JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

Haemolymph defence of an invasive herbivore: its breadth of effectiveness against predators

J. G. Lundgren[1], S. Toepfer[2,3], T. Haye[2] & U. Kuhlmann[2]

1 USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD, USA
2 CABI Europe-Switzerland, Delemont, Switzerland
3 CABI Europe, c/o Plant Health Service, CABI Europe, Hodmezovasarhely, Hungary

Keywords
Tetramorium caespitum, *Zea mays*, biological control, Carabidae, diel cycle, Lycosidae

Correspondence
Jonathan Lundgren (corresponding author), NCARL, USDA-ARS, 2923 Medary Avenue, Brookings, SD, USA. E-mail: Jonathan.Lundgren@ars.usda.gov

Received: August 5, 2009; accepted: October 28, 2009.

doi: 10.1111/j.1439-0418.2009.01478.x

Abstract

Defensive characteristics of organisms affect the trophic linkages within food webs and influence the ability of invasive species to expand their range. *Diabrotica v. virgifera* is one such invasive herbivore whose predator community is restricted by a larval haemolymph defence. The effectiveness of this haemolymph defence against a range of predator functional and taxonomic guilds from the recipient biota was evaluated in a series of experiments. Eight predator species (Carabidae, Lycosidae, Formicidae) were fed *D. v. virgifera* 3rd instars or equivalent-sized maggots in the laboratory, and the mean times spent eating, cleaning their mouthparts, resting and walking following attacks on each prey were compared. Prey species were restrained in five Hungarian maize fields for 1 h periods beginning at 09:00 and 22:00 hours. The proportion of each species attacked and the number and identity of predators consuming each prey item were recorded. All predators spent less time eating *D. v. virgifera* larvae than maggots in the laboratory, and four of the eight predator species spent more time cleaning their mouthparts. The differential responses in the predator species indicate differences in susceptibility to the *D. v. virgifera* haemolymph defence. The predator communities (numerically dominated by *Tetramorium caespitum*) in the field showed clear diel patterns in their foraging behaviour, and *D. v. virgifera* was consumed by fewer predators than maggots. The defence of *D. v. virgifera* may partly explain how invasive insects that are exposed to an extensive predator community overcome biotic resistance to the invasion process.

Introduction

Invasive insects have profound and lasting effects on the food webs of recipient habitats, and how food webs respond to the introduction of an exotic species has important implications for the ultimate establishment and integration of these invasives. One way that resident food webs confront invasion by exotic species is through biotic resistance, in part through predation of the invader (Parker et al. 2006; Nunez et al. 2008; Harwood et al. 2009). The success of an invader depends in part on its defensive capabilities against predation. But, predator diversity within even the most disturbed subterranean arthropod communities is significant (Duelli and Obrist 1998; Downie et al. 1999; Duelli et al. 1999; Lundgren et al. 2006, 2009b; Juen and Traugott 2007), and predators rely differentially on a given herbivore within these systems. Thus, a key aspect of the invasion process is how predator constituents of a food web are relatively affected by the defensive capabilities of an invasive pest.

Haemolymph-based defence (Happ and Eisner 1961; de Jong et al. 1991; Peck 2000; Boevé and Schaffner 2003; Müller and Arand 2007; Vleiger et al. 2007) in an invasive insect is one physiological factor that influences the biotic resistance to invasion, and ultimately the trophic connectedness within even endeamic food webs. These haemolymph defences can act against predators by making the prey species sticky, toxic, or distasteful (Pasteels et al. 1983; Bowers 1992; Laurent et al. 2005). Because of the intense predation pressure experienced in soil-based food webs (Lundgren et al. 2006) and reduced mobility of immature stages of herbivorous insects (relative to the adult stage), there is often a disproportionate number of subterranean, herbivorous insects that possess chemical defences against predation (Pasteels et al. 1983). One group of insects that is particularly well known for both their herbivorous lifestyle and their chemical haemolymph-based defences are beetles in the Chrysomelidae (Pasteels et al. 1982, 2004; Dettner 1987; Hilker et al. 1992; Laurent et al. 2005). The intricacies of how these anti-predator defences influence biotic resistance to invasion by exotic insects remains to be well established.

As an invasive pest of international importance, *Diabrotica virgifera virgifera* LeConte (western corn rootworm; Coleoptera: Chrysomelidae) shapes the maize ecosystem through its consumption of maize root systems. Recent research has revealed that predator taxonomic and feeding guilds consume *D. v. virgifera* immatures to varying degrees under field conditions (Lundgren et al. 2009c; Toepfer et al. 2009). In part, predation intensity is driven by the spatio-temporal occurrences of *D. virgifera* and members of its predator community (Lundgren et al. 2009b,c). But a recent discovery has shown that *D. v. virgifera* larvae possess predator-repellent properties in their haemolymph (Lundgren et al. 2009a) which are unrelated to the previously identified cucurbitacin-based defences discussed by Tallamy et al. (2005). The larval defence of *D. v. virgifera* is based on offensive chemistry and the haemolymph's tendency for rapid coagulation on predator mouthparts (Lundgren et al. 2009a). Here, in addition to describing the diverse predator community present during the day and night in Hungarian maize fields, specific experiments were designed to (i) investigate whether predators are differentially affected by the haemolymph defence of *D. v. virgifera* in the laboratory, and (ii) substantiate that *D. v. virgifera* are attacked less intensively than maggots of *C. vicina* of equal size in Central European maize fields, in part because the former are defended from predation by their haemolymph. Thus, the current research is a first step in addressing how the *D. v. virgifera* haemolymph defences affect the strength of trophic interactions that this pest has with its predator community within maize agroecosystems.

