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Source: Environmental Entomology, 46(1) : 107-117

Published By: Entomological Society of America

URL: https://doi.org/10.1093/ee/nvw157

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Environmental Entomology, 46(1), 2017, 107–117
doi: 10.1093/ee/nvw157
Advance Access Publication Date: 26 December 2016
Research

Response of *Pemphigus betae* (Hemiptera: Aphididae) and Beneficial Epigeal Arthropod Communities to Sugarbeet Plant Density and Seed-Applied Insecticide in Western Nebraska

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Subject Editor: David Onstad

Received 22 June 2016; Editorial decision 9 November 2016

Abstract

This study investigated the impact of a neonicotinoid seed-applied insecticide (Poncho Beta) and two plant densities (86,487 and 61,776 plants per hectare) on the sugarbeet root aphid (*Pemphigus betae* Doane), beneficial epigeal arthropods, and selected crop yield parameters in sugarbeet (*Beta vulgaris* L. var. *vulgaris*). Ground beetles and centipedes were the most commonly collected taxa during 2012 and 2013, respectively. Centipede, spider, and rove beetle activity densities were not affected by the seed-applied insecticide, whereas plant density had a marginal effect on centipede activity density during 2012. Ground beetle species richness, diversity, and evenness were also not impacted by the seed treatments. However, during 2013, ground beetle activity density was significantly higher in plots planted with untreated sugarbeet seeds due to the abundance of *Bembidion quadrimaculatum oppositum* Say. Sugarbeet root aphid populations were significantly higher in the untreated plots during both years. In 2012, sugarbeet tonnage and sugar yield were higher under the low plant density treatment, while higher sugar content was recorded from the seed-applied insecticide plots (2013). Seed-applied neonicotinoids and plant density had little impact on beneficial epigeal arthropod activity density. Seed treatment did result in decreased root aphid populations; however, these reductions were not sufficient to be considered as an adequate control. This limited aphid control likely contributed to inconsistent effects on yield parameters.

Key words: ground beetle, diversity, neonicotinoid

The sugarbeet root aphid, *Pemphigus betae* Doane, is a serious pest of cultivated sugarbeet (*Beta vulgaris* L. var. *vulgaris*) in North America (Hein et al. 2009), reducing both sugar and root yield (Summers and Newton 1989, Hutchison and Campbell 1994, Winter 1999, Hein et al. 2009). Unfortunately, the subterranean existence of the damaging summer populations complicates conventional chemical control measures (Winter and Patrick 1997, Dewar 2007). Sugarbeet root aphids are protected from direct contact with foliar insecticides and, with the exception of certain compounds (Jacobson and Thriugnanam 1991), most systemic compounds do not translocate within the phloem to the host's roots for adequate control (Dewar and Cooke 2006, Dewar 2007). In the absence of reliable means for aphid control, more emphasis has been placed on an integrated approach to sugarbeet root aphid management, including sanitation, irrigation scheduling, crop rotation, control by natural enemies, and the use of resistant varieties (Summers and Newton 1989, Hein et al. 2009).

The delivery of plant protectants is increasingly being done through seed application (Halmer 2000). Neonicotinoids comprise a class of insecticides that has become very important in crop protection (Elbert et al. 2008, Seagraves and Lundgren 2011, Goulson 2013). Neonicotinoid seed treatments possess lasting residual and systemic effects, and a broad-spectrum activity toward several feeding guilds, rendering them suitable for control of early-season pests (Elbert et al. 2008). Thiamethoxam, imidacloprid, and clothianidin are the neonicotinoids used as seed treatments. They are applied to

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seeds as a film coating, multilayer coating, or seed dressing to protect young plants against arthropod pests, and are used on various crops, including sugarbeet (Elbert et al. 2008).

Neonicotinoid seed treatments are renowned for their control of sugarbeet sucking insect pests and the viruses they transmit on account of their systemic action (Dewar and Read 1990, Schmeer et al. 1990, Rouchaud et al. 1994, Wauters and Dewar 1996, May 2001, Dewar et al. 2002, Dewar and Cooke 2006, Elbert et al. 2008, Strausbaugh et al. 2010). Little work has been published on the effect of neonicotinoid seed treatments on the sugarbeet root aphid. However, Dewar and Cooke (2006) indicate that seed treatments might be ineffective against root aphids, because root colonization takes place later in the season when the effect of the chemical has dissipated. Furthermore, Westwood et al. (1998) found that imidacloprid concentrations remained low in sugarbeet roots at different postplanting sampling intervals. However, a recent study suggested that, in addition to good control of the foliar bean aphid (*Aphis fabae* Scopoli), neonicotinoid seed treatments also suppressed *P. betae* incidence (Strausbaugh et al. 2010), but perhaps not consistent enough to be considered as adequate control.

Beneficial arthropods are susceptible to insecticides (Ellsbury et al. 1998), and in many cases, even more so than the target pest (Ruberson et al. 1998). This may be owing to factors such as small body size with a greater surface to volume ratio and the presence of lower levels of detoxification enzymes (Hoddle and Van Driesche 2009). However, the conservation of natural enemies of pest arthropods in agroecosystems can be key to an IPM program by preventing injurious insects from attaining pest status and reducing the damage potential of important pests (Pedigo and Rice 2009). Compared with foliar applications, neonicotinoid seed treatments are often regarded as safer to the environment owing to the decreased amount of active ingredient, lower mammalian toxicity, and reduced insecticidal contact for nontarget organisms. This provides an incentive for their use in IPM systems (Mizell and Sconyers 1992, Taylor et al. 2001, Albajes et al. 2003, Elbert et al. 2008, Jeschke and Nauen 2008). However, this should not imply that nontarget organisms would remain unaffected by seed-applied insecticides. Natural enemies can be exposed to the chemical when they supplement their diet by feeding on treated plant material (Albajes et al. 2003). Several studies conducted under laboratory conditions have found that neonicotinoid seed treatments can have an adverse effect on beneficial arthropods by causing mortality (Al-Deeb et al. 2001, Mullin et al. 2005, Moser and Obrycki 2009, Seagraves and Lundgren 2011). However, studies of seed treatments and natural enemies under field conditions have shown inconsistent results. For example, Seagraves and Lundgren (2011) reported reduced abundance of nabid bugs in thiamethoxam seed-treated soybeans and adult lacewings in imidacloprid seed-treated soybeans. In contrast, Krauter et al. (2001) did not measure a negative impact of imidacloprid seed treatments for sorghum on nabid bugs, geocorid bugs, ladybeetles in the genus *Scymnus* (Pullus), spiders, or lacewings under field conditions. Albajes et al. (2003) reported that spiders, ladybeetles, and rove beetles were not impacted negatively by imidacloprid seed-treated corn, and ground beetles were only moderately affected during one of the five years in which the study was conducted. However, these authors did report a significant negative effect of this insecticide on Heteroptera. Leslie et al. (2009) saw decreased abundance for two species of ground beetles in neonicotinoid seed-treated corn plantings. Naveed et al. (2010) reported reduced rates of parasitism of *Bemisia tabaci* (Gennadius) in cotton treated with seed-applied thiamethoxam and imidacloprid. Therefore, it is prudent to evaluate the impact of seed-applied insecticides on natural enemies for all cropping systems and natural enemy assemblages in which they are used.

