIALS AND METHODS -

The investigation took place in Southern Hungary (Csongrad county) during a 10-week period from the end of June to mid September 2000, the main feeding period of adult beetles in the field. Beetles were collected by hand from maize plants in fields selected with regard to different weed abundances in order to estimate the use of maize and weed-pollen by WCR. Six fields with a low abundance of weeds and six fields with a high abundance of weeds were used. Weedy fields were defined by containing more than three weed plant individuals of any species per transect. Beetles were also collected directly from weeds when encountered there. The maize phenology (Ritchie et al. 1992) was recorded once a week along transects within the fields to gather information on availability of different maize tissues or organs. A single transect comprised the area between two rows of maize at a length of 20 m. The maize rows were numbered and the transects were run following a random number generated by a pocket calculator. An additional randomly generated number between zero and 30 was used as the starting point for the transect, indicating the distance from the field margin. The transects were changed weekly. Additionally, the number of plants with fresh silk was recorded, serving as an indicator for silk availability. The percentage of pollen shedding maize plants was used as a measure of maize pollen availability. Furthermore, the abundance and diversity of flowering weeds was recorded weekly along the transects described above. All plant individuals were counted and determined to species level. Ten beetles were collected from each field, resulting in 120 beetles per week, 60 from each field type. These beetles were stored in the laboratory at –20°C until further processing. From the total 1200 beetles collected, 600 beetles were examined regarding their gut content and another 600 were used in the pollen analysis.

The beetles were dissected by initially cutting off the last segment of the abdomen. The abdomen and thorax were subsequently cut ventrally. After pinning the insect in a water filled wax saucer the gut was removed between the first loop of the intestine and the section between oesophagus and stomach. This piece of intestine was placed on a glass slide and examined with a light microscope. In order to differentiate between the various maize tissues found in the gut, living beetles were fed this specific tissue and were then frozen half an hour later. These voucher specimens were treated as described above, thus serving as a reference to identify the gut content of field caught specimens. Maize pollen could be distinguished clearly from non-maize pollen, silk tissue could be identified because of its characteristic tubus-like appearance. Maize leaf tissue was identified by its characteristic cuticle and stomata, while kernel tissue was recognized by its pebble-like structures.

Another 300 beetles from each field category were used in the qualitative and quantitative pollen analysis. Here only pollen and no other tissue could be identified due to the acetolysation processes. To remove pollen that was attached to the outside of the beetles, they were washed twice in 95 % alcohol. The wings and the elytra were subsequently removed and the remains were washed following the same procedure. The beetles were then placed in 1.5 ml Eppendorf tubes and 1 ml of the acetolysation solution (9:1 concentrated acetic anhydride to concentrated sulphuric acid) was added. The samples were heated in a hot block (100°C) for 15 min., and stirred with toothpicks every 5 minutes. After this time period 0.5 ml of glacial acetic acid was added and the test tubes were placed in a centrifuge (Sigma GmbH, Model 4 K 10) for 3 min. at 4000 rpm. The supernatant was decanted and discarded, the residue vortexed with 1 ml distilled water and centrifuged. This step was repeated two more times. The next washing was performed using 1 ml ethanol (95%). After centrifuging once again two drops of Safranin-0 stain were added, and the solution was centrifuged again. The supernatant was decanted and discarded and three drops of Glycerin were added to the residue. The tubes were placed in a hot block (25°C) overnight so that the ethanol could evaporate. The Glycerin droplets containing the pollen were extracted using an Eppendorf pipette and transferred to a glass slide. The pollen was counted and identified using a previously established reference collection of all weeds encountered within and alongside the margins of maize fields. We created this reference collection by hand collecting flowers in the field and storing them at –20°C until further processing. In the laboratory pollen was

removed from the flowers/anthers by submersion in KOH for 1 min., then neutralizing with distilled water and finally sieving through a 400 µm mesh. The pollen-water obtained was concentrated using a centrifuge and treated in a similar manner to the beetles in the procedure described above.

Systat 10 for Windows (SPSS Inc., 2000) was used for used for statistical analysis. For the gut content and for the pollen analyses, linear regression models were used to describe the relationship between the use of a given food item from maize pollen availability. Beetles from the two field types were compared regarding their pollen feeding and their use of host plants by repeated measures analysis of variance (RM-ANOVA) to measure within and between group variance. We used a one-way analysis of variance (ANOVA) with a Bonferroni adjustment to compare female and male use of alternative food sources from weedy and non-weedy fields to test individual samples against each other in a pair wise comparison.

RESULTS -

Gut content analysis

The use of alternative host pollen by WCR adults was dependent mainly on the availability of maize pollen. The decrease in maize pollen use was positively correlated with the reduction in maize pollen availability. Linear regression explained 54% of the variance ($y = 33.226 + 0.72x$, $P = 0.016$) for beetles from weedy fields and 72% for beetles from non-weedy fields ($y = 26.1573 + 0.79x$, $P = 0.002$; Fig. 1.a). While the percentage of beetles feeding on weed pollen increased to almost 100 % in the weedy fields, only 60 % of the beetles from non-weedy fields fed on weed pollen (Fig. 1.b). The use of weed pollen by adult WCR was negatively correlated with the availability of maize pollen (weedy fields: $y = 29.78 - 0.37x$, $R^2 = 0.38$, $P = 0.07$; non-weedy fields: $y = 27.4 - 0.41x$, $R^2 = 0.42$, $P = 0.04$). Silk feeding was observed to a large extent during the entire study period (Fig. 1.c) showing no correlation with the availability of fresh silk (weedy fields: $y = 25.84 + 0.61x$; $R^2 = 0.24$; $P = 0.15$; non-weedy fields: $y = 40.46 + 0.47x$, $R^2 = 0.11$; $P = 0.35$). Beetles fed only on leaf tissue for a short period at the beginning of the study and again towards the end of the study period. These resources were used less when pollen and silk were widely available (Fig. 1.d). Kernel feeding was especially prominent in beetles from non-weedy fields, when silk and pollen became scarce. On the other hand it was only rarely encountered in beetles from weedy fields (Fig. 1.e). Although none of the correlations were significant, a higher percentage of beetles from non-weedy fields used kernel and leaf tissue. Up to 60% of the beetles had empty guts in the first week of sampling, indicating newly hatched individuals, which had not had time to feed before sampling. This proportion dropped to zero in the second week of sampling and increased slowly again towards the end of the study. Beetles from non-weedy fields were more often found to have an empty gut (Fig. 1.f).

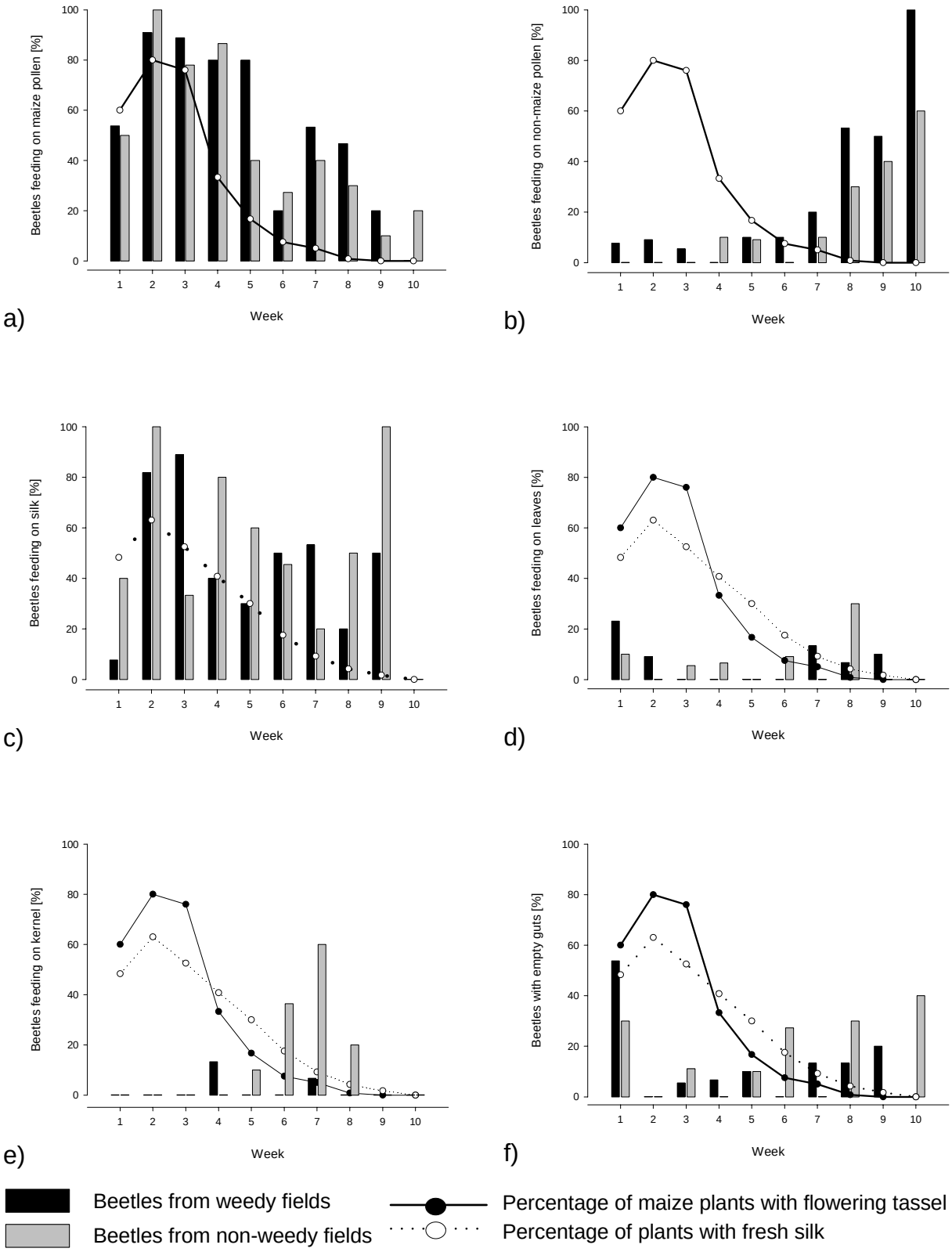

Figure 1: Use of different aboveground maize tissue. The percentage of beetles with the respective gut content is displayed against the percentage of maize plants with flowering tassel and/or fresh silk. a) Maize pollen, b) Weed pollen, c) Silk tissue, d) Leaf tissue, e) Kernel tissue and f) Empty guts. Week 1 = 23.06.2000, week 10 = 08.09.2000.

Pollen analysis

In the pollen analysis a total of 112322 pollen were counted and identified. These belonged to 19 species of plants from nine families (Tab. 1). The list of host plants found in the beetles comprised 73 % of the total weed flora found within the sampled maize fields.

The total number of pollen of different plant species ingested by WCR adults was not dependent on the frequency of these plant species in the field. Although the most common plant in maize field was maize, it was not the most common pollen ingested by WCR adults (Fig. 2).

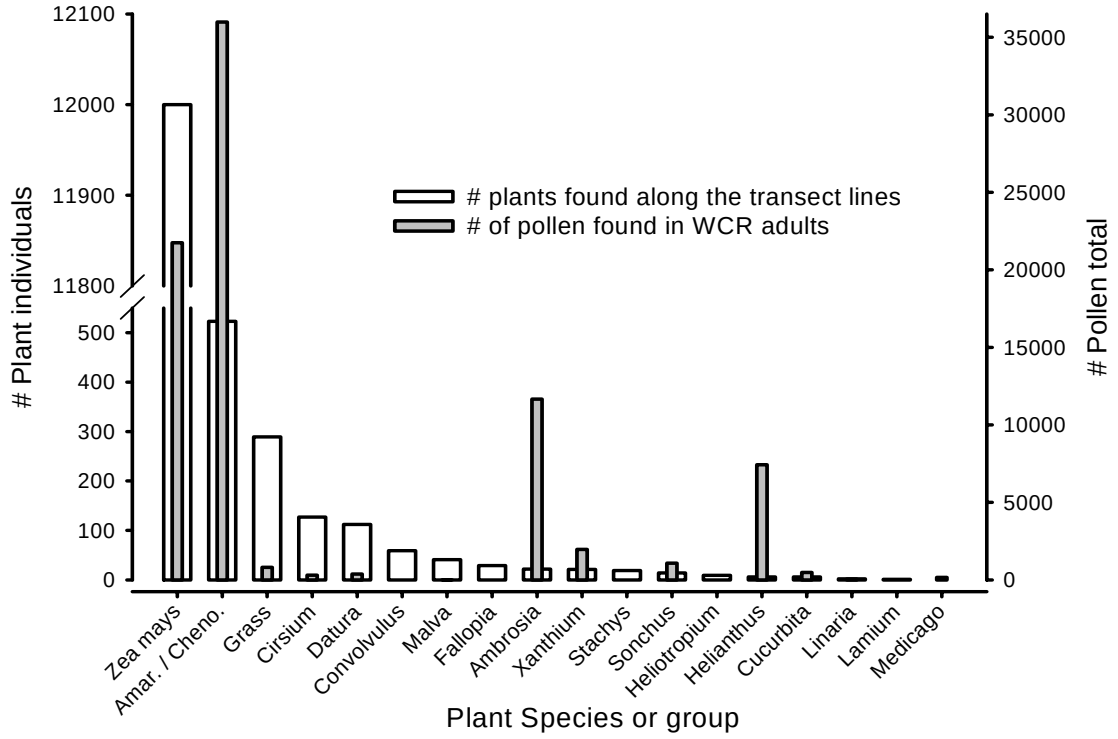

Figure 2: Plant species or species groups and cumulative number of plant individuals counted along all the transects (left Y-axis and thick white bars) and the total number of pollen found inside the guts of adult WCR (right Y-axis and the smaller grey bars)

Table 1: List of host plants used by adult *Diabrotica virgifera virgifera* as revealed by visual observations in South Hungarian fields (visual observ.) or pollen analysis. Which specific plant organ or tissue served as food for WCR is indicated.

Family	**Host plant species**	**Visual observ.**	**Pollen analysis**	**Plant organs affected**
Poaceae	*Zea mays*	yes	yes	Pollen, kernel, leaves, silk
Amaranthaceae	*Amaranthus* sp.	yes	yes*	Pollen
Chenopodiaceae	*Chenopodium album*	yes	yes*	Pollen
Asteraceae	*Ambrosia artemisiifolia*	no	yes	Pollen
	Cirsium arvense	no	yes	Pollen
	Helianthus annuus	yes	yes	Flower petals , Pollen
	Sonchus asper	yes	yes	Pollen
	Xanthium strumarium	yes	yes	Pollen
Cucurbitaceae	*Cucurbita maxima*	yes	yes	Leaves, Pollen
Fabaceae	*Medicago sativa*	yes	yes	Leaves, Pollen
	unknown Fabaceae	no	yes°	Pollen
Malvaceae	*Malva sylvestris*	yes°	yes°	Flower petals, Pollen
Poaceae	*Echinochloa crus-galli*	no	yes*	Pollen
	Setaria pumila	yes°	yes*	Pollen
	Setaria verticilaria	yes°	yes*	Pollen
	Sorghum halepense	yes°	yes*	Pollen
	Sorghum bicolor	yes°	yes*	Pollen
Scrophulariaceae	*Linaria vulgaris*	no	yes°	Pollen
Solanaceae	*Datura stramonium*	yes°	yes°	Pollen
N families = 9	N species = 19 (73%)			

° = rare event

* = impossible to distinguish within group

The use of this pollen increased significantly in both groups over time (F = 5.38;df = 9, 567; P < 0.001; Greenhouse-Geisser epsilon = 0.54; Fig. 3).

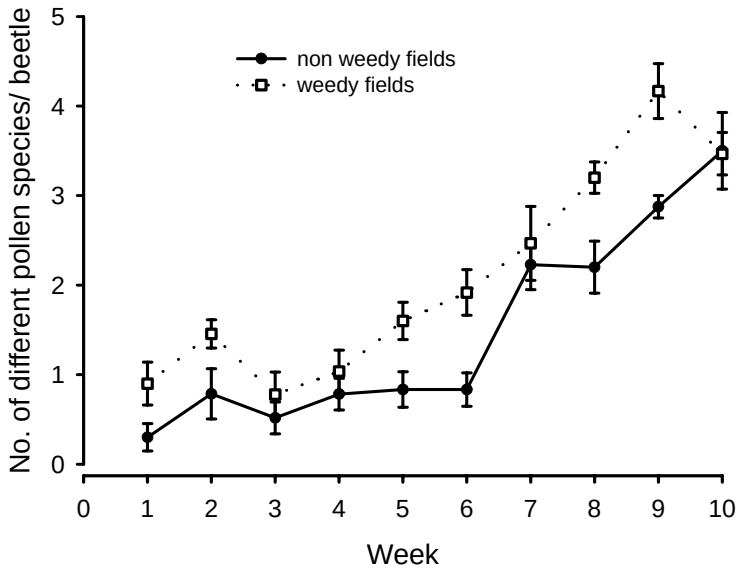

Figure 3: Number of pollen from different plant species per beetle including maize pollen. Week 1 = 23.06.2000, week 10 = 08.09.2000

Moreover, beetles from weedy fields fed significantly more on *Amaranthus / Chenopodium* pollen than the beetles from non-weedy fields (F = 46.2; df = 1,61 ; P < 0.001 Fig. 4). A linear regression between maize pollen availability and *Amaranthus / Chenopodium* pollen use explained 41% of the variance and showed a negative, significant linear relation at the 10% level for beetles from non weedy fields (y = 1.99 -2.94x, P = 0.08). The use of *Amaranthus / Chenopodium* by beetles from weedy fields showed no significant linear relation to maize pollen availability (y = 0.019 + 0.42x, R^2 = 0.05, P = 0.52).