Methods

Breadth of predators affected by *D. v. virgifera* haemolymph defence

Eight species of field-collected predators commonly found in European cornfields were selected; these predators represent a range of different feeding ecologies and natural histories present in the habitats of *D. v. virgifera* larvae. Chewing predators with different dietary specializations included *Carabus monilis* Fabricius (Carabidae), *Harpalus pensylvanicus* (DeGeer) (Carabidae), *Harpalus rufipes* (DeGeer) (Carabidae), *Poecilus cupreus* (L.) (Carabidae), *Pterostichus anthracinus* (Panzer) (Carabidae) and *Pterostichus melanarius* (Illiger) (Carabidae). Sucking predators were represented by *Trochosa ruricola* (DeGeer) (Aranae: Lycosidae). Ant workers were classified as a separate feeding category (described below). Predators were captured from maize fields in northwestern Switzerland using dry pitfall traps (except *H. pensylvanicus*, which was collected from farmland in Brookings, SD, USA). Following collection, they were maintained in the laboratory in dampened field soil and fed moistened cat food (Iam's Original with Chicken, The Iam's Company, Cincinatti, OH, USA).

Each of these predators was fed a *D. v. virgifera* 3rd instar or an equivalent-sized (based on mass) surrogate prey item (*Calliphora vicina* L. maggot; Diptera: Calliphoridae), and their feeding behaviours were monitored. *Pterostichus anthracinus* was fed 2nd instar *D. v. virgifera* and equivalent-sized maggots, as 3rd instars were too large for this predator to consistently attack. *Harpalus pensylvanicus* received maggots of *Sarcophaga bullata* Parker (Diptera: Sarcophagidae), a native North American fly of similar larval biology to the European *C. vicina*. These maggots were determined to be a suitable prey for a wide range of predators, and functioned as a control to observe predator behaviour under ideal circumstances. Twenty-four hours prior to the assay, predators were isolated into empty 100 mm diameter Petri dishes, where they were starved with only water to ensure hunger at the time of the assay (*C. monilis* was starved for 48 h, due to their large size and ability to withstand starvation). At the time of the assay, a predator was placed into a clean and empty Petri dish that contained either a 3rd instar *D. v. virgifera*, or a maggot of equivalent size. Each predator was observed for 10 min or until it attacked the prey item. The amounts of time devoted to eating, cleaning of mouthparts, resting, and walking were recorded for 2 min following the initial predation event. Cleaning of mouthparts was defined as wiping the mouthparts with the palps or legs to clear the oral cavity of congealed haemolymph, or wiping of the mouthparts on the dish. Observations were ceased after this period, because this was the minimum duration it took to entirely consume the maggot prey (*D. v. virgifera* were seldom entirely consumed). An exception to this is that the amount of time spent eating the *D. v. virgifera* larvae over the entire 10 min observation period was also recorded as a measure of a predator's likelihood to revisit the *D. v. virgifera* after the initial attack and repulsion. Predators that did not attack a prey item within 10 min were discarded from the analysis. Sample sizes for each treatment and each predator are presented in table 1.

A different procedure was applied to assess the effectiveness of the haemolymph defence against the group response of ants, a dominant predator in Central European farmland (see table 2 and Results section). A large number of *Tetramorium caespitum* (L.) (Hymenoptera: Formicidae) workers was removed from a Hungarian maize field, and maintained in a plastic tub on sugar water and moistened cat food for several weeks. For the assay, 15 worker ants were placed into a 100 mm diameter plastic Petri dish containing either a 3rd instar *D. v. virgifera* or an equivalent-sized *C. vicina* maggot. The arenas were watched constantly for 10 min, and the maximum number of ants attacking the prey item was recorded during each minute of observation. A total of 15 assays were conducted for each treatment.

Larval defence of *D. v. virgifera* under field conditions

Predation rates on sentinel *D. v. virgifera* larvae and *C. vicina* maggots were measured in five conventionally managed maize fields near Hodmezovasarhely in southern Hungary in 2007 and 2008. In 2007, three replicate plots each located in separate fields were sampled on 3-July. This time period corresponded closely with the 3rd stadium of *D. v. virgifera* (Toepfer and Kuhlmann 2006). The first of these fields (located at N 46.445°, E 20.345°; latitude, longitude) was commercially managed (with little non-crop vegetation), and of approximately 65 ha in size. The second field (located at N 46.439°, E 20.341°) was similar in size, and was commercially managed, although it was weedier than Field 1. The third field (located at N 46.439°, E 20.338°) was smaller in size (2.1 ha) than the other two. A square grid of 40 stations, four rows of 10 stations with each station

Table 1 The mean ± SEM time until first attack on *Diabrotica v. virgifera* larvae or *Calliphora vicina* maggots, and percent of predators' time spent eating, and cleaning their mouthparts during the subsequent 120 s