Apart from direct toxicity, early-season prey suppression due to seed-applied insecticides could prevent buildup of natural enemies and lessen their impact on later-season pests such as the sugarbeet root aphid. Additionally, natural enemies might also acquire the systemic insecticides indirectly from their prey, as shown by Grafton-Cardwell and Gu (2003), who observed increased toxicity in the larvae of vedalia beetles [*Rodolia cardinalis* (Mulsant)] preying on cottony cushion scale [*Icerya purchasi* (Williston)] that had fed on treated plant material. Papachristos and Milonas (2008) also measured reduced larval survival, reduced adult longevity, and reduced fecundity of a ladybeetle [*Hippodamia undecimnotata* (Schneider)] after feeding on *A. fabae* which ingested systemic imidacloprid. Because of these potential effects, seed-applied insecticides could pose a particular threat to edaphic natural enemy communities—a group that is very likely to interact with sugarbeet root aphids.

Apart from insecticides, other factors relating to the physical cropping environment are also known to influence both pest and natural enemy population dynamics. With ground beetles, for example, edaphic factors (e.g., soil moisture, soil type, etc.) and crop type have been reported to affect beetle assemblages (Holland and Luff 2000). However, little information is available on how differences in the cropping environment, such as plant spacing, affect natural enemy and pest species dynamics. For example, Honek (1988) found differing activity of ground beetles depending on vegetation density; the beetles preferred shaded soils when there was low and medium crop density and bare soil when vegetation density was highest. In sugarbeet, optimal plant spacing is needed to maximize sugar yield (Jaggard and Qi 2006, Smith et al. 2013). However, optimal plant establishment with sugarbeet is challenged by seed depth, seed placement, soil crusting, soil temperature, soil moisture, blowing soils, seed quality, damage from diseases, and insect pests and pesticides (Smith et al. 2013, Yonts et al. 2013). On an average, sugarbeet emergence in Colorado, Wyoming, and Nebraska is estimated at 65%, with fluctuations between 45 to 80% (Smith et al. 2002, 2013). With such unpredictability, it is essential to understand the responses of pests and their natural enemies to these changes to predict their impact on pest pressure and ecosystem services provided by natural enemies.

The objectives of this study were to evaluate the response of sugarbeet root aphid, resident epigeal beneficial arthropods, and several crop yield parameters to sugarbeet seed with seed-applied insecticides (Poncho Beta) planted at varying plant densities under field conditions in western Nebraska. Furthermore, we also aimed at determining whether the impact of the seed-applied insecticide on these arthropods vary across the two planting densities tested. We hypothesize that plant density, through altering within-row plant spacing, would not affect natural enemy activity density. We also hypothesize that seed-applied insecticide will affect natural enemy density through direct toxicity or by reducing prey availability.

Materials and Methods

This study was conducted during the 2012 and 2013 cropping seasons at the Mitchell research farm of the University of Nebraska–Lincoln's Panhandle Research and Extension Center (PHREC) located in the North Platte River Valley, western Nebraska (41° 56′ N; 103° 42′ W). All research plots were established in fields that produced corn in the preceding year. Sugarbeet plots were subjected to zone-tillage that resulted in a high percentage (>30%) of corn residue remaining on the soil surface. During spring, corn stalks were chopped by disking the

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field before performing the zone tillage operation. The zone tillage implement contained vertical shanks that cultivated soil to a depth of ca. 30.5 cm and a width of ca. 15–25 cm. These tilled zones constitute the new planting rows with 56-cm row spacing (Smith 2013). The zone tillage implement consisted of a large coulter in front of each vertical shank that cut corn residue. Positioned directly behind each vertical shank was a pair of wavy coulters that closed the shank mark. Finally, behind the wavy coulters, a rolling basket firmed the soil surface to ensure seed–soil contact. All plots were treated with glyphosate twice early in the season for weed control.

The study was set up in a randomized complete block design with six replications. Treatment layout was a split-plot arrangement with untreated seed and a seed-applied Poncho Beta (Bayer CropScience) at a rate of 68 g ai/unit sugarbeet seed: 60 g/ai clothianidin and 7.996 g/ai beta-cyfluthrin as the main-plot experimental treatments and a high (86,487 plants per ha) and low plant density (61,776 plants per ha) as the split-plot treatments. These two planting densities represent high and low plant populations for sugarbeet production in the area (Yonts et al. 2013). Individual plots (main-plot experimental units) contained 18 rows of sugarbeet, and measured 7 by 10 m. Seed size was regular pellet (variety: Beta 21RR25). Each plot was subsequently divided lengthwise in half so that each split-plot experimental unit measured 7 by 5 m and contained nine rows of sugarbeets. The target plant density was attained by overplanting the plots to ca. 280,000 plants per hectare (within-row plant spacing of 6.35 cm). The plots were thinned twice to the desired stand during each cropping season to ensure the correct plant population. The first round of thinning was conducted on 11–12 June 2012 and 10–13 June 2013. The second thinning was carried out on 28–30 June 2012 and 8–9 July 2013.

Beneficial Arthropod Activity Density and Ground Beetle Species Richness

Within each split plot, four pitfall traps were installed to monitor the activity density of beneficial resident epigeal arthropods. Both the population density as well as the activity of an organism can influence pitfall captures, and, therefore, the quantity obtained through these captures is defined as the activity density of the organism (Thomas et al. 1998), rather than its absolute density. Monitoring activity density of epigeal arthropods through pitfall captures is a standard procedure followed by similar studies (e.g., Lee et al. 2001, Hajek et al. 2007, Gardiner et al. 2010). The activity density data reported in this study represent the total number of arthropod individuals collected over a period of 7 d in each pitfall trap.

