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SHORT COMMUNICATION

Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae)

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The diel and seasonal activity of epigeal predators associated with pre-imaginal *Diabrotica virgifera* was described. Due to its duration, the egg stage was exposed to more predators than the larval stage. Most predators were easily categorized into day- and night-active guilds. Seasonal and diel niche partitioning may contribute to the maintenance of this diverse and abundant predator community.

Keywords: Acari; Araneae; biological control; Carabidae; corn rootworm; Gryllidae; Phalangidae; predator community; Staphylinidae

Epigeal predator communities are often quite speciose, and the niches that partition these communities and reduce direct competitive interactions remain poorly understood even within the best studied systems. Diel (Brust, Stinner, and McCartney 1986; Lundgren, Shaw, Zaborski, and Eastman 2006) and seasonal (Kromp 1999) activity patterns of individual constituents of epigeal predator communities may help these diverse assemblages to persist within highly segregated or homogeneous habitats (i.e., farmland). This niche differentiation restricts the resources that can be exploited by a predator species, and consequently not all predators contribute equally to the suppression of a target pest. Moreover, it is critical to understand when predominant predators are active in order to better study predation events and their impact on a target pest (Pfannenstiel 2005; Pfannenstiel, Booth, Vargo, and Schal 2008). Indeed, identifying which predators co-occur with each pest life stage in space and time is a critical early step in developing conservation biological control programs for a specific pest species.

A pest of broad importance that has received little attention from predator ecologists is *Diabrotica virgifera virgifera* LeConte (the western corn rootworm, referred to as *D. virgifera* hereafter). This pest is believed to have originated in Western North America, and has spread to become one of the worst pests of North American corn. Its recent introduction into Europe threatens the production of corn in this part of the world as well (Miller et al. 2005). Most of *D. virgifera*'s life cycle is spent beneath the soil. Larvae damage corn roots, which disrupts several

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physiological processes of the plant and reduces the harvestable yield (Riedell and Kim 1990; Riedell and Reese 1999).

In spite of *D. virgifera*'s economic importance, predation on the immature life stages has not been well explored (Toepfer et al., in press). While research on the top-down effects of predators on *D. virgifera* is relatively sparse, studies on this and other *Diabrotica* spp. suggest that predation may be important to the population dynamics of this pest (Toepfer et al., in press). Indeed, in their analysis of the natural factors influencing *D. virgifera* survival, Toepfer and Kuhlmann (2006) concluded that *D. virgifera* populations are likely susceptible to top-down management strategies such as biological control.

The purpose of this study is to describe the diverse predator community that co-occurs with *D. virgifera* immature stages. Additionally, time-sorting pitfall traps are used to determine how the predator community is partitioned over the diel cycle, in order to better understand when key predators are most active. This study represents the first component in a comprehensive examination of the epigeal predator community associated with *D. virgifera* immatures in South Dakota (US) cornfields, and its impact on this pest.

Research was conducted in three replicate plots of equal size (29 × 24 m) within a cornfield (4136 m²) planted to continuous no-till corn for 5 years at the Eastern South Dakota Soil and Water Research Farm in Brookings, SD (96.81W, 44.35N). Plots were fertilized with 190-67-67 kg per ha of N-P-K 2 weeks before planting. Glyphosate-tolerant field corn (Dekalb[®] 40-07, Monsanto, St. Louis, MO) was planted at 52,000 seeds per ha on 5 June 2006 (0.72 m between rows), which emerged 12 June. Herbicides (glyphosate [Round-up[®], Monsanto Co.], S-metaloclor [Dual II[®], Syngenta, Greensboro, NC], and mesotrione [Callisto[®], Syngenta]) were applied over the season 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. Corn plots were infested to functionally equalize the abundance of *D. virgifera* populations among the plots. Eggs were placed 8–10 cm deep into the center eight rows (9.1 m long) of each plot at a rate of 4900 per m using a tractor-mounted, mechanical infester (Sutter and Branson 1986) on 7 June 2006.

Temperature-based models were used to estimate the duration of each development stage of *D. virgifera* in the field. A biophenometer was placed into the center of each plot when the fields were infested with *D. virgifera* eggs, and temperatures were recorded eight times per day until *D. virgifera* adult emergence. Using the soil temperature degree-day model of Fisher, Sutter, and Branson (1990), the dates on which 50% of the population had reached each life stage (egg and 1st–3rd stadia) were determined. From the soil temperatures, a composite mean daily temperature was created for the 3–4 days surrounding each of these 50% occurrences, and the developmental model of Jackson and Elliot (1988) was applied to the data to estimate the duration of each immature stage at the observed field temperatures.

