Larval movement of Anticarsia gemmatalis (Lepidoptera: Erebidae) on vegetative and reproductive stage non-Bt soybean

Sabrina Ongaratto (✉ sabrina.ongaratto@hotmail.com)
    São Paulo State University

Edson L. L. Baldin
    São Paulo State University

Thomas E. Hunt
    University of Nebraska-Lincoln

Nicole Luhr
    University of Nebraska-Lincoln

Kory R. Heier
    University of Nebraska-Lincoln

Emily A. Robinson
    University of Nebraska-Lincoln

Research Article

Keywords:

Abstract

Integrated pest management (IPM) and insect resistance management (IRM) in a cropping system demands a comprehensive understanding of insect behavior, including larval movement. We evaluated the larval movement of velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), in a non-*Bt* soybean cultivar. Third to fifth instars were infested in different combinations in vegetative and reproductive soybean stages, and on-plant movement was characterized across select time points. The dispersion among plants in different positions and orientations was assessed by releasing 24-h-old larvae on the central plant of field plots. Results indicated that *A. gemmatalis* larvae are consistent in their movement during the day, regardless of plant section or leaf position. Over the time period of assessment, larvae gradually moved down and left the plant. Although there was not significant difference in the distance traveled by *A. gemmatalis* between orientations and positions, long distances were covered indicating that this species has nondirectional movement. This study provides relevant information regarding the movement of the velvetbean caterpillar, which can be used to improve insecticide spraying efficacy, improve probabilistic model predictive ability, and help design insect resistance management strategies.

Introduction

Soybean [*Glycine max* (L.) Merrill] is an established crop with high production value and is the most widely cultivated and used legume worldwide[1]. With the widespread adoption of soybean, several insects species have adapted to this crop and became economic pests[2,3]. Among them, the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae), is an important soybean pest occurring across the western hemisphere[4–8]. This species has been historically reported as a 'major' or 'serious' pest in the United States (U.S.), but is most common and problematic in the southern United States[5,9]. The velvetbean caterpillar is one of the fastest defoliating caterpillar species, able to strip soybean fields in five to seven days. Larvae start feeding on the new leaves, moving to the older ones, and also feeding on tender stems and pods. Larvae typically begin feeding in the upper canopy and move lower on the plant as the defoliation continues[10].

Despite the importance of this species, a greater understanding on its larval behavior is still needed, including characterization of individual on-plant and plant-to-plant movement throughout larval development[11–17]. These type of information will inform the development of integrated pest management (IPM) and insecticide resistance management (IRM) strategies in soybean and other major crops[18–20].

Based on previous studies, movement of early instar lepidopteran larvae on host plants largely determines where feeding sites become established[17,21–23]. Knowledge of on-plant movement also aids understanding of where the insects are throughout the day, when and where to sample, and when and how to treat when necessary, allowing the most efficacious spraying[11,16,23–25]. Control of lepidopteran

pests by either biotic or abiotic measures is facilitated when the precise location of the early instars known[24,26].

Insect movement among plants, classified as dispersion, is the movement away from a point that results in the dissemination of part of the original population[27]. This behavior is related to an adaptive mechanism by which insects seek energy resources, refuge, and mating places[27,28]. Another important reason to better understand larval behavior on plants arises with the advent of soybean expressing a *Bacillus thuringiensis* (*Bt*) insecticidal protein. Brazil is the leading developing country in the world planting biotech crops including soybean, while in the U.S this technology in soybean is currently being studied[29]. Large scale adoption of *Bt* corn and cotton in the United States has resulted in an intense selection pressure for the development of resistance, challenging the long-term sustainability of this **genetically modified organism** (GMO) technology[18].

The *Bt* soybean has been effective in controlling *A. gemmatalis*[30–32]; however, this technology is not available in all soybean producing regions. Therefore, this pest has a capacity to cause serious loss in conventional soybean systems, and under conditions of high infestation, foliar consumption can be total[33]. In addition, depending on the refuge areas settings, some early instars are able to feed on *Bt* plants and move to non-*Bt* plants, or move from non-*Bt* to *Bt* plants as older instars and survive[12,15,34,35]. Larvae with some tolerance to *Bt* proteins, and stimulated by *Bt* plants, may move longer distances to non-*Bt* plants[13]. Thus, larval dispersion capacity in soybean as it relates to possible resistance evolution to *Bt* toxins has increased and the adequate management depends of the pest species and its behavior, as well as the configuration and size of the refuge areas.

Regarding the growing soybean market in areas such as Brazil and U.S., and the constant challenges involving the management of leaf-feeding insects, the overall objective of this is research was to characterize the on-plant and plant-to-plant movement of *A. gemmatalis* larvae in non-*Bt* soybean. The results obtained in this research will serve as a baseline for future *A. gemmatalis* movement and management studies, optimizing insecticide spraying and deployment of natural enemies. Additionally, understanding larval movement will allow development of IRM strategy and optimal refuge design associated with *Bt* soybean by improving probabilistic models to improve their predictive ability.

