Exp Appl Acarol (2011) 55:233–248
DOI 10.1007/s10493-011-9468-y

Generalist-feeding subterranean mites as potential biological control agents of immature corn rootworms

Deirdre A. Prischmann · Eric M. Knutson · Kenton E. Dashiell · Jonathan G. Lundgren

Received: 17 August 2010 / Accepted: 21 April 2011 / Published online: 20 May 2011

Abstract Predatory mites are important components of subterranean food webs and may help regulate densities of agricultural pests, including western corn rootworms (Chrysomelidae: *Diabrotica virgifera virgifera*). Implementing conservation and/or classical biocontrol tactics could enhance densities of specialist or generalist predatory mites and lead to pest suppression, but first relevant mite species must be identified and their predatory capabilities evaluated. We conducted lab assays to quantify consumption of immature rootworms and oviposition rates of various mite species. Our study indicates that rootworms are a sub-optimal food source for the mite taxa tested. However, all mite species fed upon rootworms to some degree, although consumption by nematophagous *Eviphis ostrinus* was extremely low. Predators consumed more rootworm larvae than eggs, and mite size was correlated with prey consumption, with larger predators eating more prey. Four mite taxa (*Gaeolaelaps* sp., *S. miles*, *Gl. americana*, and *G. aculeifer*) had detrimental effects on survival of rootworm larvae, and the latter two species also had negative impacts on densities of pest eggs. Although it is unlikely that any of these mite species by itself has a major impact on rootworm control, the community of generalist soil-dwelling mites may play an important role in regulating immature rootworm populations in the field.

Keywords Biological control · Soil mite · Generalist predator · Rootworm · Acari · *Diabrotica*

D. A. Prischmann (✉) · E. M. Knutson
Entomology Department, North Dakota State University, Fargo, ND, USA
e-mail: Deirdre.Prischmann@ndsu.edu

K. E. Dashiell · J. G. Lundgren
North Central Agricultural Research Laboratory, USDA-ARS, Brooking, SD, USA

K. E. Dashiell
Tropical Soil Biology and Fertility Institute, CIAT, Nairobi, Kenya

Introduction

Information on the role predatory mites play in regulating subterranean agricultural pests is limited. Soil-dwelling predatory mites have primarily been investigated for control of thrips and fungus gnats in greenhouses (Wright and Chambers 1994; Enkegaard et al. 1997; Berndt et al. 2004; Wiethoff et al. 2004), and dung flies (Axtell 1963; Rodriguez and Wade 1961; Wade and Rodriguez 1961; Wallace and Holm 1983). Native soil mites are potential candidates for conservation biocontrol strategies and as bioindicators (Karg 1968; Ruf 1998) because they are intimately linked to and affected by their environment. Crop management practices (e.g. crop rotation, tillage, soil amendments, insecticide use, etc.) impact soil mite densities (Bedano and Ruf 2007), and thus can be manipulated to maximize predator densities and pest control. In addition, mites are attractive for augmentative biocontrol because they can be mass produced and some are available commercially. Before effective pest management strategies can be implemented, key predatory mite species for a particular pest management system must be identified and evaluated.

Corn rootworms (Chrysomelidae: *Diabrotica* spp.) are economic pests of maize throughout corn producing regions of the United States and Europe (Gray et al. 2009; Spencer et al. 2009). The majority of damage is caused by larvae, whose feeding on root tissue disrupts several physiological processes within the plant (Riedell 1990) and can cause severe yield losses if not controlled (Sutter et al. 1990; Spike and Tollefson 1991; Godfrey et al. 1993). Current rootworm management practices include non-selective soil insecticides, crop rotation, and rootworm-specific Bt maize hybrids, but each is limited in its scope and effectiveness. Broad-spectrum insecticides applied as seed treatments, directly to the soil, and for adult rootworm control pose environmental and human health concerns, and rootworms have developed resistance to some of these chemicals (Miller et al. 2009; van Rosen and Ester 2010). Crop rotation is challenged by the proliferation of rotation-resistant rootworm strains (Krysan et al. 1984; Levine et al. 2002; Schroeder et al. 2005) and the widespread planting of continuous corn driven by high maize prices. Non-transgenic hybrids are desirable for use in certain situations, including refuges required for insect resistance management, organic fields, and when producers are hesitant or unable to use genetically modified organisms. The end result is that incorporating additional management tools into rootworm IPM programs will help preserve existing control strategies and increase the sustainability and profitability of maize production.

Biological control as a rootworm management tool remains poorly understood, largely because few studies have quantified how predators limit pest populations and subsequently protect crops. However, many arthropods feed on immature rootworms, including meso- and astigmatid mites (Chiang 1970; Mihm 1972; Mihm and Chiang 1976; Brust and House 1988, 1989; Lundgren et al. 2009a; Toepfer et al. 2009; Lundgren et al. 2010). Even so, virtually nothing is known about subterranean trophic interactions or the potential use of predatory mites in rootworm IPM programs. Given that mites have the potential to lower rootworm populations and could be used in both augmentative and conservation biocontrol programs, they may play a key role in existing IPM frameworks.

Our overall goal was to identify soil-dwelling predatory mites (native and commercially available species) that may be important natural enemies of immature rootworms. Specific objectives included: (1) investigating the effects of predatory mite identity and size on predation of rootworm eggs and first instar larvae, and (2) quantifying impacts of prey type (rootworm eggs, larvae, or no prey) on mite oviposition and mortality.

Materials and methods

Mite species used in predation experiments

We focused on two commercially available predaceous mite species and native mite species that were most numerous in our soil samples and known to be predaceous from the literature. We also examined a mite species in the nematophagous family Eviphididae (Mašán and Halliday 2010) to explore potential artifacts of the no-choice design on predation.

The four indigenous mesostigmatid mite species used in predation experiments were: *Gaeolaelaps* sp. (Laelapidae), *Macrocheles insignitus* Berlese (Macrochelidae), *Glyptholaspis* (= *Holostaspis*) *americana* (Berlese) (Macrochelidae), and *Eviphis ostrinus* (C.L. Koch) (Eviphididae). Two species of commercially available laelapid soil mites, *Gaeolaelaps* (*Hypoaspis*) *aculeifer* (Canestrini) and *Stratiolaelaps* (*Hypoaspis*) *miles* (Berlese) (but see below), were purchased from Koppert Biological Systems (Romulus, MI, USA) and Biocontrol Network (Brentwood, TN, USA), respectively. We focused primarily on testing females (with the exception of *G. aculeifer* where both males and females were tested).