Time-sorting pitfall traps were used to collect predators from the cornfields during pre-pupal stages of *D. virgifera*. A total of 22 sample days were conducted from 29 May to 20 July, 5 days of which occurred before the *D. virgifera* eggs were artificially placed in the field. Trapping ceased when 50% of the *D. virgifera* population was in the 3rd stadium. To increase the capture efficiency of the pitfall traps, a metal barrier (1.5 m × 14.5 cm, long × tall) was placed between two pitfall

traps, such that insects which encountered the barrier would travel along its length and fall into a trap at either end. The barrier was placed at a 45° angle with the corn row, such that insects traveling between and along rows would have equal opportunity for capture. Traps designed to intercept a larger area have been shown to magnify the capture relative to traditional hole pitfalls, thereby reducing the total number of traps required in a given habitat (Luff 1975). A set of traps were placed in the inter-row areas near the centers of the three plots, and the barrier crossed over two corn rows.

Time-sorting pitfall traps allowed the monitoring of diel activity patterns in each predator group. An electronically-timed, rotating trap mechanism was modified from a commercially available unit (Collection Bottle Rotator, Model 1512, John W. Hock Co., Gainesville, FL). Arthropods were collected into one of eight PVC tubes (7.6 cm inner diameter) that were attached to metal rotator support rings. A coffee cup liner (Solo, No. 806A) with the bottom cut off served as a pitfall funnel. The timer mechanism was set to segregate pitfall collections into eight 3-h intervals over a diel cycle. Each of the rotating pitfall traps was placed into two plastic tubs that were buried in the soil; this tub-in-tub design facilitated removing the traps for cleaning and maintenance. The large traps (80 cm diameter) were covered and concealed by soil and ground litter from the site so that only the pitfall opening was visible at the surface. Trap openings were covered when not active. The collection receptacles were filled with 100% ethylene glycol, and the trap contents were collected at approximately 09:00 h on each trapping date.

Predators were identified and grouped into operational taxonomic units, many of which are at the species level. To facilitate analyses, only predators that represented more than 1% of the total captures were included in the community analyses. Whenever possible, the numerous taxa that were collected infrequently were grouped under a larger taxonomic category (i.e., Other Carabidae, Other Lycosidae, Other spiders, and Other mites). Predators were stored individually in 70% EtOH at –20°C until they could be identified and their DNA extracted. *Diabrotica virgifera*-specific DNA sequences contained within the guts of these predators were amplified using PCR, and results of this gut content analysis, and relative importance of these taxa as *D. virgifera* predators, are published elsewhere (Lundgren, Ellsbury, and Prischmann, submitted).

All sampling methods are accompanied by inherent biases in the arthropods they collect. Although pitfall traps provide questionable information about the composition of epigeal predator communities, their relative densities, and their impact on target pests (Koivula, Kotze, Hiisivuori, and Rita 2003; Lundgren et al. 2006), they are a cost- and time-effective method for collecting large quantities of active predators. Thus, the data presented in this manuscript pertains primarily to the active epigeal predator community.

A mean (SEM) of 617 ± 58 (1876 total) predators per plot were collected in the pitfall traps over the experimental period. This community consisted of 85 taxa; 17 of the least frequently collected taxa (95 specimens total) were omitted from these analyses. The remaining 68 taxa were consolidated into 17 taxonomic classes for phenological representation (Figure 1).

A temporally dynamic predator community was found in association with *D. virgifera* immatures in the field (Figure 1). The egg stage of *D. virgifera* is much longer than the larval stage, and is thus exposed to a much broader predator