Predator	Time eating (%)		Time cleaning (%)		Duration to 1st attack (s)	
	D. v. virgifera	Maggot	*D. v. virgifera*	Maggot	*D. v. virgifera*	Maggot
Pterostichus anthracinus	70.21 ± 8.79 (20) A	94.91 ± 5.09 (19)	10.75 ± 5.19	0	261 ± 36	177 ± 28
	$\chi^2_1 = 5.75$, P = 0.02		$\chi^2_1 = 4.11$, P = 0.04		$\chi^2_1 = 2.80$, P = 0.10	
Pterostichus melanarius	59.28 ± 12.46 (15) AB	100 ± 0 (14)	12.00 ± 6.34	0	281 ± 41	267 ± 40
	$\chi^2_1 = 8.13$, P = 0.004		$\chi^2_1 = 4.15$, P = 0.04		$\chi^2_1 = 0.08$, P = 0.77	
Poecilus cupreus	44.09 ± 9.19 (23) BC	86.11 ± 8.25 (15)	51.74 ± 8.70	0	148 ± 27	177 ± 27
	$\chi^2_1 = 9.09$, P = 0.003		$\chi^2_1 = 17.46$, P < 0.001		$\chi^2_1 = 0.11$, P = 0.74	
Carabus monilis	43.97 ± 13.00 (13) AC	91.81 ± 5.38 (17)	25.77 ± 9.40	0	400 ± 42	211 ± 36
	$\chi^2_1 = 8.60$, P = 0.003		$\chi^2_1 = 9.33$, P = 0.002		$\chi^2_1 = 8.95$, P = 0.003	
Harpalus rufipes	19.17 ± 7.04 (16) CD	87.84 ± 6.43 (17)	55.00 ± 8.79	0	140 ± 24	275 ± 42
	$\chi^2_1 = 19.41$, P < 0.001		$\chi^2_1 = 20.38$, P < 0.001		$\chi^2_1 = 6.09$, P = 0.01	
Harpalus pensylvanicus	7.86 ± 3.10 (14) D	79.64 ± 9.17 (14)	67.61 ± 7.69	0	235± 44	254 ± 49
	$\chi^2_1 = 20.72$, P < 0.001		$\chi^2_1 = 23.68$, P < 0.001		$\chi^2_1 = 0.01$, P = 0.93	
Trochosa ruricolis	54.44 ± 12.69 (15) AC	100 ± 0 (16)	6.83 ± 4.63	0	172 ± 49	57 ± 13
	$\chi^2_1 = 10.82$, P = 0.001		$\chi^2_1 = 3.42$, P = 0.06		$\chi^2_1 = 1.60$, P = 0.21	

Sample sizes are denoted in parentheses in the columns indicating time spent eating. Percentage values within the columns indicating time spent eating by *D. v. virgifera* followed by different letters are significantly different from one another. Contrasts between the prey items for each behaviour are statistically represented beneath each comparison.

Table 2 Ants and total numbers of predators observed feeding on *Diabrotica virgifera* larvae or *Calliphora vicina* maggots during day and night sampling periods in Hungarian maize fields during 2007 and 2008

		No of feeding on		No of feeding during		
	Predator species[1]	*D. v. virgifera*	*C. vicina*	Day	Night	Grand total
2007	*Tetramorium caespitum* (Hymenoptera: Formicidae)	90 ± 22	231 ± 81	195 ± 62	126 ± 42	963
	Annual total per plot; mean ± SEM 20 species	96 ± 21 (289 total specimens; 14 species)	238 ± 81 (713 total specimens; 11 species)	198 ± 61 (595 total specimens; 4 species)	136 ± 70 (407 total specimens; 18 species)	1002 total specimens (21 species)
2008	*Tetramorium caespitum*	17 ± 5	58 ± 1	8 ± 4	67 ± 8	150
	Annual total per plot; mean ± SEM 11 species	25 ± 7 (50 total specimens; 8 species)	66 ± 2 (135 total specimens; 8 species)	12 ± 4 (23 total specimens; 4 species)	80 ± 8 (162 total specimens; 9 species)	185 total specimens (11 species)
Grand total; mean ± SEM	28 species	339	848	618	569	1187

spaced 5 m apart, was established at the margin of each field.

In 2008, two replicate plots located in separate fields were sampled on three dates (20-June, 24-June, and 2-July). These dates approximately correspond to the occurrence of 1st, 2nd and 3rd instars of *D. v. virgifera* during 2008 (Toepfer and Kuhlmann 2006). The first of these maize fields (located at N 46.438°, E 20.343°) was approximately 10 ha in size; the second field (located at N 46.437°, E 20.350°) was approximately 15 ha in size. In each plot, a square grid of 25 stations, five rows of five stations with each station spaced 3 m apart, was established at the margin of each field.

At each station (marked by flags), a set of sentinel larvae was placed in the fields at approximately 09:00 (day) and 22:00 (night). Each set consisted of a 3rd instar *D. v. virgifera* and a *C. vicina* maggot of similar size. The posterior segment of each prey item was impaled on a size 0 insect pin, which was then stuck into a 2-cm ball of clay. Larvae restrained in this manner can live for at least several hours, and seldom escape; for example, *C. vicina* maggots were known to over time pupate and eclose while impaled on a pin! At each station, the clay balls that the prey were stuck into were buried at or just beneath the soil surface at the base of maize plants. The two prey species were separated by the inter-row distance at each station (approximately 0.75 m; their placement at each station was randomly assigned).

After being left in the field for 1 h, the condition of the prey at each station was checked. Quietly, an observer would approach each station, and record whether the larva was alive and well, dead and present, missing from the pin, or in the act of being eaten. Flashlights were employed for the night samples. When predators were observed feeding on the prey, representatives were captured for identification. The number of predators feeding at a prey item was noted. A prey item was regarded as ''attacked'' if it was dead, missing, or in the act of being eaten.