Representative mite specimens were mounted in Hoyer's medium on glass slides and identified using a compound microscope and relevant keys (Karg 1979; Evans and Till 1979; Hyatt and Emberson 1988; Krantz and Ainscough 1990; Lindquist et al. 2009; Mašán and Halliday 2010). Although we refer to *Stratiolaelaps miles* in this manuscript, our specimens were identified as *Stratiolaelaps scimitus* (Womersley) (Walter and Campbell 2003). And readers should note that *Gaeolaelaps* is frequently misspelled in the literature (*Geolaelaps*) (Halliday and Lindquist 2007).

Mite collection and culture for predation experiments

Indigenous subterranean mites used in lab assays were obtained from maize fields with a history of maize production and no or infrequent soil insecticide use near (Hankinson, ND, USA). In 2008 and 2009, top soil (30.5 cm deep) was collected using a spade and transferred to 18.9 -l plastic buckets with lids. Buckets were stored in a cooler (ca. 5°C) until processed. Berlese funnels with 25 watt bulbs were used to extract living mites into moistened plaster-lined glass jars. Soil was placed in each funnel and mites removed from collection jars using a small paintbrush after 2, 4, and 7 days. Mite species were initially separated based on physical characteristics, such as body shape, size, and coloration.

Mites were cultured in 540 ml plastic containers (Fabri-Kal, Kalamazoo MI). Rearing containers had approximately 2.5 cm of plaster of Paris (DAP®, DAP Products, Baltimore, MD, USA) in the bottom that was periodically moistened to maintain humidity. Approximately 25 g of potting soil and two to three moist cotton wicks were added to each container. Mites were transferred into rearing containers using a small paintbrush, with different species kept in separate containers. Mites were maintained on a mixed-prey diet of Collembola, nematodes, acarid mites (Astigmata), and western corn rootworm larvae (*Diabrotica virgifera virgifera* LeConte). Collembola and nematodes were obtained from the collected field soil, acarid mites were cultured from shipments of commercially available predatory mites, and corn rootworms were supplied by the USDA-ARS North Central Agricultural Research Lab (Brookings, SD, USA). A small amount of active dry yeast (Red Star, Lesaffre Yeast, Milwaukee, WI, USA) was added to each container in a plastic cap (7.4 ml vial lid) to sustain non-rootworm prey populations. Mites were

periodically mounted in Hoyer's medium to confirm colony identity and purity (Karg 1979; Evans and Till 1979; Hyatt and Emberson 1988; Krantz and Ainscough 1990; Lindquist et al. 2009; Mašán and Halliday 2010).

Although some species did well in culture for short periods of time (ca. 1–2 months), we were not successful in continuously rearing native mite species, in some cases due to disease. Inadequate food and environmental conditions could also have contributed to rearing issues. In addition, although we attempted to provide oviposition sites (cotton wicks, pieces of corn cobs, soil particles, holes in the plaster), we were unable to find or recover mite eggs, although mite larvae and nymphs were seen in some colonies. Therefore, most indigenous mites used in predation experiments were used within 1 week after being extracted from soil samples.

Rootworms used in predation experiments

Non-diapausing western corn rootworm eggs were obtained weekly (USDA-ARS, Brookings, SD) and were stored at constant dark (25 ± 2°C, 40–60% RH). Eggs were separated from the soil using a 60-mesh sieve and a Büchner funnel. Unfed, first instar larvae were collected from within the original Petri dishes after eggs hatched.

Predation experiments

Experiments were conducted in the lab in arenas consisting of 2.0 ml microtubes filled with 1.0 ml of moist plaster of Paris (DAP®). Background mortality (and reproduction of starved predators) was assessed using control arenas with non-fed predators ($n = 10$) and rootworms in arenas without predators ($n = 10$ for eggs and $n = 10$ for larvae). For most predators, there were initially 20 replicates of each rootworm treatment (i.e. $n = 20$ for eggs and larvae). Exceptions were *M. insignitus* ($n = 8$ for all control treatments, $n = 12$ for eggs, $n = 16$ for larvae), *Gaeolaelaps* sp. ($n = 8$ for all control treatments, $n = 8$ for eggs and larvae), and *Gl. americana* ($n = 20$ for rootworm control treatments, $n = 40$ for larvae). Only predation of rootworm eggs was assessed for *G. aculeifer* males.

For experimental arenas, one starved mite (starved for 24–72 h) and five rootworm eggs or newly hatched, starved first instars from the same age cohort were transferred to each arena using a small paintbrush. This prey density was based on preliminary experiments, and provided predators with excess prey. Age and reproductive status of mites could not be standardized because inadequate rearing techniques forced us to use field-collected mites. Arenas were maintained in an incubator (constant darkness, 25 ± 2°C, 40–60% RH), and a uniform amount of distilled water was added to all arenas as needed (5–10 µl). Arenas were checked daily and the following recorded: number of intact eggs, live larvae, prey consumed, other (i.e. diseased eggs, hatched eggs, non-consumed dead larvae), live adult mites, and immature mites. Consumed eggs were identified based on the presence of a hole or slit and a shrunken or hollow appearance. Only diseased or consumed eggs were replaced daily. However, if any eggs exhibited signs of advanced development (larval head capsules visible beneath the chorion) or began hatching, all eggs in all arenas were immediately replaced. Larvae were considered consumed if they were shriveled or shrunken. Because predators could cause indirect larval mortality by wounding, data on dead non-consumed larvae (including diseased larvae which were pink or yellow) were collected. All larvae were replaced on a daily basis, regardless of their status. One exception was for *M. insignitus*, where larvae were not replaced daily for a period of 2 days due to a lack of experimental organisms. Immature mites were removed from arenas

immediately after detection to prevent cannibalism, although cannibalism is rare for *G. aculeifer* and *S. miles* (Berndt et al. 2003). Experiments were maintained for 7–14 days, depending on the mite species (Table 2).

After experiments were ended, a representative sample of mites were preserved in 70% ethanol and their sex verified by examining their ventral shields using a dissecting microscope (Evans and Till 1979; Lindquist et al. 2009). In addition, mite size was quantified by measuring the length of the sclerotized dorsal shield using a compound microscope (Olympus, Model AHBT). Voucher specimens were deposited in the North Dakota State Insect Reference Collection housed in the North Dakota State University Entomology Department (Fargo, ND).

Statistical analysis

Because predation experiments were maintained for different lengths of time, we conducted separate analyses for each mite species. Data normality and equality of variance among groups was assessed by visual inspection and Levene's test. We used a square root $(X + 0.5)$ transformation to normalize data distributions, and data from arenas where mites did not survive until the end of the experiment were omitted from predation and reproduction analyses.