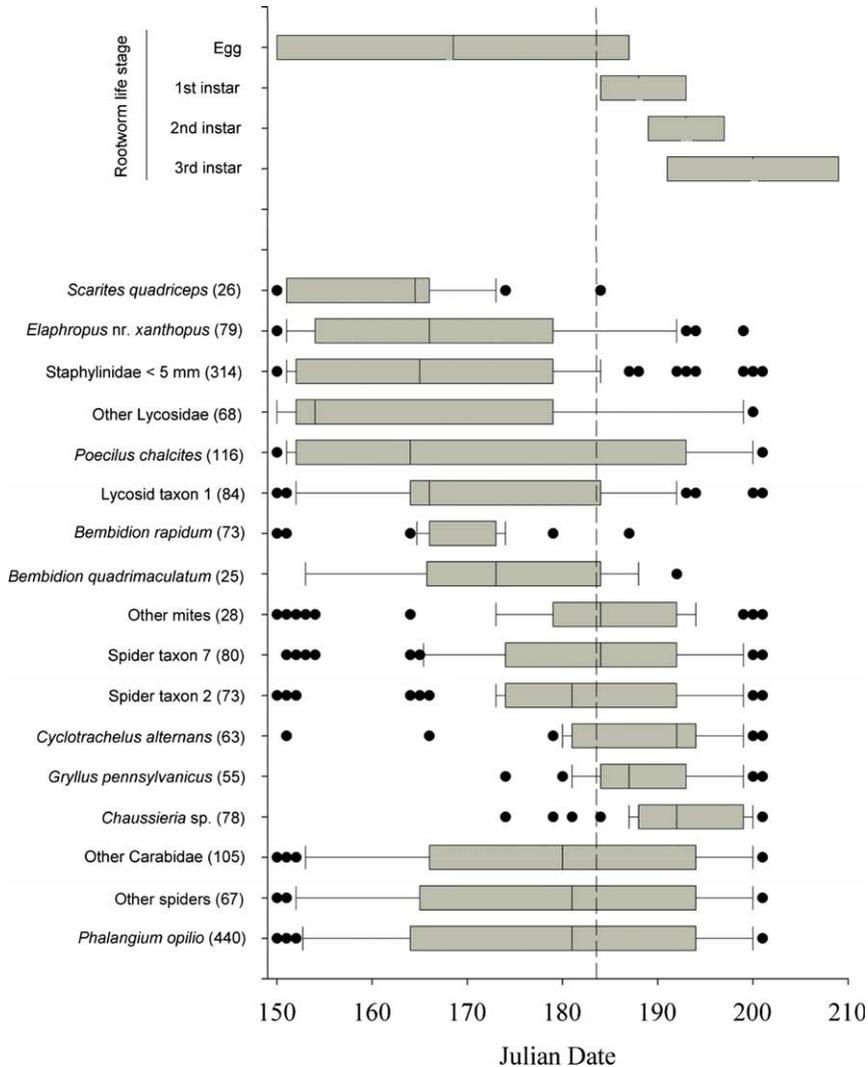


Figure 1. Seasonal phenology of *Diabrotica virgifera* immatures, and the temporally overlapping active predator community in corn. *Diabrotica virgifera* phenology was estimated from degree day models and soil thermal units (see text for more information). Box and whisker plots were created for each predator taxon using pooled data from three replicate plots, with the middle line of the box indicating the date on which the median number of specimens was captured; the shaded boxes represent the median 50% of datapoints, and the dots represent datapoints in the highest and lowest quartiles. Sample size for each predator taxon is indicated in parentheses. The vertical dashed line indicates the beginning of the larval stage.

community and for a relatively extended period of time. Indeed, eggs are laid in late summer and are also exposed to predators during the autumn and winter. At least half the individuals of 14 of these taxa were captured during the *D. virgifera* egg stage. *Scarites quadriceps* Chaudoir (Coleoptera: Carabidae), *Elaphropus* nr.

xanthopus Dejean (Carabidae), small staphylinids (<5 mm long) (Coleoptera: Staphylinidae), lycosid taxon 1 (Araneae: Lycosidae), ‘Other Lycosidae’ (Araneae), *Poecilus chalcites* (Say) (Carabidae), *Bembidion rapidum* (LeConte) and *B. quadrimaculatum* LeConte (Carabidae) were found most frequently in association with the *D. virgifera* egg stage.

Several predators were captured almost exclusively during the larval stage. Ten predator taxa overlapped with the larval stages of *D. virgifera*. Of these, *Cyclotrachelus alternans* (Casey) (Carabidae), *G. pennsylvanicus* (Orthoptera: Gryllidae), and *Chaussieria* sp. (Acari: Anystidae) were captured almost exclusively during the larval stage of *D. virgifera*. Although some predator groups were distinctly captured during specific *D. virgifera* life stages, a few were prevalent the entire season, including *Ph. opilio* L., *Po. chalcites*, and a handful of other carabids and spider species.