One pair of traps was oriented across the rows of each subplot, whereas the other pair was oriented lengthwise between the two center rows of each subplot. A sheet of metal flashing was installed between the two traps in each position, thereby linking pairs of traps. Flashings were installed to increase the rate of capture of beneficial arthropods. These metal flashings each measured ca. 165 by 30 cm, with ca. 15 cm buried below soil level. Pitfall traps were constructed by making a hole in the soil with a 107-mm-diameter golf hole cutter and inserting a section of PVC piping (76 mm diameter and 150 mm high) into each hole to prevent soil from collapsing into the samples. A small disposable plastic cup (147 ml capacity), containing a mixture (ca. 38 ml) of ethylene glycol and water (1:3 ratio) as a killing and preservation agent, was placed into each hole at the time of trap activation. A small amount of dishwashing liquid was added to the preservation agent master mix (10 ml/3.78 liter) to reduce surface tension. A tight-fitting plastic funnel (75 mm diameter on top and 25 mm diameter at the bottom) was placed on top of each cup to ensure capture of soil arthropods wandering into the traps. Each pitfall trap was subsequently covered with a custom-manufactured plastic lid (250 mm diameter), leaving ca. 10-cm space between the lid and soil surface for arthropods to enter. The lids were affixed to a 40.7- by 8.9-cm piece of wood with 12.7-cm bolts attached to each end, which were used to anchor the lid to the soil surface. Pitfall traps were left in the field for the duration of the growing season and capped with a tight-fitting lid when not activated.

Beneficial arthropod activity density was measured three times during the latter part of each growing season. Samples were removed on 3 July, 27 July, and 29 August during 2012, and 5 July, 29 July, and 30 August during 2013, and the traps were left open in the field for 7 d at a time. The taxa of beneficial epigeal arthropods sampled included selected beetle families (Carabidae, Staphylinidae, and Coccinellidae), spiders (Order: Araneae), harvestmen (Order: Opiliones), and centipedes (Class: Chilopoda). All of these are considered important predators of arthropod pests in agroecosystems (e.g., Weibull et al. 2003, Brewer and Elliot 2004, Eitzinger and Traugott 2011).

Due to the abundance and diversity of ground beetles in the samples and their significance in agroecosystems (Holland and Luff 2000), they were identified to species (Lindroth 1961–1969, Bousquet 2012). A reference collection containing voucher specimens of these ground beetles is housed at the University of Nebraska–Lincoln's Panhandle Research and Extension Center (405 Avenue I, Scottsbluff NE 69361). Furthermore, on account of significant differences in ground beetle activity density observed between the untreated and treated plots during 2013, three diversity indices (apart from measuring activity density) were calculated for this taxon: species richness (species count), Simpson's diversity index, and Simpson's evenness. These diversity indices were calculated for each pitfall trap separately, where the number of beetles collected in each trap represents the cumulative total over the three collecting dates.

Simpson's diversity index (D) quantified ground beetle diversity within the treated and untreated plots. This index is calculated by:

$$D = \sum p_i^2$$

where p_i is the proportion (from the total count of all species) of individuals collected for the ith ground beetle species (Magurran 2004). This diversity index accounts for both species richness (the number of species in a sample) as well as evenness (the relative abundance of each species in a sample). The reciprocal of the Simpson's diversity index ($1/D$) was used to calculate the diversity of ground beetles found in both the treated and untreated plots. The reciprocal index ranges on a scale from one to a maximum equal to the total number of species collected within the sampled habitat. The higher the value of this index, the more even and diverse the species assemblage of the sample or habitat (Magurran 2004).

Simpson's evenness was calculated as:

$$E_{1/D} = (1/D)/S$$

where S (species richness) represents the number of species in the sample or habitat. Simpson's evenness ranges on a scale from 0–1, with one indicating complete evenness (i.e., the proportions of each ground beetle species are equal).

Sugarbeet Root Aphid Ratings and Crop Parameters

To encourage the establishment of sugarbeet root aphid populations, plots were infested with these aphids adjacent to the two metal flashings within each plot. Aphid colonies were reared in a greenhouse on sugarbeets grown in tall tree pots (Stuewe & Sons, Inc., Tangent, OR). Each tree pot measured 10 cm wide by 36 cm

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high and had a 2.83-liter volume. A total of five sugarbeet seeds were planted in each pot and subsequently thinned to two sugarbeets per pot. Following this, five mature, apterous root aphids were introduced into each of the three holes (16 cm diameter) made next to the sugarbeet seedlings in each pot. Subsequently, the tree pots were incubated in a greenhouse at 23°C for 3 wk to allow buildup of sugarbeet root aphid populations. They were then removed and field infestations commenced. All plots were inoculated with the soil from four pots at each flashing for a total of eight pots per subplot. Following the last arthropod sampling, four beets were removed next to the metal flashings and the level of sugarbeet root aphid infestation visually rated according to the 0–5 root rating scale developed by Hutchison and Campbell (1994). With this scale, a value of 0 indicates that no root aphid colonies are present; a value of 1 indicates the presence of a single colony of 2.54 cm in diameter or less; a value of 2 indicates the presence of two or more colonies, each with a diameter of 2.54 cm or less; a value of 3 indicates the presence of colonies >2.54 cm in diameter, covering <50% of the root surface; a value of 4 indicates the presence of colonies covering 50–90% of the root surface; while a value of 5 indicates colonies covering 90–100% of a root's surface.

During both 2012 and 2013, root ratings were conducted on 4 September. All plots were machine harvested using a two-row sugarbeet harvester. Only rows four and five from each subplot were harvested. A total of 7.62 m was harvested from each of the two-row harvest sample, and two subsamples were collected from each ($n = 8$–10 beets per subsample). For each subplot, sugarbeet yield (tons per hectare), percentage sugar loss to molasses (SLM), sugar yield (kg/ha), and percentage sugar content were recorded. The two subsamples from each subplot were used to calculate both the percentage SLM and percentage sugar content at Western Sugar's tare laboratory (Scottsbluff), using standard industry procedures. Tonnage and kg sugar per ha were quantified for each subplot as a whole (i.e., one sample per subplot).