Factorial repeated measures ANOVA was used to assess intact rootworm eggs and live rootworm larvae. Predator presence (control arena—no mite, experimental arena—mite present) and prey (rootworm eggs, larvae) were the independent variables and density of undamaged rootworms was the dependent variable (intact, non-diseased eggs and live larvae). *P*-values were adjusted using the Greenhouse-Geisser statistic to correct for sphericity violations (SYSTAT Software 2007). If *time* × *treatment* interactions were significant, a profile analysis was done for each time period. For analyses of undamaged (intact or live rootworms), because our primary interest was examining effects of predator presence on rootworm density (i.e. comparing control arenas with no mites and experimental arenas with mites), we conducted separate analyses for rootworm eggs and larvae on each date using Games-Howell tests, which do not require balanced designs or equal variance between groups (Games and Howell 1976; SYSTAT).

When assessing predation, rootworm life stage (egg, larva) was the independent variable, and prey consumed (eaten) was the dependent variable. When assessing mite reproduction, rootworm treatment (no prey, rootworm eggs, rootworm larvae) was the independent variable and mite immatures produced was the dependent variable. For predation and reproduction analyses, if *time* × *treatment* interactions were significant, a profile analysis was done at each time period using Kruskal–Wallis (reproduction analyses only) and/or Games-Howell tests. When the presence of zeros precluded the use of repeated measures ANOVA (e.g. few rootworms were eaten or few mite immatures produced during the course of the experiment), data from each time period was analyzed using Kruskal–Wallis (reproduction analyses only) and/or Games-Howell tests.

Kaplan–Meier nonparametric survival analysis and the log-rank (Mantel–Haenszel) test were used to investigate treatment effects on mite survival (SYSTAT). Arena designation was the independent stratification variable (control with mite only, mites and rootworm eggs, mites and rootworm larvae) and days alive was the survival variable. Because experiments ran for different lengths of time, data for each mite species was analyzed separately.

Correlations between mean mite size (i.e. length of the dorsal shield) and mean number of rootworm prey (eggs or larvae) eaten per day over the duration of the experiment were

examined using Pearson's correlation coefficients and Barlett's Chi-square statistic (SYSTAT). Data from *G. aculeifer* males were only used in correlations with rootworm eggs, as no experiments using rootworm larvae were performed.

Results

Because of limitations in availability of experimental organisms, developmental stage of rootworm eggs and quality of larvae varied to some degree over the course of each experiment. This caused the density of live rootworms in both control and experimental tubes to periodically decrease (e.g. *G. aculeifer*♀ day 5 and *E. ostrinus* day 3, Fig. 1) due to egg hatching and/or enhanced larval death likely due to starvation. However, differences in densities of rootworm prey between control and experimental arenas can be attributed to the presence of predators (prey consumption and mortality from wounding). Incidence of the other causes of mortality (hatched or diseased eggs, larvae drown in condensation or dying from starvation) on each date were comparable between control and experimental arenas since they were provisioned with prey from similar age cohorts.

For all other mite taxa, regardless of variations in prey development or quality, effects of treatments on density of live rootworms (intact eggs and live larvae) were not consistent through time, as evidenced by significant *time* × *treatment* interactions (Table 1).

Throughout the *Gaeolaelaps* sp. experiment, densities of intact eggs were relatively stable in both control and experimental arenas, although impacts of predator presence on densities of live rootworm larvae was not consistent, leading to a significant *time* × *mite* × *prey* interaction (Table 1). Densities of intact rootworm eggs were similar between control and experimental arenas on all relevant dates (i.e. days when intact egg densities were below the maximum of $n = 5$) ($P > 0.055$; Fig. 1). There were significantly fewer live larvae when mites were present on day 2 ($P = 0.020$) and day 10 ($P = 0.012$), which is likely due to mortality from wounding, as no larvae were eaten on those days (Fig. 2a). *Gaeolaelaps* sp. did not consume any rootworm eggs and ate few rootworm larvae per day (0.13 ± 0.04 across the entire experiment; Fig. 2a). The number of rootworm larvae consumed was only statistically higher than eggs eaten on day 4 ($P = 0.042$). This mite species did not reproduce at all, regardless of experimental treatment (Fig. 2b).

For *M. insignitus*, regardless of predator treatment, densities of intact rootworm eggs were relatively stable while densities of live rootworm larvae varied through time, leading to a significant *time* × *prey* interaction (Table 1). There was consistently no effect of predator presence on intact/live rootworms (*time* × *mite* × *prey*, $P = 0.831$; *time* × *mite*, $P = 0.678$). Thus, when examining each date separately, there were no significant differences in densities of intact rootworm eggs or live larvae between control and experimental arenas for all relevant dates (eggs, $P > 0.055$; larvae, $P > 0.055$). *Macrocheles insignitus* did not consume any rootworm eggs and ate an average of 0.13 ± 0.06 rootworm larvae per day (Fig. 2a). However, there were no statistically significant differences in densities of rootworm eggs and larvae eaten on any day ($P > 0.055$). *Macrocheles insignitus* reproduced to a limited degree in arenas provisioned with rootworm larvae (0.03 ± 0.02 immatures per day on average; Fig. 2b). However, there were no statistically significant differences among treatments (day 4, Kruskal–Wallis $H = 2.431$, $P = 0.297$; day 7, $H = 1.333$, $P = 0.513$).

Densities of live rootworm larvae in arenas with *Glyptholaspis americana* increased over time, while changes in densities of larvae in control tubes and rootworm eggs in both arenas types were not as dramatic, leading to a significant *time* × *mite* × *prey* interaction