Data analysis

In the laboratory assays, Kruskal–Wallis non-parametric ANOVA were used to compare the non-normal mean proportions of time spent eating, walking, resting, and cleaning between treatments fed maggots or *D. v. virgifera* larvae; this test was also used to compare the non-normal mean times to initial attack on the two prey types by each predator species. The amount of time spent eating *D. v. virgifera* larvae over 2 and 10 min observations among species was compared using parametric ANOVA (this data conformed to the assumptions of the model). The maximum number of ants attacking prey per minute were compared between the two prey treatments using rm-ANOVA, with prey species as the between subject comparison, and 10 levels of time as the within-subject variable.

In the field observations, the mean (log+1) proportions of maggots and *D. v. virgifera* larvae attacked per plot during 2007 and 2008 were compared using two-factor ANOVA (year and prey species as factors). The log number of predators found consuming each prey species per plot during 2007 and 2008 was compared using two-factor ANOVA (year and prey species as factors). Likewise, the log+1 mean proportions of both prey items (pooled) consumed per plot and the log number of predators attacking both prey items per plot were compared during the day and night sample periods using two-factor ANOVA (year and time period as factors).

Results

Breadth of predators affected by *D. v. virgifera* haemolymph defence

Although feeding durations on *D. v. virgifera* larvae were reduced for all predators relative to those fed maggots, the predators were repelled to different degrees. This was evidenced by the significantly different amounts of time spent eating by the different predator species during the 2 min directly after the first attack (fig. 1). Predators displayed different propensities for returning to the *D. v. virgifera* larva after their initial attack, as is evidenced by the duration spent eating over the entire 10 min observation period (fig. 1). Specifically, *Pt. anthracinus, Pt. melanarius, Po. chalcites,* and *Tr. ruricola* spent additional time eating after the initial attack, while *C. monilis, H. pensylvanicus, H. rufipes,* and *Te. caespitum* did not return to the *D. virgifera* prey (figs 1 & 2).

Of the other parameters/behaviours monitored, cleaning was the most consistently affected by prey type. All predators spent significantly more time cleaning their mouthparts when fed *D. v. virgifera* larvae relative to control maggots (table 1). However, only three of the seven species (*H. rufipes, H. pensylvanicus,* and *Poecilus cupreus*) spent more than 50% of their time cleaning their mouthparts following attack of *D. v. virgifera* larvae (table 1). None of the predators attacking maggots cleaned their mouthparts. Of the seven species of predator, only two species attacked one prey more quickly than the others. *Harpalus rufipes* attacked maggots more quickly than *D. v. virgifera* and the opposite pattern was observed in *C. monilis* (table 1). For the most part, predators fed *D. v. virgifera* or maggots often spent similar amounts of time walking (*C. monilis*: $\chi^2_1 = 0.58$, $P = 0.45$; *H. rufipes*: $\chi^2_1 = 0.33$, $P = 0.57$; *Po. cupreus*: $\chi^2_1 = 2.09$, $P = 0.15$: *Pt. melanarius*: $\chi^2_1 = 8.11$, $P = 0.004$; *Pt. anthracinus*: $\chi^2_1 = 4.62$, $P = 0.03$; *Tr. ruricola*: $\chi^2_1 = 2.20$, $P = 0.14$), and resting (*C. monilis*: $\chi^2_1 = 3.17$, $P = 0.08$; *H. rufipes*: $\chi^2_1 = 2.19$, $P = 0.14$; *Po. cupreus*: $\chi^2_1 = 1.53$, $P = 0.21$; *Pt. melanarius*: $\chi^2_1 = 0.93$, $P = 0.33$; *Pt. anthracinus*: $\chi^2_1 = 0$, $P = 1.00$; *Tr. ruricola*: $\chi^2_1 = 9.14$, $P = 0.003$). The only exceptions to this pattern is that *Tr. ruricola*, *Pt. melanarius* and *Pt. anthracinus* fed *D. v. virgifera* larvae spent significantly more time walking or resting than those fed maggots. Mean ± SEM percent of times spent walking by *D. v. virgifera*-fed and maggot-fed *Pt. melanarius* were 27.72 ± 0.94 and 0%, respectively; percent of times spent walking by *D. v. virgifera*-fed and maggot-fed *Pt. anthracinus* were 16.46 ± 6.03 and 3.68 ± 3.68%, respectively. *Trochosa ruricola* fed *D. v. virgifera* or maggots spent mean SEM of 35.50 ± 11.18 and 0% of their time resting, respectively.

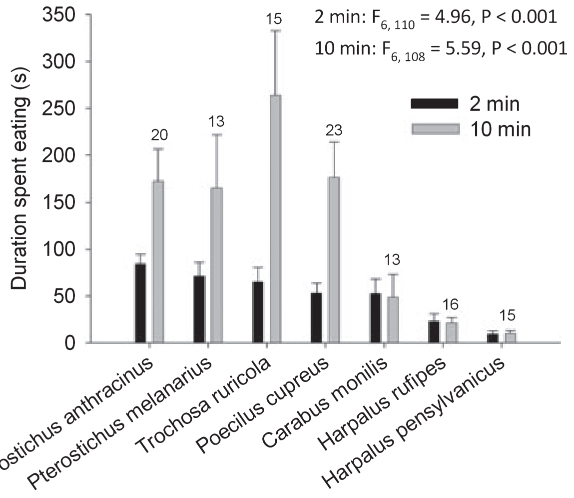

Fig. 1 A comparison of predators' propensity to return to *D. v. virgifera* 3rd instars over a 10 min observation period under laboratory conditions. The amount of time spent eating was recorded for the first 2 min post-attack interval, and for the entire 10 min observation period. All bars represent mean values, error bars represent SEM. Samples sizes for the 2 min observations are listed in table 1; sample sizes for the 10 min observations are presented as arabic numerals above the bar.