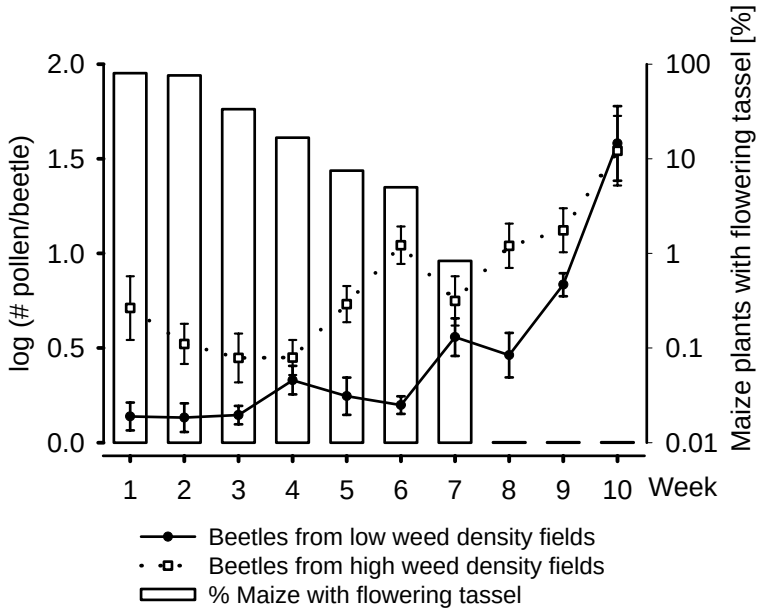

Figure 4: Amount of *Amaranthus/Chenopodium* pollen per beetle (Log transformed data) influenced by time (week 1-10) and habitat (weedy vs. non-weedy fields). Bars indicate the availability of maize pollen (= percentage of flowering maize plants; Week 1 = 23.06.2000, week 10 = 08.09.2000)

No significant difference could be observed in the use of pollen from *A. artemisiifolia* between beetles collected in the two field types (F = 0.27; df = 1. 61; P = 0.61) (Fig. 5). In beetles from both fields a significant increase of *A. artemisiifolia* pollen was found over time (F = 18.82; df = 9. 567; P < 0.001; Greenhouse-Geisser epsilon = 0.32). Even in those fields where the abundance of *A. artemisiifolia* was low, a similar amount of pollen was found in the beetle guts, as in those beetles from the fields that had a high abundance of this weed. The use of *A. artemisiifolia* pollen was due mainly to the decreasing availability of maize pollen (y = 0.68 - 0.37x, R^2 = 0.81, P < 0.001).

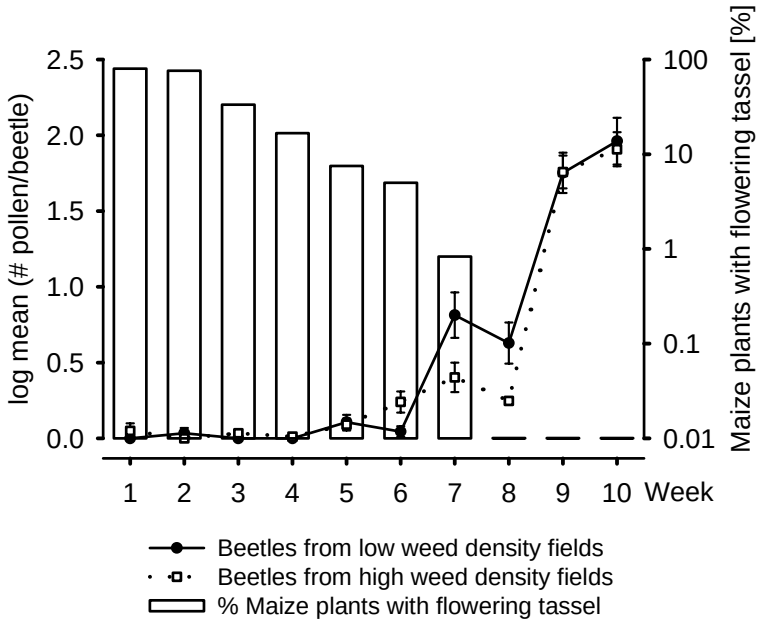

Figure 5: Log transformed data of number of *Ambrosia artemisiifolia* pollen per beetle and the availability of maize pollen (= percentage of flowering maize plants during the study period from week 1 to 10). Week 1 = 23.06.2000, week 10 = 08.09.2000)

Field type as well as sex played a prominent role in influencing the feeding ecology of WCR during the entire study period. Male and female beetles, grouped together from weedy fields, fed significantly more on weed pollen (F = 6.686; df = 1. 599; P = 0.01) and used a bigger array of host plants (F = 29.385; df = 1. 599; P < 0.001) than the beetles from non-weedy fields. There were no differences regarding the use of maize pollen between the two field types (F = 0.853; df = 1. 599; P = 0.356).

However, the differences observed between the sexes were not straightforward. When data from both field types was pooled females were found to feed more frequently on maize and weed pollen than males, although the differences were not significant (F = 2.933; df = 1. 599; P = 0.087 for maize pollen and F = 0.371; df = 1. 599; P = 0.543 for weed pollen).

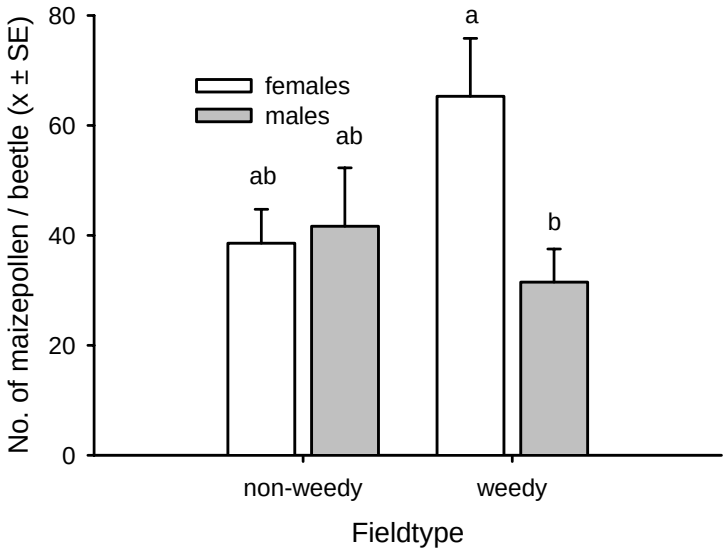

Figure 6: Number of maize pollen per beetle. Female (white bars) and male beetles (grey bars) are displayed separately by field type (weedy vs. non weedy). Same numbers above bars indicate no significant differences between samples (ANOVA).

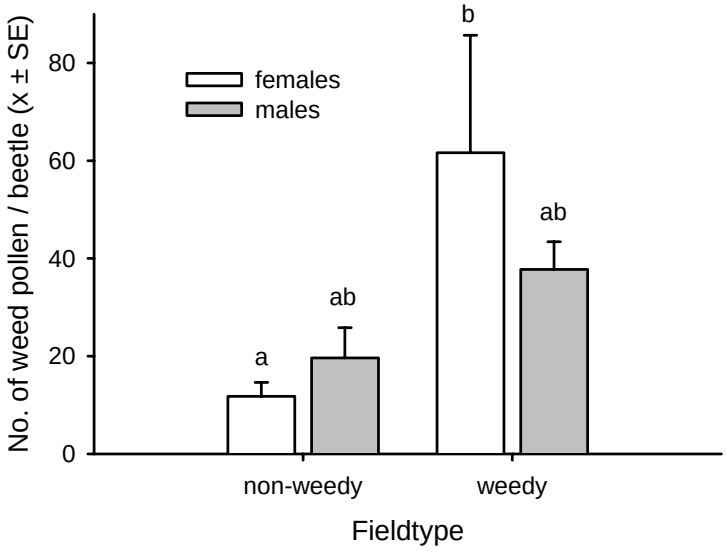

Figure 7: Number of weed pollen per beetle. Female (white bars) and male beetles (grey bars) are displayed separately by field type (weedy vs. non weedy). Same numbers above bars indicate no significant differences between samples (ANOVA, Bonferroni adjustment).

There was a strong interaction between field type and sex (F = 4.236; df = 1. 599; P = 0.04). These interactions could be mainly attributed to the females from weedy fields that fed significantly (F = 2.849; df = 1. 599; P = 0.037) more on maize pollen than the males in weedy fields and beetles of both sexes from non-weedy fields (Fig. 6). Females from weedy fields also fed significantly more on weed pollen than males in weedy fields and beetles of both sexes from non-weedy fields (F = 2.827; df = 1. 599; P = 0.038; Fig. 7).

Generally beetles caught in weedy fields contained a more diverse array of pollen than the beetles from non-weedy fields (F = 29.4; df = 1. 599; P < 0.001; Fig. 8). Furthermore males fed on a significantly higher number of plant species than female beetles (F = 3.876; df = 1. 599; P = 0.05). No significant interaction between field type and sex could be observed.

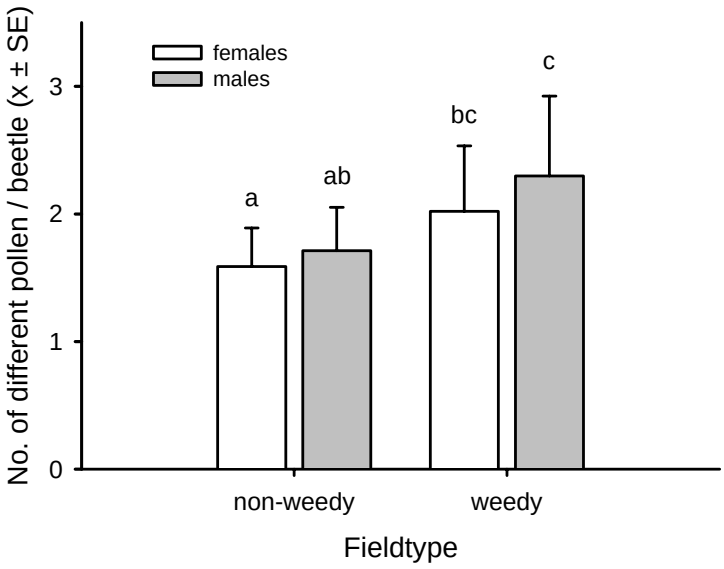

Figure 8: Number of pollen from different plant species per beetle. Female (white bars) and male beetles (grey bars) are displayed separately by field type (weedy vs. non weedy). Same numbers above bars indicate no significant differences between samples. (ANOVA, Bonferroni adjustment).

DISCUSSION:

We were the first to demonstrate that changing maize phenology profoundly influenced food use by WCR in Europe. We were able to show that WCR used more pollen sources in a more diverse habitat. Moreover, we found that female beetles used alternative food resources to a larger extent than male beetles. The feeding

biology of adult WCR exhibited a high plasticity, and was influenced mainly by the three factors discussed below:

Impact of maize phenology changing over time:

Gut content analysis:

Ball (1957) had already hypothesized that the nutritional ecology of adult WCR was based on the availability of maize tissue changing in time. The results of our gut content analysis support his idea. Beetles started feeding on leaves, then on pollen and silk and then finally on kernel and weeds. After the depletion of their primary food source, maize pollen and silk, beetles started to feed on other maize tissue or weed pollen.

Pollen analysis:

Maize pollen was only available during a few weeks at the beginning of the study period, while most other plants flowered during the entire period. Weeds thus provided pollen for a longer time, which explains the finding that WCR fed more on *Amaranthus/ Chenopodium* pollen than on maize pollen. The use of alternative pollen resources such as *Amaranthus/ Chenopodium* or *Ambrosia* increased after maize pollen became scarce. The beetles from both field types fed on more plant species towards the end of the study, when maize pollen availability was close to zero.

Ludwig and Hill (1975) described the different maize tissues used by WCR but did this only for a single sampling date at the end of July. They encountered only two species of weed pollen: *Amaranthus* sp. and *Ambrosia* sp., where *Amaranthus* sp. was used by almost 100% of all beetles sampled, while *Ambrosia* sp. played only a minor role, being absent from beetles caught on weeds and being present only in 6-8% of all beetles caught on maize. Furthermore they proposed a change in food use after pollen shedding and silking of maize. They contrasted the behavior of the closely related *Diabrotica barberi* Smith & Lawrence (Northern Corn Rootworm = NCR), which started to feed more on weed pollen after maize became unsuitable as a food source, while WCR was supposedly feeding more on other maize tissue. Cinereski and Chiang (1968) also observed an increase in non-maize pollen over time in NCR. Here we demonstrated that the feeding behavior of WCR, at least in Southern Europe, is much more plastic than described by Ludwig and Hill (1975), and showed a comparable response to the depletion of maize pollen as NCR. This

was also reported by O´Neal et al. (2002). They found that the influence of maize phenology led to feeding on soybean foliage in the presence of older maize, indicating the possibility of highly variable responses with regard to different environments. Elliot et al. (1990) found that survival in WCR decreases with plant age, more so for older than for younger beetles. However, whether survival of the beetles increases in the presence of flowering weeds acting as alternative pollen sources remains to be investigated.

Impact of habitat:

Gut content analysis

Weedy fields provided alternative pollen resources that were used to a large extent by adult *D. v. virgifera*. Beetles from fields with a low abundance of weeds may have to exploit less suitable food sources such as maize leaves, or an even higher proportion will be found to have an empty gut. WCR tends to feed on those items that are most available (Ludwig and Hill, 1975). They found that silk was the food most available in non-weedy fields, while in weedy fields weed pollen was the most prominent food for WCR. We were able to support their data in so far, as that beetles in weedy fields were using significantly more weed pollen, although beetles were feeding equally on silk tissue in both field categories.

D. barberi does not feed on maize leaves, it leaves the maize field to feed on other pollen when pollen and silk are too dry. (Ludwig and Hill, 1975). Similar behavior could also be observed for WCR that were caught in weedy fields. They fed less on maize leaves and more on weed pollen.

Pollen analysis

In comparison to the closely related subspecies *D. v. zeae* Krysan and Smith, which was found to feed on 45 different plant families and 63 different genera (Jones and Coppedge, 2000), the list of host plants compiled in this study seems less impressive , however they comprise 73% of all plant species present within the sampled maize fields in southern Hungary.

In some cases weeds acted as additional pollen sources. This could be observed for beetles from weedy fields that fed on *Amaranthus/Chenopodium* pollen during the entire study period to an equal extent regardless of maize pollen availability. Beetles from non-weedy fields fed on these plants only after maize pollen

became scarce. Feeding on *Ambrosia* describes an alternative use of this host plant, following the decrease in maize pollen availability. Beetles from both field types fed to a similar extend on Ambrosia pollen. Thus, WCR exhibited a certain preference for *Ambrosia*, partly explained also by the late flowering of this weed that started around the 4th week of July. *Ambrosia* was the only plant in maize fields which provided a lot of pollen during this vegetation period.

For a long time WCR was regarded as a beetle that neither leaves maize fields (Branson and Krysan, 1981) nor flies as actively between maize fields when edible maize tissue becomes scarce; behavior which is well known from the closely related species *D. barberi* (Naranjo, 1991). However, our results clearly demonstrate that WCR did leave the fields to feed outside on weed pollen to a large extent, when maize was not a useable resource anymore. We suggest short distance flights along the margins, to forage, for example, on *Ambrosia*, or to adjacent fields to feed on sunflower pollen.

Hill and Mayo (1980) found practically no WCR beetles on weeds but mentioned *Amaranthus* sp., *Ambrosia* sp., *Setaria* sp. and *Sorghum* sp. to be host plants without showing any data. We found all these plant species occurring in or near maize fields to be alternative pollen sources for this pest. Cinereski and Chiang (1968) found pollen from maize, Gramineae, Compositae, Leguminosae and Cucurbitaceae in the guts of the closely related species *D.barberi*, which is though to feed on a wider array of host plants than *D. v. virgifera.* However, our results provide evidence that WCR is feeding on a wider host range than was realized up to now. Studies on phagostimulation due to amino acids present in pollen (Hollister and Mullin, 1999) revealed that WCR fed more on maize and squash pollen than on sunflower and goldenrod in a no-choice experiment. They attributed these findings to the presence of a combination of specific amino acids. However, they did not include further data on other pollen that we found in our study. We therefore speculate that there may be either more general cues leading to pollen feeding in adult WCR than previously known or that pollen feeding is limited by just the presence or absence of flowering weeds.

Preferences for specific weed plants which we discovered in beetles from non-weedy fields which had fed on *Ambrosia artemisiifolia* pollen have not been reported up to now. However, phagostimulatory cues, which could explain these findings, remain to be analyzed. McKone et al. (2001) found only *D. barberi* to feed on

sunflower but not *D. v. virgifera*. Mullin et al. (1991) even isolated and identified antifeedants from sunflower and *Solidago canadensis* pollen, suggesting that Asteraceae are not beneficial food sources for *D. v. virgifera*. In addition they described a decreased longevity when adults fed on floral parts of sunflower as compared to maize. However, this data originates from no-choice tests with beetles feeding exclusively on sunflower for their entire life span.

Impact of sex:

Pollen analysis

Female beetles fed more on weed pollen than males. This may be explained by the necessity of females to find nitrogen rich food for egg maturation (Wheeler, 1996). As oviposition takes place during the entire life span of a female, alternative pollen sources are extremely valuable when maize pollen is not longer available. The best food for egg production in WCR is maize pollen and green silk (Elliot et al., 1990) but weed pollen may contribute as well. If however the more extensive use of alternative food resources by females leads to a higher fecundity, a higher population density or faster population buildup remains to be investigated. Males fed on a wider array of host plants than females, although each individual had less pollen in its gut compared to females. We hypothesize that males are more mobile than females thus encountering more weeds as they fly around in the maize fields. This idea is supported by Naranjo (1991) who found males to be more active flyers than females. In maize, Ludwig and Hill (1975) found that more males than females had maize plus weed pollen in their guts, which also favors our mobility hypothesis.

Pavuk and Stinner (1994) concluded from their studies that weeds in maize fields had no significant effect on WCR populations, although higher numbers of beetles were encountered in mixed weeds plots. Hungarian population densities in our study were too low to obtain a reliable estimate if weeds support a higher population density or not. As Siegfried and Mullin (1990) pointed out, the longevity of females is significantly reduced when fed exclusively on alternative food such as squash blossom or sunflower inflorescences compared to females maintained on maize ears, although the former diet keeps them alive enabling the production of viable eggs. This scenario does not happen in natural settings and alternative food sources are mainly used additionally, not exclusively. The same conclusion holds

true for Mullin et al. (1991) who argue that Asteraceae pollen is not a food source for WCR. Contrary to these findings we could clearly demonstrate, that WCR in Europe fed to a large extent on Asteraceae pollen from several plant species.