Acceptance of Sugarbeet Root Aphids as Prey

Because predation on sugarbeet root aphid colonies occurring on sugarbeet remains poorly understood, a controlled experiment was conducted to determine if the dominant ground beetle species observed from this study accept this aphid species as prey. In 2013, six individual beetles (replicates) from 10 commonly observed ground beetle species, observed during previous seasons (2010–2012), were captured live in sugarbeet fields at the Mitchell research farm. Each beetle was starved for 24 h, with only a moistened cotton wick provided as a source of moisture. Evidence suggests that ground beetle foraging depends directly on hunger levels (Fournier and Loreau 2002), and starving these beetles (for a period of a few hours up to 2 wk—with 24 h being the norm) before conducting prey determination experiments, is a standard procedure followed in such studies (e.g., Andersen et al. 1983, Baines et al. 1990, Floate et al. 1990, Bilde and Toft 1997, Jørgensen and Toft 1997, Mundy et al. 2000, Harwood et al. 2003, Lang and Gsöl 2001, Calder et al. 2005, Hatteland et al. 2010, Monzó et al. 2011, Lee and Edwards 2012, Davey et al. 2013, Okrouhlik and Foltan 2015, Morrison et al. 2016). The rationale for doing so is to avoid misleading results where satiated beetles will not feed on any prey being offered to them.

Six field-collected apterous root aphids of varying ages were subsequently placed into a 20-ml glass scintillation vial with a single beetle. Vials were placed on their side to enable free movement of the beetles and prey, capped with a 70-mesh material that was affixed with a rubber band. These vials were used because previous experimentation (unpublished data) indicated that the aphids are unable to scale the sides of the glass vials, which would have allowed them to avoid predation. Vials containing the aphids and beetles were placed in a growth chamber at 23 °C (a photoperiod of 16:8 [L:D] h) for 24 h, when the beetles were removed and the remaining aphids recorded.

Statistical Procedures

The effects of plant density and seed-applied insecticides on beneficial arthropod activity density, root aphid populations, and crop parameters were evaluated by means of a two-way ANOVA implemented with PROC GLIMMIX (SAS Institute 2008). This procedure was used to test for differences between the main-plot factors (seed treatment) and the split-plot factors (plant density), as well as any interactions that might exist between the two. For comparing beneficial arthropod activity density between the four treatments, the data from each pitfall trap were pooled (cumulative) over the three sampling dates of each year separately. Three sampling dates were chosen to collect a sufficient number of beneficial arthropods from each taxon for carrying out statistical analyses. This study did not aim at determining the seasonal activity and species composition of beneficial arthropods, but rather aimed at evaluating the overall impact of plant density and insecticide-treated sugarbeet seeds on these arthropods during the latter half of the season. Because the beneficial arthropod data represented direct counts, the data were fitted to either a Poisson or negative binomial distribution. Significantly different means among the treatments were separated using a Tukey ad hoc mean comparison test at the $\alpha = 0.05$ level of significance.

A one-way ANOVA was used to compare the three diversity indices for ground beetles between the treated and untreated plots (PROC GLIMMIX, SAS Institute 2008). The same procedure was used to test for differences in the number of sugarbeet root aphids consumed between the 10 most dominant ground beetle species. A Pearson's correlation was calculated (PROC CORR, SAS Institute 2008), to test the relationship between sugarbeet root aphid ratings and those crop yield parameters that showed a significant response to seed-applied insecticides. Correlations were also used to determine the relationship between sugarbeet root aphid ratings and those ground beetle species that were significantly affected by the seed-applied insecticide.

Results

Beneficial Arthropod Activity Density and Ground Beetle Species Richness

A total of 3,673 and 6,274 beneficial epigeal arthropods were collected in 2012 and 2013, respectively (Table 1). Very few

Table 1. Total number of beneficial arthropods (by taxon) collected by means of pitfall trapping during 2012 and 2013

Beneficial arthropod taxon	Total number collected[a]	
	2012	2013
Araneae (spiders)	411	558
Carabidae (ground beetles)	2,205	1,720
Chilopoda (centipedes)	574	2,607
Coccinellidae (lady beetles)	7	0
Opiliones (harvestmen)	3	12
Staphylinidae (rove beetles)	473	1,377
Total	3,673	6,274

[a] Total number of individuals collected from $n = 288$ pitfalls.

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Table 2. Effect of seed-applied insecticide (Poncho Beta) and plant density of sugarbeet on mean (± SEM) centipede, spider, rove beetle, and ground beetle activity density (individuals per trap per 7-d trapping period), during 2012 as revealed by a two-way ANOVA (means separated by a post hoc Tukey mean comparison test at $\alpha = 0.05$)

Taxon	Seed treatment	Plant density		Seed treatment (ST)		Plant density (PD)		ST × PD	
		Low	High	$F_{1, 5}$	P	$F_{1, 10}$	P	$F_{1, 10}$	P
Chilopoda	Untreated	10.71 ± 2.48	14.25 ± 2.62	0.62	0.47	4.24	0.07	0.23	0.64
	Treated	10.07 ± 1.79	12.09 ± 1.86						
Araneae	Untreated	7.75 ± 0.98	7.92 ± 0.99	1.83	0.23	0.87	0.37	0.58	0.47
	Treated	8.33 ± 1.02	10.25 ± 1.18						
Staphylinidae	Untreated	9.58 ± 0.98	11.24 ± 1.07	1.23	0.32	0.33	0.58	1.05	0.33
	Treated	9.49 ± 0.97	9.08 ± 0.95						
Carabidae	Untreated	39.85 ± 5.54	42.16 ± 5.84	1.32	0.30	0.74	0.41	0.02	0.89
	Treated	44.71 ± 6.18	48.37 ± 6.65						

Treatments were: 1) untreated seed planted at a low plant density (86,487 plants per ha), 2) untreated seed planted at a high plant density (61,776 plants per ha), 3) treated seed planted at a low plant density, and 4) treated seed planted at a high plant density

Table 3. Effect of seed-applied insecticide (Poncho Beta) and plant density of sugarbeet on mean (± SEM) centipede, spider, rove beetle, and ground beetle activity density (individuals per trap per 7-d trapping period), during 2013 as revealed by a two-way ANOVA (means separated by a post hoc Tukey mean comparison test at $\alpha = 0.05$)

Taxon	Seed treatment	Plant density		Seed treatment (ST)		Plant density (PD)		ST × PD	
		Low	High	$F_{1, 5}$	P	$F_{1, 10}$	P	$F_{1, 10}$	P
Chilopoda	Untreated	50.00 ± 7.63	49.63 ± 8.39	2.80	0.16	1.26	0.29	0.98	0.35
	Treated	51.54 ± 9.77	64.13 ± 8.44						
Araneae	Untreated	10.90 ± 1.95	11.99 ± 1.72	0.03	0.86	0.11	0.74	0.19	0.67
	Treated	11.27 ± 1.63	11.09 ± 1.78						
Staphylinidae	Untreated	29.86 ± 22.09	27.94 ± 12.09	0.01	0.91	0.09	0.77	0.81	0.39
	Treated	24.49 ± 3.10	29.66 ± 3.83						
Carabidae	Untreated	40.73 ± 2.33	38.91 ± 2.76	11.22	0.02	0.03	0.87	0.38	0.55
	Treated	30.74 ± 3.23	31.49 ± 3.13						

