Ecological Applications, 19(8), 2009, pp. 2157–2166

Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction

Jonathan G. Lundgren,[1] Michael E. Ellsbury, and Deirdre A. Prischmann[2]

North Central Agricultural Research Laboratory, USDA Agricultural Research Service, 2923 Medary Avenue, Brookings, South Dakota 57006 USA

Abstract. The identity and impact of trophic linkages within subterranean arthropod communities are challenging to establish, a fact that hinders the development of conservation biological control programs of subterranean herbivores. *Diabrotica virgifera* (the western corn rootworm) is a severe agricultural pest that lives subterraneously during its pre-imaginal stages and succumbs to high levels of pre-imaginal mortality from unknown agents. The guts of 1500 field-collected arthropod predators were analyzed for *D. virgifera*-specific DNA sequences using quantitative polymerase chain reaction (qPCR). These gut analyses were used to generate relative and taxon-specific prey consumption indices for the major predator taxa and to determine relative consumption levels during *D. virgifera* egg and larval stages by predator feeding guilds. Laboratory feeding assays were used to determine the meal size consumed during 5 min and digestion rates of *D. virgifera* DNA of four predators abundant in *D. virgifera*-infested cornfields. More than 17 taxa consumed *D. virgifera* in the field. Harvestmen and small rove beetles were the most abundant predators captured, and the most frequent predators within the community to consume *D. virgifera*. The largest proportions of individual species' populations testing positive for *D. virgifera* DNA were found in ground beetles (*Scarites quadriceps* and *Poecilus chalcites*) and spiders, wolf spiders, and predaceous mites. Because of the longer duration of the egg stage, significantly more predators consumed *D. virgifera* eggs than larvae, but a similar proportion of the predator community fed on eggs and larvae. Predators with sucking mouthparts had a higher consumption index than chewing predators. Laboratory assays confirmed that sucking predators consume more *D. virgifera* DNA during 5 min than the chewing predators, and all four predators digested this DNA at a similar rate. This research substantiates that a diverse community of soil-dwelling and subterranean predators contribute to the high level of mortality incurred by *D. virgifera* in cornfields (~99% pre-adult mortality). Moreover, qPCR is a useful tool for describing trophic relationships within subterranean food webs, a crucial step in determining the relative contributions of a diverse predator community to the population dynamics of an herbivorous arthropod.

Key words: *biological control;* Diabrotica virgifera; *DNA analysis; feeding guilds; generalist predators; gut content analysis; predator conservation; qPCR; western corn rootworm.*

Introduction

Soil-based food webs are critical to nutrient cycling and soil function and are even linked with aboveground ecosystem processes (Hedlund et al. 2004, Hättenschwiler and Gasser 2005, Wardle et al. 2005, Sánchez-Moreno and Ferris 2007). Polyphagous soil-dwelling arthropod predators are a diverse and abundant component of most belowground food webs, where they contribute to ecosystem processes by exerting top-down effects on food webs (Riechert and Lockley 1984, Symondson et al. 2002, Lundgren et al. 2006). Their ability to consume a wide range of foods allows these predators to persist within ecosystems even when preferred prey populations are low (Riechert and Lockley 1984, Chang and Kareiva 1999). Not all members of a generalist predator community contribute equally to the regulation of an arthropod population (Moya-Laraño and Wise 2007, Sanders and Platner 2007). In part, this stems from differential susceptibility of various herbivore life stages to predation, but also from inherent differences in the foraging and feeding behavior of the predators themselves. Given these constraints, knowledge about which arthropod life stages incur the most predation and relative contributions of different predator species and functional guilds is crucial when establishing the strength of trophic linkages within subterranean food webs.

Studies on arthropod predator–prey interactions in subterranean systems are challenged by an inability to observe predation events and often necessitate the use of

Manuscript received 9 October 2008; revised 27 February 2009; accepted 10 March 2009. Corresponding Editor: R. A. Hufbauer.

[1] E-mail: Jonathan.Lundgren@ars.usda.gov

[2] Present address: North Dakota State University, Entomology Department 7650, P.O. Box 6050, Fargo, North Dakota 58108-6050 USA.

indirect methods to uncover key relationships (Luck et al. 1988). Genetic analyses provide an accessible method for examining the interactions between predators and prey (Symondson 2002, Gariepy et al. 2007). Polymerase chain reaction (PCR) has been used to study the feeding behavior of an array of arthropod predators, including spiders (Agusti et al. 2003, Greenstone and Shufran 2003), coccinellids (Hoogendoorn and Heimpel 2001, Greenstone et al. 2007, Weber and Lundgren 2009), predaceous mites (Read et al. 2006), carabids (Zaidi et al. 1999, Juen and Traugott 2005), lacewings (Chen et al. 2000), and predaceous hemipterans (Greenstone et al. 2007, Harwood et al. 2007, 2009). While genetic gut content analysis is a powerful tool, there has been limited application of this method under field conditions (Harwood and Greenstone 2008). The use of PCR has largely been restricted to identifying the relative frequencies of predation, from which the detectability half-life (sensu Greenstone et al. 2007) of a meal can be determined as an index of the digestion efficiency of a predator for a given prey. This metric may be useful in ranking the strength of trophic linkages among an herbivore and each member of its predator community.

In addition to measuring frequency of predation, serological gut analyses (i.e., enzyme-linked immunosorbent assay [ELISA]) (Greenstone 1996, Sunderland 1996, Naranjo and Hagler 2001) and quantitative PCR (qPCR) (Nejstgaard et al. 2007, Zhang et al. 2007, Durbin et al. 2008, Weber and Lundgren 2009) may be used to analyze gut contents semi-quantitatively and add further information to the detectability half-life index. In qPCR (the technique applied in the current study), a molecular tag (i.e., SYBR Green) with a high affinity for double-stranded DNA is included in the reaction mixture, and a fluorometric reading is recorded at each cycle of the PCR. When it binds to DNA, the tag fluoresces and the intensity of this fluorescence is correlated with the amount of target DNA present in a sample. Essentially, greater quantities of DNA are detected sooner during the amplification process. Thus, it is possible to estimate the amount of prey DNA within the stomachs of numerous predator species and to generate a consumption index that is based on both the frequency of detection and the amount of prey DNA consumed by members of a predator community.