Our results apply only to southern Hungary. As weed composition changes feeding ecology will change, too. Therefore, as *Ambrosia* is not present all over central Europe it would be of particular interest, to investigate if WCR would exhibit similar preferences for other late flowering weeds in other parts of Europe. Moreover it would be interesting to know, how the feeding ecology of WCR varies with the flowering weeds present.

We clearly demonstrated the large plasticity of adult WCR nutritional ecology. The observed adaptability of WCR to the nutritional resources of European agro-ecosystems could be one important factor which may contribute to the invasion potential and spreading capability of WCR of up to 100 km per year (Baufeld, 2001). We conclude that a high abundance of alternative pollen sources may facilitate spreading, may lead to a higher survival and fecundity and subsequently to higher levels of damage as well as higher population densities in the following year.

ACKNOWLEDGMENTS:

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REFERENCES CITED:

Ball, H. J., 1957. On the biology and egg-laying habits of the Western Corn Rootworm. J. Econ. Entomol. 50, 126-128.

Baufeld, P, Enzian, S., 2001. Simulation model spreading scenarios for Western Corn Rootworm (*Diabrotica virgifera virgifera*) in case of Germany. Proceedings of the XXI IWGO Conference, Legnaro, Italy. Veneto Agricoltura. pp 63-67.

Branson, T. F., Krysan, J. L., 1981. Feeding and oviposition behavior and life cycle strategies of Diabrotica: An evolutionary view with implications for pest management. Environ. Entomol. 10, 826-831.

Chiang, H. C., 1973. Bionomics of the northern and western corn rootworm. Annu. Rev Entomol. 18, 47-72.

Cinereski, J. E., Chiang, H. C., 1968. The pattern of movement of adults of the Northern Corn Rootworm inside and outside of corn fields. J. Econ. Entomol. 61, 1531-1536.

Elliot, N. C., Gustin, R. D., Hanson, S. L., 1990. Influence of adult diet on the reproductive biology and survival of the western corn rootworm, *Diabrotica virgifera virgifera*. Entomol. Exp. Appl. 56, 15-21.

EPPO, 2003. http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_ virgifera.html.

EU Commission, 2003. http://europe.eu.int/comm/agriculture/

Hill, R. E., Mayo, Z. B., 1980. Distribution and abundance of corn rootworm species as influenced by topography and crop rotation in eastern Nebraska. Environ. Entomol. 9,122-127.

Hollister, B., Mullin, C. A., 1999. Isolation and identification of primary metabolite feeding stimulants for adult Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte, from host pollen. J. Chem. Ecol. 25, 1263-1280.

Jones, G. D., Coppedge, J. R., 2000. Foraging resources of adult Mexican corn rootworm (Coleoptera: Chrysomelidae) in Bell County, Texas. J. Econ. Entomol. 93, 636-643.

Krysan, J. L., Miller, T. A., 1986. Methods for the study of pest *Diabrotica*. Springer Verlag, New York.

Ludwig, K. A., Hill, R. E., 1975. Comparison of gut content of adult Western and Northern Corn Rootworm in Northeast Nebraska. Environ. Entomol. 4, 435-438.

McKone, M. J., McLauchlan, K. K., Lebrun, E. G., McCalls, A. C., 2001. An edge effect caused by adult corn-rootworm beetles on sunflower in tallgrass prairie remnants. Conservation Biology 15, 1315-1324.

Mullin, C. A., Alfatafta, A. A., Harman, J. L., Serino, A. A., Everett, S. L., 1991. Corn rootworm feeding on sunflower and other Compositae. In P. A. Hedin (Ed.) Naturally occurring pest bioregulators. Symposium series 449. American Chemical Society, Washington, DC. pp. 278-292.

Naranjo, S. E., 1991. Movement of Corn Rootworm beetles, *Diabrotica* spp. (Coleoptera: Chrysomelidae), at cornfield boundaries in relation to sex, reproductive status, and crop phenology. Environ. Entomol. 20, 230-240.

O´Neal, M E., DiFonzo, C. D., Landis, D. A., 2002. Western Corn Rootworm (Coleoptera: Chrysomelidae) feeding on corn and soybean leaves affected by corn phenology. Environ. Entomol. 31, 285-292.

Pavuk, D. M., Stinner, B. R., 1994. Influence of weeds within *Zea mays* crop plantings on populations of adult *Diabrotica barberi* and *Diabrotica virgifera virgifera*. Agricult. Ecosyst. Environm. 50, 165-175.

Pimentel, D., Lach, L., Zuniga, R., Morrison, D., 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50, 53-65.

Ritchie, W. W., Hanway, J. J., Benson, G. O.. 1992. How a corn plant develops. Iowa State University of Science and Technology Cooperative Extension Service, Special Report 48.

Siegfried, B. D., Mullin, C. A., 1990. Effects of alternative host plants on longevity, oviposition, and emergence of Western and Northern Corn Rootworms (Coleoptera: Chrysomelidae). Environ. Entomol. 19, 474-480.

Simberloff, D., 2003. How much information on population biology is needed to manage introduced species? Conserv. Biol. 17, 83-92.

SPSS Inc., 2000. Systat 10 for Windows; SPSS Inc., Chicago.

USDA, 2003. http://NASS.USDA.GOV/CENSUS/

Walker, B., Steffen, W., 1997. An overview of the implications of global change for natural and managed terrestrial ecosystems. Conserv. Ecol. 1: http://www.consecol.org/vol1/iss2/art2.

Wheeler, D.,1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41, 407-431.

Williamson, M., 1996. Biological Invasions. Chapman & Hall, UK. 244 pp.

How to measure the food utilization of subterranean insects: a case study with the Western Corn Rootworm (*Diabrotica virgifera virgifera*).

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Keywords: Corn Rootworm, food conversion efficiency, ECI, food utilization, soil insects

Introduction

Studies of food conversion efficiency are used to determine the suitability of a particular food item for the development, growth or maintenance of animals (Waldbauer, 1968). When carried out on insects these studies on food conversion efficiency were up to now always limited to aboveground mostly leaf or shoot feeding insects. Insects which feed belowground or on the roots were neglected on account of methodological difficulties in handling the insects and because direct observations were not possible. The following description provides information on an experimental design which allows to measure feeding and to subsequently calculate food conversion efficiency for belowground feeding insect larvae of the maize pest Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte). This method was developed in order to acquire knowledge on the impact of different maize varieties and possible alternative host plants on the larval development. Because this species invaded Europe in the beginning of the 1990´s, it is of vital interest to determine how suitable European maize varieties and weeds are as food sources, thus facilitating the spread and the buildup of economically relevant populations.

Material and Methods

Like most studies on food conversion efficiency the method described here is also dependent on gravimetric measurements (Waldbauer, 1986). In order to determine

the efficiency with which an herbivorous insect converts plant biomass into own body biomass it is necessary to acquire not only the initial weight of the larvae and of the food item but also the final weight of both. The larval weight should thereby increase whereas the weight of the food item will usually decrease. This relationship is calculated as follows:

ECI = weight gain of larvae / weight loss of roots * 100

In order to achieve a better comparability the calculations are performed with dry weights. Aliquots are required to estimate the initial weight of the larvae and the food items. For the final calculation it is crucial that the aliquots are obtained with high precision. Applying the method presented here the efficiency of conversion of ingested food (ECI) is measured. Other calculations, such as the approximate digestibility index (AD) or the efficiency of digested food (ECD) require the measurement of the weight of faeces which is virtually impossible for subterranean insects.

While the calculation of the ECI is regarded an analysis of covariance (ANCOVA) can be performed using weight gain of the larvae and the amount of ingested food as the dependent variables and initial fresh weight as the covariate to correct for an eventual bias due to different initial weights (Rabenheimer and Simpson, 1992; Horton and Redak, 1993).

The test tubes used in this experimental device were 10cm long, 1cm wide and were closed with plastic plugs. The test tubes were half filled with plaster of Paris mixed with activated charcoal. This charcoal acted as an indicator for humidity: if the moisture level was sufficient the plaster of Paris retained its dark gray color whereas it turned almost white when dry. Vermiculit (an expanded Aluminum-Iron-Magnesium-Silicate usually used for isolation purposes or in gardening as a soil substitute) was the material used to simulate a subterranean environment. The mineral, puffed by heat and pressure to form granules with a layered structure, was sieved to obtain particles of 0.5-2.5mm size. This mineral acted both as a moisture buffer and as a substitute for the missing soil surroundings. Preliminary experiments revealed that the larvae of the Western Corn Rootworm are apparently thigmotactic, thus a

substrate was needed to simulate an underground environment which (1) could be added and removed easily from the set up, (2) held moisture to some extent and (3) was chemically inert. The vermiculit used in our experiments fulfilled these requirements but any other granular inert substance could also be used. Finally, a fine scale (Sartorius GmbH, Micro MC5 / SC2) was used to measure differences of up to 0.001mg in weight.

The larvae used in the experiments were obtained by the following protocol derived from Jackson (1986): The eggs of *D. v. virgifera* were obtained from females which had been caught in the field and which were kept in cages where they could oviposit for 2.5 months. The eggs were stored for a minimum period of 5 months at 8°C. At the beginning of each experiment the required number of eggs was incubated for 2 weeks at 26°C and 60% RH. Five days before the first larvae were expected to hatch, 50 g of maize seeds were mixed with 200 g regular potting earth and thoroughly moistened. The growing maize plants served as food for the larvae until they were extracted using a modified Berlese funnel (approximately 16 days after first hatch). This modified extraction method comprised of a sieve with 0.7 cm mesh size which was placed over a water container. The earth from the small containers with the plants and larvae was placed in the sieve and a light bulb was placed on top. The heat and moisture gradient forced the larvae to move downwards and to finally fall into the water container. They were then skimmed from the water surface and used in the experiments.

The maize plants were grown in a greenhouse for 7 weeks. The substrate was half sand and half regular potting earth. This mixture was used because it could be easily removed from the roots by washing. The roots obtained were cleaned and only the primary roots were used. From these the upper 5 cm were discarded and from the rest root pieces with a diameter of 1 to 2.5 mm and a fresh weight ranging from 0.6 to 0.9 g were placed in the test tubes. The L2 of the Western Corn Rootworm which we used exhibited a clear preference for this size class. 80% preferred thicker to smaller roots and also medium sized to smaller roots, as we demonstrated in two-choice experiments. The medium sized roots of 1-2.5 mm diameter were available in larger quantities than the thicker roots. Thus we always used this size class in our experiments. After introducing the roots into the experimental test tube, sufficient

vermiculite was added to surround and cover the roots completely. The moisture content was subsequently adjusted to the level of a moist but not saturated environment (about 2.5 ml water in this design). Free water droplets were avoided, because larvae got trapped in these droplets, which then increased in size as the larvae moved around and finally led to immobility and suffocation. Only L2 larvae of a weight ranging from 1.0 to 2.0 mg fresh weight were used in the experiments. The restriction to one age/size class was necessary because larvae from this particular class were most suitable as regards the results of these experiments (Fig. 1). They showed significantly higher increase in weight compared to the other size classes (ANOVA: $F_{4:256} = 13.08$, $p < 0.001$).

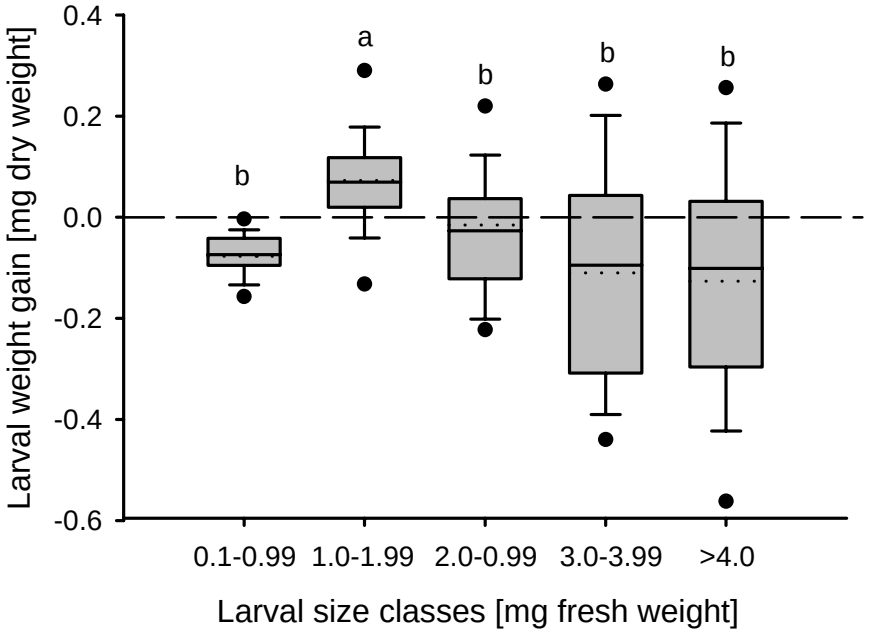

Figure 1: Weight gain of different size classes of larvae of *Diabrotica virgifera virgifera*. Full lines in the box plots indicate the median, while the dotted lines indicate the mean value. Only 5 and 95% quartiles are shown by the outlying points. The dashed line indicates zero weight gain. Same numbers above bars indicate no significant difference between size classes (ANOVA; Bonferroni adjustment for pairwise comparison).

Moreover, the first larval instars were not used because the larvae proved to be too sensitive to changes in their environment, such as moisture or food. The extracted

larvae were weighed and placed inside the test tubes on top of the vermiculit embedded root pieces. The tubes were closed and kept in darkness at 26°C and 60% RH. After 6 days the larvae and roots were extracted, dried for 3 days at 80°C and weighed.

Discussion

This method provides for the first time an opportunity of examining food suitability for subterranean insects not only qualitatively by measuring survival of larvae but also quantitatively by measuring the growth of each individual larva. Using this method it was possible to evaluate gradual differences between varieties of the main host plant maize and alternative host plants (Moeser and Vidal in prep.). The method also allows for a relatively quick assessment or screening as to the suitability of different host plants.

Acknowledgements:

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References

Horton, D. R. and R. A. Redak, 1993. Further comments on analysis of covariance in insect dietary studies. Entomol. Exp. Appl. 69: 263-275

Jackson, J. J., 1986. Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi*. In: J. L Krysan and T. A. Miller (eds.) Methods for the study of pest *Diabrotica*. Springer-Verlag, New York: pp. 25-47

Raubenheimer, D. and S. J. Simpson, 1992. Analysis of covariance: an alternative to nutritional indices. Entomol. Exp. Appl. 62: 221-231

Waldbauer, G. P., 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288

Highly variable response of larvae of the invasive maize pest *Diabrotica virgifera virgifera* (Coleoptera, Chrysomelidae) to European maize varieties.

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Abstract -

We studied the performance of larvae of *Diabrotica virgifera virgifera* LeConte (Chrysomelidae, Galerucinae) on 17 different maize varieties from 6 European countries. We performed food conversion efficiency studies using a newly established method. The growth of *D. v. virgifera* (western corn rootworm) larvae and the amount of ingested food was measured and the food conversion efficiency (ECI) was calculated. In addition to this we analyzed the C/N ratio and the phytosterol content of the different varieties. Significant differences between the maize varieties with regard to larval weight gain, amount of ingested food and food conversion efficiency were encountered. The efficiency of *D. v. virgifera* in converting root biomass into own biomass was positively correlated with the amount of nitrogen in the plant tissue. Furthermore the phytosterol content had a strong influence on larval weight gain and the amount of ingested food. It was possible to group the varieties into suitable and unsuitable cultivars with regard to *D. v. virgifera* larval performance on the basis of the phytosterol content. Our results provide first evidence of the high variability of European maize varieties with respect to *D. v. virgifera* nutrition. The use of less suitable maize varieties is discussed with respect to integrated pest management strategies.

Keywords: Western corn rootworm, root feeding, food conversion efficiency, nutritional ecology, plant-herbivore interactions.

Introduction

INVASIVE SPECIES pose a heavy threat to national economies (Pimentel et al. 2000) in particular with regard to agricultural production systems. Whereas US agriculture has to deal with non-native arthropod pests causing damages of 14 billion USD per year (Pimentel et al. 2003), European agro-ecosystems have only rarely been invaded (Elton, 1958). However, after the establishment of the Colorado potato beetle at the beginning of the 20th century, Europe now has to face an invasion of similar magnitude by the worst maize pest originating from the US.

Since the first discovery of the leaf beetle *Diabrotica virgifera virgifera* LeConte (Western corn rootworm) 1992 near Belgrade, Yugoslavia, the insect has spread considerably and is now encountered in more than 14 European countries (EPPO 2003). The number of beetles and infested countries are increasing each year. *D. v. virgifera* has been known in the USA since the beginning of the 20th century. However it was not until after 1950 that it became the most important pest in maize, now causing economic losses of about 1 billion USD per year (Krysan and Miller 1986). *D. v. virgifera* larvae are considered to be specialized maize root feeders. The larvae initially feed externally on the root system and mine inside the primary roots (Chiang 1973).

This maize plant– *D. v. virgifera* interaction may be considered from both sides: Namely, the reaction of the differing maize varieties to larval damage and the performance of the larvae on the different varieties. Maize breeders have been mainly successful on the first part of this interaction, especially the breeding for maize varieties which tolerate larval damage by root-re-growth (Ortman et al. 1974). On the other side of this interaction research concentrated on breeding for maize varieties with antibiosis; however, have met with limited success up to now (Levine and Oloumi-Sadeghi 1991). If varieties with reduced damage cannot be produced a different approach might be taken. Varieties which are not resistant but are however less suitable for *D. v. virgifera* development would yield similar results with regard to *D. v. virgifera* control. This would lead to reduced population densities due to delayed development and decreased survival.