Treatments were: 1) untreated seed planted at a low plant density (86,487 plants per ha), 2) untreated seed planted at a high plant density (61,776 plants per ha), 3) treated seed planted at a low plant density, and 4) treated seed planted at a high plant density.

ladybeetles (total $n = 7$) and harvestmen (total $n = 15$) were collected during both seasons; therefore, these taxa were not analyzed further. During 2012, the highest activity density was recorded for ground beetles, whereas centipedes were most abundant during 2013. Centipede activity showed a marginal response to plant density during 2012, but not in 2013. Furthermore, they were not impacted by the seed-applied insecticides during either season (Tables 2 and 3). During both seasons, seed-applied insecticide and plant density did not affect spider or rove beetle activity (Tables 2 and 3). The same was true for ground beetle activity density during the 2012 cropping season (Table 2). However, mean (± SEM) ground beetle activity during the 2013 cropping season (Table 3) was significantly higher in the untreated plots (39.81 ± 2.13) compared with the insecticide-treated plots (31.11 ± 2.82). No interactions between the seed-applied insecticide and plant density were observed for any of the taxa.

Throughout this study, a total of 3,925 ground beetle specimens were collected in the pitfall traps, containing 36 species in 18 genera (Table 4). However, their numbers were slightly lower during the 2013 field season. Eight species made up ca. 90% of the total ground beetle abundance during 2012, while only five species constituted the same percentage during 2013 (Table 4). The most commonly collected species were *Harpalus erraticus* Say and *Bembidion quadrimaculatum oppositum* Say during the 2012 and 2013 seasons, respectively. During 2012, *H. erraticus* accounted for 30% of the total number of ground beetle specimens collected, but only 2% during the following year. In contrast, *B. quadrimaculatum oppositum* comprised 16% of the total number of ground beetles collected in 2012, but 58% of the total in 2013.

The number of ground beetle species caught in both the seed-applied insecticide treatment and untreated plots were similar during both seasons (Table 5). In addition, there were no significant differences in Simpson's diversity index or Simpson's evenness between the treated and untreated plots during either year (Table 5). There was also little difference between the treated and untreated plots in the activity density of the most dominant ground beetle species (Table 6). However, one species, *B. quadrimaculatum oppositum*, had a significantly higher activity density ($P = 0.01$) in the untreated plots (23.51 ± 1.75) compared with the treated plots (17.62 ± 1.38) during the 2013 season (Table 6). This largely accounted for the observed difference in overall ground beetle activity density between these two treatments during this season. The dominance of this species also led to lower Simpson's diversity and evenness values this year.

Sugarbeet Root Aphid Ratings and Crop Parameters

During both years, the effect of seed treatment on root aphid populations was significant, whereas the effect of plant density was marginally significant only during 2013 (Tables 7 and 8). Sugarbeet root aphid populations were significantly greater in untreated plots compared with the treated plots during both seasons (2012: 2.85 ± 0.18

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Table 4. Percentage abundance of ground beetle (Coleoptera: Carabidae) species collected during the 2012 and 2013 field seasons

Species	% Total	
	2012[a]	2013[b]
Acupalpus partiarius (Say)	–	0.06
Agonum placidum (Say)	0.27	0.58
Amara carinata (LeConte)	†9.98	1.86
Amara farcta LeConte	†11.61	–
Amara quenseli quenseli (Schönherr)	–	0.06
Anisodactylus carbonarius (Say)	0.05	–
Bembidion nitidum (Kirby)	0.32	0.41
Bembidion obscurellum obscurellum (Motschulsky)	–	0.06
Bembidion quadrimaculatum oppositum Say	†16.01	†57.79
Bembidion rapidum (LeConte)	1.50	†4.30
Bembidion tetracolum tetracolum Say	2.54	†16.16
Bracdycellus congener (LeConte)	0.05	–
Bradycellus rupestris (Say)	0.05	–
Chlaenius tricolor tricolor Dejean	0.05	1.98
Cicindela punctulata punctulata Olivier	1.90	0.35
Cratacanthus dubius (Palisot de Beauvois)	–	0.06
Dicheirotrichus cognatus (Gyllenhal)	0.05	–
Dyschirius globulosus (Say)	–	0.06
Elaphropus anceps (LeConte)	†5.26	†8.66
Harpalus amputatus amputatus Say	†2.68	0.23
Harpalus caliginosus (F.)	0.23	0.17
Harpalus erraticus Say	†29.89	1.63
Harpalus herbivagus Say	0.18	0.17
Harpalus pensylvanicus (DeGeer)	†5.40	†2.15
Harpalus reversus Casey	0.59	0.23
Harpalus somnulentus Dejean	0.09	–
Lebia bivittata (F.)	0.05	–
Microlestes linearis (LeConte)	0.45	1.63
Poecilus chalcites (Say)	0.05	–
Poecilus lucublandus (Say)	1.22	0.23
Poecilus scitulus LeConte	0.45	–
Pterostichus femoralis (Kirby)	0.05	–
Pterostichus melanarius melanarius (Illiger)	–	0.06
Pterostichus permundus (Say)	0.27	0.06
Stenolophus comma (F.)	†8.75	1.05
Stenolophus lineola (F.)	0.05	–
Sum	2,205	1,720
No. of species	30	25

[a] A total of 2,205 ground beetles collected during three sampling dates.

[b] A total of 1,720 ground beetles collected during three sampling dates.

† Ground beetle species making up ca. 90% of the total captures within a specified year.

untreated vs. 2.31 ± 0.18 treated plots; 2013: 2.93 ± 0.19 untreated vs. 2.10 ± 0.19 treated plots). No interaction between the seed-applied insecticide and plant density was observed for either year. The correlation between the abundance of *B. quadrimaculatum oppositum* and sugarbeet root aphid ratings during both 2012 and 2013 was nonsignificant (2012: $r = -0.18$, $n = 48$, $P = 0.23$; 2013: $r = 0.23$, $n = 48$, $P = 0.12$).