An ecologically and economically important insect that functions as an excellent model system for exploring trophic interactions within subterranean food webs is *Diabrotica virgifera virgifera* LeConte (the western corn rootworm; Coleoptera: Chrysomelidae). *Diabrotica virgifera* is one of the most severe agricultural pests in the world (Krysan 1986, Moeser and Hibbard 2005). Most of *D. vigifera*'s life cycle is spent beneath the soil, where larvae destroy corn roots. This damage disrupts several physiological processes of the plant and reduces the harvestable yield (Riedell 1990, 1993, Riedell and Kim 1990, Riedell and Evenson 1993, Riedell and Reese 1999). Its ability to shape the aboveground plant community and to adapt to new environments and management tactics have made this species a model for understanding invasion ecology and the genetic forces underlying evolution in insects (Miller et al. 2005, Sappington et al. 2006). In spite of *D. virgifera*'s economic importance (Metcalf 1986) and usefulness to understanding insect ecology, natural enemies of *D. virgifera* are poorly understood, especially with respect to the generalist predators that consume immature stages (Kuhlmann and van der Burgt 1998, Toepfer et al. 2009). A better concept of how predators function as consumers of *D. virgifera* would not only allow the development of more sustainable pest management for this pest, but would grant a better understanding of how biotic mortality factors contribute to the population dynamics, invasion ecology, and evolution of arthropods.

Here we ask three questions of crucial importance in establishing the trophic linkages within subterranean arthropod food webs. These are: (1) Are subterranean egg and larval stages of an arthropod equally susceptible to predation by generalist predators? (2) Do predator species within diverse subterranean communities vary in their use of a specific prey species? (3) Do chewing and sucking predator feeding guilds consume subterranean arthropods at similar rates? We address these questions using genetic gut content analysis of field-collected predators spatiotemporally coincident with eggs and larvae of the target herbivore, along with supporting laboratory feeding assays. In addition to the ecological value of developing tools that describe subterranean food webs within terrestrial ecosystems, there is a clear application of this technology in implementing conservation biological control programs within agroecosystems.

Materials and Methods

Field site

The field design and maintenance briefly described here is presented in detail in Lundgren et al. (2009). Three replicate plots of equal size (29 × 24 m) were embedded into a no-till field (4136 m^2) planted continuously to corn for five years at the Eastern South Dakota Soil and Water Research Farm in Brookings, South Dakota, USA (44°21′ N, 96°48′36″ W). Glyphosate-tolerant field corn (52 000 seeds per ha; 0.72 m between rows) emerged 12 June, and herbicides were applied as needed to ensure minimal weed populations.

Eggs of *D. virgifera* were produced at the Insect Rearing Facility of the North Central Agricultural Research Laboratory (NCARL) in Brookings, South Dakota. Plots were infested to ensure that *D. virgifera* immatures were present at fairly equal densities. Eggs were placed 8–10 cm deep into the center eight rows (9.1 m long) of each plot at a rate of 4900 eggs/m using a tractor-pulled mechanical infester (Sutter and Branson 1986) two days after corn was planted. Under natural conditions, the majority of *D. virgifera* eggs are

deposited in the top 10 cm of the soil profile in close proximity to maize plants (Pruess et al. 1968, Ruesink 1986). Larval densities resulting from this egg infestation level are intended to represent a moderate infestation of *D. virgifera* within North American maize fields (Sutter and Branson 1986), although under a more uniform distribution than what occurs naturally (Park and Tollefson 2006, Toepfer et al. 2007). Temperature-based degree-day models (Jackson and Elliott 1988, Fisher et al. 1990) were used to estimate when 50% of the *D. virgifera* population entered each stadium (methods and results presented in Lundgren et al. [2009]).

Mortality incurred by *D. virgifera* populations was estimated by monitoring the adult emergence from the infested areas. Six *D. virgifera* emergence cages (1.03×0.64 m) were placed into each *D. virgifera*-infested area on 26 July, and they were checked weekly until 6 September. The total number of *D. virgifera* adults recovered from each cage was counted, and a mean emergence per meter per cage was calculated. An additional two cages were placed in uninfested areas of each plot to determine background *D. virgifera* populations. Mean numbers of *D. virgifera* captured per cage in the uninfested areas (1.00 ± 0.44 beetles/m^2 [mean $\pm$ SE]) were subtracted from the number captured in each adjacent infested area to determine the number of infested *D. virgifera* eggs that survived to adulthood.

Predator sampling

Barrier-linked, time-sorting pitfall traps (as described in Lundgren et al. [2009]) were used to collect predators during the pre-pupal stages of *D. virgifera*. A total of 22 sample days were conducted from 29 May to 20 July; this final sample corresponded to when 50% of the *D. virgifera* populations were in the third stadium. The collection receptacles contained 100% ethylene glycol, which preserves DNA for genetic analyses (Leal-Klevezas et al. 2000, Rubink et al. 2003, Vink et al. 2005, Weber and Lundgren 2009). The trap contents were collected at approximately 09:00 on each trapping date.

Predators were identified and grouped into morphotaxa, many of which are at the species level. To facilitate analyses, only predators that represented >1% of the total captures were included in the community analyses. Whenever possible, the numerous morphotaxa that were collected infrequently were grouped under a larger taxon (i.e., "other Carabidae," "other Lycosidae," "other spiders," and "other mites"). Predators were stored individually in 70% ethanol (EtOH) at −20°C until they could be identified and their DNA extracted.

Pitfall traps are legitimately criticized as a poor measure of the true composition of soil-dwelling predator communities and their relative densities (Greenslade 1964, Adis 1979, Koivula et al. 2003, Lundgren et al. 2006), but they are unparalleled in both their economy and ability to collect large quantities of surface-active predators for gut content analysis (Harwood 2008), which is a major requisite of initial predator surveys such as this one. Thus, the results reported in this manuscript pertain primarily to the surface-active predator community.

Procedures for genetic gut content analysis

The DNA extractions were performed using DNeasy tissue extraction kits (QIAGEN, Valencia, California, USA) according to product instructions. The digestive tracts of predators longer than 1 cm were dissected, since excess tissues occasionally clog the filter on the DNeasy spin column. The samples were macerated in ATL buffer using autoclaved pestles and incubated with proteinase K for 3 h. The final double-stranded DNA yield was quantified from each extraction using the absorbance ratio of 260/280 nm (BioPhotometer, Eppendorf, New York, New York, USA). Total DNA yield from the predators ranged from 5 to 630 μg/mL. All extractions were stored at −20°C.