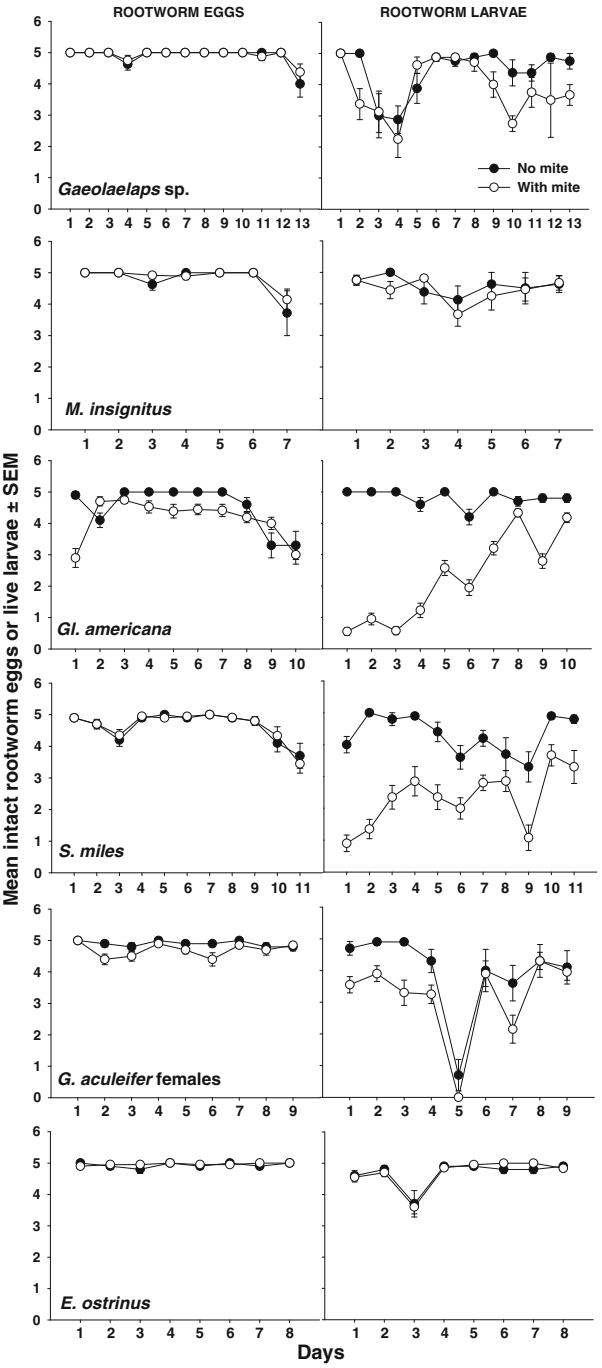

Fig. 1 Mean densities (±SEM) of intact rootworm eggs and live rootworm larvae over time in arenas with and without predatory mites

(Table 1). For most of the experiment, densities of intact eggs were significantly lower when *Gl. americana* was present (day 1, $P < 0.001$; day 3, $P = 0.021$; day 4, $P = 0.029$; day 5, $P = 0.014$; day 6, $P = 0.004$; day 7, $P = 0.008$; days 8–10, $P > 0.055$; Fig. 1). On

Table 1 *P* values from factorial repeated-measures analyses investigating mite presence (*mite*) and rootworm life stage (*prey*) on intact rootworm eggs and live larvae

	Mite species					
	Gaeolaelaps	*M. insignitus*	*Gl. americana*	*S. miles*	*G. aculeifer*♀	*E. ostrinus*
Time[a]	<0.001	0.174	<0.001	0.004	<0.001	<0.001
Time × mite[a]	0.031	0.678	<0.001	0.049	0.218	0.702
Time × prey[a]	0.002	0.041	<0.001	<0.001	<0.001	<0.001
Time × mite × prey[a]	0.027	0.831	<0.001	0.046	0.224	0.864

[a] *P*-values adjusted using the Greenhouse-Geisser statistic

day 2, densities of intact eggs were significantly lower when mites were absent ($P = 0.051$). This was because the previous day mature eggs had erroneously not been replaced in control arenas, whereas experimental arenas had been provisioned with new eggs. With the exception of day 8 ($P = 0.065$), densities of live larvae were significantly lower when *Gl. americana* was present (days 1–7 and 9, $P < 0.001$; day 10, $P = 0.004$; Fig. 1). *Glyptholaspis americana* frequently consumed rootworm immatures, although the number of rootworm eggs versus larvae consumed was not consistent throughout the experiment (*time* × *prey*, $P < 0.001$; *time*, $P < 0.001$; Fig. 2a). Initially, *Gl. americana* ate significantly more rootworm larvae than eggs (days 1–4 and 9, $P < 0.001$; day 5, $P = 0.005$; day 6, $P = 0.003$), but this changed towards the end of the experiment (day 7, $P = 0.065$; day 8, $P = 0.772$; day 10, $P = 0.486$). On average, *Gl. americana* consumed more rootworm eggs (0.61 ± 0.09) and larvae (1.56 ± 0.08) per day than any other mite species (Fig. 2a). Even so, the average number of *Gl. americana* immatures produced per day was low in arenas with rootworm eggs (0.06 ± 0.02) and larvae (0.08 ± 0.02). Starved mites did not reproduce, and there were no significant differences in densities of *Gl. americana* immatures among treatments on any day ($P > 0.055$).

The impact of *S. miles* presence on densities of live rootworm larvae was not constant through time, although this was not the case for intact rootworm eggs, leading to a significant *time* × *mite* × *prey* interaction (Table 1). There were no significant differences in the density of intact rootworm eggs between control and experimental arenas (all days, $P > 0.055$; Fig. 1). With the exception of day 8 ($P = 0.320$), densities of intact larvae were always significantly lower when *S. miles* was present (days 1–3 and 5, $P < 0.001$; days 4, 7, 9, $P = 0.001$; days 6, 10, $P = 0.004$; day 11, $P = 0.022$). With the exception of day 8 ($P = 0.320$), *S. miles* consistently ate significantly more rootworm larvae per day (0.75 ± 0.09 on average) than eggs (0.17 ± 0.03 on average) (*time* × *prey*, $P = 0.095$; *time*, $P = 0.337$; days 1–3 and 5, $P < 0.001$; days 4, 7, 9, $P = 0.001$; days 6, 10, $P = 0.004$; day 11, $P = 0.022$; Fig. 2a). Starved *S. miles* did not reproduce. Average *S. miles* reproduction over the entire experiment was highest when arenas were provisioned with rootworm larvae (0.28 ± 0.09), followed by rootworm eggs (0.04 ± 0.02). However, differences were only significant on day 2 (Kruskal–Wallis $H = 9.257$, $P = 0.010$) and day 3 ($H = 11.894$, $P = 0.003$) (days 1 and 4–11, $P > 0.055$; Fig. 2b). On day 2 and 3, *S. miles* reproduction was significantly higher in arenas with rootworm larvae compared to eggs (day 2, $P = 0.039$; day 3, $P = 0.017$) and no prey (day 2, $P = 0.025$; day 3, $P = 0.017$).

The repeated measures analysis indicated that *G. aculeifer*♀ had a consistent impact on densities of intact rootworm eggs and live rootworm larvae (*time* × *mite*, $P = 0.613$;