The formicid, *Te. caespitum,* attacked the maggots much more intensively than the *D. v. virgifera* larvae (rm- ANOVA results; treatment: $F_{1,28} = 76.48$, $P < 0.001$; time: $F_{9,252} = 15.57$, $P < 0.001$; treatment × time: $F_{9,252} = 10.87$, $P < 0.001$) (fig. 2). More ants attacked the maggots, and they sustained their attack for longer periods of time. After an initial attack on *D. v. virgifera* by an individual, we observed the attacking ant antennating with several

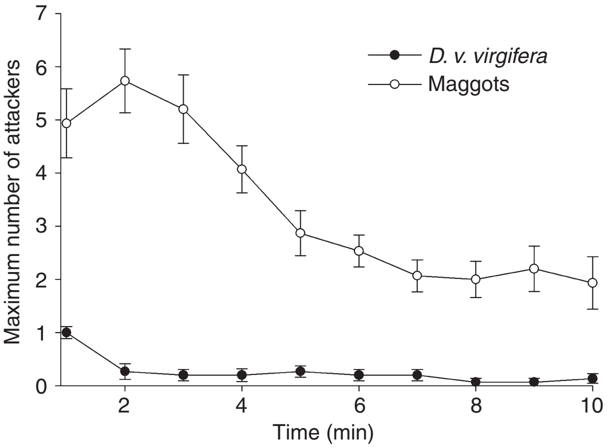

Fig. 2 The attack intensity of the ant *Tetramorium caespitum* (n = 15 ants per experimental unit) on *Diabrotica v. virgifera* 3rd instars and *Calliphora vicina* maggots of equivalent size over a 10 min observation period. The *y*-axis represents the maximum number of simultaneous attackers observed per 1 min period. Sample sizes were 15 ants per treatments, dots indicate the mean, and error bars indicate SEM.

of the other workers, and few additional attacks were subsequently observed by any of the ants.

Larval defence of *D. v. virgifera* under field conditions

In 2007, there were 1002 predators found at 202 (42%) sentinel prey after 1 h of their placement. In 2008, 182 predators were found consuming 74 (14%) sentinel prey (table 2). A total of 28 species was found consuming the prey items (19 species consumed the *D. v. virgifera* larvae; 15 species consumed *C. vicina* maggots; table 2). *Tetramorium caespitum* (Formicidae) dominated the predator communities during both years. Approximately 33.76 ± 8.68 and 26.44 ± 6.83% of maggots and *D. v. virgifera* larvae per plot were attacked, respectively.

There was significantly more predators found attacking the maggots than *D. v. virgifera* larvae, and more predators were found attacking the larvae per plot during 2007 than in 2008 (fig. 3). Predation was less common during 2008 than during 2007, but there was no significant difference in the frequencies of predation per plot on the two prey species during either year (prey: $F_{1,6} = 2.80$, $P = 0.15$; year: $F_{1,6} = 64.27$, $P < 0.0001$; interactions: $F_{1,6} = 0.73$, $P = 0.73$).There were strong diel activity patterns of predators, but these patterns were distinctly different between study years. Nocturnal predator communities were more species rich than the diurnal communities (table 2, Supplementary material). Mean ± SEM frequencies of predation per plot during the day and night were 28.08 ± 9.52 and 32.08 ± 5.66%, respectively. In addition to fewer predators being captured during 2008 than during 2007, there were significant differences in the number of predators attacking during the day and night, but these patterns were different during 2007 and 2008 (fig. 4). When day-active ants were excluded from the analysis, diel predation patterns were consistent between years; the mean ± SEM number of

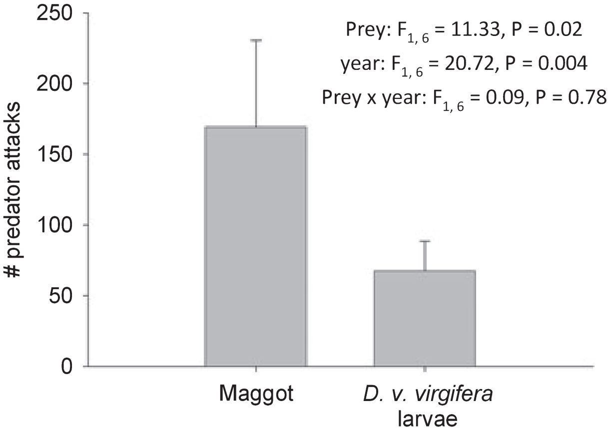

Fig. 3 The number of predators (mostly ants) found attacking *Diabrotica v. virgifera* 3rd instars and *Calliphora vicina* maggot (n = 537 each species) in five Hungarian maize fields (n = 5 plots). Bars represent mean number of predators found attacking sentinel prey items, and error bars represent SEM. Means between treatments are significantly different (α = 0.05).