Primers that amplify a *D. virgifera*-specific DNA sequence were developed and their specificity screened against numerous nontarget arthropods. Primers (forward 5′-TAGTTCCCTTAATAATTGGTGCTC-3′; reverse 5′-CCCCCTTTCTACTATCCTTCTTA-3′) were designed using the PrimerSelect feature of Lasergene 7 software (DNASTAR, Madison, Wisconsin, USA) based on sequences in GenBank published by Clark et al. (2001; accession number AF278549). These primers amplified a 119-bp segment of the COI and tRNA-Leu genes of *Diabrotica virgifera virgifera*. Primers were screened for nontarget interactions against nearly 100 different arthropod species found in soil- and foliar-dwelling corn communities, including the predators captured during this study (Appendix). The only other species in the study system whose DNA these primers amplify are *Gryllus pennsylvanicus* Burmeister (only occasionally) (Orthoptera: Gryllidae) and *Diabrotica barberi* Smith & Lawrence, but the resulting products are easily separated from the *D. virgifera* sequence since they dissociate (or melt) at different temperatures (*G. pennsylvanicus*, 85.9°C; *D. barberi*, 70.5°C; the PCR product of *D. virgifera* dissociates uni-modally at 73.5°C).

The PCR reactions (25 μL) were composed of 12.5 μL 2× Brilliant SYBR Green qPCR Master Mix (Qiagen), 225 nmol/L of each primer, 1 μL template DNA, and 9.5 μL of molecular-grade water (Sigma-Aldrich, St. Louis, Missouri, USA). Well-to-well variation in detection was normalized using the ROX dye. Extractions were amplified using an MX3000P qPCR system (Stratagene, La Jolla, California, USA) under the following conditions: 95°C for 15 min, followed by 45 cycles of 94°C for 15 s, 56°C for 30 s, and 72°C for 30 s. Fluorescence was recorded at 492 nm (for SYBR Green) and 582 nm (for ROX, for normalization) during the annealing step of each cycle. The fluorescence threshold of each positive detection was adjusted manually to bring the dRn

(baseline-corrected normalized fluorescence) just above the background fluorescence. A series ($n = 3$–5) of positive controls (*D. virgifera* DNA from a single larva) and three negative (no-template) controls were run on each 96-well plate. The Ct values (which is the PCR cycle at which the sample's fluorescence can be detected over background fluorescence) of the *D. virgifera*-positive predators were adjusted by the difference that the specific plate's control series varied from the composite mean value of all the control series. To ensure that positive values originated from the desired product (and not a cross-reaction), a dissociation curve was produced for each sample by heating the samples to 95°C for 1 min, then dropping the temperature to 55°C and ramping up at 0.2°C/s to 95°C, monitoring fluorescence continuously.

Gut analysis of field-collected predators

Two measures of the frequency of *D. virgifera* consumption were calculated for the 17 most abundant taxa based on the results of the qPCR. The relative frequency (number of positives in a taxon/total number of predators analyzed) and taxon-specific frequency (number of positives in a taxon/number of this taxon analyzed) of consumption were calculated separately for each species in each of the three replicate plots. The former is a measure of the relative frequency of consumption within the predator community. The latter calculation indicates how frequently individuals within a taxon consume *D. virgifera* (essentially, it is a relative measure of the proportion of each species' population that consumes the prey item, ignoring the relative abundances of the different predator species). The frequencies of consumption per plot were compared among the taxa using independent Kruskal-Wallis nonparametric ANOVA (SYSTAT 2004).

Using the relative and taxon-specific frequencies of consumption described above, two predation indices were created: a relative consumption index and a taxon-specific consumption index. For each predator species the relative frequency and taxon-specific frequencies of consumption were multiplied by a transformed Ct value ($Ct^{-1} \times 1000$) for each positive predator. The mean transformed Ct values and relative and taxon-specific consumption indices were compared among the taxa using Kruskal-Wallis nonparametric ANOVA (note that the sample sizes for these indices were the same as in the quantification analyses).

The frequencies of predation by sucking and chewing predators during the egg and larval stages of the herbivore were calculated for each plot. The mean numbers of predators testing positive per plot and frequencies of consumption per plot were compared between egg and larval stages and chewing and sucking predators using independent Kruskal-Wallis ANOVA. Each individual transformed Ct value was multiplied by the respective frequency of predation in each category (i.e., egg, larva, chewing, sucking) to generate a prey consumption index. Mean transformed Ct values and prey consumption indices were compared between categories using independent two-factor ANOVA (with life stage and feeding guild as factors).

Digestion rates of predators

Laboratory assays on the retention times of the amplified *D. virgifera* DNA segment were performed on four predator species that were abundant in *D. virgifera*-infested cornfields: *Poecilus chalcites* (Say) and *Cyclotrachelus alternans* (Casey) (Coleoptera: Carabidae) represented chewing predators, and *Phalangium opilio* L. (Opiliones: Phalangiidae) and lycosids (Aranae: Lycosidae) represented sucking predators. Field-collected predators were maintained on moistened cat food (Iams Original, Iams, Cincinnati, Ohio, USA) until the feeding assays, which were performed within three weeks of collection. Predators were confined to individual Petri dishes and were provided with only water for 48 h prior to the assay. Individual predators were observed until they attacked an early third-instar *D. virgifera* (laboratory produced). These assays were performed between 07:30 and 09:30; the predation observations for the carabids were conducted in a darkened room using headlamps. Each predator was allowed to feed for 5 min; those that consumed the entire larva in less than 5 min were provided an additional larva.

After the feeding bout, the remaining prey were removed, and the predator was randomly assigned to one of seven treatment groups. Predators in these treatments were frozen in prechilled 70% EtOH at −20°C at 0, 0.5, 1, 2, 3, 4, or 6 h after feeding. Ambient temperature during digestion was 23°C. Sample sizes for each kill time varied depending on the degree of success in capturing each predator species in the field, but a minimum of seven observations were recorded for each time period. DNA was extracted from each predator, the *D. virgifera* DNA was amplified, and linear regressions of Ct^{-1} over time were created for each predator.

Consumption and digestion rates of *D. virgifera* by the four predators were compared in two ways. First, the mean meal sizes consumed during 5 min (mean Ct^{-1} at $T = 0$) were compared among the four predators using ANOVA. The initial meal sizes were normalized among the four predator species, and ANCOVA (with species as the main effect and time as the covariate) was used to compare the rates of digestion among the four species.

Results

Frequency of prey consumption

A total of 1550 predators were analyzed and 166 specimens tested positive for *D. virgifera* DNA. The frequencies of consumption by the most abundant taxa captured in the pitfalls are presented in Table 1. In addition to the results in Table 1, cantharid larvae (Coleoptera: Cantharidae; three of nine specimens positive), a nabid nymph (Hemiptera: Nabidae; one of one specimen), large staphylinids (>5 mm; four of 17

TABLE 1. Frequency of detection and quantity of western corn rootworm (*Diabrotica virgifera*) DNA in the guts of major predator taxa per plot.