The nitrogen content (Slansky and Sriber 1985) and the phytosterol content (Svoboda and Thompson 1985) are two parameters used to explain the performance of herbivores on a specific host plant. The influence of nitrogen on insect performance has been well documented by more than 200 studies revised by Scriber (1984). The C/N ratio may be considered as a parameter to explain the performance of herbivore insects on different host plants. A further group of plant derived secondary compounds, which play an important role in insect performance, are phytosterols. These isoprenoid derived plant compounds are essential components of cell membranes and serve as precursors of molting hormones (ecdysteroids) in many insects (Svoboda 1984). Insects, and many other invertebrates, are unable to synthesize the steroid nucleus. Metabolic constraints may limit which sterols support normal growth and development (Behmer and Elias 2000). We therefore analyzed the C/N ratio and the phytosterol content of the plants fed to the larvae in order to correlate larval performance with these parameters derived from different European maize cultivars.

Material and Methods -

Similar to most studies on food conversion efficiency we also applied gravimetric measurements (Waldbauer 1968). In order to determine the efficiency with which an herbivorous insect converts plant biomass into own body biomass it was necessary to acquire not only the initial weight of the larvae and of the food item, but also the final weight of both. The larval weight should thereby increase whereas the weight of the food item should usually decrease. This relationship (the efficiency of conversion of ingested food = ECI) is calculated as follows:

ECI = weight gain of larvae / weight loss of roots * 100

An important prerequisite when calculating an ECI in feeding studies is a linear relationship between the initial and the final weight of larvae (Raubenheimer and Simpson 1992). We therefore plotted our data with respect to this assumption before calculating the ECI for each host plant. In order to achieve a better comparability the calculations were performed using dry weights. Aliquots were required to estimate the initial weight of the larvae and of the food items. For the final calculation it was crucial that the aliquots had been obtained with high precision. Applying the method

presented here the efficiency of conversion of ingested food (ECI) was measured. Other calculations, such as the approximate digestibility index (AD) or the efficiency of digested food (ECD) would require the measurement of the weight of faeces which is virtually impossible for subterranean insects.

An experimental device was designed from test tubes which were 10 cm long, 1 cm wide and were closed with plastic plugs. The test tubes were half filled with plaster of Paris mixed with activated charcoal (Merck GmbH, Germany). The charcoal acted as an indicator for humidity: if the moisture level was sufficient the plaster of Paris retained its dark gray color whereas it turned almost white when dry. Vermiculite (Klein GmbH, Zellertal, Germany) was used to simulate a subterranean environment. The mineral, puffed by heat and pressure to form granules with a layered structure, was sieved to obtain particles of 0.5-2.5 mm size. It acted as both a moisture buffer and a substitute for the missing soil surroundings. Preliminary experiments revealed that the larvae of the Western Corn Rootworm are apparently thigmotactic, thus a substrate was required to simulate an underground environment. Finally, a fine scale (Sartorius GmbH, Goettingen, Germany; Model: Micro MC5 / SC2) was used to measure differences of up to 0.001mg in weight.

A total of 17 maize varieties from 6 European countries were tested (Table 1). The majority were modern hybrid lines while one was an inbred line (LG 2447) and three were open pollinated varieties (Reid’s Yellow Dent, Green Fields and Krug).

Table 1: Maize varieties tested, country of origin, seed company, product name and abbreviation used further on are indicated. Ranking of the varieties follows the one given in the figures.

Country	Seed company	Variety name	Abbreviation
Hungary	Pioneer	Colomba	CL
France	Pioneer	PR 34 FO 2	PR 34
Germany	LG Nickerson	Banguy	BA
France	Mais Angevin	Anjou 258	A 258
Hungary	Martonvasar	Norma	NO
Italy	DeKalb/Monsanto	DK 440	DK 440
Italy	Istituto di Cerealicoltura Bergamo	Marano	MA
Croatia	Pioneer	Florencia	P 73
France	DeKalb/Monsanto	DK 312	DK 312
Croatia	Agricultural Institute Osijek	OSSK 602	OSSK 602
Germany	Euralis	Earlystar	EA
France	LG Nickerson	LG 2447	LG 2447
Croatia	Agricultural Institute Osijek	OSSK 617	OSSK 617
France	Mais Adour	Panama	PA
Portugal	Greenfield farms	Reid’s Yellow Dent	RYD
Portugal	Greenfield farms	Krug	KRUG
Portugal	Greenfield farms	Green Fields	GF

The maize plants were grown in the greenhouse for seven weeks. The substrate was half sand and half potting soil (RTS spezial, Oekohum GmbH, Dransfled, Germany). This mixture was used because it could be easily removed from the roots by washing. The roots obtained were cleaned and only the primary roots which originated from the plant base and no secondary root branches which originated from others roots were used. Second instar larvae are more frequently found in the first 10 cm of the roots from the plant base (Strnad and Bergman 1987 and pers. observ.). From these first 10 cm the upper 5 cm were discarded and from the rest root pieces with a diameter from 1 to 2.5 mm and a fresh weight ranging from 0.6 to 0.9 g were placed in the test tubes. After introducing the roots into the experimental test tube, sufficient vermiculite was added to surround and cover the roots completely. Afterwards, the moisture content was adjusted to the level of a

moist but not saturated environment (about 2.5 ml water in this design). Free water droplets were avoided, because larvae got trapped in these droplets, which increased in size as the larvae moved around and finally led to immobility and suffocation.

The larvae used in the experiments were obtained by the following protocol derived from Jackson (1986): The eggs of *D. v. virgifera* were obtained from females caught in the field in Southern Hungary and kept in cages for 2.5 months with substrate for oviposition. The eggs were stored for a minimum period of five months at 8°C. At the beginning of each experiment the required number of eggs were incubated for 2 weeks at 26°C and 60% RH. Five days before the first larvae were expected to hatch 50 g of maize seeds were mixed with 200 g of regular potting earth and thoroughly moistened. The growing maize plants served as food for the larvae until they were extracted by a modified Berlese funnel (approximately 16 d after first hatch). This modified extraction method comprised of a sieve of 0.7 cm mesh size which was placed over a water container. The soil from the small containers with the plants and larvae was placed in the sieve and a light bulb was positioned above on top. The heat and moisture gradient forced the larvae to move downwards and to finally fall in the water container. They were then skimmed off the water surface and used in the experiment. Only second instar larvae of a weight ranging from 1.0 to 2.9 mg fresh weight were used in the experiments. The restriction to a single age/size class was necessary because larvae from this particular class proved most suitable with regard to the results in these experiments (Moeser and Vidal 2003a). The extracted larvae were weighed and placed inside the test tubes on top of the vermiculite embedded root pieces. The tubes were closed and kept in darkness at 26°C and 60% RH. After six days the larvae and roots were extracted, dried for three days at 80°C and weighed.

C/N Analyses.

In order to examine the carbon and nitrogen content of the different varieties we performed a C/N analysis. The roots were dried at 80°C for three days. 30 mg of dry roots of each variety were pooled because single roots provide sufficient biomass for the analysis to be examined individually. The 30 mg were finely ground and three samples of 5 mg dry weight were taken. These were compared to a standard of 5 mg

Acetanilide (Merck GmbH, Germany) every 20 samples in a C/N analyzer (Elementar Analysensysteme Hanau GmbH, Germany; Model: Vario EL III). The known quantity of C and N in the standard allowed for the calculation of the amount of these elements in each root sample. Subsequently the C/N ratio was determined.

Sterol analyses.

While preparing the roots for the feeding trials described above, three samples of roots per plant species weighing 0.5 g (fresh weight) each were deeply frozen in liquid nitrogen and stored at -20°C until further processing. To extract the phytosterols the roots were ground to a fine powder under liquid nitrogen using a mortar. To each sample a mixture of 5 ml 10 M potassium hydroxide solution, 15 ml 96% ethanol and 0.3% Pyrogallol (Merck GmbH, Germany) was added. An ultrasonic homogenizer (Model: Sonoplus HG 2200 / UW 2200, 200 W, 20 kHz, Bandelin GmbH, Germany) was used for 30 sec to enhance further cell breakdown and to free the solution from microscopic air bubbles trapped inside the vial. The samples were subsequently kept in a shaker water bath at 80°C for 2.5 h. After cooling the samples to room temperature, 40 µl of an internal standard were added (Cholesterol 4 mg / ml chloroform, Merck GmbH, Germany). The sterols were extracted by applying 10 ml hexane to each sample. After thorough shaking for 10 sec the hexane fraction was transferred to a rotary evaporator flask with an Eppendorf pipette. This extraction step was repeated once. The total of 20 ml hexane solution was washed with 1 ml de-mineralized water, which was then extracted with an Eppendorf pipette. The samples were distilled using a rotary evaporator and a water bath of 42°C. The pertaining sterols were resolved in 1.5 ml hexane through gentle shaking and transferred to 1.5 ml Eppendorf cups. After centrifugation with 10,000 rpm for 10 min. the supernatant was transferred to vials and the hexane was evaporated overnight at 50°C in a hot block. The sterols were then resolved in 240 µl hexane and 60 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, Fluka / Riedel-deHaen GmbH, Germany) and incubated at 70°C for 20 min. 1 µl of each sample with a split of 1:50 of extracted sterols were analyzed using a gas chromatograph (Shimadzu GmbH, Model: GC14/15A) with a flame ionization detector. The samples were run on a fused silica column (SPB-1; 1.3m x 0.32mm, 0.25µm film thickness, Supelco Inc./ Sigma-Aldrich, Germany). Helium was used as a carrier gas, the make-up gas was synthetic air with a linear velocity of 35cm/s. The temperature program was initially 5 min at

180°C then increase to 290°C at 4°C/min with a 20 min postrun time. The detector temperature was 300°C. Peak areas were calculated using an integrator and the internal standard. The sterols represented by each peak were identified beforehand using GC-MS with synthetic sterols as comparisons. The individual peak areas were summed up which resulted in the total sterol content.

Statistics.

Systat 10 for Windows (SPSS inc. 2000) was used for the statistical analysis. To estimate the differences between the varieties with regard to larval performance an analysis of covariance (ANCOVA) was performed. We used the weight gain of the larvae and the amount of ingested food as the dependent variable and the initial fresh weight as the covariate to correct for an eventual bias due to different initial larval weights (Rabenheimer and Simpson 1992; Horton and Redak 1993). For a pair wise comparison between varieties an analysis of variance (ANOVA) with a Bonferroni adjustment derived from GLM was performed.

Results -

The weight gain of *D. v. virgifera* larvae feeding on the different maize varieties showed gradual differences ranging from positive to negative values. There were significant differences in weight gain between the varieties Colomba and Panama/Green Fields/OSSK 617 (F = 3.05; df = 16, 469; P < 0.001). Additionally, the initial fresh weight as a covariate had a significant influence on the differences in weight gain (F = 34.04; df = 16, 469; P < 0.001). Furthermore positive weight gain was observed in only 14 of the 17 varieties and only 11 varieties showed a positive mean larval weight gain (Fig. 1). The other varieties showed a mean negative weight gain, or a net weight loss. The three open pollinated varieties displayed results ranging from almost no weight gain to similar values found within the hybrids.

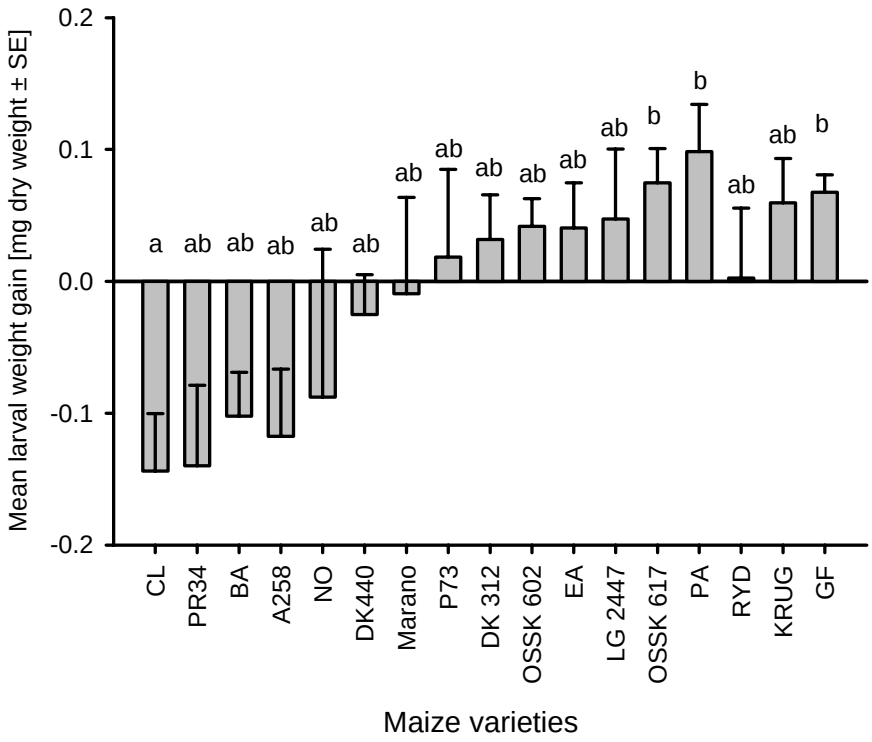

Figure 1: Mean weight gain of *D. v. virgifera* larvae feeding on different maize varieties. Identical) letters above bars indicate no significant difference (ANOVA, Bonferroni adjustment).

The larvae did not feed to the same extent on all varieties tested (Fig. 2). They fed most on those varieties were they also gained weight. An exception was the variety “Marano” where the highest food consumption was found, but no weight gain of larvae could be observed. Lowest feeding was observed for the varieties were the larvae were not able to gain weight. There were several varieties which exhibited significant differences with regard to the amount of food consumed by the larvae (F = 5.23; df = 16, 485; P < 0.001). Here the initial fresh weight of the larvae had no significant influence on the amount of ingested food (F = 0.28; df = 16, 485; P = 0.59). The three open pollinated varieties showed an inverse feeding pattern: the varieties which were fed on most were the varieties with the lowest weight gain for the larvae.

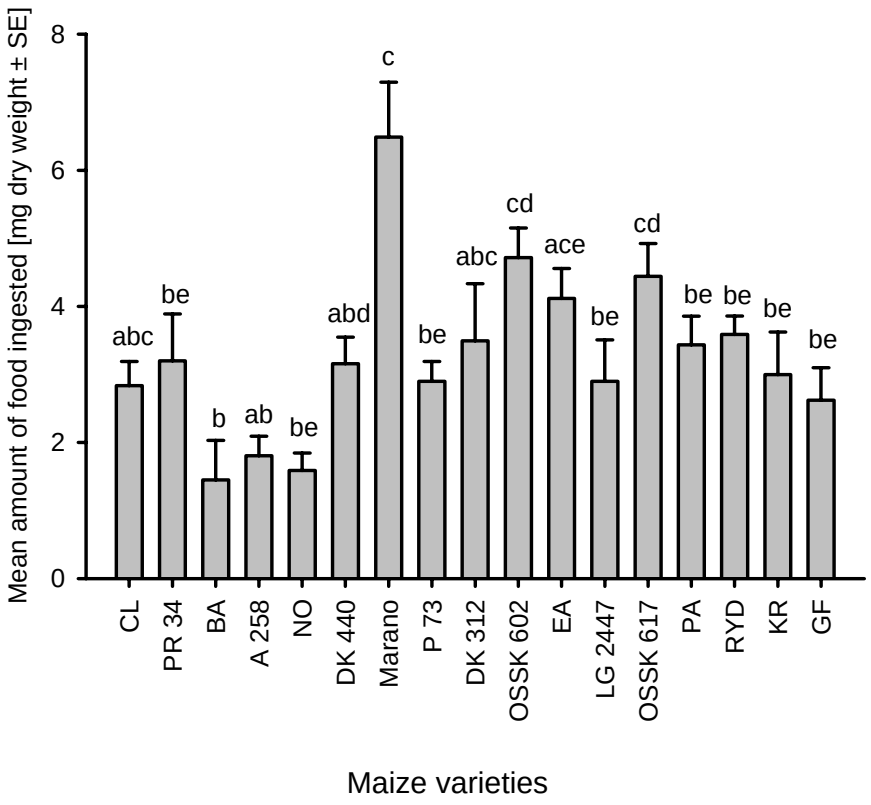

Figure 2: Mean amount of ingested food by *D. v. virgifera* larvae. Identical letters above bars indicate no significant differences (ANOVA, Bonferroni adjustment). The varieties are in the same order as in Figure1.

The calculations on food conversion efficiency revealed a similar pattern as compared to the results of larval weight gain (Fig. 3). The larvae with the highest food

conversion efficiency showed the highest weight gain. Significant differences were found between varieties (F = 2.34; df = 16, 469; P = 0.003), while the initial fresh weight of the larvae used as a covariate had a significant influence on this result (F= 16.22; df = 16, 469; P < 0.001). Larval weight loss resulted in a negative ECI index for several varieties.

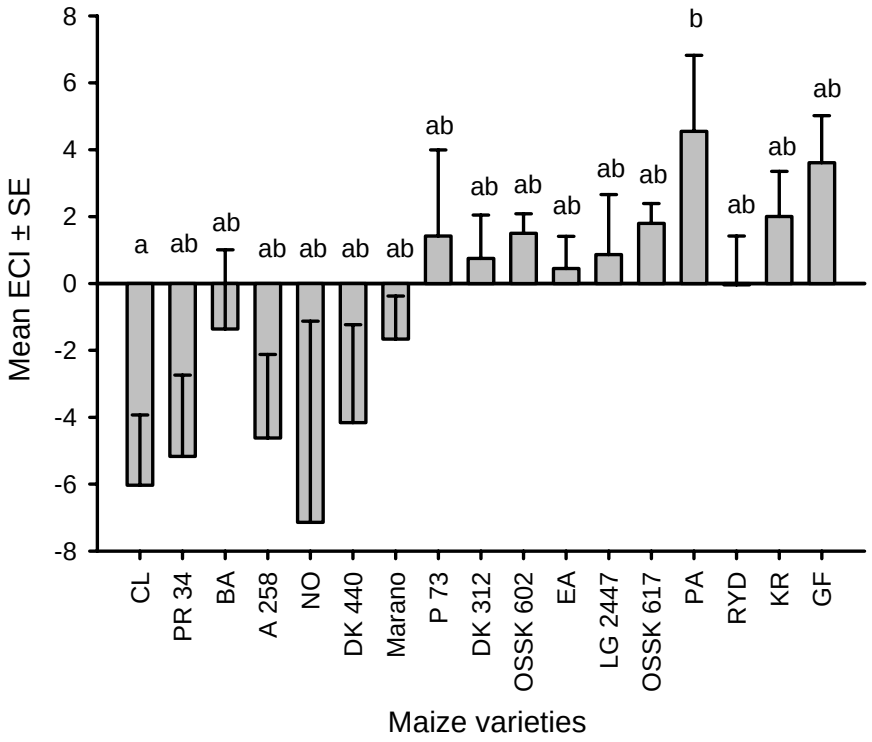

Figure 3: Mean ECI index for *D. v. virgifera* larvae feeding on different maize varieties. Identical letters above bars indicate no significant difference (ANOVA, Bonferroni adjustment). The varieties are ranked in the same order as in Figure1.