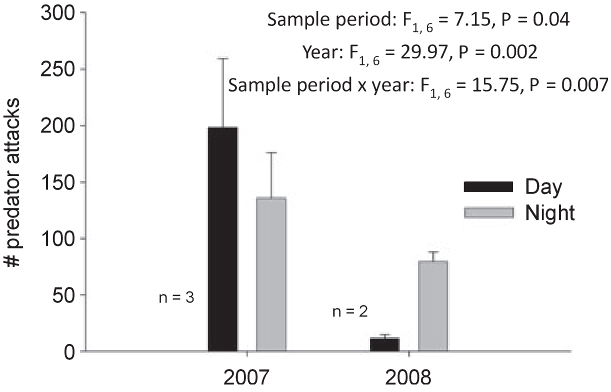

Fig. 4 Diel predation patterns on *Calliphora vicina* and *Diabrotica v. virgifera* sentinel larvae in Hungarian maize fields (mostly by ants). Observations were conducted from 9 to 10 AM (day) and from 10 to 11 PM (night) in early July of both years. Sample sizes are presented above the bars for each year. Bars represent the mean number of predators captured attacking the sentinel prey per field (error bars represent SEM). There was a significant effect of sample year, diel period, and interaction (α = 0.05).

predation events observed per plot during the day and night were consistent among years 3.2 ± 0.66 and 11.2 ± 1.56, respectively (sample period: $F_{1,6} = 21.08$, $P = 0.004$; year: $F_{1,6} = 0.95$, $P = 0.37$; sample period × year: $F_{1,6} = 0.48$, $P = 0.51$).

Discussion

Although all predators were repelled by *D. v. virgifera* larvae in the laboratory, this defence functioned to varying degrees in the different predators. First, the different predators spent differential amounts of time eating *D. v. virgifera* during the initial 2 min after the first attack (table 1). Predators invariably fed on maggots for longer than they did on *D. v. virgifera*. The haemolymph defence was particularly repulsive to *H. pensylvanicus*, *H. rufipes*, and *Te. caespitum*. We observed that the coagulation and chemical aspects of the haemolymph defence operated with varying effectiveness in the different predators. For example, although repelled by the defence, *C. monilis*, *Pt. melanarius*, and the fluid-feeding *Tr. ruricola* and *Te. caespitum* did not spend significantly different amounts of time cleaning their mouthparts after consuming maggots and *D. v. virgifera* larvae, suggesting that coagulation of the *D. v. virgifera* larvae was an ineffective defensive tactic against these predators. The other predators spent significantly more time cleaning their mouthparts when fed the *D. v. virgifera* larvae. Predators also varied in their propensity to return to *D. v. virgifera* larvae over the 10 min observation period. *Carabus monilis*, *H. pensylvanicus*, *H. rufipes* and *Te. caespitum* did not return to *D. v. virgifera* after their initial repulsion, whereas *Pt. anthracinus*, *Pt. melanarius*, and *Po. cupreus* (all voracious generalist predators) returned to eat *D. v. virgifera* larvae soon after their initial response.

The idiosyncratic behaviours practiced by different predators are difficult to quantify, but clearly underlie the results uncovered in this assay. For example, the large (>2 cm in length) *C. monilis* pierced the larva with their relatively long mandibles, and the haemolymph of *D. v. virgifera* never came in contact with the oral cavity; the beetles simply dropped the *D. v. virgifera* larva and did not return to it. This behaviour led to a minimal feeding duration, and little cleaning of the mouthparts; moreover, it suggests chemoreceptors located on the external mouthparts are important in the decision of whether to ingest a food item. In contrast to *C. monilis*, *Tr. ruricola* seldom left the *D. v. virgifera* larva unattended; even between feeding bouts they kept a leg near the prey item and repeatedly bit it over the 10 min observation period; presumably the attendance was to ensure it would not escape. Incidentally, previous research in the laboratory and field have shown that sucking predators like wolf spiders are better adapted to preying upon *D. v. virgifera* under field conditions than chewing predators, and consume more *D. v. virgifera* tissue over a 5 min feeding period than some chewing carabids (Lundgren et al. 2009c). Finally, after the initial attack by *Te. caespitum*, a message was somehow conveyed to the remaining workers (either by the attacking ant, or by volatile infochemicals within the haemolymph), and few additional attempts were made to attack the *D. v. virgifera* larva. Because of their incredible effects on their environments (Hölldobler and Wilson 1990; Beattie and Hughes 2002), ants are often the target of haemolymph defences in insects (Boevé and Schaffner 2003; Petre et al. 2007), but laboratory results are seldom reinforced under field situations.

Although *D. v. virgifera* larvae experienced reduced predation in Hungarian maize fields, the haemolymph defence did not entirely preclude predators from consuming this herbivore. A similar number of predator species attacked sentinel *D. v. virgifera* larvae and *C. vicina* maggots in maize fields, and a similar frequency of these prey items were attacked per field. However, substantially more ants were found attacking the *C. vicina* maggots during both years of study. One explanation for this pattern is that once the *D. v. virgifera* larva is attacked, the attacking predator is sometimes repelled and leaves the prey item. Predators that attack the *C. vicina* maggots remain feeding, and are thus more abundant on the prey item 1 h after placement when observations were made. Indeed, this very case was observed during one nocturnal observation, when a *H. rufipes* was seen running from the *D. v. virgifera* station across the row to the maggot station, where it began to feed. *Tetramorium caespitum* was by far the most abundant predator attacking both prey items, although they attacked the maggots more intensively. This result shows a degree of contrast with the laboratory results, which revealed that *Te. caespitum* was strongly repelled by *D. v. virgifera* larvae. The repellency may have been intensified within the Petri dish laboratory assay because of increased contact among the workers, or the constraining of potentially volatile infochemicals within the enclosed arena. Although, we recognize that maggots and *D. v. virgifera* larvae differ in many ways that could affect their suitability as prey (e.g. reaction to attack, nutrition, general mobility, etc.), the field observations are in line with the hypothesis that *D. v. virgifera* larvae are defended against predation by their haemolymph defence, but also suggest that laboratory assays provide a more severe estimate of the intensity of this effectiveness than is realized under natural conditions.