Predator taxa	Relative frequency of detection (‰)	Taxon-specific frequency of detection (%)	Quantity of DNA per predator taxon ($[Ct^{-1}] \times 1000$)
Acari			
Chaussieria sp.	**2.15 ± 0.43**	13.49 ± 1.39	26.32 ± 0.68 (10)
Other mites	0.86 ± 0.43	**16.67 ± 9.62**	26.66 ± 0.26 (4)
Aranae			
Spider taxon 2	1.72 ± 0.57	10.77 ± 3.49	**27.08 ± 0.69 (8)**
Spider taxon 7	**1.93 ± 0.64**	11.54 ± 3.63	26.32 ± 0.68 (9)
Other spiders	**2.15 ± 1.14**	**17.01 ± 10.95**	26.19 ± 0.62 (9)
Lycosid taxon 1	1.07 ± 0.21	7.61 ± 2.22	26.40 ± 0.79 (5)
Other Lycosidae	1.07 ± 0.21	**18.63 ± 7.98**	**27.53 ± 0.95 (4)**
Opiliones: Phalangiidae			
Phalangium opilio	**6.65 ± 1.55**	8.39 ± 1.33	**27.19 ± 0.42 (30)**
Coleoptera: Carabidae			
Bembidion rapidum	1.29 ± 0	8.42 ± 0.61	**27.63 ± 1.04 (5)**
Bembidion quadrimaculatum	0.64 ± 0.37	9.26 ± 4.90	26.27 ± 0.54 (3)
Cyclotrachelus alternans	1.72 ± 0.86	13.18 ± 6.94	25.79 ± 0.96 (8)
Elaphropus nr. *xanthopus*	1.07 ± 0.21	8.37 ± 2.57	26.23 ± 0.65 (5)
Poecilus chalcites	1.72 ± 0.21	**17.46 ± 10.13**	**26.78 ± 0.61 (6)**
Scarites quadriceps (larvae and adults)	0.86 ± 0.57	**20.37 ± 15.16**	26.69 ± 1.51 (4)
Other Carabidae	**1.93 ± 0.74**	7.88 ± 1.70	25.67 ± 0.55 (8)
Coleoptera: Staphylinidae			
Staphylinids <5 mm	**5.15 ± 1.34**	10.07 ± 2.48	26.54 ± 0.43 (24)

Notes: Values are expressed as mean ± SE. The top five ranking predators in each column appear in boldface. For detection frequencies, $n = 3$ replicate plots. For the quantity of DNA analyzed, numbers in parentheses represent the number of predators analyzed. Ct is the PCR cycle at which the sample's fluorescence can be detected over background fluorescence. The field study was conducted at the Eastern South Dakota Soil and Water Research Farm in Brookings, South Dakota, USA.

specimens), velvet mites (Acari: Trombidiidae; four of four specimens), and a centipede (Chilopoda; one of two specimens) also tested positive for *D. virgifera* DNA. All of the positive *Scarites quadriceps* were larvae, four out of five of which tested positive for *D. virgifera* DNA. The taxon-specific frequency of consumption did not differ among the taxa ($\chi^2 = 6.47$, df = 16, $P = 0.98$), nor did the relative frequency of consumption found in these taxa ($\chi^2 = 25.19$, df = 16, $P = 0.07$).

Quantification of prey consumption

Of those predators that were analyzed in the frequency analyses, only 142 positive detections representing 33 morphotaxa could be used in the quantitative analysis. The Ct value of the *D. virgifera* standard was 12.91 ± 0.82 (mean ± SE), indicating high sensitivity for the target sequence and low plate-to-plate variability. The mean quantity of *D. virgifera* DNA found in the predators was not significantly different among the taxa ($\chi^2 = 14.08$, df = 15, $P = 0.52$; Table 1). The mean relative consumption indices differed substantially among the predators ($\chi^2 = 136.00$, df = 15, $P < 0.0001$), as did the taxon-specific consumption indices ($\chi^2 = 133.27$, df = 15, $P < 0.0001$; Table 2).

Prey consumption by predator feeding guilds during different pest life stages

Significantly more predators tested positive for *D. virgifera* DNA during the egg stage of the herbivore than during the larval stage ($\chi^2 = 3.86$, df = 1, $P = 0.05$; 30.67 ± 2.16 and 19.00 ± 2.31 predators during the egg and larval stages, respectively), but similar proportions of the predator community captured during the egg and larval stages tested positive ($\chi^2 = 0.43$, df = 1, $P = 0.43$; 0.10 ± 0.02 and 0.12 ± 0.02 of the predator samples during the egg and larval stages, respectively).

Similar numbers of sucking and chewing predators consumed *D. virgifera* DNA ($\chi^2 = 1.19$, df = 1, $P = 0.28$; 24.33 ± 4.67 and 18.33 ± 2.03 for sucking and chewing predators, respectively), and frequencies of consumption were also similar between chewing and sucking predators ($\chi^2 = 1.19$, df = 1, $P = 0.28$; 0.10 ± 0.01 and 0.08 ± 0.01 for sucking and chewing predators, respectively).

Life stage of the herbivore did not affect the consumption indices of predators, but chewing predators had a significantly lower prey consumption index than sucking predators (life stage, $F_{1, 124} = 0.01$, $P = 0.93$; feeding style, $F_{1, 124} = 17.01$, $P < 0.0001$; interaction, $F_{1, 124} = 0.18$, $P = 0.67$; Fig. 1). Neither life stage of the pest nor feeding guild of the predators affected the estimated amount of *D. virgifera* DNA detected in predators (life stage, $F_{1, 124} = 1.06$, $P = 0.30$; feeding style, $F_{1, 124} = 2.68$, $P = 0.10$; interaction, $F_{1, 124} = 0.02$, $P = 0.88$; transformed Ct values, egg stage, 26.44 ± 0.23 and 26.99 ± 0.26 for chewing and sucking predators, respectively; larval stage, 26.17 ± 0.39 and 26.63 ± 0.26).

TABLE 2. Consumption indices of *Diabrotica virgifera* DNA within each predator.