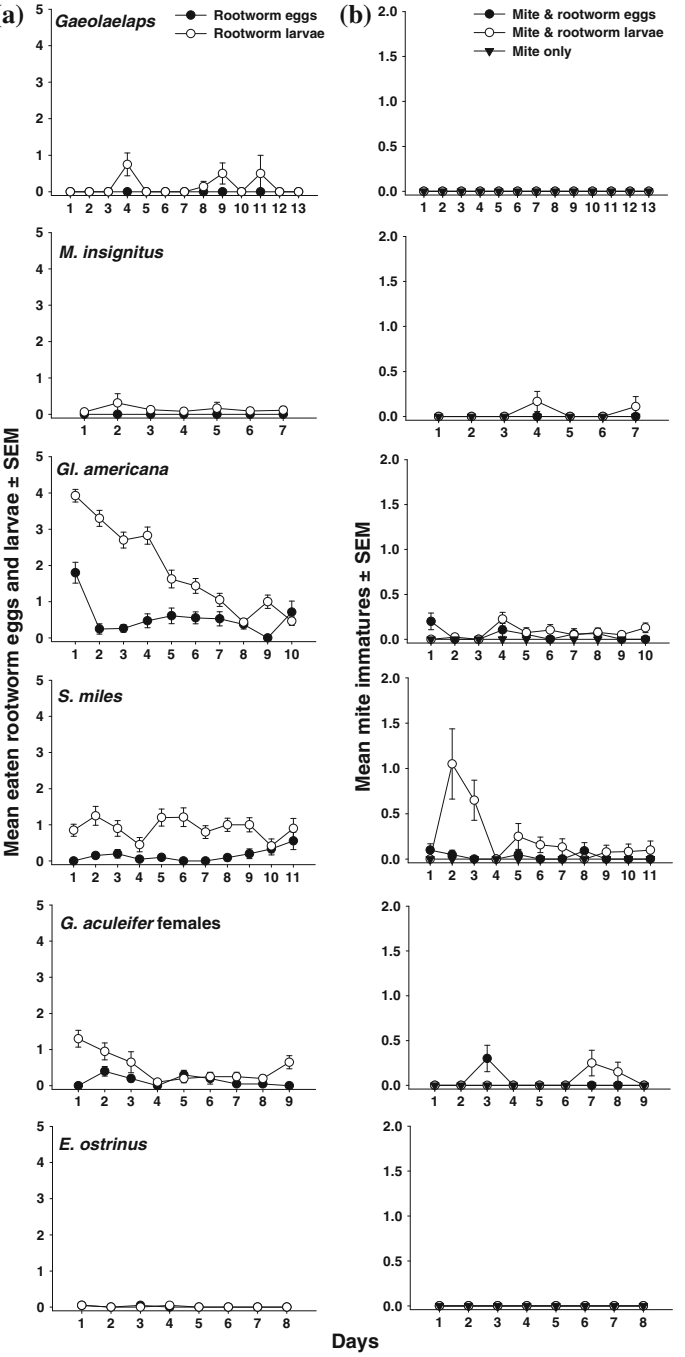

Fig. 2 **a** Mean densities (±SEM) of rootworm eggs and larvae eaten by predatory mites over time, **b** Mean densities (±SEM) of mite immatures produced over time in arenas with different prey

time, $P = 0.021$), although the impact of prey identity was not uniform, with densities of intact eggs and live larvae varying from the maximum $n = 5$ (*time* × *prey*, $P < 0.001$; Fig. 1). During the subsequent profile analysis, densities of intact eggs were only significantly lower in arenas with mites on day 2 ($P = 0.017$) and day 6 ($P = 0.041$). On most days, densities of live larvae were significantly lower when *G. aculeifer*♀ were present, especially at the start of the experiment (day 1 and 3, $P = 0.002$; day 2, $P = 0.001$; day 4, $P = 0.035$; day 7, $P = 0.052$; remaining days, $P > 0.055$; Fig. 1). Quality of rootworm larvae was substantially reduced in both control and experimental arenas on day 5. The effect of prey identity on rootworms consumed by *G. aculeifer*♀ was not consistent over time. *Gaeolaelaps aculeifer*♀ ate significantly more rootworm larvae than eggs at the beginning and end of the experiment (*time* × *mite*, $P < 0.001$; *time*, $P < 0.001$; day 1, $P < 0.001$, day 2, $P = 0.066$, day 9, $P = 0.001$, days 3-8, $P > 0.055$; Fig. 2a). When averaged across dates, *G. aculeifer*♀ consumed 0.51 ± 0.07 rootworm larvae versus 0.13 ± 0.03 rootworm eggs per day. Reproduction by *G. aculeifer*♀ was extremely limited, and there was no significant effect of prey treatment on production of mite immatures on any relevant date ($P > 0.055$; Fig. 2b). When averaged for the entire experiment, *G. aculeifer*♀ produced 0.12 ± 0.05 immatures per day in arenas with rootworm eggs and 0.04 ± 0.02 in arenas with rootworm larvae. Starved *G. aculeifer*♀ did not reproduce.

With regard to *G. aculeifer*♂, we only conducted experiments using rootworm eggs due to a lack of experimental organisms. Throughout the experiment, there was no significant impact of predator presence on the density of intact rootworm eggs (*time* × *mite*, $P = 0.613$; *time*, $P = 0.021$; *mite*, all dates, $P > 0.055$; data not shown), and *G. aculeifer*♂ did not consume any rootworm eggs (data not shown).

For *E. ostrinus*, the variation in quality of rootworm larvae on day 3 (Fig. 1) led to a significant *time* × *prey* interaction (Table 1). However, this interaction became non-significant when this date was removed from the analysis (*time* × *mite* × *prey*, $P = 0.753$; *time* × *prey*, $P = 0.110$; *time* × *mite*, $P = 0.517$; *time*, $P = 0.059$), and so data were combined among dates. Overall, between control and experimental arenas there were no significant differences in mean density of intact rootworm eggs (no mite, 4.94 ± 0.03; with mite, 4.96 ± 0.02; $P = 0.449$) or live rootworm larvae (no mite, 4.68 ± 0.06; with mite, 4.66 ± 0.06; $P = 0.833$). *Eviphis ostrinus* consumed few rootworms per day, regardless of prey life stage (eggs, 0.01 ± 0.01; larvae 0.02 ± 0.01; $P = 0.643$; Fig. 2a), and no females in any arenas laid eggs (Fig. 2b).

There were no significant differences in mite survival among prey treatment (no prey, rootworm eggs, rootworm larvae) for most mite species (Table 2). All *G. aculeifer*♀ and ♂ survived until the end of the experiment. Survival of *Gaeolaelaps* sp. was significantly lower in arenas with rootworm larvae compared to controls with no prey ($\chi^2 = 6.882$, $P = 0.009$) and rootworm eggs ($\chi^2 = 6.882$, $P = 0.009$).

Glyptholaspis americana had the longest dorsal shield ($n = 10$, 1.173 ± 0.076 mm), followed by *S. miles* ($n = 18$, 0.656 ± 0.007 mm), *G. aculeifer*♀ ($n = 48$, 0.648 ± 0.005 mm), *G. aculeifer*♂ ($n = 30$, 0.515 ± 0.004 mm), *E. ostrinus* ($n = 50$, 0.470 ± 0.004 mm), *Gaeolaelaps* sp. ($n = 1$, 0.435 mm; non-voucher specimens were disposed of accidentally) and *M. insignitus* ($n = 11$, 0.434 ± 0.015 mm). There was a significant correlation between the length of a mite's dorsal shield (i.e. mite size) and the mean amount of rootworm prey consumed daily during the duration of the experiment (rootworm eggs, $P < 0.001$; rootworm larvae, $P = 0.001$; Fig. 3). The longer the dorsal shield (i.e. the larger the mite), the more rootworm prey was consumed.