Our data support the hypothesis that diverse and abundant arthropod predator communities within agroecosystems are maintained in part by the temporal partitioning of the diel cycle (Park 1941; Luff 1978; Dennison and Hodkinson 1984; Lundgren et al. 2006, 2009b; Weber et al. 2009). In both sample years, the predator community was more diverse during the night than during the day, but relative predation patterns during the day and night observations shifted from year to year. Several lines of evidence suggest that patterns in predation and in predator community structure over the diel cycle were driven by weather and the weather-affected abundance and activity patterns of *Te. caespitum*. Observations on predation from 2007 support the current understanding that *Te. caespitum* is primarily day active (Deffernez et al. 1990). This species is a strong competitor, and other predator species may have restricted their foraging to night hours to reduce direct competitive interactions with *Te. caespitum*. Temperatures during the sample periods in 2008 were often above 30°C (S. Toepfer, personal observation), and we believe this reduced foraging and disrupted normal foraging patterns in *Te. caespitum*. Diel and seasonal foraging patterns in ants are particularly well documented as being influenced by temperature (Pol and Lopez de Casenave 2004; Drees et al. 2007). Field data from Hungary reinforces the importance of considering both the day and night active predator communities when exploring key interactions of predators and a given prey item (Pfannenstiel 2005).

Defensive characteristics of specific members within a food web affect its connectedness, and ultimately influence many of the ecological processes (e.g. top-down management of herbivore populations, nutrient cycling, etc.) underway in most systems. It is clear that the haemolymph defence of *D. v. virgifera* influences the relative strengths of trophic linkages that ultimately influence its ability to invade new regions. Not only was the haemolymph of *D. v. virgifera* larvae repellent to all predators tested in the laboratory assays (but to varying degrees), it likely contributed to the observed reduced predation of this species under field conditions. Subterranean *D. v. virgifera* immatures succumb to predation by a community of belowground predators, but species and functional guilds vary in the degree to which they exploit this prey resource (as demonstrated by Lundgren et al. 2009c). Future research will help to address how these defences contribute to the success of *D. v. virgifera* as a colonizer and invader, and develop ways that land managers can overcome these defences in search of sustainable, ecologically based pest management.

Acknowledgements

We thank Samantha Magnus (University of Victoria, Canada), Michael Wogin (Simon Fraser University, Vancouver, Canada), and Hongmei Li (CABI Europe-Switzerland) for technical assistance in the laboratory; Christina Pilz (BOKU, Austria) and Marianne Baroni (University of Neuchatel, Switzerland) assisted with field predation observations. Lars Andraesssen (University of Manitoba, Winnipeg, Canada) provided helpful advice on *C. vicina* larva rearing. Markó Balínt (University of Clui Napoca, Romania) identified the ants collected in Hungary, Ferenc Tóth and Kinga Fetyko (University of Godollo, Hungary) identified the spiders, and Henryk Luka, (FiBL, Frick, Switzerland) identified the Carabidae and Staphylinidae. John Tooker (Pennsylvania State University) and Bruce Hibbard (USDA-ARS) provided valuable comments on earlier drafts of this manuscript. Mention of any proprietary product does not constitute endorsement by the USDA. This project was funded in part by an OECD Fellowship awarded to Dr Lundgren.

References

Beattie AJ, Hughes L, 2002. Ant-plant interactions. In: Plant-animal interactions: an evolutionary approach. Ed. by MHerrera C, Pellmyr O, Blackwell Publishing, Oxford, UK, 211–235.

Boevé J-L, Schaffner U, 2003. Why does the larval integument of some sawfly species disrupt so easily? The harmful haemolymph hypothesis. Oecologia 134, 104–111.

Bowers MD, 1992. The evolution of unpalatability and the cost of chemical defense in insects. In: Insect chemical ecology. Ed. by Roitberg BD, Isman MB, Springer, Dordrecht, The Netherlands, 216–244.

Deffernez L, Champagne P, Verhaeghe J-C, Josens G, Loreau M, 1990. Analysis of the spatio-temporal niche of foraging grassland ants in the field. Insect. Soc. 37, 1–13.

Dennison DF, Hodkinson ID, 1984. Structure of the predatory beetle community in a woodland soil ecosystem. V. Summary and conclusions. Pedobiologia 26, 171–177.

Dettner K, 1987. Chemosystematics and evolution of beetle chemical defenses. Annu. Rev. Entomol. 32, 17–48.

Downie IS, Wilson WL, Abernethy VJ, McCracken DI, Foster GN, Ribera I, Murphy KJ, Waterhouse A, 1999. The impact of different agricultural land-uses on epigeal spider diversity in scotland. J. Insect Conserv. 3, 273–286.

Drees BBM, Summerlin B, Vinson SB, 2007. Foraging activity and temperature relationship for the red imported fire ant. Southwest. Entomol. 32, 149–155.

Duelli P, Obrist MK, 1998. In search of the best correlates for local organismal biodiversity in cultivated areas. Biodivers. Conserv. 7, 297–309.

Duelli P, Obrist MK, Schmatz DR, 1999. Biodiversity evaluation in agricultural landscapes: above-ground insects. Agric. Ecosyst. Environ. 74, 33–64.

Happ GM, Eisner T, 1961. Hemorrhage in a coccinellid beetle and its repellent effect on ants. Science 134, 329–331.

Harwood JD, Yoo HJS, Rowley DL, Greenstone MH, O' Neil RJ, 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. Biol. Invasions 11, 895–903.