Predator taxa	Relative consumption index	Taxon-specific consumption index
Acari		
Chaussieria sp.	**5.66 ± 0.09**	3.55 ± 0.06
Other mites	2.29 ± 0.02	**4.44 ± 0.03**
Aranae		
Spider taxon 2	4.66 ± 0.09	2.92 ± 0.06
Spider taxon 7	**5.08 ± 0.09**	3.04 ± 0.06
Other spiders	**5.63 ± 0.09**	**4.45 ± 0.07**
Lycosid taxon 1	2.82 ± 0.06	2.01 ± 0.04
Other Lycosidae	2.95 ± 0.08	**5.13 ± 0.14**
Opiliones: Phalangiidae		
Phalangium opilio	**18.09 ± 0.22**	2.28 ± 0.03
Coleoptera: Carabidae		
Bembidion rapidum	3.56 ± 0.11	2.33 ± 0.07
Bembidion quadrimaculatum	1.68 ± 0.02	2.43 ± 0.04
Cyclotrachelus alternans	4.44 ± 0.11	3.40 ± 0.08
Elaphropus nr. *xanthopus*	2.81 ± 0.05	2.20 ± 0.04
Poecilus chalcites	4.61 ± 0.08	**4.68 ± 0.08**
Scarites quadriceps (larvae and adults)	2.30 ± 0.09	**5.44 ± 0.22**
Other Carabidae	4.95 ± 0.07	2.02 ± 0.03
Coleoptera: Staphylinidae		
Staphylinids <5 mm	**13.67 ± 0.16**	2.67 ± 0.03

Notes: Values are expressed as mean ± SE. The top five ranking predators in each column appear in boldface. The relative consumption index is the DNA quantity ($Ct^{-1} \times 1000$) × the proportion of the entire predator community at which each taxon tested positive (×100). The taxon-specific consumption index is the DNA quantity (PCR cycles to initial detection; $Ct^{-1} \times 1000$) × proportion of each taxon population testing positive for *D. virgifera* DNA. Ct is the PCR (polymerase chain reaction) cycle at which the sample's fluorescence can be detected over background fluorescence.

Digestion rates of predators

The meal sizes consumed during 5 min of feeding varied significantly among the predators, but the rates at which they digested the *D. virgifera* DNA were statistically similar (Fig. 2). The sucking predators (*Phalangium opilio* and lycosids) consumed more *D. virgifera* DNA in 5 min than the chewing predators (*Poecilus chalcites* and *C. alternans*), although the amounts consumed by *P. chalcites* and lycosids were statistically similar (Fig. 2). Time was correlated with digestion for three of the four predators (*C. alternans* was the exception), and they all digested *D.virgifera* DNA at similar rates (Fig. 2). One-third of the *C. alternans* regurgitated their meals when they were placed in the prechilled 70% EtOH (these observations were included in the analysis).

Diabrotica virgifera *mortality*

Only 19.38 ± 4.20 of the 4900 eggs per meter of row survived until adulthood. Thus, 99.6% of eggs died before adult eclosion. From the information on adult emergence based on temperature, the traps were in place eight calendar days or 130 degree-days before 50% male emergence, and emergence phenology was in line with what was predicted from the pre-imaginal temperature models.

DISCUSSION

Quantification of the relative and taxon-specific metrics of consumption by field-collected predators using genetic gut content analyses are a powerful tool for better understanding the relative contributions of members of a diverse predator community to mortality of a specific arthropod population. Returning to our original questions, the results presented here indicate (1) that predation levels on eggs and larvae of an arthropod within a soil ecosystem differ and are likely affected by their relative exposure to predation and the predators present. Additionally, (2) different predator species within a diverse predator community clearly vary in their consumption of a given herbivore, and (3) it appears that feeding style of the predator at least partially contributes to their propensity to use a given prey species. Although qPCR-based gut analysis is a powerful indirect method for establishing relative trophic linkages, particularly within subterranean food webs, there are several caveats involved with this experimental system that need to be recognized.

Relative consumption rates of different herbivore life stages are largely tied to their relative degrees of

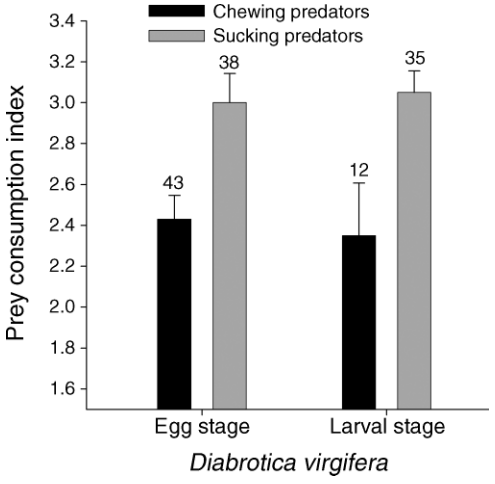

FIG. 1. Prey consumption levels (mean + SE) by sucking and chewing predators captured during the egg and larval stages of the western corn rootworm (*Diabrotica virgifera*). The predation index is the mean frequency of predation for each category ([number positive/number collected] × the amount of DNA in the predator stomach [Ct^{-1}]). Ct is the PCR cycle at which the sample's fluorescence can be detected over background fluorescence. Sucking predators had a significantly higher consumption index than the chewing predators, and pest life stage had no effect on prey consumption indices (see *Results: Prey consumption by predator* ... for supporting statistics). Sample size (number of predators testing positive for *D. virgifera* DNA) is indicated above the bars. The field study was conducted at the Eastern South Dakota Soil and Water Research Farm in Brookings, South Dakota, USA.