Table 2 Kaplan–Meier analysis of impact of prey on mite survival

Species	Length of experiment (days)	% of mites surviving to the end of experiment			Chi-square statistic	*P* value
		No prey	Rootworm eggs	Rootworm larvae		
Gaeolaelaps sp.[a]	13	87.5	100	37.5	13.159	0.001
M. insignitus[a]	7	83.3	61.5	56.3	0.961	0.618
Gl. americana[a]	10	77.7	70.0	87.5	2.993	0.224
S. miles[b]	11	30.0	45.5	50.0	0.343	0.842
G. aculeifer ♀[b]	9	100	100	100	[c]	[c]
G. aculeifer ♂[b]	14	100	100	n/a	[c]	[c]
E. ostrinus[a]	8	90.0	95.0	90.0	0.433	0.805

[a] Indigenous field-collected species

[b] Commercially-available species

[c] Tests could not be run because all mites survived until the end of the experiment

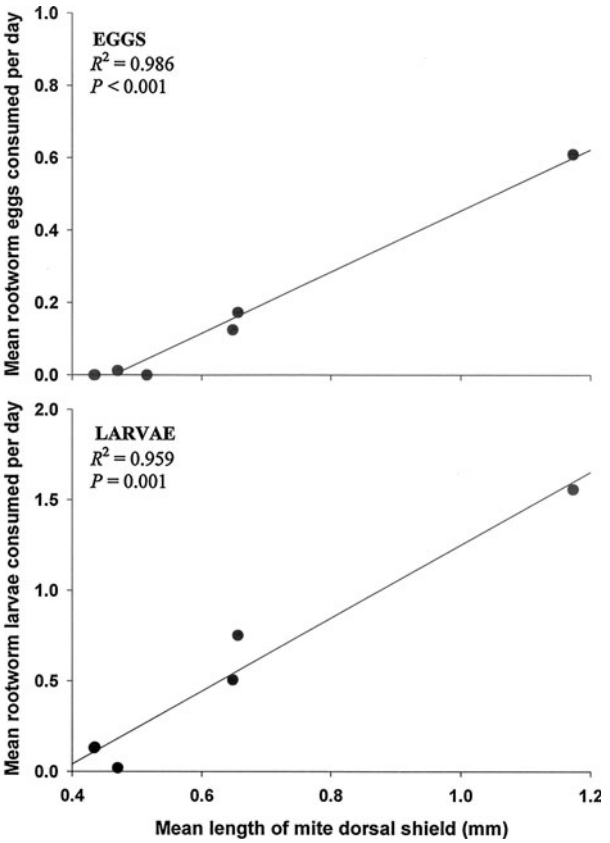

Fig. 3 Correlations between mean rootworms consumed per day (averaged across the entire experiment) and mean mite dorsal shield length (mm)

Discussion

Mites used in lab experiments are commonly found in soil environments, and with the exception of *E. ostrinus*, are generalist predators that feed on a variety of organisms, including nematodes, Collembola, mites, and insect larvae (Evans and Till 1979; Sardar and Murphy 1987; Hyatt and Emberson 1988; Moore et al. 1988; Lindquist et al. 2009). *Gaeolaelaps aculeifer*, *S. miles*, and *Macrocheles* species have been studied for biocontrol of dung flies (Axtell 1963; Rodriguez and Wade 1961; Wade and Rodriguez 1961; Krantz 1983; Wallace and Holm 1983; Halliday and Holm 1987), fungus gnat larvae (Wright and Chambers 1994), sciarid fly larvae (Enkegaard et al. 1997), and thrips (Berndt et al. 2004; Wiethoff et al. 2004). Mites in the family Eviphididae (e.g. *E. ostrinus*) primarily feed on nematodes (Mašán and Halliday 2010).

Under laboratory conditions, there were clear differences in the acceptability and suitability of rootworm eggs and larvae for the mite taxa examined. This was clearly related to predator size, with larger predators consuming more rootworm prey. Other studies have also found mite size influences predation efficacy (Messelink and van Holstein-Saj 2006). Smaller mite species rarely ate rootworm eggs, and they were infrequently consumed by mid-sized predators. Oophagous mites often have specialized chelicerae (Evans and Till 1979), and the generalist-feeders tested in this study may have been limited in their ability to penetrate the egg chorion, especially the smaller mites. Additionally, predatory mites may be more likely to attack moving prey, which has been documented for *S. miles* (Shereef et al. 1980).

With two exceptions (i.e. *M. insignitus* and *E. ostrinus*), mite presence had a negative impact on rootworm larvae, either via successful predation events or increased larval mortality (due to wounding, facilitating pathogen entry, or enhancing starvation due to increased prey movement and/or utilization of defenses). *Gaeolaelaps* sp. appeared to primarily cause mortality indirectly, as this species did not consume many rootworm larvae. *Glyptholaspis americana*, *G. aculeifer*♀, and *S. miles* ate the most rootworm larvae, and the nematophagous mite *E. ostrinus* only consumed a small number of rootworms, indicating that predation rates in our no-choice tests are likely only slightly inflated compared to the field. Initially, mean predation rates for the most effective predator (*Gl. americana*) were 3.93 ± 0.17 rootworm larvae (day 1), although this steadily declined over time (day 10, 0.46 ± 0.12). When averaged across the entire experiment, *S. miles* and *G. aculeifer*♀ ate 0.75 and 0.51 rootworm larvae per day, respectively, which is lower than daily predation rates on similar-sized insects (fly larvae: 0.9–7.7, thrips immatures: 1.6–3.5; Wright and Chambers 1994; Enkegaard et al. 1997; Berndt et al. 2004).

One reason for lower rates of mite predation on rootworm larvae could be related to prey defense mechanisms. Larval hemolymph of some *Diabrotica* spp. is sticky and has chemical properties that deter or repel predators (Wallace and Blum 1971; Lundgren et al. 2009b, 2010). While observing *G. aculeifer* and *S. miles* prey upon rootworm larvae, after piercing the cuticle and beginning to feed predators would often stop and clean their chelicerae, which were ensnared in coagulated hemolymph. This type of behavior was also noticed in lab experiments with carabid beetles, spiders, and ants (Lundgren et al. 2009b, 2010). In addition, survival of *Gaeolaelaps* sp. was lower in arenas provisioned with rootworm larvae, although this was not the case for other mite species.