The influence of initial weight was best described by the negative correlation between the initial fresh weight and the weight gain (y = 2.03 - 1.79x; R^2 = 0.17; P <0.0001). There was no linear relationship between initial fresh weight and the amount of food eaten by the larvae (y = 3.21 + 0.04x; R^2 = 0.0004; P = 0.73). Similar to weight gain, the ECI was also negatively correlated to the initial fresh weight (y = 3.85 - 1.83x; R^2 = 0.084; P <0.0001).

C/N ratio.

The parameters of carbon and nitrogen content of the food revealed a significant positive relation between the amount of ingested food and the C/N ratio and also between the ECI index and the nitrogen content (Table 2). No other significant correlations could be observed between the carbon or nitrogen content and the measured variables.

Table 2: Correlation between the three measured variables, larval weight gain, amount of ingested food and food conversion efficiency and the Carbon and Nitrogen content or the respective ratio.

Variables	Regression	r^2	p-value
Weight gain vs. C/N ratio	y = 55.39 - 2.28x	0.0002	n. s.
Weight gain vs. C content	y = 1.1 - 0.025x	0.054	n. s.
Weight gain vs. N content	y = 0.84 + 0.27x	0.017	n. s.
Ingested food vs. C/N ratio	y = 42.14 + 4.65x	0.21	*
Ingested food vs. C content	y = 44.78 + -0.035x	0.003	n. s.
Ingested food vs. N content	y = 0.99 - 0.054x	0.14	n. s.
ECI vs. C/N ratio	y = 55.17 - 0.34x	0.011	n. s.
ECI vs. C content	y = 44.66 - 0.018x	0.008	n. s.
ECI vs. N content	y = 0.89 + 0.019x	0.24	**

n. s. = not significant

* = significant at the 10% level

** = significant at the 5 % level

Phytosterols.

Four major sterols were detected and summed up to result in the total sterol content: Brassicasterol, Campesterol, Sitosterol and Stigmasterol. The individual amounts were summed up after integrating the peak areas to result in the total sterol content. When all varieties were taken together no relationship between each varieties total sterol content and larval weight gain could be revealed ($y = 0.034 - 0.0007x$; $R^2 = 0.06$; $P = 0.32$; Fig. 4). Furthermore no correlation could be found between the total sterol content and the amount of ingested food ($y = 2.12 - 0.0003x$; $R^2 = 0.0001$; $P = 0.96$; Fig. 5).

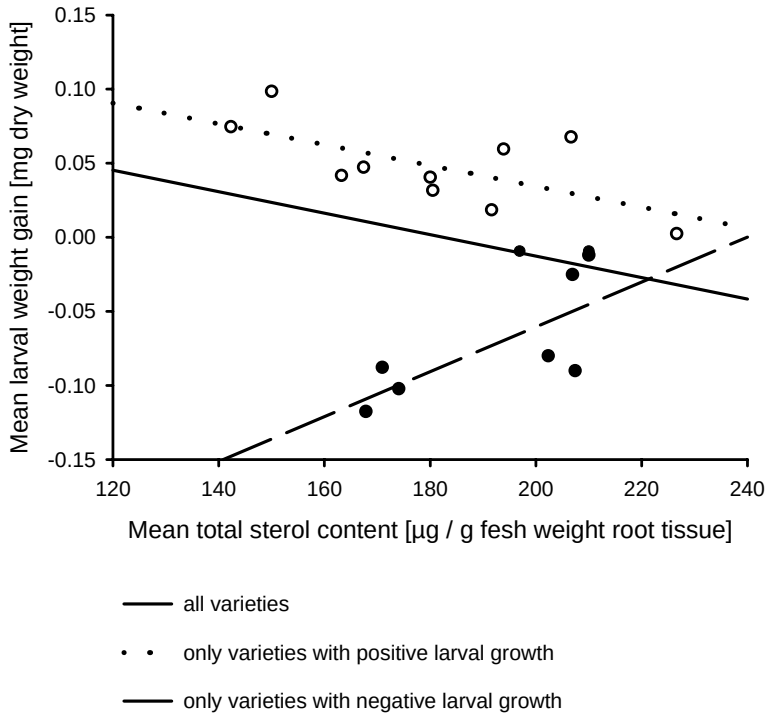

Figure 4: Relationship between the mean total sterol content and the mean larval weight gain. White points represent varieties with positive larval growth, black points indicate varieties with negative larval growth.

When those varieties which yielded either positive or negative larval growth were considered separately, a significant correlation between these parameters was encountered. A significant negative relation was discovered between the sterol content and the larval growth in varieties with positive larval weight gain ($y = 0.15 + 0.0007x$; $R^2 = 0.42$; $P = 0.04$; Fig. 4). Within these varieties a negative correlation was determined additionally between the sterol content and the amount of ingested food ($y = 5.66 - 0.011x$; $R^2 = 0.31$; $P = 0.07$) which was significant at the 10% level.

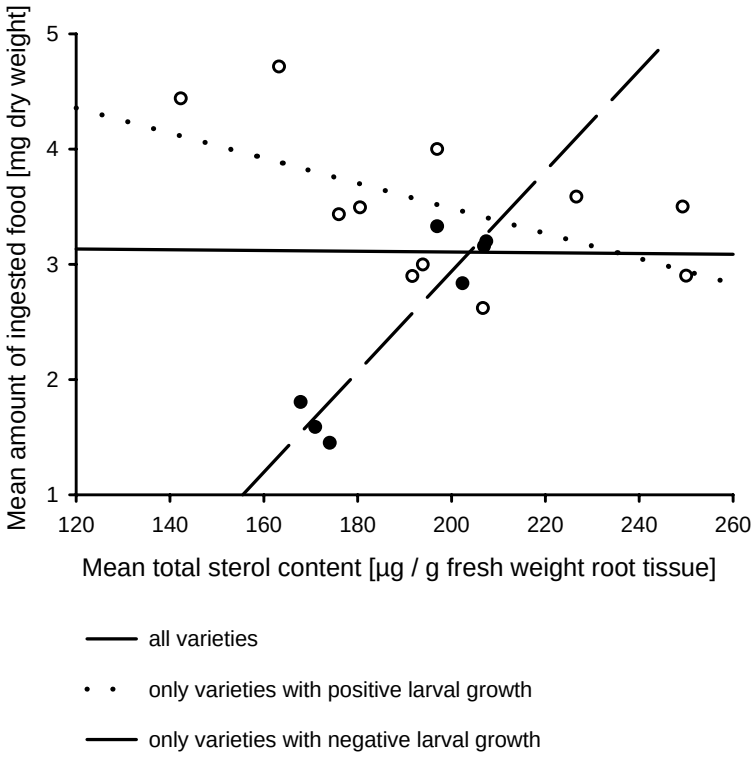

Figure 5: Relationship between mean total sterol content of the roots and the mean amount of ingested food. White points represent varieties with positive larval growth, black points indicate varieties with negative larval growth.

For those varieties which yielded a negative larval weight gain the sterol content was positively correlated to weight gain. This relation was significant at the 10% level ($y = -0.33 + 0.002x$; $R^2 = 0.47$; $P = 0.06$; Fig. 5). Within these varieties a highly significant positive relationship was encountered between the sterol content and the amount of ingested food ($y = -5.78 + 0.05x$; $R^2 = 0.87$; $P = 0.002$).

Discussion -

D. v. virgifera larvae exhibited a highly variable response with regard to weight gain, amount of ingested food and food conversion efficiency when fed on different European maize varieties.

Larvae of different ages and size show a preference for different parts of the root (Chiang 1973). Under field conditions larvae would thus search for more appropriate feeding sites when a given root is unpalatable on account of physical or chemical

cues. However as we did not carry out choice experiments we could not verify the possibility of increased weight gain in larvae feeding on more appropriate roots. Therefore, varieties which yielded weight loss for *D. v. virgifera* should be examined further in order to determine which factors led to this apparent unsuitability. Studying the mechanisms which result in larval weight loss could lead to the discovery of mechanisms useful in plant breeding against larval damage. The case of the variety “Marano” showed that although the larvae did not gain weight on average they fed to a considerable extent, which under field conditions would lead to massive root damage and subsequent malnutrition or plant lodging. This variety is an old landrace used locally in the Veneto region of Northern Italy. A similar pattern could be observed with Reid’s Yellow Dent where heavy feeding was observed but weight gain of the larvae was minimal. The other two open pollinated varieties showed no such contradictory results.

When larvae fed substantially on roots but did not gain weight, postingestive effects of the different food items are a possible explanation (see “Phytosterols”). No preingestive effects like antibiosis could be observed, because *D. v. virgifera* fed on all varieties (Horton and Redak 1993). Hydroxyamic acids are considered to act as antibiosis factors for *D. v. virgifera* (Assabgui et al. 1995). We could observe no such behavior in the larvae and thus suggest this to be of minor importance. Resistance to *D. v. virgifera* (measured as reduced larval damage) has been encountered in some experimental maize hybrids at high egg densities though no explanation was given for this result (Branson et al. 1983). These authors were also unable to discriminate between post- and pre-ingestive effects.

Differing degrees of larval feeding are of importance, as they point to a previously neglected part within the *D. v. virgifera* -maize interaction: varieties which yield low larval weight gain might still show huge larval damage if food conversion efficiency is low. On the other hand, varieties which display little damage could still be suitable resources for larval development, when food conversion efficiency is high. Thus the ECI is a good measure of indicating the suitability of the different maize varieties for larval development and should be considered in future research concerning *D. v. virgifera* -maize interaction. As we were the first to collect quantitative data on the suitability of host plants for root feeders, no other literature is as yet available for further comparisons (Slansky and Scriber 1982).

C/N ratio.

The carbon-nutrient balance hypothesis (CNBH) is orientated mainly towards the plants point of view in a herbivore-host plant interaction (Hamilton et al. 2001). We used this hypothesis to explain the performance of *D. v. virgifera* larvae on different host plant varieties due to differences in the C/N ratio. Our results suggest that there are no carbon- or nitrogen-based defence compounds acting on *D. v. virgifera* performance when feeding on maize roots. Nitrogen has to be considered rather as a nutritive component, because a higher N content of a given variety led to higher efficiency of conversion of ingested food. Roots are considered to be an extremely nutrient poor food (Slansky and Scriber 1985). Therefore, root tissue of varieties with a higher N content could be converted more efficiently into own biomass than varieties with a low N content. Under natural conditions a nitrogen treatment of maize plants in the field led to an increase in larval damage and increased emergence rates of *D. v. virgifera* (Spike and Tollefson 1988), which supports our findings. Similarly Scriber (1984) revised more than 200 studies regarding the response of insects to nitrogen fertilization and found an increase in insect growth and damage in the majority of the papers analysed.

Phytosterols.

Sterol content had a strong influence on *D. v. virgifera* feeding behavior and larval performance. Sterols are essential nutrients for insects, but metabolic constraints may limit which phytosterols support normal growth and development (Behmer and Elias 1999). Thus a relative decrease in one sterol compound compared to others would lead to an increase in feeding to compensate for the deficient component. Maize tissue is known to contain mainly sitosterol, stigmasterol, campesterol and 24-epicampestreol (Guo et al. 1995). It is unknown which of these phytosterols can be metabolized by *D. v. virgifera*.

However, we suggest that those varieties which yielded positive larval weight gain provided sufficient sterols for regular growth. A higher overall sterol content in these varieties may be due to an increase of those sterol components which are unsuitable or even detrimental to larval growth. The total sterol content may contain a mixture of suitable and unsuitable compounds. When the grasshopper *Schistocerca americana* Drury was confronted with sterols of spinach (*Spinacia oleracea* L.) the results revealed that unsuitable phytosterols may even act as feeding deterrents

(Behmer and Elias 1999). It is therefore possible that an altered sterol composition led to decreased feeding and finally relatively less weight gain compared to those varieties with less total sterol content.

Heliothis zea Hübner reared on an artificial diet displayed a huge variety of responses, when fed different sterols at different compositions (Nes et al. 1997). It is possible that those maize varieties unsuitable for larval growth in our study were deficient of a certain sterol component. This deficiency would result in increased feeding to compensate for this given compound. However, even with increased uptake of root tissue the larvae did not gain weight but displayed an overall weight loss. We therefore conclude that either the sterol composition was inadequate or other factors not covered by this study had an additional influence on the larval performance. As shown with *Phyllotreta cruciferae* Goeze altered phytosterol content in *Brassica napus* L. resulted in reduced survival and prolonged larval stages (Bodnaryk et al. 1997). In our study phytosterols had a strong impact on the larval performance of *D. v. virgifera*, though the results underline that further experiments are necessary, in particular with regard to the individual sterols involved in insect development.

We conclude that larval performance varies to a great extent on the differing European maize varieties. The future search for a maize variety which is worth planting even in the presence of *D. v. virgifera* should focus on varieties which suffer little damage or which are able to tolerate damage but also yield decreased larval growth. Research into plants with an altered or unsuitable sterol composition impeding larval growth could be a valuable approach in breeding programs to counteract *D. v. virgifera*.

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References Cited

Assabgui, R. A., J. T. Arnason and R. I. Hamilton. 1993. Field evaluation of hydroamic acids as antibiosis factors in elite maize inbreds to the western corn rootworm (Coleoptera : Chrysomelidae). J. Econ. Entomol. 88: 1482-1493.

Behmer, S. T. and D. O. Elias. 1999. Phytosterol structure and its impact on feeding behaviour in the generalist grasshopper *Schistocerca americana*. Phys. Entomol 24: 18-27.

Behmer, S. T. and D. O. Elias. 2000. Sterol metabolic constraints as a factor contributing to the maintenance of diet mixing in grasshoppers (Orthoptera: Acrididae). Phys. and Bioche. Zool. 73: 219-230.

Behmer, S. T. and R. J. Grebenok. 1998. Impact of dietary sterols on life history traits of a caterpillar. Phys. Entomol. 23: 165-175.

Branson, T. F., V. A. Welch, G. R. Sutter and J. R. Fisher. 1983. Resistance to larvae of *Diabrotica virgifera virgifera* in three experimental maize hybrids. Environ. Entomol. 12: 1509-1512.

Bodnaryk, R. P. , M. Luo and L. Kudryk. 1997. Effects of modifying the phytosterol profile of canola, *Brassica napus* L., on growth, development, and survival of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) and the aphids *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Can. J. Plant Sci. 77: 677-683.

Chiang, H. C.. 1973. Bionomics of the northern and western corn rootworm. Ann. Rev Entomol. 18: 47-72.

Elton, C. S. 1958. The ecology of invasions by animals and plants. University of Chicago Press, 181 pp.

EPPO. 2003. http://www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_ virgifera.html

Guo, D., M. Venkatramesh and W. D. Nes. 1995. Developmental regulation of sterol biosynthesis in *Zea mays*. Lipids 30: 203-219.

Hamilton, J., A. Zangerl, E. DeLucia and M. Berenbaum. 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecol. Letters 4: 86-95.

Horton, D. R. and R. A. Redak. 1993. Further comments on analysis of covariance in insect dietary studies. Entomol. Exp. Appl. 69: 263-275.

Jackson, J. J. 1986. Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi*. In: J. L Krysan and T. A. Miller (eds.) Methods for the study of pest *Diabrotica*. Springer-Verlag, New York: pp. 25-47.

Krysan, J. L. and T. A. Miller. 1986. Methods for the study of pest *Diabrotica*. Springer Verlag, New York, 260 pp.

Lerdau, M and P. D. Coley. 2002. Benefits of the carbon-nutrient balance hypothesis. Oikos 98: 533-535.

Levin, E. and H. Oloumi-Sadeghi. 1991. Management of Diabroticite rootworms in corn. Ann. Rev. Entomol. 36: 229-255.

Moeser, J. and S. Vidal. 2003a. How to measure the food utilization of subterranean insects: a case study with the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Submitted to Journal of Applied Entomology

Nes, W. D., M. Lopez, W. Zhou, D. Guo, P. F. Dowd and R. A. Norton. 1997. Sterol utilization and metabolism by *Heliothis zea*. Lipids 32: 1317-1322.

Ortman, E. E., D.C. Peters and E. D. Gerloff. 1974. Techniques, accomplishments, and future potential of host plant resistance to *Diabrotica*. In F.G. Maxwell and F. A. Harris (eds.) Proceedings of the Summer Institute on Biological Control of Plant Insects and Diseases. University Press, Mississippi, pp. 344-358.

Pimentel, D., L. Lach, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50: 53-65.

Pimentel, D., L. Lach, R. Zuniga and D. Morrison. 2003. Environmental and economic costs of alien arthropods and other organisms in the United States. In G. J. Hallman and C. P. Schwalbe (eds.) Invasive arthropods in agriculture: Problems and solutions. Science Publishers Inc., Enfield, NH. pp. 107-117.

Raubenheimer, D. and S. J. Simpson. 1992. Analysis of covariance: an alternative to nutritional indices. Entomol. Exp. Appl. 62: 221-231.

Scriber, J. M. 1984. Nitrogen nutrition of plants and insect invasion. In R. D. Hauck (ed.) Nitrogen in crop production. Amer. Soc. Agron. Publ., Madison, Wisc. Chapter 34.