Seed treatment and plant density did not affect the percentage of sugar loss to molasses in either of the two seasons. In addition, no interaction was detected (Tables 7 and 8). Sugar content was not impacted by the treatment factors during 2012 (Table 7); however, there was a marginal effect for seed treatment during 2013 (Table 8) that resulted in a higher sugar content in insecticide-treated plots (11.30 ± 0.24 untreated vs. 12.13 ± 0.24 treated). There was a marginally significant negative correlation between sugar content and sugarbeet root aphid ratings during this season ($r = -0.36$, $n = 24$, $P = 0.08$), showing decreased sugar content with increased sugarbeet root aphid ratings. In 2012, plant density affected tonnage with no interaction with insecticide (Table 7). Higher root weights per plot were observed with lower plant density in 2012 (47.07 ± 4.90 tons per ha low density vs. 36.30 ± 4.90 tons per ha high density). However, neither plant density nor seed treatment affected tonnage during 2013, but there was a marginal interaction owing to a slight reduction in yield for the treated plots and increase in yield for the untreated plots when moving from low to high plant density (Table 8). In 2012, the effect of plant density on sugar yield (kg/ha) was significant, while sugar yield was unaffected by plant density and seed treatment during 2013 (Tables 7 and 8). As with tonnage, sugar yield was significantly higher in the lower plant density plots during 2012 (6,105 ± 535 kg/ha low population vs. 4,704 ± 535 kg/ha high population).

Acceptance of Sugarbeet Root Aphids as Prey by Selected Ground Beetle Species

All 10 most-abundant ground beetle species tested readily accepted sugarbeet root aphids as prey. There were no significant differences observed between the various species in their capacity to consume this aphid ($F_{9,45} = 0.48$, $P = 0.48$; range 4.2–6.0 aphids consumed).

Discussion

Contrary to the experimental hypothesis, insecticide seed treatments did not impact centipede, spider, or rove beetle activity. Furthermore, the diversity, species richness, and evenness of ground beetles were not affected by the seed-applied insecticides. However, it did affect one ground beetle species, *B. quadrimaculatum oppositum*, with a 25% population reduction in the treated plots in 2013. This reduction was not seen in 2012, perhaps owing to the lower activity of this species. A reduction in prey numbers early in the season when insecticide seed treatments are reportedly most effective, and the indirect or direct toxicity would explain a reduction in predatory numbers later in the season (Albajes et al. 2003). However, no such effect was observed in this study for centipedes, spiders, or rove beetles, despite lower sugarbeet root aphid ratings in the treated plots. The results obtained are similar to those of Albajes et al. (2003), who did not observe differences in the abundance of spiders or ground beetles caught via pitfall trapping between imidacloprid-treated and untreated plots under corn production; however, lower numbers of Staphylinidae were observed in their treated plots. These results are also in accordance with those of Krauter et al. (2001), who saw no impact of Gaucho (imidacloprid) seed treatments on the late-season abundance of natural enemies sampled in sorghum. Seagraves and Lundgren (2011) also did not observe an effect of soybean seeds treated with thiamethoxam on spider abundance. However, other predatory taxa (Chrysopidae and Nabidae), as well as the overall predatory abundance, were reduced by the seed treatments in their study.

With this study, a complex of generalist natural enemies was sampled. While all individuals from the selected taxa were collected and enumerated, it is noteworthy that not all of the species in each taxon are strictly predatory. For example, rove beetles, certain ground beetle species, and even some spider species consume non animal food (e.g., seeds, pollen, and fungi). The fact that the sampled arthropods remained largely unaffected by the insecticide seed treatments suggests that either the omnivorous and phytophagous species did not supplement their diet with sugarbeet vegetable matter to any significant degree, or, if this was the case, the impact

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Table 5. Comparisons of mean (± SEM) ground beetle (Coleoptera: Carabidae) species richness, Simpson's diversity index (reciprocal), and Simpson's evenness between seed-applied insecticide sugarbeet and untreated sugarbeet during 2012 and 2013

	Species richness (*S*)		Simpson's diversity (1/*D*)		Simpson's evenness (*E*)	
	2012	2013	2012	2013	2012	2013
Untreated	10.46 ± 0.38	7.17 ± 0.32	5.62 ± 0.23	2.55 ± 0.17	0.54 ± 0.02	0.36 ± 0.02
Treated	10.13 ± 0.38	7.04 ± 0.32	4.99 ± 0.23	2.74 ± 0.17	0.50 ± 0.02	0.40 ± 0.02
$F_{1, 5}$	0.38	0.09	3.73	0.99	2.54	2.35
P	0.56	0.78	0.11	0.37	0.17	0.19

Table 6. Mean (± SEM) activity density (individuals per trap per 7-d trapping period) of the most abundant ground beetle (Coleoptera: Carabidae) species collected by means of pitfall trapping in seed-applied insecticide sugarbeet and untreated sugarbeet in western Nebraska

	Treatment		$F_{1, 5}$	*P*
	Untreated	Treated		
2012				
Amara carinata (LeConte)	4.58 ± 0.58	4.52 ± 0.57	0.01	0.94
Amara farcta LeConte	3.77 ± 0.89	5.96 ± 1.35	3.42	0.12
Bembidion quadrimaculatum oppositum Say	7.13 ± 0.88	7.37 ± 0.90	0.04	0.86
Elaphropus anceps (LeConte)	2.50 ± 0.85	1.50 ± 0.53	3.34	0.13
Harpalus amputatus amputatus Say	0.95 ± 0.31	1.25 ± 0.39	0.37	0.57
Harpalus erraticus Say	11.36 ± 2.51	13.52 ± 2.97	0.37	0.57
Harpalus pensylvanicus (DeGeer)	2.63 ± 0.44	2.26 ± 0.40	0.38	0.56
Stenolophus comma (F.)	1.95 ± 0.92	3.25 ± 1.50	0.61	0.47
Other	5.08 ± 0.60	4.42 ± 0.54	1.11	0.34
2013				
Bembidion quadrimaculatum oppositum Say	23.51 ± 1.75	17.62 ± 1.38	13.92	0.01
Bembidion rapidum (LeConte)	1.26 ± 0.44	1.36 ± 0.47	0.03	0.86
Bembidion tetracolum tetracolum Say	6.36 ± 1.17	4.69 ± 0.89	2.69	0.16
Elaphropus anceps (LeConte)	3.48 ± 0.63	2.51 ± 0.48	1.91	0.23
Harpalus pensylvanicus (DeGeer)	1.04 ± 0.21	0.50 ± 0.15	4.37	0.09
Other	3.69 ± 0.46	4.10 ± 0.49	0.53	0.50

of the systemic insecticide had diminished by the time the beneficial arthropods were first sampled (Westwood et al. 1998). This study was designed to measure beneficial arthropod activity density during mid-season when migrating sugarbeet root aphids colonize sugarbeets and initiate colonies. Therefore, the early-season effects of seed treatments on beneficial arthropods were not assessed. This could have been significant because direct toxicity of the insecticide would be highest early in the season. The plots used in this study were also relatively small in their dimensions, and it is possible that recolonization by beneficial arthropods could happen rapidly following initial declines in their numbers. Finally, seed dressings are reportedly less toxic to natural enemies, in general, as opposed to insecticide foliar sprays (Croft 1990).