Rootworms supported limited reproduction of four mite species, with *S. miles* having the highest average oviposition rate (0.2 eggs per day). However, this reproductive rate is substantially lower than rates on other food types. With thrips as a food source, *G. aculeifer* and *S. miles* produced 2.5 and 0.8 eggs per day, respectively (Berndt et al. 2004) and the

latter species laid 2–3 eggs per day with flour mites as prey (Acaridae: *Acarus siro* L.; Wright and Chambers 1994).

Predatory mites have previously been considered to have a minor role in rootworm biocontrol because of their polyphagous feeding habits (Kuhlmann and van der Burgt 1998). However, although they utilize numerous food sources, generalist predators can contribute to pest suppression (Chang and Kareiva 1999; Symondson et al. 2002), including generalist mites (McMurtry 1992; Prischmann et al. 2006; Beaulieu and Weeks 2007), especially when conservation tactics increase their densities within cropping systems (Lundgren and Fergen 2010). The literature indicates that a variety of arthropods consume immature rootworms, including astigmatid and mesostigmatid mites (Mihm 1972; Mihm and Chiang 1976; Brust and House 1988, 1989; Lundgren et al. 2009a; Toepfer et al. 2009). In field studies, Chiang (1970) calculated that mite predation (Laelapidae) reduced adult rootworm emergence by 20%, which increased to 63% after amending the soil with manure. Further studies revealed that predatory mites (*Androlaelaps* sp., *Stratiolaelaps* sp.) had higher abundances in manured plots, were spatially associated with rootworms within the soil, and consumed western and northern corn rootworm (*Diabrotica barberi* Smith & Lawrence) eggs and larvae in the lab (Mihm 1972; Mihm and Chiang 1976). Brust and House (1988, 1989) found *Tyrophagus putrescentiae* (Astigmata: Acaridae) were attracted to and readily consumed southern corn rootworm eggs (*Diabrotica undecimpunctata howardi* Barber), lowered adult emergence from infested greenhouse pots, and contributed to reduced damage to peanut roots in the field. Based on DNA analyses, *Chaussieria* (Anystidae) and velvet mites (Trombidiidae) also feed on rootworms within agricultural fields, with the former primarily sampled when rootworms were in the larval stage (Lundgren et al. 2009a, c).

Our study indicates that rootworms appear to be a sub-optimal food source for the mite taxa tested. However, all mite species consumed rootworms to some degree, with larger predators eating more prey. Additionally, the presence of four predator taxa (*Gl. americana*, *G. aculeifer*, *S. miles*, and *Gaeolaelaps* sp.) was associated with a significantly higher incidence of prey mortality, primarily with regard to rootworm larvae. Immature rootworms experience a high degree of mortality in the field, and even though the egg stage is susceptible to predation for several months, the greatest levels of mortality is sustained by first instar larvae (Toepfer and Kuhlmann 2006). Given that other studies have also shown various mite taxa to consume rootworms (Chiang 1970; Mihm 1972; Mihm and Chiang 1976; Brust and House 1988, 1989; Lundgren et al. 2009a), the overall community of generalist predatory mites may have a significant impact on rootworm densities, especially if predators target first instars when they are temporally abundant. Manipulative experiments are needed in order to determine potential levels of pest suppression in a field setting. Improving biocontrol of immature rootworms would aid farmers, especially organic producers, reduce the reliance on harmful soil insecticides and Bt varieties with costly technology fees, and contribute to the development of ecologically-based, sustainable corn rootworm management programs.

Acknowledgments Thanks to David Schneider, Amber Hammerbeck, Jeff Olsen, and Gene Schmidt for field and lab assistance; Chad Nielson and Wade French for providing rootworm eggs; and the anonymous reviewers. This project was funded by the North Central Integrated Pest Management Center, Subaward No. 2007-04967-16.

References

Axtell RC (1963) Effect of Macrochelidae (Acarina: Mesostigmata) on house fly production from dairy cattle manure. J Econ Entomol 56:317–321

Beaulieu F, Weeks AR (2007) Free-living mesostigmatic mites in Australia: their roles in biological control and bioindication. Aust J Exp Agric 47:460–478
Bedano JC, Ruf A (2007) Soil predatory mite communities (Acari: Gamasina) in agroecosystems of central Argentina. Appl Soil Ecol 36:22–31
Berndt O, Meyhöfer R, Poehling H-M (2003) Propensity towards cannibalism among *Hypoaspis aculeifer* and *H. miles*, two soil-dwelling predatory mite species. Exp Appl Acarol 31:1–14
Berndt O, Poehling H-M, Meyhöfer R (2004) Predation capacity of two predatory laelapid mites on soil-dwelling thrips stages. Entomol Exp Appl 112:107–115
Brust GE, House GJ (1988) A study of *Tyrophagous putrescentiae* (Acari: Acaridae) as a facultative predator of southern corn rootworm eggs. Exp Appl Acarol 4:335–344
Brust GE, House GJ (1989) Effects of soil moisture, no-tillage and predators on southern corn rootworm (*Diabrotica undecimpunctata howardi*) survival in corn agroecosystems. Agric Ecosys Environ 31:199–216
Chang GC, Kareiva P (1999) The case for indigenous generalists in biological control. In: Hawkins BA, Cornell HV (eds) Theoretical approaches to biological control. Cambridge University Press, Cambridge, UK, pp 103–115
Chiang HC (1970) Effects of manure applications and mite predation on corn rootworm populations in Minnesota. J Econ Entomol 63:934–936
Enkegaard A, Sardar MA, Brødsgaard HF (1997) The predatory mite *Hypoaspis miles*: biological and demographic characteristics on two prey species, the mushroom sciarid fly, *Lycoriella solani*, and the mould mite, *Tyrophagus putrescentiae*. Entomol Exp Appl 82:135–146
Evans GO, Till WM (1979) Mesostigmatic mites of Britain and Ireland (Chelicerata: Acari-Parasitiformes): an introduction to their external morphology and classification. Trans Zool Soc Lond 35:139–270
Games PA, Howell JF (1976) Pairwise multiple comparison procedures with unequal N's and/or variances: a Monte Carlo study. J Educ Stat 1:113–125
Godfrey LD, Meinke LJ, Wright RJ (1993) Vegetative and reproductive biomass accumulation in field corn: response to root injury by western corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 86:1557–1573
Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO (2009) Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu Rev Entomol 54:303–321
Halliday RB, Holm E (1987) Mites of the family Macrochelidae as predators of two species of dung-breeding pest flies. Biocontrol 32:333–338
Halliday RB, Lindquist EE (2007) Nomenclatural notes on the names Gaeolaelaps and Geolaelaps (Acari: Laelapidae). Zootaxa 1621:65–67
Hyatt KH, Emberson RM (1988) A review of the Macrochelidae (Acari: Mesostigmata) of the British Isles. Bull Br Mus Nat Hist (Zool) 54:63–125
Karg VW (1968) Soil biological investigation of the suitability of mites, particularly the predatory type as indicators. Pedobiologia 8:30–49
Karg VW (1979) The genus *Hypoaspis* Canestrini, 1884 (Acarina, Parasitiformes). Zool Jb Syst 106:65–104
Krantz GW (1983) Mites as biological control agents of dung-breeding flies, with special reference to the Macrochelidae. In: Hoy MA, Cunningham GL, Knutson L (eds) Biological control of pests by mites. University of California, Berkeley, pp 91–98
Krantz GW, Ainscough BD (1990) Acarina: Mesostigmata (Gamasida). In: Dindal DL (ed) Soil biology guide. Wiley, New York, pp 583–665
Krysan JL, Jackson JJ, Lew AC (1984) Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). Environ Entomol 13:1237–1240
Kuhlmann U, van der Burgt WACM (1998) Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. Biocontrol News Info 19:59–68N
Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME (2002) Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Am Entomol 48:94–107
Lindquist EE, Krantz GW, Walter DE (2009) Order Mesostigmata. In: Krantz GW, Walter DE (eds) A manual of acarology, 3rd edn. Texas Tech University Press, Lubbock, pp 124–232
Lundgren JG, Fergen JK (2010) The effects of a winter cover crop on *Diabrotica virgifera* (Coleoptera: Chrysomelidae) populations and beneficial arthropod communities in no-till maize. Environ Entomol 39:1816–1828
Lundgren JG, Ellsbury ME, Prischmann DA (2009a) PCR-based analysis of the predator community associated with *Diabrotica virgifera* immatures. Ecol Appl 19:2157–2166
Lundgren JG, Haye T, Toepfer S, Kuhlmann U (2009b) A multifaceted hemolymph defense against predation in *Diabrotica virgifera virgifera* larvae. Biocontrol Sci Techn 19:871–880