Slansky, F. Jr. and J. M. Scriber. 1982. Selected bibliography and summary of quantitative food utilization by immature insects. ESA Bulletin 28: 43-55.

Slansky, F. Jr. and J. M. Scriber. 1985. Food consumption and utilization. In G. A. Kerkut and L. I. Gilbert (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 88-163.

Strnad, S. P. and M. K. Bergman. 1987. Distribution and orientation of western corn rootworm (Coleoptera: Chrysomelidae) larvae in corn roots. Environ. Entomol. 16: 1193-1198.

Spike, B. P. and J. J. Tollefson. 1988. Western corn rootworm (Coleoptera: Chrysomelidae) larval survival and damage potential to corn subjected to nitrogen and plant density treatments. J. Econ. Entomol. 81: 1450-1455.

SPSS Inc. 2000. Systat 10 for Windows; SPSS Inc., Chicago.

Svoboda, J. A. 1984. Insect steroids: Metabolism and function. In W. D. Nes, G. Fuller and L. S. Tsai (eds.) Isoterpenoids in plants: Biochemistry and function. Marcel Dekker Inc. New York. pp. 367-388.

Svoboda, J. A. and M. J. Thompson. 1985. Steroids. In G. A. Kerkut and L. I. Gilbert (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 137-175.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Do alternative host plants enhance the invasion of the maize pest *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae, Galerucinae) in Europe?

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ABSTRACT -

We investigated the performance of larvae of the invasive maize pest *Diabrotica virgifera virgifera* LeConte (western corn rootworm) on roots of alternative host plants. During laboratory feeding trials we measured growth, amount of ingested food and determined the food conversion efficiency of second instar larvae. We tested eight species of weeds (seven monocot and one dicot) and three monocot crops with regard to host plant suitability employing a newly established method. We additionally examined the C/N ratio and the phytosterol content of the different plant species as parameters to interpret larval performance. Larval growth, the amount of ingested food, and the food conversion efficiency differed significantly between plant species. Plant species with a high nitrogen content were less suitable for *D. v. virgifera* development. The phytosterol content had a significant influence on the amount of ingested food, but not on larval weight gain. The performance of *D. v. virgifera* larvae on alternative hosts was comparable to their performance on maize. The ability to use alternative hosts for larval development may contribute to the invasion potential of *D. v. virgifera* and has important implications for integrated pest management.

KEYWORDS - Western corn rootworm, invasive species, nutritional ecology, root feeding, food conversion efficiency, ECI, C/N ratio, phytosterols

Introduction

INVASIVE SPECIES are regarded as the second most important factor responsible for biodiversity loss (Walker and Steffen 1997). They also pose heavy monetary losses on national economies (Pimentel et al. 2000). Although the parameters which determine the success or failure of an invasive species are largely unknown, some factors responsible for a successful invasion have already been determined such as propagule pressure, spreading potential and the capability of the species to adapt to the biotic and abiotic conditions in its new range (Williamson 1996). For invasive herbivorous insects one of the most important prerequisites for successful invasion is to find suitable host plants (Worner 2003).

When *Diabrotica virgifera virgifera* LeConte (Chrysomelidae, Galerucinae) was first detected in Europe at the beginning of the 1990s, its main host plant, maize (*Zea mays* L.), had already been established as a crop for several hundred years. Therefore, conditions for this leaf beetle to colonize new areas in Europe were excellent. Since its first detection, *D. v. virgifera* (western corn rootworm) has become established in 14 European countries (EPPO 2003). *D. v. virgifera* is considered to be specialized on maize as a host plant (Chiang 1973). As *D. v. virgifera* larvae develop best when feeding on maize roots (Branson and Ortman 1967, 1970), one of the most widely used cultural practices in order to prevent larval damage in the USA is crop rotation (Gray et al. 1998). Female beetles lay their eggs in maize fields, where the eggs diapause over winter (Chiang 1973). When a different crop other than maize is planted the following year, hatching larvae encounter only unsuitable host plants and thus cannot survive. At the beginning of the 1990's, a strain of *D. v. virgifera* became established in the US corn belt that seemed to have adapted to this rotation practice by oviposition in soybean fields, which would then be rotated to maize fields the following year (Gray et al. 1998). *D. v. virgifera* females are known to prefer to oviposit near clumps of monocot grasses (*Setaria* sp.) rather than in bare soil or near the stalks of harvested maize (Johnson and Turpin 1985). Larval movement is limited and larvae are attracted to carbon dioxide not by specific volatile substances (Krysan and Miller 1986). They are unable to discriminate between the roots of different plant species from a distance but only after contact (Strnad and Dunn 1989). Thus they either have to feed on the roots of those plants, which they encounter first or risk starvation during the search for a more appropriate host plant. However, *D. v. virgifera* larvae are known to feed and survive on several

monocot species (Branson and Ortman 1970), although knowledge regarding their performance on alternative host plants is limited. Weeds or monocot crops may thus act as reservoir host plants when maize is not available. If European weed species, typically encountered in maize fields, serve as alternative host plants, this could enhance the survival of *D. v. virgifera* in areas recently invaded in Europe and could undermine control measures such as crop rotation. In order to evaluate the performance of *D. v. virgifera* on alternative host plants we carried out food conversion efficiency studies using common weed species and monocot crops.

The nitrogen content (Slansky and Sriber 1985) and the phytosterol content (Svoboda and Thompson 1985) are two parameters used to explain the performance of herbivores on a specific host plant. The influence of nitrogen on insect performance has been well documented by more than 200 studies reviewed by Scriber (1984). The C/N ratio may be considered as a parameter to explain the performance of herbivore insects on different host plants. A further group of plant derived secondary compounds, which play an important role in insect performance, are phytosterols. These isoprenoid derived plant compounds are essential components of cell membranes and serve as precursors of molting hormones (ecdysteroids) in many insects (Svoboda 1984). Insects, and many other invertebrates, are unable to synthesize the steroid nucleus. Metabolic constraints may limit which sterols support normal growth and development (Behmer and Elias 2000). We therefore analyzed the C/N ratio and the phytosterol content of the plants fed to the *D. v. virgifera* larvae in order to correlate larval performance with these plant parameters.

Material and methods

We used gravimetric methods to determine the efficiency of converting plant biomass into body biomass for *D. v. virgifera* larvae. Following the method established by Waldbauer (1968) it was necessary to first obtain both the initial fresh weight of the larvae and of the food and also the final dry weight of both. Usually larval weight will increase with decreasing weight of the food item. This relationship (the efficiency of conversion of ingested food = ECI) is calculated as follows:

ECI = weight gain of larvae / weight loss of roots * 100

An important prerequisite when calculating an ECI in feeding studies is a linear relationship between the initial and the final weight of larvae (Raubenheimer and Simpson 1992). Therefore, we plotted our data with respect to this assumption before calculating the ECI for each host plant. Several samples, however, revealed a negative value for the amount of ingested food, we were not able to use all data (compare to the equation above). In order to achieve an improved comparability, the calculations were performed with dry weights. This method requires aliquots to estimate the initial dry weights of larvae and food items. Twenty samples from each host plant species and 30 samples for larvae aliquots were used.

We performed the feeding trials using 11 plant species. All but one were members of the Poaceae family. We selected the weed species to be tested in our design on the basis of weed abundance in Hungarian maize fields. During a study on the use of alternative host plants by adult *D. v. virgifera* we sampled the diversity and abundance of weeds in maize fields in southern Hungary (Moeser and Vidal 2003b). The sampling was carried out for 10 weeks in 12 fields with one transect per field, which was altered weekly. A single transect comprised the area between two rows of maize at a length of 20 m. The maize rows were numbered and the transects were run following a random number generated by a pocket calculator. An additional randomly generated number between zero and 30 was used as the starting point for the transect, indicating the distance from the field margin. In each transect the number of individual plants per species was counted. *Amaranthus* sp. was the most common weed, while grasses occupied the second rank in weed abundance. Because *D. v. virgifera* larvae are known to survive only on roots of monocot plants we concentrated on the different grass species (Table 1), rather than testing other dicot weeds. All weed seeds were collected from weeds in the sampled maize fields. The seeds were stored at 15°C for eight months before being used. Additionally, we obtained seeds of monocot crops, which are grown in southern Hungary (*Sorghum halepense* L. and *S. bicolor* L.). We also included winter wheat (*Triticum aestivum* L.) in our tests, because it is the most common monocot crop in Central Europe. The same experiments as described below were also performed using 17 european maize varieties (Moeser and Vidal 2003c). The two highest values for mean larval weight gain, mean amount of ingested food and mean ECI are given in the results for comparison.

Table 1. Host plants used in feeding trials with *D. v. virgifera* larvae. The order is the same as in the figures 1 - 3. The origin of the seeds is indicated.

Family	Species	Source
Poaceae	Cynodon dactylon	Collected in Hungarian maize fields
Poaceae	*Sorghum halepense*	Cereal Research Company, Szeged
Poaceae	Echinochloa crus-galli	Collected in Hungarian maize fields
Poaceae	Setaria italica	Collected in Hungarian maize fields
Amaranthaceae	*Amaranthus* sp.	Collected in Hungarian maize fields
Poaceae	*Eragrostis* sp.	Dreschflegel GmbH, Germany
Poaceae	*Sorghum bicolor*	Cereal Research Company, Szeged
Poaceae	*Triticum aestivum* var. "Bussard"	Lochow-Petkus GmbH, Germany
Poaceae	Setaria verticilaria	Collected in Hungarian maize fields
Poaceae	*Setaria glauca*	Collected in Hungarian maize fields
Poaceae	*Panicum miliaceum*	Collected in Hungarian maize fields

The plants were grown in a greenhouse for 10 weeks. The winter wheat was vernalized for two months at 4°C before planting. The substrate was half sand and half potting soil (RTS spezial, Oekohum GmbH, Dransfled, Germany). This mixture was used because it could be easily removed from the roots by washing. The roots obtained were cleaned thoroughly and cut into pieces ranging from 0.8 to 0.9 g fresh weight. The amount of roots used in the experiments was calculated from simultaneous experiments using maize roots. According to data obtained from these experiments *D. v. virgifera* larvae are able to consume up to 0.7 g fresh weight of roots in a six day feeding trial. After introducing the roots into the experimental test tubes, sufficient vermiculite (Klein GmbH, Zellertal, Germany) was added to completely surround and cover the roots. The moisture content was subsequently adjusted to a level of a moist but not saturated environment (about 2.5 ml water in this design). Free water droplets were avoided, because larvae got trapped in these droplets, which increased in size as the larvae moved around and finally led to immobility and suffocation. Details for the experimental design are given in Moeser and Vidal (2003a).

The larvae used in the experiments were obtained by the following protocol derived from Jackson (1986): The eggs of *D. v. virgifera* were acquired from females caught

in the field in Southern Hungary. They were kept in cages and were allowed to oviposit for 2.5 months. The eggs were stored for a minimum period of five months at 8°C. At the beginning of each experiment the required number of eggs were incubated for two weeks at 26°C and 60% RH. Five days before the first larvae were expected to hatch 50 g of maize seeds were mixed with 200 g of regular potting earth and thoroughly moistened. The growing maize plants served as food for the larvae until they were extracted by a modified Berlese funnel (approximately 16 d after first hatch). This modified extraction method comprised of a sieve of 0.7 cm mesh size which was placed over a water container. The earth from the small containers with the plants and larvae was placed into the sieve and a light bulb was positioned above. The heat and moisture gradient forced the larvae to move downwards and to finally fall into the water container. They were then skimmed off the water surface and used in the experiment. Only second instar larvae of a weight ranging from 1.0 to 2.9 mg fresh weight were used in the experiments. The restriction to a single age/size class was necessary because larvae from this particular class proved most suitable regarding the results of these experiments (Moeser and Vidal 2003a). The first instars were not used because the larvae were too sensitive to changes in their environment, such as moisture or food. The extracted larvae were weighed and placed inside the test tubes on top of the vermiculite embedded root pieces. The tubes were closed and kept in darkness at 26°C and 60% RH. After six days the larvae and roots were extracted, dried for three days at 80°C and weighed. A fine scale (Sartorius GmbH, Goettingen, Germany; Model: Micro MC5 / SC2) was used to measure up to 0.001 mg differences in larval weight.

C/N Analyses.

For C/N Analyses a sufficient amount of roots, which were also obtained during the preparations for the feeding trials, were dried at 80°C for three days. Thirty mg of dry roots of each variety were then pooled because single roots did not have sufficient weight to be examined individually. The 30 mg were finely ground and three samples of 5 mg dry weight were taken. These were compared to a standard of 5 mg Acetanilide (Merck GmbH, Germany) every 20 samples in a C/N analyzer (Model: Vario EL III, Elementar Analysensysteme Hanau GmbH, Germany). The known quantity of C and N in the standard allowed for the calculation of the amount of these elements present in each root sample. The C/N ratio was subsequently determined.

Sterol analyses.

While preparing the roots for the feeding trials described above, three samples of roots per plant species weighing 0.5 g (fresh weight) each were deeply frozen in liquid nitrogen and stored at -20°C until further processing. To extract the phytosterols the roots were ground to a fine powder under liquid nitrogen using a mortar. To each sample a mixture of 5 ml 10 M potassium hydroxide solution, 15 ml 96% ethanol and 0.3% Pyrogallol (Merck GmbH, Germany) was added. An ultrasonic homogenizer (Model: Sonoplus HG 2200 / UW 2200, 200 W, 20 kHz, Bandelin GmbH, Germany) was used for 30 sec to enhance further cell breakdown and to free the solution from microscopic air bubbles trapped inside the vial. The samples were subsequently kept in a shaker water bath at 80°C for 2.5 h. After cooling the samples to room temperature, 40 µl of an internal standard were added (Cholesterol 4 mg / ml chloroform, Merck GmbH, Germany). The sterols were extracted by applying 10 ml hexane to each sample. After thorough shaking for 10 sec the hexane fraction was transferred to a rotary evaporator flask with an Eppendorf pipette. This extraction step was repeated once. The total of 20 ml hexane solution was washed with 1 ml de-mineralized water, which was then extracted with an Eppendorf pipette. The samples were distilled using a rotary evaporator and a water bath of 42°C. The pertaining sterols were resolved in 1.5 ml hexane through gentle shaking and transferred to 1.5 ml Eppendorf cups. After centrifugation with 10,000 rpm for 10 min. the supernatant was transferred to vials and the hexane was evaporated overnight at 50°C in a hot block. The sterols were then resolved in 240 µl hexane and 60 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA, Fluka / Riedel-deHaen GmbH, Germany) and incubated at 70°C for 20 min. 1 µl of each sample with a split of 1:50 of extracted sterols were analyzed using a gas chromatograph (Shimadzu GmbH, Model: GC14/15A) with a flame ionization detector. The samples were run on a fused silica column (SPB-1; 1.3m x 0.32mm, 0.25µm film thickness, Supelco Inc./ Sigma-Aldrich, Germany). Helium was used as a carrier gas, the make-up gas was synthetic air with a linear velocity of 35cm/s. The temperature program was initially 5 min at 180°C then increase to 290°C at 4°C/min with a 20 min postrun time. The detector temperature was 300°C. Peak areas were calculated using an integrator and the internal standard. The sterols represented by each peak were identified beforehand

using GC-MS with synthetic sterols as comparisons. The individual peak areas were summed up which resulted in the total sterol content.

Statistics.

To estimate differences regarding the larval weight gain and the amount of ingested food an analysis of covariance (ANCOVA) was performed. The weight gain of the larvae or the amount of ingested food was used as the dependent variable, while the initial fresh weight of the larvae served as the covariate to correct for an eventual bias due to different initial weights (Raubenheimer and Simpson 1992; Horton and Redak 1993). For a pairwise comparison between the host plants an analysis of variance (ANOVA) with a Bonferroni adjustment was performed. Systat 10 for Windows (SPSS Inc. 2000) was used for computation. To correlate two variables we performed linear regression analysis using SigmaPlot 2000 for Windows 6.0 (SPSS Inc. 2000).

Results-

The weight gain of *D. v. virgifera* larvae on alternative host plants ranged from positive to negative values (Fig. 1). There were significant differences between the different plant species with regard to larval weight gain (F = 2.99; df = 10, 265; P = 0.002). Additionally the initial fresh weight of the larvae used as the covariate had a significant influence on weight gain (F = 18.63; df = 10, 265; P < 0.001). Three common weeds and winter wheat yielded positive mean weight gain, all other plant species tested yielded a mean weight loss. However, in *Eragrostis* sp. and *Sorghum bicolor* several larvae of *D. v. virgifera* displayed considerable weight gain as indicated by the positive standard error.

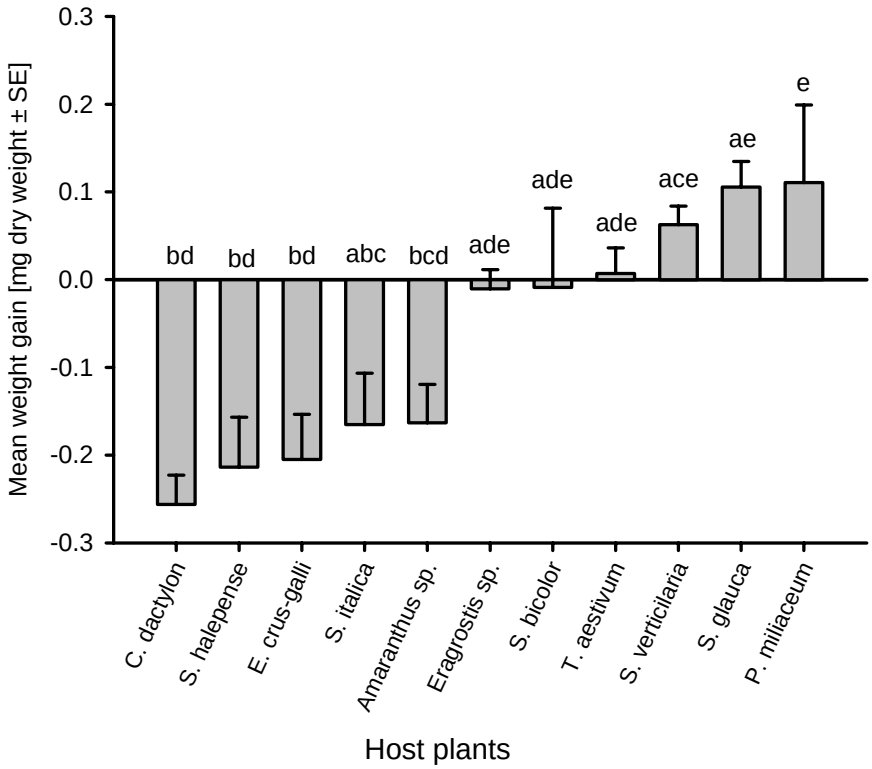

Figure 1. Weight gain of *D. v. virgifera* larvae feeding on roots of alternative host plants. Identical letters above bars indicate no significant difference (ANOVA, Bonferroni adjustment).