Hilker M, Eschbach U, Dettner K, 1992. Occurrence of anthraquinones in eggs and larvae of several Galerucinae (Coleoptera: Chrysomelidae). Naturwissenschaften 79, 271–274.

Hölldobler B, Wilson EO, 1990. The ants. Belknap Press, Cambridge, MA.

de Jong PW, Holloway GJ, Brakefield PM, de VosH, 1991. Chemical defence in ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (adalia bipunctata). Chemoecology 2, 15–19.

Juen A, Traugott M, 2007. Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. Mol. Ecol. 16, 1545–1557.

Laurent P, Braekman J-C, Daloze D, 2005. Insect chemical defense. Top. Curr. Chem. 240, 167–229.

Luff ML, 1978. Diel activity patterns of some field Carabidae. Ecol. Entomol. 3, 53–62.

Lundgren JG, Shaw JT, Zaborski ER, Eastman CE, 2006. The influence of organic transition systems on beneficial ground-dwelling arthropods and predation of insects and weed seeds. Renewable Agriculture and Food Systems 21, 227–237.

Lundgren JG, Haye T, Toepfer S, Kuhlmann U, 2009a. A multi-faceted haemolymph defense against predation in *Diabrotica virgifera virgifera* larvae. Biocontrol Sci. Technol. 19, 871–880.

Lundgren JG, Nichols S, Prischmann DA, Ellsbury MM, 2009b. Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae). Biocontrol Sci. Technol. 19, 327–333.

Lundgren JG, Prischmann DA, Ellsbury MM, 2009c. Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. Ecol. Appl. 19, 2157–2166.

Müller C, Arand K, 2007. Trade-offs in oviposition choice? Food-dependent performance and defence against predators of a herbivorous sawfly. Entomol. Exp. Appl. 124, 153–159.

Nunez MA, Simberloff D, Relva MA, 2008. Seed predation as a barrier to alien conifer invasions. Biol. Invasions 10, 1389–1398.

Park O, 1941. Concerning community symmetry. Ecology 22, 164–167.

Parker JD, Burkepile DE, Hay ME, 2006. Opposing effects of native and exotic herbivores on plant invasions. Science 311, 1459–1461.

Pasteels JM, Braekman JC, Daloze D, Ottinger R, 1982. Chemical defence in chrysomelid larvae and adults. Tetrahedron 38, 1891–1897.

Pasteels JM, Grégoire J-C, Rowell-Rahier M, 1983. The chemical ecology of defense in arthropods. Annu. Rev. Entomol. 28, 263–289.

Pasteels JM, Daloze D, de Bisseau J-C, Termonia A, Windsor DM, 2004. Patterns in host-plant association and defensive toxins produced by neotropical chrysomeline beetles. In: New developments in the biology of Chrysomelidae. Ed. by Jolivet P, Santiago-Blay JA, Schmitt M, SPB Academic Publishing, The Hague, The Netherlands, 669–676.

Peck DC, 2000. Reflex bleeding in froghoppers (Homoptera: Cercopidae): variation in behavior and taxonomic distribution. Ann. Entomol. Soc. Am. 93, 1186–1194.

Petre C-A, Detrain C, Boevé J-L, 2007. Anti-predator defence mechanisms in sawfly larvae of *Arge* (Hymenoptera, Argidae). J. Insect Physiol. 53, 668–675.

Pfannenstiel RS, 2005. Nocturnal predators and their impact on lepidopteran eggs in annual crops: what we don't see does help us! In: Proceedings of the 2nd international symposium on biological control of arthropods. Ed. by Hoddle MS, USDA Forest Service Publication FHTET-2005-08, Washington, USA, 463–471.

Pol R, Lopez de Casenave J, 2004. Activity patterns of harvester ants *Pogonomyrmex pronotalis* and *Pogonomyrmex rastratus* in the central Monte desert, Argentina. J. Insect Behav. 17, 647–661.

Tallamy DW, Hibbard BE, Clark TL, Gillespie JJ, 2005. Western corn rootworm, cucurbits and cucurbitacins. In: Western corn rootworm: ecology and management. Ed. by Vidal S, Kuhlmann U, Edwards CR, CAB International, Wallingford, UK, 67–94.

Toepfer S, Kuhlmann U, 2006. Constructing life-tables for the invasive maize pest *Diabrotica virgifera virgifera*

(Col., Chrysomelidae) in Europe. J. Appl. Entomol. 130, 193–205.

Toepfer S, Haye T, Erlandson M, Goettel M, Lundgren JG, Kleespies RG, Weber DC, Cabrera Walsh G, Peters A, Ehlers R-U, Strasser H, Moore D, Keller S, Vidal S, Kuhlmann U, 2009. A review of the natural enemies of beetles in the subtribe Diabroticina (Coleoptera: Chrysomelidae): implications for sustainable pest management. Biocontrol Sci. Technol. 19, 1–65.

Vleiger L, Brakefield PM, Müller C, 2007. Effectiveness of the defence mechanism of the turnip sawfly, *Athalia rosae* (Hymenoptera: Tenthredinidae), against predation by lizards. Bull. Entomol. Res. 94, 283–289.

Weber DC, Pfannenstiel RS, Lundgren JG, 2009. Diel predation pattern assessment and exploitation of sentinel prey: new interpretations of community & individual behaviors. In: Proceedings of the Third ISBCA, Christchurch, New Zealand.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Predators observed feeding on *Diabrotica virgifera* larvae or *Calliphora vicina* maggots during day and night sampling periods in Hungarian maize fields during 2007 and 2008.