Ground beetles comprised a large component of the total number of epigeal beneficial arthropods collected during this study, especially during the first year. There is considerable interest in this group because of their contributions to pest and weed management in agroecosystems (Luff 2002). In their review on the impact of agriculture on ground beetle assemblages in temperate agroecosystems, Holland and Luff (2000) concluded that ground beetle assemblages within cropping systems are usually composed of ca. 30 species, of which usually <10 species dominate. This was supported by our results as 30 and 25 species were collected during 2012 and 2013, respectively. Furthermore, we found <10 dominant species during both years. It was this dominance by only a few species each year that led to low Simpson's diversity and evenness values.

Several of the most abundant ground beetle species collected in this study have been previously reported as abundant in agroecosystems in North America and elsewhere, highlighting their importance to agroecosystems. Examples include *B. quadrimaculatum* in alfalfa, carrots, corn, potatoes, soybeans, and wheat (Esau and Peters 1975, Best and Beegle 1977, Hsin et al. 1979, Boivin and Hance 1994, Ellsbury et al. 1998, Kinnunen and Tiainen 1999, Melnychuk et al. 2003, Floate et al. 2007, Bourassa et al. 2008, Bourassa et al. 2010); *Bembidion rapidum* (LeConte) in corn, soybeans, and wheat (Best and Beegle 1977, Hsin et al. 1979, Clark et al. 2006); *Harpalus pensylvanicus* (DeGeer) in alfalfa, corn, millet, pasture grass, sorghum, soybeans, sunflowers, and wheat (Rivard 1966, Kirk 1971, Best and Beegle 1977, Hsin et al. 1979, Weiss et al. 1990, Tonhasca 1993, Pavuk et al. 1997, Ellsbury et al. 1998, Clark et al. 2006, Miller and Peairs 2008); *Stenolophus comma* (F.) in alfalfa, beans, corn, potatoes, sainfoin, and wheat (Hsin et al. 1979, Lester and Morrill 1989, Bourassa et al. 2008); *Elaphropus anceps* (LeConte) in corn, soybeans, and wheat (Clark et al. 2006), *H. erraticus* in corn (Kirk 1971); *Amara carinata* (LeConte) in beans, corn, and potatoes (Kirk 1971, Floate et al. 2007, Bourassa et al. 2008); *Amara farcta* LeConte in alfalfa, beans, corn, potatoes, sainfoin, and wheat (Lester and Morrill 1989, Bourassa et al. 2008, Bourassa et al. 2010); *Bembidion tetracolum* Say in cabbage (Armstrong and McKinlay 1997, Prasad and Snyder 2004); and *Harpalus amputatus* Say in alfalfa, corn, millet, sainfoin, sorghum, sunflower, and wheat (Lester and Morrill 1989, Miller and Peairs 2008).

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Table 7. Effect of seed-applied insecticide (Poncho Beta) and plant density of sugarbeet on mean (± SEM) sugarbeet root aphid (SBRA) rating, sugar loss to molasses, sugar content, tonnage, and sugar yield during 2012 as revealed by a two-way ANOVA (means separated by a post hoc Tukey mean comparison test at $\alpha = 0.05$)

Yield parameter	Seed treatment	Plant density		Seed treatment (ST)		Plant density (PD)		ST × PD	
		Low	High	$F_{1, 5}$	P	$F_{1, 10}$	P	$F_{1, 10}$	P
SBRA rating	Untreated	2.63 ± 0.22	3.08 ± 0.22	9.35	0.03	0.50	0.50	3.54	0.09
	Treated	2.42 ± 0.22	2.21 ± 0.22						
SLM (%)	Untreated	1.73 ± 0.11	1.65 ± 0.11	1.43	0.29	0.56	0.47	0.18	0.68
	Treated	1.58 ± 0.11	1.56 ± 0.11						
Sugar content (%)	Untreated	12.68 ± 0.55	13.21 ± 0.55	2.08	0.21	0.18	0.68	0.78	0.40
	Treated	13.63 ± 0.55	13.44 ± 0.55						
Tons/ha	Untreated	49.28 ± 5.89	38.87 ± 5.89	0.78	0.42	8.55	0.02	0.01	0.92
	Treated	44.86 ± 5.89	33.72 ± 5.89						
Sugar yield (kg/ha)	Untreated	6176.24 ± 666.33	5007.91 ± 666.33	0.34	0.58	8.99	0.01	0.25	0.63
	Treated	6033.12 ± 666.33	4400.70 ± 666.33						

Treatments were: 1) untreated seed planted at a low plant density (86,487 plants per ha), 2) untreated seed planted at a high plant density (61,776 plants per ha), 3) treated seed planted at a low plant density, and 4) treated seed planted at a high plant density

Table 8. Effect of seed-applied insecticide (Poncho Beta) and plant density of sugarbeet on mean (± SEM) sugarbeet root aphid (SBRA) rating, sugar loss to molasses, sugar content, tonnage, and sugar yield during 2013 as revealed by a two-way ANOVA (means separated by a post hoc Tukey mean comparison test at $\alpha = 0.05$)

Yield parameter	Seed treatment	Plant density		Seed treatment (ST)		Plant density (PD)		ST × PD	
		Low	High	$F_{1, 5}$	P	$F_{1, 10}$	P	$F_{1, 10}$	P
SBRA rating	Untreated	2.56 ± 0.26	3.29 ± 0.26	9.06	0.03	4.44	0.06	0.82	0.39
	Treated	1.96 ± 0.26	2.25 ± 0.26						
SLM (%)	Untreated	1.43 ± 0.08	1.61 ± 0.08	1.90	0.23	0.98	0.35	1.69	0.22
	Treated	1.42 ± 0.08	1.40 ± 0.08						
Sugar content (%)	Untreated	11.62 ± 0.34	10.98 ± 0.34	5.87	0.06	0.40	0.54	1.55	0.24
	Treated	12.02 ± 0.34	12.24 ± 0.34						
Tons/ha	Untreated	45.56 ± 2.75	52.13 ± 2.75	0.25	0.64	0.56	0.47	4.14	0.07
	Treated	51.83 ± 2.75	48.81 ± 2.75						
Sugar yield (kg/ha)	Untreated	5306.79 ± 365.50	5728.04 ± 365.50	1.70	0.25	0.08	0.78	1.67	0.23
	Treated	6229.37 ± 365.50	5958.54 ± 365.50						

Treatments were: 1) untreated seed planted at a low plant density (86,487 plants per ha), 2) untreated seed planted at a high plant density (61,776 plants per ha), 3) treated seed planted at a low plant density, and 4) treated seed planted at a high plant density.