The mean weight gain of larvae feeding on *S. glauca* (0.11 mg dry weight ± 0.03 SE) and *P. miliaceum* (0.11 mg dry weight ± 0.08 SE) were exceeding the highest weight

gain of larvae feeding on maize roots. The highest values feeding on maize roots were 0.1 mg dry weight ± 0.03 SE and 0.08 mg dry weight ± 0.02 SE.

Significant differences were measured with respect to the amount of ingested food on the different host plants (ANCOVA: $F = 6.82$; df = 10, 265; $P < 0.001$; Fig. 2). The initial fresh weight of larvae used as a covariate had no significant influence on the amount of feeding (ANCOVA: $F = 0.38$; df = 10, 265; $P = 0.58$). The host plants, which yielded the highest larval weight gain, were not the ones which were fed on most.

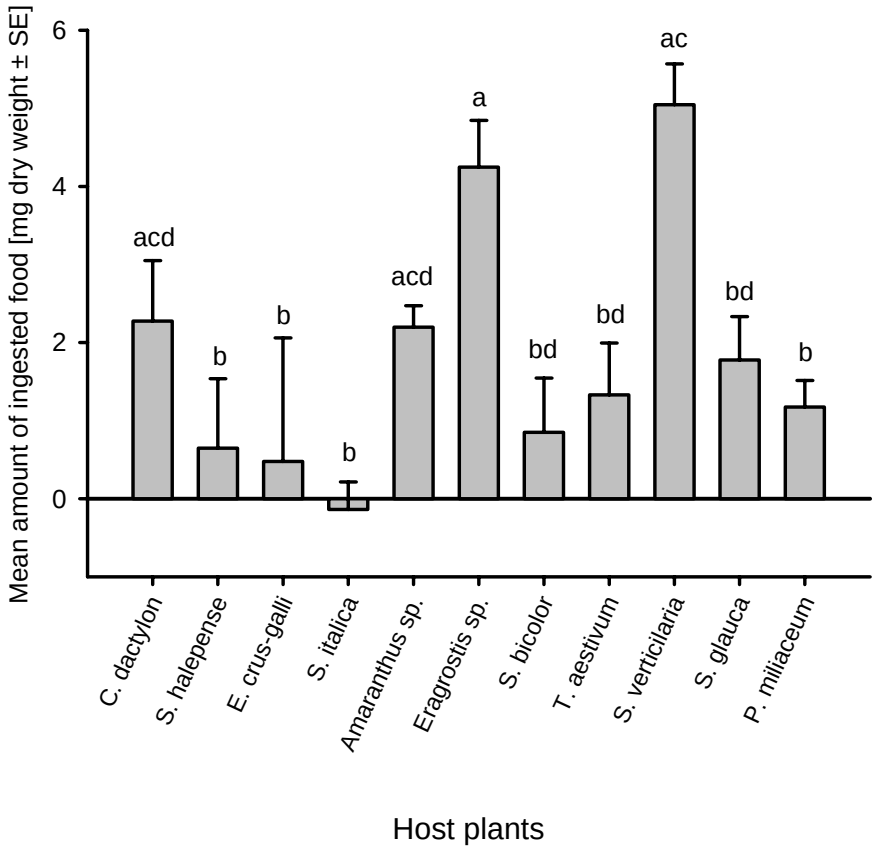

Figure 2. Amount of ingested food of alternative host plants by *D. v. virgifera* larvae. Identical letters above bars indicate no significant differences between plant species (ANOVA, Bonferroni adjustment).

The most pronounced feeding occurred on roots of *Setaria verticilaria*, which resulted in considerable weight gain, followed by *Eragrosti*s sp. which led to a mean weight loss although some individuals showed an increase in weight. *C. dactylon* was ranked third but was the most unsuitable plant species with regard to larval weight gain. The lowest amount of feeding occurred in those plant species, which were

unsuitable for larval development. The other species showed intermediate feeding rates.

The highest mean feeding rates (*S. verticilaria*: 5.04 mg dry weight ± 0.46 SE and *Eragrostis* sp.: 4.24 mg dry weight ± 0.55 SE) were in the same range as the highest mean feeding rates on maize roots (6.4 mg dry weight ± 0.75 SE and 4.71 mg dry weight ± 0.41 SE).

The larval weight gain was significantly correlated to the amount of ingested food; however the linear regression accounted for only 4% of the variability (R^2 = 0.039; *F* = -0.016 + 0.012x; *P* = 0.02).

A highly significant linear relation was found between the initial and the final weight of the larvae in our study (R^2 = 0.92; *F* = 0.07 +0.81x; *P* <0.0001; compare “Material and Methods” for linear relationship as prerequisite for ECI).

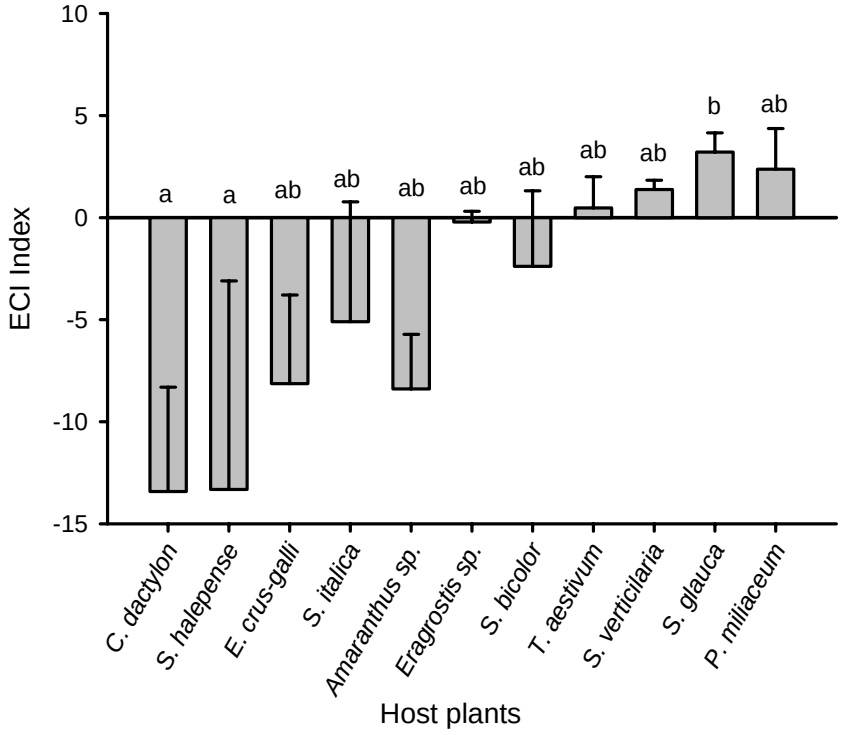

Figure 3. Food conversion efficiency index (ECI) of *D. v. virgifera* larvae on different host plants. Identical letters above bars indicate no significant difference between plant species (ANOVA, Bonferroni adjustment).

The ECI showed a similar pattern (Fig. 3) compared to the values for weight gain of *D. v. virgifera* larvae. Those plant species on which larvae gained weight had a positive ECI, while those plants which showed a decrease in larval weight had a negative ECI.

Significant differences could be observed between the host plants which allowed for highest food conversion efficiency (*S. glauca*) and the two host plants which resulted in the lowest efficiency (*C. dactylon* and *S. halepense*) (F = 1.97; df = 10, 180; P = 0.04). The initial fresh weight of the larvae used as the covariate had no significant influence on this result (F = 0.21; df = 10, 180; P = 0.65). The highest mean efficiency was reached when feeding on *S. glauca* (3.41 ± 0.93 SE) and on *P. miliaceum* (2.37 ± 1.98 SE). These ECI values were in the same range as the highest efficiencies reached when feeding on maize tissue (4.55 ± 1.85 SE and 3.61 ± 1.24 SE).

C/N ratio

The weight gain of *D. v. virgifera* larva was not significantly correlated (P = 0.07) to the amount of nitrogen determined in the root tissue (Table 2). Furthermore it was also not correlated to the carbon content and the C/N ratio. Significant linear relationships were observed between the amount of ingested food and the carbon and the nitrogen content as well as the C/N ratio (Table 2).

Table 2: Correlations between larval weight gain, the amount of ingested food and the amounts of carbon, nitrogen and their respective ratio.

Variables	Regression	R^2	significance
Weight gain vs. C/N ratio	F = 49.72 + 16.52x	0.08	n. s.
Weight gain vs. C content	F = 44.70 + 1.86x	0.09	n. s.
Weight gain vs. N content	F = 0.09 + -0.64x	0.44	*
Ingested food vs. C/N ratio	F = 42.94 + 3.18x	0.49	**
Ingested food vs. C content	F = 43.87 + 0.39x	0.68	**
Ingested food vs. N content	F = 1.03 + -0.05x	0.58	**

n. s. = not significant.

* = significant at the 10% level.

** = significant at the 5 % level.

Phytosterols

Five major sterols were detected: Brassicasterol, Campesterol, Methylcholesterol Sitosterol and Stigmasterol. The individual amounts were summed up after integrating the peak areas to result in the total sterol content. There was no linear relationship between the overall sterol content and the mean larval weight gain (R^2 = 0.015; *F* = 0.03 -0.0004x; *P* = 0.72; Fig. 4), but we encountered a significant relationship between the overall sterol content and the amount of ingested food (R^2 = 0.33; *F* =-3.05+0.024x; *P* = 0.05; Fig. 4).

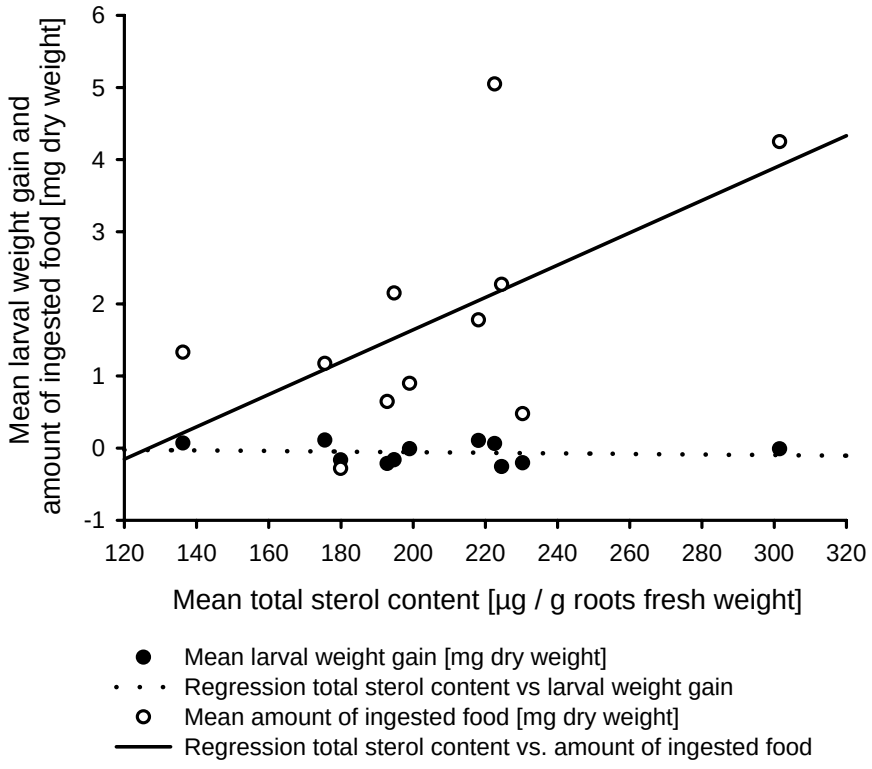

Figure 4. Correlations between the overall sterol content and the mean larval weight gain and the mean amount of ingested food.

Discussion

D. v. virgifera larvae were able to survive and grow on various monocot host plants other than maize. Our data suggests that several weed species as well as winter wheat are as suitable as maize roots for larval development (Moeser and Vidal 2003c).

D. v. virgifera larvae are able to develop on several other grasses from the Poaceae family but exhibit a drastically reduced survival rate (Branson and Ortman 1967, 1970). Our results do not contradict these findings. However, growth and feeding on grass weeds and crops is comparable in magnitude to maize (Moeser and Vidal 2003c). As we provided excess food to avoid starvation of the larvae, it seems that in some cases such as *S. glauca* or *T. aestivum* it is not the quality of the food but the quantity that limits *D. v. virgifera* survival. Root distribution and the three-dimensional geometry in the soil under natural conditions may not be advantageous for *D. v. virgifera* larvae. We suggest that monocot host plants other than maize may indeed provide sufficient food to support substantial larval feeding when grown at high densities.

The potential of *D. v. virgifera* to develop on *T. aestivum* has been clearly demonstrated. Thus a crop rotation including maize and winter wheat could lead to *D. v. virgifera* populations adapted to winter wheat as a host plant in certain situations where the phenology of wheat is appropriate for larval growth like it is in Central Europe. The adaptation of *D. v. virgifera* to the US soybean–maize crop rotation system has led to damage on first year corn planted after soybean ,but not to soybean. Rotation with winter wheat would offer *D. v. virgifera* a potentially suitable host, so even host switching may be selected for.

While *Eragrostis* sp. and *S. italica* were considered suitable for larval development (Branson and Ortman 1970), we only found some individuals to be able to grow on *Eragrostis* sp. and none at all which developed on *S. italica*. However, some larvae were able to grow on *S. bicolor*, which was considered to be unsuitable for *D. v. virgifera*, because of the hydrocyanic acid present in the its roots (Assabgui et al. 1993).

We encountered pre as well as postingestive effects of host plants on *D. v. virgifera* in our feeding trials. When the larvae did not feed on a given host (*S. italica*) or fed only very little (*S. halepense* and *E. crus-galli*) pre-ingestive effects like antibiosis may be attributable for this interaction. In other cases, *D. v. virgifera* larvae were feeding substantially on weed species such as *C. dactylon* but did not gain any weight. Here postingestive effects on *D. v. virgifera* larvae have to be considered, where essential nutritive compounds may be missing (see “Phytosterols section below”) or substances are present which prove detrimental for larval development after ingestion.

C/N ratio.

The carbon-nutrient balance hypothesis (CNBH) aims at interpreting the plants point of view of an herbivore-plant interaction (Hamilton et al. 2001). The CNBH predicts an increase of nitrogen based defense compounds such as alkaloids (Lerdau and Coley 2002) if nutrients are abundant, but light is limited. As the plants we used in the feeding trials were grown in the greenhouse with sufficient nutrients available we prefer to suggest light to be the limiting factor. As *D. v. virgifera* fed less and gained less weight on those varieties with a high nitrogen content we conclude that N-based defense compounds may be responsible. The hypothesis of the existence of a carbon-based defense could be rejected, because *D. v. virgifera* larvae were feeding more on those host plants which contained more carbon.

Phytosterols

Feeding behavior of *D. v. virgifera* was strongly influenced by phytosterol content. While the larvae were feeding more on those plants with a high phytosterol content they did not gain more weight on these plants. Sterols are essential nutrients for insects, but metabolic constraints can limit which phytosterols support normal growth and development (Behmer and Elias 1999). Thus, a relative decrease in one sterol compared to others would lead to an increase in feeding to compensate for the deficient component. It is unknown which phytosterols are metabolised by *D. v. virgifera*. As could be shown for *Phyllotreta cruciferae* Goeze, altered phytosterol content in *Brassica napus* L. resulted in reduced survival and prolonged larval stages (Bodnaryk et al. 1997). *Heliothis zea* Hübner reared on an artificial diet displayed a huge variety of responses, when fed different sterols at different compositions (Nes et al. 1997). When the grasshopper *Schistocerca americana* Drury was confronted with sterols of Spinach (*Spinacia oleracea* L.) the results revealed a mixture of suitable and unsuitable sterol components, where the latter acted as feeding deterrents (Behmer and Elias 1999). This may explain the antibiosis effect we demonstrated with several plant species in our study. When confronted with non-host phytosterols the specialist herbivore *Plutella xylostella* L. exhibited reduced growth while growing best when the composition of sterols were similar to the composition of sterols in its host plant (Behmer and Grebenok 1998). Whether the sterol

composition of the host plants we tested resembles the sterol composition in maize remains to be investigated.

We conclude that *D. v. virgifera* larvae can and most likely will use alternative hosts for larval development. Weeds will be used as reservoirs for *D. v. virgifera* larvae to develop on in the absence of maize. Other monocot crops such as winter wheat are likely to be affected as well. To what extent these host plants are used by *D. v. virgifera* will be determined by the selection pressure imposed, for example by crop rotation. If used incorrectly this cultural practice could in fact select for *D. v. virgifera* using other monocot crops like winter wheat or monocot weeds. The success of eradication programs, even in situations with no maize production, seems unlikely in the light of our results.

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References Cited

Assabgui, R. A., J. T. Arnason and R. I. Hamilton. 1993. Field evaluation of hydroamic acids as antibiosis factors in elite maize inbreds to the western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 88: 1482-1493.

Behmer, S. T. and D. O. Elias. 1999. Phytosterol structure and its impact on feeding behaviour in the generalist grasshopper *Schistocerca americana*. Phys. Entomol 24: 18-27.

Behmer, S. T. and D. O. Elias. 2000. Sterol metabolic constraints as a factor contributing to the maintenance of diet mixing in grasshoppers (Orthoptera: Acrididae). Phys. and Bioche. Zool. 73: 219-230.