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

Lundgren JG, Toepfer S, Haye T, Kuhlmann U (2010) Haemolymph defence of an invasive herbivore: its breadth of effectiveness against predators. J Appl Entomol 134:439–448

Mašán P, Halliday B (2010) Review of the European genera of Eviphididae (Acari: Mesostigmata) and the species occurring in Slovakia. Zootaxa 2585:1–122

McMurtry JA (1992) Dynamics and potential impact of 'generalist' phytoseiids in agroecosystems and possibilities for establishment of exotic species. Exp Appl Acarol 14:371–382

Messelink GJ, van Holstein-Saj R (2006) Potential for biological control of the bulb scale mite (Acari: Tarsonemidae) by predatory mites in amaryllis. Proc Neth Entomol Soc Meet 17:113–118

Mihm JA (1972) Ecological observations on predatory mites in relation to northern and western corn rootworms. MS thesis, University of Minnesota, MN

Mihm JA, Chiang HC (1976) Laboratory studies of the life cycle and reproduction of some soil-and manure-inhabiting predatory mites (Acarina: Laelapidae). Pedobiologia 16:353–363

Miller NJ, Guillemaud T, Giordano R, Siegfried BD, Gray ME, Meinke LJ, Sappington TW (2009) Genes, gene flow and adaptation of *Diabrotica virgifera virgifera*. Agric For Entomol 11:47–60

Moore JC, Walter DE, Hunt HW (1988) Arthropod regulation of micro- and mesobiota in below-ground detrital food webs. Annu Rev Entomol 33:419–439

Prischmann DA, James DG, Wright LC, Snyder WE (2006) Effects of generalist phytoseiid mites and grapevine canopy structure on spider mite (Acari: Tetranychidae) biocontrol. Environ Entomol 35: 56–67

Riedell WE (1990) Rootworm and mechanical damage effects on root morphology and water relations in maize. Crop Sci 30:628–631

Rodriguez JG, Wade CF (1961) The nutrition of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) in relation to its predatory action on the house fly egg. Ann Entomol Soc Amer 54:782–788

Ruf A (1998) A maturity index for predatory soil mites (Mesostigmata: Gamasina) as an indicator of environmental impacts of pollution on forest soils. Appl Soil Ecol 9:447–452

Sardar MA, Murphy PW (1987) Feeding tests of grassland soil-inhabiting gamasine predators. Acarologia 28:117–121

Schroeder JB, Ratcliffe ST, Gray ME (2005) Effect of four cropping systems on variant western corn rootworm (Coleoptera: Chrysomelidae) adult and egg densities and subsequent larval injury in rotated maize. J Econ Entomol 98:1587–1593

Shereef GM, Soliman ZR, Afifi AM (1980) Economic importance of the mite, *Hypoaspis miles* (Berlese) (Mesostigmata: Laelapidae) and its life history. Bull Zool Soc Egypt 30:130–138

Spencer JL, Hibbard BE, Moeser J, Onstad DW (2009) Behavior and ecology of the western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Agric For Entomol 11:9–27

Spike BP, Tollefson JJ (1991) Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J Econ Entomol 84:1585–1590

Sutter GR, Fisher JR, Elliott NC, Branson TF (1990) Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn rootworms (Coleoptera: Chrysomelidae). J Econ Entomol 83:2414–2420

Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? Annu Rev Entomol 47:561–594

SYSTAT Software Inc. (2007) SYSTAT version 12.02. SYSTAT Software Inc., Chicago

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, Jackson J, 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 Techn 19:1–65

van Rosen K, Ester A (2010) Chemical control of *Diabrotica virgifera virgifera* LeConte. J Appl Entomol 134:376–384

Wade CF, Rodriguez JG (1961) Life history of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), a predator of the house fly. Ann Entomol Soc Am 54:776–781

Wallace JB, Blum MS (1971) Reflex bleeding: a highly refined defensive mechanism in *Diabrotica* larvae (Coleoptera: Chrysomelidae). Ann Ent Soc Am 64:1021–1024

Wallace MMH, Holm E (1983) Establishment and dispersal of the introduced predatory mite, *Macrocheles peregrinus* Krantz, in Australia. J Aust Entomol Soc 22:345–348

Walter DE, Campbell NJH (2003) Exotic vs endemic biocontrol agents: would the real *Stratiolaelaps miles* (Berlese) (Acari: Mesostigmata: Laelapidae), please stand up? Biol Control 26:253–269

Wiethoff J, Poehling H-M, Meyhöfer R (2004) Combining plant- and soil-dwelling predatory mites to optimize biological control of thrips. Exp Appl Acarol 34:239–261

Wright EM, Chambers RJ (1994) The biology of the predatory mite *Hypoaspis miles* (Acari: Laelapidae), a potential biological control agent of *Bradysia pauper* (Dipt. Sciaridae). Entomophaga 39:225–235