With the exception of *B. quadrimaculatum oppositum* in 2013, the activity of the remaining ground beetle species was not influenced by seed treatments. Furthermore, the fact that the majority of the ground beetle species collected in this study (as well as the remaining beneficial taxa) did not exhibit higher numbers in the untreated plots where sugarbeet root aphid abundance was higher is not surprising, considering the fact that generalist natural enemies rarely respond numerically to any single prey species (Symondson et al. 2002).

The high rate of capture of *B. quadrimaculatum oppositum* during 2013 accounted for both the overall difference in ground beetle activity between the treated and untreated plots, as well as for the lower biodiversity indices (both treatments) relative to 2012. This demonstrates that beneficial arthropod activity density and species assemblage can vary greatly between localities and seasons (Albajes et al. 2003, Bourassa et al. 2008). It also highlights the importance of evaluating key taxa on the species (or generic) level, rather than on an ordinal or family level. *Bembidion quadrimaculatum oppositum* is a well-documented predator of arthropods (Best and Beegle 1977, Grafius and Warner 1989, Baines et al. 1990); therefore, it is reasonable to hypothesize that it responded to the elevated sugarbeet root aphid numbers in the untreated plots. However, the results from the correlation analyses between this species and sugarbeet root aphid ratings during both 2012 and 2013 did not support this hypothesis, suggesting that it might have responded to a different suite of arthropod prey that was affected by the seed treatments in the treated plots. Alternatively, the limited range in sugarbeet root aphid ratings (i.e., ratings >2 in treated plots) observed throughout this study, might also have led to the observed weak correlations between root aphids and *B. quadrimaculatum oppositum*.

With the exception of centipedes in 2012, we found no effect of plant density on the activity density of epigeal beneficial arthropods. The observations from this study agree with those made by Boiteau (1984) who observed no difference in the abundance of ground beetles, spiders, and rove beetles between 15- and 36-cm within-row seed spacings in potatoes. Mayse (1978) did observe higher natural enemy abundance in high-density soybeans compared to low-density soybeans, but they manipulated between-row spacing, which led to different microclimatic conditions between these two treatments due to differences in the degree of soil coverage by foliage (i.e., more open spaces with increased row spacing, which led to higher soil temperatures). In addition, they sampled a different natural enemy complex, mainly confined to the above-soil parts of the crop. All plots in this study were subjected to the same agricultural practices

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and both plant densities tested in this study are common for the area of research (Yonts et al. 2013). Nonetheless, due to the leafy structure of the crop and the fact that between-row spacing was not altered, sugarbeet plants in the low population plots were still likely able to compensate and produce enough leaf biomass to cover the soil surface as it would in the high plant density plots, thus leading to minimal microclimatic differences.

Similar to the findings of Strausbaugh et al. (2010), insecticide seed treatments reduced sugarbeet root aphid populations during both seasons in this study. However, infestation levels remained moderately high (>2.0 on the root rating scale) in the treated plots. These levels of sugarbeet root aphid infestation would still contribute to yield loss (Hutchison and Campbell 1994); therefore, our results support the statement made by Dewar and Cooke (2006) that seed treatments will be less effective against late-season pests such as the sugarbeet root aphids. Low sugar content (range: 11.3–13.6%) was observed throughout this study in all treatment combinations. The most likely cause for this was the high level of sugarbeet root aphid infestations observed in both the insecticide-treated and untreated plots. Indeed, a correlation between sugarbeet root aphid ratings and this parameter during 2013 indicated a moderately strong negative relationship, suggesting that these aphids, at least in part, contributed to the decreased sugar content. Lower sugar content is expected as a consequence of higher sugarbeet root aphid pressure (Hutchison and Campbell 1994).

For the remaining yield parameters, sugarbeet root aphids appeared to have little impact as illustrated by the lack of significant differences between the treated and untreated plots, despite higher aphid pressure in the untreated plots. Differences in root yield and sugar yield between the low and high plant densities in 2012 were opposite to what was expected (i.e., higher yields under higher beet populations). During this season, the general area of the field in which the research plots for this study were established showed signs of water stress in the sugarbeet crop. It is possible that increased drainage (or some other unknown factor) resulted in higher competition for moisture between individual plants, which would have put plants in the lower plant population at an advantage.

No-choice prey experiments have been criticized as being unrealistic, because potential predators are starved ahead of time; therefore, they are more likely to accept prey they would normally not prefer under natural conditions. However, a study conducted by Lang and Gsöl (2001) have shown that different levels of ground beetle saturation affected the number of prey consumed, but not prey preference. The high rate of predation observed for all ground beetle species tested (with no differences in their consumption), indicate that these generalist predators are capable of feeding on sugarbeet root aphids. This warrants further investigation into the contribution of these predators to sugarbeet root aphid management, especially under natural conditions using subterranean root aphid colonies.

Seed-applied neonicotinoids appear to reduce root aphids populations with a minimal impact on edaphic beneficial arthropods in sugarbeets. The only exception to this was observed with the ground beetle species, *B. quadrimaculatum oppositum*, which was significantly suppressed by seed-applied insecticides during one year of the study. However, the level of sugarbeet root aphid suppression cannot be considered adequate for aphid management in practice. With minor exceptions, plant density also had little impact on these organisms, while its effect on the various yield parameters was inconsistent from year to year.

Acknowledgments

Thanks to Henda Pretorius, Rick Patrick, Susan Harvey, and summer student workers for their help with field work, and for helping with sorting and identifying the pitfall captures. We are also grateful toward Gene Kizzire and his crew for their help in preparing and maintaining the research plots. Lastly, we thank Western Sugar for the funding provided to undertake this research, and the University of Nebraska–Lincoln's Panhandle Research and Extension Center for making the study site available.

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