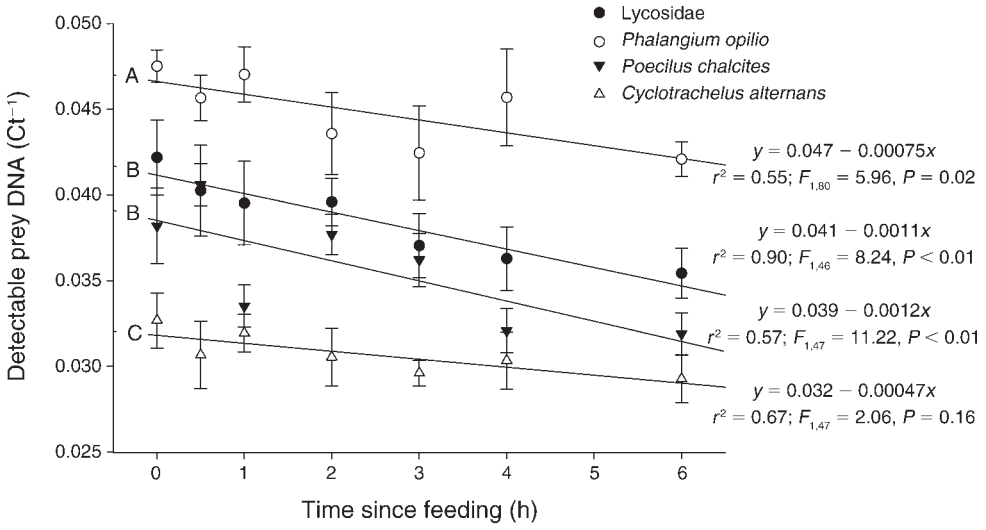

FIG. 2. Digestion rates (mean ± SE) of *Diabrotica virgifera* DNA by four predator species collected in association with *D. virgifera*-infested cornfields. Lycosids and *Phalangium opilio* represent sucking predators, and *Poecilus chalcites* and *Cyclotrachelus alternans* represent chewing predators. All insects consumed third-instar *D. virgifera* for 5 min; mean initial meal sizes consumed per insect (represented by Ct^{-1}) associated with different letters are significantly different ($\alpha = 0.05$). Ct is the PCR cycle at which the sample's fluorescence can be detected over background fluorescence.

exposure to predator community, particularly temporally. For example, the egg stage of *D. virgifera* is not an inherently more attractive food source to predators, but likely suffers greater predation because of its increased duration. Significantly more predators tested positive for *D. virgifera* DNA during the egg stage of the pest than during the larval stage. However, the amount of DNA consumed, the frequency of consumption, and the consumption index were similar between pest life stages. Lundgren et al. (2009) document that the egg stage of *D. virgifera* is substantially longer than the larval stage, and consequently the egg stage is exposed to more predators and greater predator diversity than the larval stage.

Genetic gut content analysis established that predator species within a diverse community consume a given arthropod differentially, and different metrics derived from qPCR-based analyses allow a broader picture of the relative strengths of these trophic linkages than conventional PCR-based analyses. When evaluating the relative consumption frequencies and indices, *Phalangium opilio* and small staphylinids (<5 mm) are ranked substantially higher than the other predators. Although only 8.4% and 10.1% of individuals within these species consumed *D. virgifera* DNA (the taxon-specific frequency of consumption), their sheer abundance resulted in the highest relative frequency of detection of all predators (Table 1). It should be noted that the relative frequency of consumption calculated for the index reflects the relative abundance of predator taxa in the pitfall traps. The relative abundance in the field, however, is likely to differ (Adis 1979, Halsall and Wratten 1988, Spence and Niemala 1994, Lundgren et al. 2006). Still, the large numbers of *P. opilio* and small staphylinids captured in pitfalls necessitate that the importance of these taxa should not be discounted until other sampling methods, such as quadrat and core samples, can affirm the true relative densities of predator taxa.

From the taxon-specific metrics, it is clear that some taxa consume *D. virgifera* immatures more often than others. Of the tested taxa, *S. quadriceps* (20.4% of individuals within the species), "other Lycosidae" (18.6%), *Poecilus chalcites* (17.5%), "other spiders" (17.0%), and "other mites" (16.7%) had the highest taxon-specific consumption indices and predation frequencies (Table 2). Most of the remaining taxa had a relatively low taxon-specific consumption rate (<50% of the consumption indices of *S. quadriceps*), including the taxa ranking highest in the relative consumption indices, *Phalangium opilio* and small staphylinids. Carabids and mites are documented as feeding on *Diabrotica*, particularly under laboratory conditions (Toepfer et al. 2009), but little is known of the interactions of *P. opilio*, staphylinids, and spiders with *D. virgifera*.

The feeding style of a predator species partially defines the boundaries of their feeding niche within a food web. Fluid-feeding predators had a substantially higher consumption index relative to those predators with chewing mouthparts (Figs. 1 and 2). This observation from the field-collected predators was substantiated using the laboratory feeding assays. Specifically, sucking predators consumed more *D. virgifera* DNA under laboratory conditions than the chewing predators, and all four predators assayed digested the *D. virgifera* DNA at similar rates (Fig. 2). Predator species vary in their ability to locate, manipulate, and digest different food items. While the mechanisms that underlie the greater capabilities of

sucking predators to consume *D. virgifera* immatures in the laboratory and the field remain unknown, additional attention should focus on how their efficacy as biological control agents can be improved.

Gut content analysis as presented here is best applied as a first indication of the relative predation capabilities of arthropods; additional analysis is necessary to fully interpret the implications of this initial description of the predator community. First, the final amount of a food found in a predator gut, be it revealed through microscopic, serological, or genetic means, is affected by the relative digestion rate by the predator (or detectability half-life), temperature, the initial amount of prey consumed, the size and types of meals following initial consumption of the target prey, activity level of the predator, time since collection of the predator, technical conditions of the molecular analysis, physiological status of the prey and predator at consumption, and the preservation method of field-collected arthropods (Lövei et al. 1985, 1990, Greenstone 1996, Sunderland 1996, Hagler 1998, Hoogendoorn and Heimpel 2001, Naranjo and Hagler 2001, Symondson 2002, Foltan et al. 2005, De Leon et al. 2006, Greenstone et al. 2007, Weber and Lundgren 2009). With these limitations to genetic gut content analysis in mind, our laboratory retention studies, as well as personal observations by the authors, confirm the patterns observed in field-collected predator taxa and feeding guilds.

Assigning ecological functions to soil organisms is challenging, especially given the tremendous biotic diversity residing within the soil column (Coleman 2008). The qPCR analysis of the gut contents of a predator community provides an undisrupted snapshot of the dynamic interactions of an insect and members of its predator community and establishes key linkages within soil food webs. Clearly, one of the reasons that such a diverse arthropod community can persist within a spatially simple habitat such as the soil is that resources are finely partitioned within the community. In the *D. virgifera* system, different life stages of the herbivore were variably exposed to predation, and the predator community was clearly partitioned in their likelihood to consume this abundant herbivore. Now that key trophic linkages have been established in this model system, additional research on how spatial distribution and defensive traits of an herbivore further partition the relevant predator community will allow ecologists to better understand how soil food webs contribute to ecosystem functions. Moreover, this technique for establishing trophic linkages can be used to examine relationships at other levels of the soil food chain to help resolve the tangle often associated with complex food webs (Polis 1991).