Almost invariably, the predators surveyed were easily categorized as either day- or night-active guilds (Figure 2). *Phalangium opilio* L., lycosid taxon 1, *C. alternans*, and *S. quadriceps* were captured during the night. The remainder of the taxa was mostly captured during daylight hours. *Chaussieria* sp. had a particularly brief collection period, being collected almost exclusively between 12:00 and 18:00 h (Figure 2). *Gryllus pennsylvanicus* and spider taxon 2 were active throughout most of the day, with reduced activity during the morning hours. In general, it is often the case that highly managed monocultures of row crops are frequently associated with a

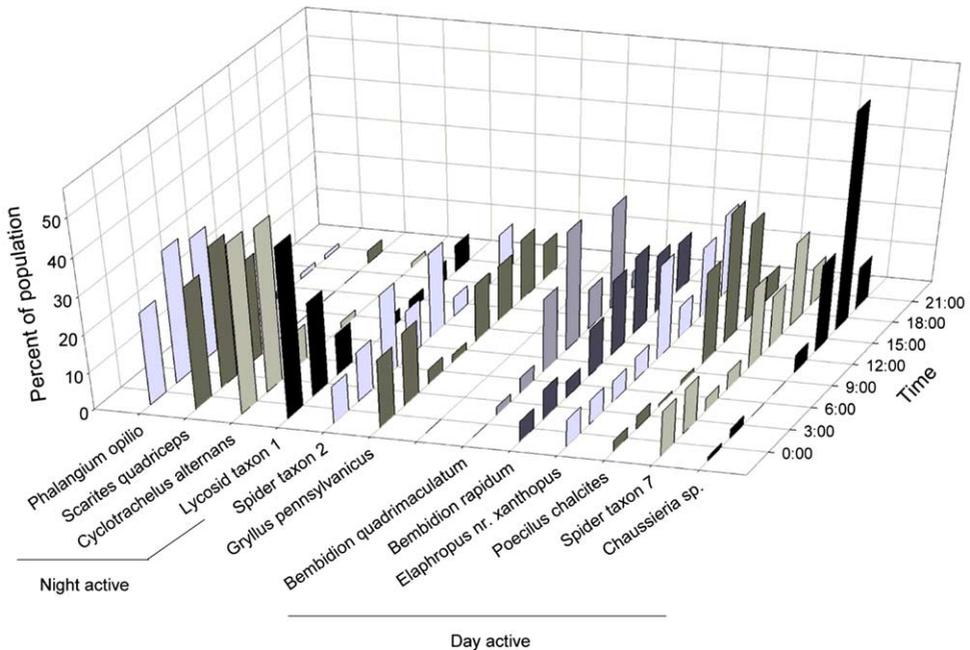


Figure 2. The diel activity patterns of major predators associated with *Diabrotica virgifera* immatures. Each bar represents the mean percent of the total number of each taxon captured during the preceding 3-h interval ($n = 3$; error bars omitted for presentation clarity). The most taxonomically heterogeneous taxa are not included in this presentation. Primarily day and night active taxa are indicated on the X-axis.

large complement of nocturnally active predators (Brust et al. 1986). Lundgren et al. (2006) found that the predator communities residing in more stable agroecosystems (perennial hayfields) were active and predated sentinel prey more evenly throughout the diel cycle than in more highly managed vegetable and cash grain systems (Chapman, Armstrong, and McKinlay 1999 report similar findings with *Pterostichus melanarius*). A firm understanding of when key predators consume target pests is a critical but oft neglected step in describing the importance of endemic generalist predator communities during the initial development of conservation biological control programs. Barrier-linked, rotating pitfall traps offer a powerful tool for studying the diel activity patterns of active predator communities (Blumberg and Crossley 1988; Chapman and Armstrong 1997).

In the case of the epigeal predator community associated with *D. virgifera* immatures, directed efforts to observe predation events must capture both the day and night active components of this diverse predator community. More, but not necessarily more effective, predator taxa co-occur with the egg stage of *D. virgifera* than the larval stage. A next step in the examination of this community should focus on the relative efficacy of the predators associated with *D. virgifera* immatures as biological control agents, and how farmland can be manipulated to maximize the impact of predators on this important pest.

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