Behmer, S. T. and R. J. Grebenok. 1998. Impact of dietary sterols on life history traits of a caterpillar. Phys. Entomol. 23: 165-175.

Bodnaryk, R. P., M. Luo and L. Kudryk. 1997. Effects of modifying the phytosterol profile of canola, *Brassica napus* L., on growth, development, and survival of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) and the aphids *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Can. J. Plant Sci. 77: 677-683.

Branson, T. F. 1982. Olfactory response of larvae of *Diabrotica virgifera virgifera* to plant roots. Ent. Exp. Appl. 31: 303-307.

Branson, T. F. and E. E. Ortman, 1967. Host range of larvae of the western corn rootworm. J. Econ. Entomol. 60: 201-203.

Branson, T. F. and E. E. Ortman. 1970. The host range of larvae of the western corn rootworm: Further studies. J. Econ. Entomol 63: 800-803.

Chiang, H. C. 1973. Bionomics of the northern and western corn rootworm. Annu. Rev. Entomol. 18: 47-72.

EPPO. 2003. http:// www.eppo.org/QUARANTINE/Diabrotica_virgifera/diabrotica_ virgifera.html

Gray, M. E., E. Levine and H. Oloumi-Sadeghi. 1998. Adaptation to crop rotation: Western and northern rootworms respond uniquely to a cultural practice. Recent Res. Devel. in Entomology 2: 19-31.

Hamilton, J., A. Zangerl, E. DeLucia and M. Berenbaum. 2001. The carbon-nutrient bilance hypothesis: its rise and fall. Ecol. Letters 4: 86-95.

Horton, D. R. and R. A. Redak, 1993. Further comments on analysis of covariance in insect dietary studies. Entomol. Exp. Appl. 69: 263-275.

Jackson, J. J. 1986. Rearing and handling of *Diabrotica virgifera* and *Diabrotica undecimpunctata howardi*. *In* J. L Krysan and T. A. Miller (eds.), Methods for the study of pest *Diabrotica*. Springer-Verlag, New York: pp. 25-47.

Johnson T. B. and F. T. Turpin. 1985. Northern and western corn rootworm (Coleoptera: Chrysomelidae) oviposition in corn as influenced by foxtail populations and tillage systems. J. Econ. Entomol. 78: 57-60.

Krysan, J. L. and T. A. Miller. 1986. Methods for the study of pest *Diabrotica*. Springer Verlag, New York, 260 pp.

Lerdau, M and P. D. Coley. 2002. Benefits of the Carbon-Nutrient Balance Hypothesis. Oikos 98: 533-535.

Moeser, J. and S. Vidal. 2003a. How to measure the food utilization of subterranean insects: a case study with the western corn rootworm (*Diabrotica virgifera virgifera*). Submitted to Journal of Applied Entomology

Moeser, J. and S. Vidal. 2003b. Does plasticity in adult feeding behavior facilitate the invasion of Europe by the maize pest *Diabrotica virgifera virgifera*? Submitted to Agric. Ecosyst. and Environment.

Moeser, J. and S. Vidal. 2003c. Highly variable response of larvae of the invasive maize pest *Diabrotica virgifera virgifera* to European maize varieties. Submitted to Journal of Economic Entomology

Nes, W. D., M. Lopez, W. Zhou, D. Guo, P. F. Dowd and R. A. Norton. 1997. Sterol utilization and metabolism by *Heliothis zea*. Lipids 32: 1317-1322.

Pimentel, D., L. Lach, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50: 53-65.

Raubenheimer, D. and S. J. Simpson. 1992. Analysis of covariance: an alternative to nutritional indices. Entomol. Exp. Appl. 62: 221-231.

Scriber, J. M. 1984. Nitrogen nutrition of plants and insect invasion. *In* R. D. Hauck (ed.), Nitrogen in crop production. Amer. Soc. Agron. Publ., Madison, Wisc. Chapter 34.

Scriber, J. M. and F. Slansky Jr. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183-211.

Slansky, F. Jr. and J. M. Scriber. 1985. Food consumption and utilization. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 88-163.

Strnad S. P. and P.E. Dunn. 1989. Host search behavior of neonate western corn rootworm (*Diabrotica virgifera virgifera*). J. Insect Physiol. 36: 201-205

SPSS Inc. 2000. Systat 10 for Windows; SPSS Inc., Chicago.

Svoboda, J. A. 1984. Insect steroids: Metabolism and function. *In* W. D. Nes, G. Fuller and L. S. Tsai (eds.), Isoterpenoids in plants: Biochemistry and function. Marcel Dekker Inc. New York. pp. 367-388.

Svoboda, J. A. and M. J. Thompson. 1985. Steroids. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 137-175.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

Walker, B. and W. Steffen. 1997. An overview of the implications of global change for natural and managed terrestrial ecosystems. Conservation ecology 1(2): http://www.consecol.org/vol1/iss2/art2

Williamson, M. 1996. Biological invasions. Chapman and Hall, UK. 244pp.

Worner, S. P. 2003. Predicting the invasive potential of exotic insects. *In* G. J. Hallman and C. P. Schwalbe (eds.), Invasive arthropods in agriculture: Problems and solutions. Science Publishers Inc., Enfield, NH. pp. 120-137.

Discussion:

When an insect invades new areas it has to meet different challenges. Besides other factors the invasive potential of an insect is determined by its nutritional ecology. All life stages in the life cycle have to find appropriate food items to succeed in the invasion. If only one stage fails, the invasions stops. If a maize specialist insect herbivore invades an area where maize is grown (like it is happening in Europe just now with regard to the Western Corn Rootworm [**WCR**], *Diabrotica virgifera virgifera*), it should not be faced with too much difficulties for the insect to find suitable food patches. However, due to the specialization of WCR in using an annual crop, the beetle has to cope with temporally limited availability of its main food sources. Furthermore harvest and cultural practices may put up high selection pressures on WCR to use alternative food resources. Both adults as well as larvae have to cope with these challenges. European maize production systems differ from US agro-ecosystems in having maize fields distributed more patchy, in most areas an at least 3 year rotational system and a more diverse landscape (Messner, pers. com).

Phenotypic plasticity of an invasive species is known to facilitate biological invasions (Agrawal, 2001). We were able to demonstrate the great plasticity of WCR nutritional ecology with regard to larval and adult food utilization and performance. The adult beetles exhibited a high adaptability with regard to their nutritional ecology in their new range. In advance of our studies it was suggested that food use in WCR should depend on maize phenology (Ball, 1957; O'Neal et al., 2002) We were able to corroborate this hypothesis for the first time, by demonstrating the use of maize tissue in adult WCR according to maize phenology. WCR was known as a maize pollen and silk feeders in general until now (Ludwig and Hill, 1975). However, in Europe the beetles use a significantly larger array of resources compared to beetles from the US (Ludwig and Hill, 1975). A preference for maize pollen and silk could also be shown in the study presented here, but adults used other food sources as well. In Europe, the majority of all flowering weeds present in the maize fields in Southern Hungary were used as alternative pollen sources by WCR. The use of pollen from alternative host plants by adult beetles was depending on maize

phenology. Moreover, we demonstrated a strong influence of the diversity of flowering weeds in a given habitat on WCR feeding behavior.

The use of alternative food resources may facilitate spreading and even accelerate spreading speed of WCR in Europe. Female and male beetles use alternative food resources differently. Because female beetles were using weed pollen more extensively than males we suggest that alternative pollen resources have to be considered especially valuable for fecundity and thus propagule pressure.

The results from this study have also implications for integrated pest management measures: For example, in Southern Hungary the intercropping of maize and squash is a common cropping system. This cultural practice should be avoided as it adds to the resources used by WCR. Moreover, dicot weeds should be omitted from the field, especially *Amaranthus* sp. and *Chenopodium* sp. Additionally, to reduce pollen sources which are important for the beetles towards the end of the vegetation period *Ambrosia* sp. should be also removed from the fields.

Until now, larval performance in WCR was measured indirectly by root damage ratings (Ksyan and Miller, 1986). In the present study a newly developed method was used to examine the food conversion efficiency of WCR feeding on different European maize varieties.

WCR larvae showed highly variable responses when feeding on different European maize varieties. The different phytosterol content and the C/N ratio had significant influences on the suitability for WCR development. In the past research on resistant maize varieties mainly focused on pre-ingestive effects like deterrent substances (Assabgui et al., 1995). We suggest future search for unsuitable maize varieties to focus on altered composition of essential nutritional compounds like phytosterols to interfere with regular larval development.

Until now, survival of larvae was the only measure to estimate larval performance on alternative host. This qualitative measure may now be supplemented by the quantitative data from this study. By using the newly developed method we were able to show that larvae of WCR perform as well on several alternative host plants as on maize. Especially, they performed comparably well on some monocot grasses and crops, but not on dicot plants.

The implications for integrated pest management of these findings are various: As WCR larvae have the ability to survive on monocot crops including winter wheat,. any crop rotation practice should avoid rotating maize and any other monocot crop the next year. Similarly, monocot weeds should be avoided in maize fields because they will allow WCR development the following year even in case of crop rotation. WCR has already shown its adaptation capability by changing the oviposition preference in the maize-soy bean rotation in the US corn belt. When strong selection pressures like crop rotation act on WCR populations this pest species can be expected to adapt to use other available resources.

The nutritional ecology of WCR is most likely to change as the invasion progresses throughout Europe according to the resources which are available. The diversity of weeds which may act as additional food resources for the adults is different in different parts of Europe. The abundance of weeds will be influenced by differing production intensities (e.g. the use of herbicides). Moreover, the diversity and abundance of monocot weeds and crops for larval development will further impact the ability of WCR to maintain the invasion. In Europe more diverse maize varieties are used. Especially local ones, which are grown only on a small scale, may provide useful sources of resistance breeding programs.

Besides the direct interaction with the host plant, also indirect interactions with other organisms are likely to occur: If e.g. the colonization of the roots by microorganism has an impact on larval performance will be a future field for research. Also the study of competition with other maize pests or pathogens may provide useful insights into invasion processes.

References:

Assabgui, R. A., J. T. Arnason and R. I. Hamilton, 1983. Field Evaluation of Hydroamic Acids as Antibiosis Factors in Elite Maize Inbreds to the Western Corn Rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 88: 1482-1493

Agrawal, A. A., 2001. Phenotypic Plasticity in the Interactions and Evolution of Species. Science, 294: 321-326

Ball, H. J., 1957. On the biology and egg-laying habits of the Western Corn Rootworm. J. of Econ. Entomol. 50: 126-128

Baufeld, P and S. Enzian, 2001. Simulation model spreading scenarios for Western Corn Rootworm (*Diabrotica virgifera virgifera*) in case of Germany. Proceedings of the XXI IWGO Conference, Legnaro, Italy. Veneto Agricoltura. pp. 63-67

Behmer, S. T. and D. O. Elias, 1999. Phytosterol structure and its impact on feeding behaviour in the generalist grasshopper *Schistocerca americana*. Phys. Entomol 24: 18-27

Behmer, S. T. and D. O. Elias, 2000. Sterol Metabolic Constraints as a Factor Contributing to the Maintenance of Diet Mixing in Grasshoppers (Orthoptera: Acrididae). Phys. and Bioche. Zool. 73: 219-230

Behmer, S. T. and R. J. Grebenok, 1998. Impact of dietary sterols on life history traits of a caterpillar. Phys. Entomol. 23: 165-175

Branson, T. F. and E. E. Ortman, 1967. Fertility of Western Corn Rootworm Reared as Larvae on Alternate Hosts. J. Econ. Entomol. 60: 595

Branson, T. F. and E. E. Ortman, 1970. The Host Range of Larvae of the Western Corn Rootworm: Further Studies. J. Econ. Entomol 63: 800-803

Branson, T. F. and J. L. Krysan, 1981. Feeding and Oviposition Behavior and Life Cycle Strategies of *Diabrotica*: An Evolutionary View with Implications for Pest Management. Environ. Entomol. 10: 826-831

Chiang, H. C., 1973. Bionomics of the northern and western corn rootworm. Annu. Rev Entomol. 18: 47-72

Eben, A., M. E. Barbercheck and Martín Aluja S., 1997. Mexican diabroticite beetles: I. Laboratory test on host breadth of *Acalymma* and *Diabrotica* spp. Ent. Exp. Appl. 82: 53-62

Elton, C. S., 1958. The Ecology of Invasions by Animals and Plants. University of Chicago Press, 181 pp.

Hamilton, J., A. Zangerl, E. DeLucia and M. Berenbaum, 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecol. Letters 4: 86-95

Johnson T. B. and F. T. Turpin, 1985. Northern and Western Corn Rootworm (Coleoptera: Chrysomelidae) Oviposition in Corn as Influenced by Foxtail Populations and Tillage Systems. J. Econ. Entomol. 78: 57-60

Krysan, J. L. and T. A. Miller, 1986. Methods for the study of pest *Diabrotica*. Springer Verlag, New York, 260 pp.

Lerdau, M and P. D. Coley, 2002. Benefits of the Carbon-Nutrient Balance Hypothesis. Oikos 98: 533-535

Ludwig, K. A. and R. E. Hill, 1975. Comparison of gut content of adult Western and Northern Corn Rootworm in Northeast Nebraska. Environ. Entomol. 4: 435-438

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout and F. A. Bazzaz, 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol. Appl. 10: 689-710

Mooney, H. A: and E. E. Cleland, 2001. The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. USA, 98: 5446-5451

Nes, W. D., M. Lopez, W. Zhou, D. Guo, P. F. Dowd and R. A. Norton, 1997. Sterol utilization and metabolism by *Heliothis zea*. Lipids 32: 1317-1322.

O'Neal, M. E., C. D. DiFonzo and D. A. Landis, 2002. Western Corn Rootworm (Coleoptera: Chrysomelidae) Feeling on Corn And Soybean Leaves Affected by Corn Phenology. Environ. Entomol. 31: 285-292

Pimentel, D., L. Lach, R. Zuniga and D. Morrison, 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50: 53-65.

Pimentel, D., L. Lach, R. Zuniga and D. Morrison, 2003. Environmental and economic costs of alien arthropods and other organisms in the United States. In G. J. Hallman and C. P. Schwalbe (eds.) Invasive arthropods in agriculture: Problems and solutions. Science Publishers Inc., Enfield, NH. pp. 107-117

Scriber, J. M., 1984. Nitrogen nutrition of plants and insect invasion. In R. D. Hauck (ed.) Nitrogen in crop production. Amer. Soc. Agron. Publ., Madison, Wisc. Chapter 34

Scriber, J. M. and F. Slansky Jr., 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183-211

Simberloff, D., 2003. How much information on population biology is needed to manage introduced species? Conservation Biology, 17: 83-92

Slansky, F. Jr. and J. M. Scriber, 1985. Food consumption and utilization. In G. A. Kerkut and L. I. Gilbert (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 88-163.

Svoboda, J. A., 1984. Insect Steroids: Metabolism and Function. In W. D. Nes, G. Fuller and L. S. Tsai (eds.) Isoterpenoids in Plants: Biochemistry and function. Marcel Dekker Inc. New York. pp. 367-388

Svoboda, J. A. and M. J. Thompson, 1985. Steroids. In G. A. Kerkut and L. I. Gilbert (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Vol. 4. Pergamon Press, New York. pp. 137-175.

Walker, B̃ and W. Steffen, 1997. An overview of the implications of global change for natural and managed terrestrial ecosystems. Conservation ecology 1: http://www.consecol.org/vol1/iss2/art2

Wheeler, D.,1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41: 407-431

Williamson, M, 1996. Biological Invasions. Chapman and Hall, UK. 244 pp.

Publications:

Articles (submitted):

Moeser, J. and S. Vidal, 2003b. Does food use by adults of the maize pest *Diabrotica virgifera virgifera* facilitate its invasion in Europe? Submitted to Agriculture, Ecosystems and Environment

Moeser, J. and S. Vidal, 2003a. How to measure the food utilization of subterranean insects: a case study with the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Submitted to Journal of Applied Entomology

Moeser, J. and S. Vidal, 2003c. Highly variable response of larvae of the invasive maize pest *Diabrotica virgifera virgifera* to European maize varieties. Submitted to Journal of Economic Entomology

Moeser, J. and S. Vidal, 2003d. Do alternative host plants enhance the invasion of the maize pest *Diabrotica virgifera virgifera* in Europe? Submitted to Environmental Entomology

Articles (published):

Moeser, J. and S. Vidal, 2001. Nahrungsquellen von adulten *Diabrotica virgifera virgifera* in Süd-Ungarn. Welche Rolle spielen Unkräuter?. Mitt. Dtsch. Ges. Allg. Angew. Ent. 13: 539-542

Moeser, J. and S. Vidal, 2002. Alternative food resources for adult *Diabrotica virgifera virgifera* in southern Hungary. Proc. of the XXI IWGO Conference, Padova, Italy19-24

Presentations at conferences or workshops:

Moeser, J. and S. Vidal, 2000. Threat to European maize production by the invasive quarantine pest Western Corn Rootworm (*Diabrotica virgifera virgifera*): a new sustainable crop management approach. Presentation at the workshop on “Research activities on maize production”, Brussels, Belgium

Moeser, J. and S. Vidal, 2000. Food resources used by *Diabrotica virgifera virgifera* in southern Hungary. Presentation at the VII Diabrotica subgroup meeting of the IWGO, Stuttgart, Germany

Moeser, J. and S. Vidal, 2001. Alternative food resources for adult *Diabrotica virgifera virgifera* . Are they opportunistic feeders?. Presentation at the XXI IWGO conference, Legnaro, Italy

Moeser, J. and S. Vidal, 2002. Europäische Maissorten gegen einen invasiven Maisschädling (*Diabrotica virgifera virgifera*) aus der Neuen Welt? Presentation at the 53. Plant protection Meeting , Bonn, Germany

Moeser, J. and S. Vidal, 2002. How adaptive is the invasive maize pest *Diabrotica virgifera virgifera* in European agroecosystems? Presentation at the workshop from the European Science Foundation “Evolution in invasive species”, Halle Germany

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