Acknowledgments

We thank Sharon Nichols, Matt Jones, and John Anderson for their help in collecting and sorting specimens and data. Carson Dinger, Janet Fergen, and Mallory Johnson provided indispensible observation skills in the laboratory assays, and Max Pravacek maintained the field plots. Tim Haye, Ulrich Kuhlmann, and Don Weber provided helpful comments on earlier drafts of this manuscript. Mention of a commercial or proprietary product does not constitute endorsement by the USDA.

Literature Cited

Adis, J. 1979. Problems of interpreting arthropod sampling with pitfall traps. Zoologischer Anzeiger Jena 202:177–184.

Agusti, N., P. Shayler, J. D. Harwood, I. P. Vaughan, K. D. Sunderland, and W. O. C. Symondson. 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Molecular Ecology 12:3467–3475.

Chang, G. C., and P. M. Kareiva. 1999. The case for indigenous generalists in biological control. Pages 103–115 *in* B. A. Hawkins and H. V. Cornell, editors. Theoretical approaches to biological control. Cambridge University Press, Cambridge, UK.

Chen, Y., K. L. Giles, M. E. Payton, and M. H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Molecular Ecology 9:1887–1898.

Clark, T. L., L. J. Meinke, and J. E. Foster. 2001. Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. Insect Molecular Biology 10:303–314.

Coleman, D. C. 2008. From peds to paradoxes: linkages between soil biota and their influences on ecological processes. Soil Biology and Biochemistry 40:271–289.

De Leon, J. H., V. Fournier, J. R. Hagler, and K. M. Daane. 2006. Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *Homalodisca liturata* for use in predator gut content examinations. Entomologia Experimentalis et Applicata 119:109–119.

Durbin, E. G., M. C. Casas, T. A. Rynearson, and D. C. Smith. 2008. Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. Marine Biology 153: 699–707.

Fisher, J. R., G. R. Sutter, and T. F. Branson. 1990. Influence of corn planting date on the life stage development and phenology of *Diabrotica virgifera virgifera*. Entomologia Experimentalis et Applicata 54:219–224.

Foltan, P., S. Sheppard, M. Konvicka, and W. O. C. Symondson. 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. Molecular Ecology 14:4147–4158.

Gariepy, T. D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. Journal of Applied Entomology 131:225–240.

Greenslade, P. J. M. 1964. Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). Journal of Animal Ecology 33:301–310.

Greenstone, M. H. 1996. Serological analysis of arthropod predation: past, present and future. Pages 265–300 *in* W. O. C. Symondson and J. E. Liddell, editors. The ecology of agricultural pests: biochemical approaches. Chapman and Hall, London, UK.

Greenstone, M. H., D. L. Rowley, D. C. Weber, M. E. Payton, and D. J. Hawthorne. 2007. Feeding mode and prey detectability half-lives in moelcular gut-content analysis: an example with two predators of the Colorado potato beetle. Bulletin of Entomological Research 97:201–209.

Greenstone, M. H., and K. A. Shufran. 2003. Spider predation: species-specific identification of gut contents by polymerase chain reaction. Journal of Arachnology 31:131–134.

Hagler, J. R. 1998. Variation in the efficacy of several predator gut content immunoassays. Biological Control 12:25–32.

Halsall, N. B., and S. D. Wratten. 1988. The efficiency of pitfall trapping for polyphagous predatory Carabidae. Ecological Entomology 13:293–299.

Harwood, J. D. 2008. Are sweep net sampling and pitfall trapping compatible with molecular analysis of predation? Environmental Entomology 37:990–995.

Harwood, J. D., N. Desneux, H. J. S. Yoo, D. L. Rowley, M. H. Greenstone, J. J. Obrycki, and R. J. O'Neil. 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Molecular Ecology 16:4390–4400.

Harwood, J. D., and M. H. Greenstone. 2008. Molecular diagnosis of natural enemy–host interactions. Pages 41–57 *in* N. Liu, editor. Recent advances in insect physiology, toxicology and molecular biology. Research Signpost, Trivandrum, Kerala, India.

Harwood, J. D., H. J. S. Yoo, D. L. Rowley, M. H. Greenstone, and R. J. O'Neil. 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. Biological Invasions 11:895–903.

Hättenschwiler, S., and P. Gasser. 2005. Soil animals alter plant litter diversity effects on decomposition. Proceedings of the National Academy of Sciences (USA) 102:1519–1524.

Hedlund, K., B. Griffeths, S. Christensen, S. Scheu, H. Setala, T. Tscharntke, and H. Verhoef. 2004. Trophic interactions in changing landscapes: responses in soil food webs. Basic and Applied Ecology 5:495–503.

Hoogendoorn, M., and G. E. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Molecular Ecology 10:2059–2067.

Jackson, J. J., and N. C. Elliott. 1988. Temperature-dependent development of immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Environmental Entomology 17:166–177.

Juen, A., and M. Traugott. 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator–prey system. Oecologia 142:344–352.

Koivula, M., D. J. Kotze, L. Hiisivuori, and H. Rita. 2003. Pitfall trap efficiency: Do trap size, collecting fluid and vegetation structure matter? Entomologica Fennica 14:1–14.

Krysan, J. L. 1986. Introduction: Biology, distribution, and identification of pest *Diabrotica*. Pages 1–23 *in* J. L. Krysan and T. A. Miller, editors. Methods for the study of pest *Diabrotica*. Springer-Verlag, New York, New York, USA.

Kuhlmann, U., and W. A. C. M. van der Burgt. 1998. Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. Biocontrol News and Information 19:59N–68N.

Leal-Klevezas, D. S., I. O. Martinez-Vazquez, B. Cuevas-Hernandez, and J. P. Martinez-Soriano. 2000. Antifreeze solution improves DNA recovery by preserving the integrity of pathogen-infected blood and other tissues. Clinical and Diagnostic Laboratory Immunology 7:945–946.

Lövei, G. L., E. Monostori, and I. Ando. 1985. Digestion rate in relation to starvation in the larva of a carabid predator, *Poecilus cupreus*. Entomologia Experimentalis et Applicata 37:123–127.

Lövei, G. L., P. I. Sopp, and K. D. Sunderland. 1990. Digestion rate in relation to alternative feeding in three species of polyphagous predators. Ecological Entomology 15:293–300.

Luck, R. F., B. M. Shepard, and P. E. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Annual Review of Entomology 33:367–391.

Lundgren, J. G., S. Nichols, D. A. Prischmann, and M. M. Ellsbury. 2009. Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae). Biocontrol Science and Technology 19:327–333.

Lundgren, J. G., J. T. Shaw, E. R. Zaborski, and C. E. Eastman. 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.

Metcalf, R. L. 1986. Foreward. Pages vii–xv *in* J. L. Krysan and T. A. Miller, editors. Methods for the study of pest *Diabrotica*. Springer-Verlag, New York, New York, USA.

Miller, N., A. Estoup, S. Toepfer, D. Bourguet, L. Lapchin, S. Derridj, K. S. Kim, P. Reynaud, L. Furlan, and T. Guillemaud. 2005. Multiple transatlantic introductions of the western corn rootworm. Science 310:992.

Moeser, J., and B. E. Hibbard. 2005. A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (LeConte) in the new and old world-nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? Pages 41–67 *in* S. Vidal, U. Kuhlmann, and C. R. Edwards, editors. Western corn rootworm: ecology and management. CABI Publishing, Wallingford, UK.

Moya-Laraño, J., and D. H. Wise. 2007. Direct and indirect effects of ants on a forest-floor food web. Ecology 86:1454–1465.

Naranjo, S. E., and J. R. Hagler. 2001. Toward the quantification of predation with predator gut immunoassays: a new approach integrating functional response behavior. Biological Control 20:175–189.

Nejstgaard, J. C., M. E. Frischer, P. Simonelli, C. Troedsson, M. Brakel, F. Adiyaman, A. F. Sazhin, and L. F. Artigas. 2007. Quantitative PCR to estimate copepod feeding. Marine Biology 153:565–577.

Park, Y.-L., and J. J. Tollefson. 2006. Spatial distributions of corn rootworm (Coleoptera: Chrysomelidae) eggs and larvae: implications for sampling. Journal of the Kansas Entomological Society 79:129–135.

Polis, G. A. 1991. Complex trophic interactions in deserts: an empirical critique of food-web theory. American Naturalist 138:123–155.

Pruess, K. P., G. T. Weekman, and B. R. Somerhalder. 1968. Western corn rootworm egg distribution and adult emergence under two corn tillage systems. Journal of Economic Entomology 61:1424–1427.

Read, D. S., S. K. Sheppard, M. W. Bruford, D. M. Glen, and W. O. C. Symondson. 2006. Molecular detection of predation by soil micro-arthropods on nematodes. Molecular Ecology 15:1963–1972.

Riechert, S. E., and T. Lockley. 1984. Spiders as biological control agents. Annual Review of Entomology 29:299–320.

Riedell, W. E. 1990. Western corn rootworm damage or mechanical root cutting: effects on root morphology and water relations in maize. Crop Science 30:628–631.

Riedell, W. E. 1993. Advances in understanding corn rootworm damage effects on maize physiology. Pages 76–90 *in* D. Wilkinson, editor. Proceedings of the Corn Sorghum Research Conference. American Seed Trade Association, Washington, D.C., USA.

Riedell, W. E., and P. D. Evenson. 1993. Rootworm feeding tolerance in single-cross maize hybrids from different eras. Crop Science 33:951–955.

Riedell, W. E., and A. Y. Kim. 1990. Anatomical characterization of western corn rootworm damage in adventitious roots of maize. Journal of the Iowa Academy of Sciences 97: 15–17.

Riedell, W. E., and R. N. Reese. 1999. Maize morphology and shoot CO_2 assimilation after root damage by western corn rootworm larvae. Crop Science 39:1332–1340.

Rubink, W. L., K. D. Murray, K. A. Baum, and M. A. Pinto. 2003. Long term preservation of DNA from honey bees (*Apis mellifera*) collected in aerial pitfall traps. Texas Journal of Science 55:159–168.

Ruesink, W. G. 1986. Egg sampling techniques. Pages 83–100 *in* J. L. Krysan and T. A. Miller, editors. Methods for the study of pest Diabrotica. Springer-Verlag, New York, New York, USA.

Sánchez-Moreno, S., and H. Ferris. 2007. Suppressive service of the soil food web: effects of environmental management. Agriculture, Ecosystems, and Environment 119:75–87.

Sanders, D., and C. Platner. 2007. Intraguild interactions between spiders and ants and top-down control in a grassland food web. Oecologia 150:611–624.

Sappington, T. W., B. D. Siegfried, and T. Guillamaud. 2006. Coordinated *Diabrotica* genetics research: accelerating progress on an urgent insect pest problem. American Entomologist 52:90–97.

Spence, J. R., and J. K. Niemala. 1994. Sampling carabid assemblages with pitfall traps: the madness and the method. Canadian Entomologist 126:881–894.

Sunderland, K. D. 1996. Progress in quantifying predation using antibody techniques. Pages 419–456 *in* W. O. C. Symondson and J. E. Liddell, editors. The ecology of agricultural pests: biochemical approaches. Chapman and Hall, London, UK.

Sutter, G. R., and T. F. Branson. 1986. Artificial infestation of field plots. Pages 147–157 *in* J. L. Krysan and T. A. Miller, editors. Methods for the study of pest Diabrotica. Springer-Verlag, New York, New York, USA.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology 11:627–641.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biological control agents? Annual Review of Entomology 47:561–594.

SYSTAT. 2004. SYSTAT 11. SYSTAT, Richmond, California, USA.

Toepfer, S., M. M. Ellsbury, R. Eschen, and U. Kuhlmann. 2007. Spatial clustering of *Diabrotica virgifera virgifera* and *Agriotes ustulatus* in small-scale maize fields without topographic relief drift. Entomologia Experimentalis et Applicata 124:61–75.

Toepfer, S., et al. 2009. A review of the natural enemies of beetles in the subtribe Diabroticina (Coleoptera: Chrysomelidae): implications for sustainable pest management. Biocontrol Science and Technology 19:1–65.

Vink, C. J., S. M. Thomas, P. Paquin, C. Y. Hayashi, and M. Hedin. 2005. The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics 19:99–104.

Wardle, D. A., W. M. Williamson, G. W. Yeates, and K. I. Bonner. 2005. Trickle-down effects of aboveground trophic cascades on the soil food web. Oikos 111:348–358.

Weber, D. C., and J. G. Lundgren. 2009. Quantification of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation. Journal of Insect Science 9:41.

Zaidi, R. H., Z. Jaal, N. J. Hawkes, J. Hemingway, and W. O. C. Symondson. 1999. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Molecular Ecology 8:2081–2087.

Zhang, G.-F., Z.-C. Lü, F.-H. Wan, and G. Lövei. 2007. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Molecular Ecology 7:947–954.

APPENDIX

Species against which the *Diabrotica virgifera*-specific PCR primers were tested without positive detection (*Ecological Archives* A019-091-A1).