Results

On-plant larval movement

Considering plant location, no significant differences were observed for the estimated proportion for 3rd instar *A. gemmatalis* larvae in V7 soybean on the top ($F = 0.97$; $df = 9$; $P = 0.4815$), middle ($F = 1.04$; $df = 9$; $P = 0.4320$) and lower sections of the plant ($F = 0.47$; $df = 9$; $P = 0.8860$, Fig. 1A). However, the time effect was significant ($F = 3.01$; $df = 9$; $P = 0.0111$) on the larvae number that left the plant, since the proportion rate of larvae started with 0.35 and had an increase to 0.60. For the 4th instar larvae/R3, we observed that larvae did not congregate in the lower section of the plants ($F = 1.41$; $df = 4$; $P = 0.2794$, Fig.

1B). On the other hand, as day 1 progressed, the larvae that moved off the plant significantly increased from 0.17 at 8 h to 0.87 at 44 h (F = 15.70; df = 9; P< 0.0001). The larvae in the top (F = 5.00; df = 9; P = 0.0004) and middle (F = 8.08; df = 9; P< 0.0001) sections was off after 20 h point (Fig. 1B). Significant differences were observed for 5th instar larvae/V7,R1 on and before 16 h. The estimated number of larvae found in the top part of the soybean plants (proportion of 0.20 − 0.07 larvae) (F = 4.17; df = 4; P = 0.0182) differed from the middle section (0.02 − 0.00 larvae) (F = 0.47; df = 4; P = 0.7583) and larvae off the plant (0.73 − 0.90 larvae) (F = 2.22; df = 4; P = 0.1156). There was not enough data to obtain results for the lower portion (Fig. 1C). For the 5th instar/R3,R4 assay, we found significant differences on and before point time 18 h in the top (F = 7.50; df = 5; P = 0.0006), middle (F = 8.39; df = 5; P = 0.0003) and off plant (F = 19.42; df = 5; P< 0.0001). The proportion of larvae in the lower part of the plant was not significant (F = 0.49; df = 5; P = 0.7779). Throughout the evaluation, the estimate proportion of larvae reduced in the different plant sections, while it had an increase for larvae off plant (Fig.1D).

Regarding leaf location, the proportion of 3rd instar larvae in V7 soybean stage significantly changes over time on the adaxial (P< 0.0001), abaxial part (P<0.0001), and for larvae that left the plant (P = 0.0111, Table 1). The distribution was inversely proportional, since in the first time point, with a rate of 0.39, 0.25 and 0.35, changing to 0.19, 0.20 and 0.60, respectively, in the last evaluation time. We found a difference in the proportion of 4th instar larvae in R3 soybean in adaxial, abaxial, and off plant (P< 0.0001, Table 2). While in the beginning, a rate of 0.53 (adaxial) and 0.28 (abaxial) were on the plant, the proportion rate of larvae that left the plants gradually increased through time, reaching 0.87 at the point 44 (hour). For the 5th instar/V7,R1 assay, there were differences only in the time points on and before 16 hours. All insects after time point 16 were off the plant. For the adaxial part of the leaf, there was little to no data after time point 8, which is why we did not have enough data to run the model. There was no difference in the ratio of larvae counts for different time points for the abaxial part of the leaf (P = 0.8288) and for larvae counts for different time points off the plant (P = 0.1156, Table 3). Significant differences were observed in the adaxial (P = 0.0057) and abaxial part of the leaf (P = 0.0027) with rates of 0.30 and 0.25, respectively for 5th instar larvae in R3,R4 soybean. From the first evaluation, an estimated proportion of 0.44 insects were off plant, reaching 100.00% of larvae off at the time point 20 (P< 0.0001, Table 4).

Plant-to-plant larval movement

There were no statistical differences (P > 0.05) for mean and maximum distanced moved by *A. gemmatalis* larvae among the different quadrants (NE, SE, NW, and SW), N and S orientations, within infested row (N and S axes), and across rows (E and W axes, Table 5). Numerically, the mean (104.72 cm) and maximum distance (142.52 cm) covered by *A. gemmatalis* were greater at NE direction and taking the interval distance, one larvae reaching out 202.62 cm. Following the orientation, both mean and maximum distances were observed at N (77.25 and 102.30 cm, respectively). In the infested row, the values related to the mean distance were similar [51.54 cm (N) to 56.16 cm (S)] and maximum distance were 62.25 cm (N) and 87.50 cm (S). Across rows, the distances moved were around 40.00 cm.

Regarding the quadrants, the highest larvae number was observed in the SE (13 larvae), while across row, 2 larvae were found in the W. The proportion of larvae found within infested row was higher in the N position (9 larvae). However, in total, more larvae (25) were found in the S orientation (Table 5). The larval frequency varied from 37.14% (SE quadrant) to 17.14% (NW quadrants). With respect to N and S orientations, the larvae were distributed similarly, with frequencies of 48.98 and 51.02%, respectively. Within the infested row (N and S axes), more than 60.00% of the larvae moved North. Considering the movement of larvae across rows (W and E axes), twice the larvae were recovered on the Western axis (Table 5). A visual distribution of larvae in the plots, 12 days after release, indicating nondirectional movement is provided in Fig. 2.

Discussion

There is little information on *A. gemmatalis* larval movement on soybean plants. In the current study we assessed for the first time the movement of this species on soybean under field conditions and observed that in all the on-plant movement scenarios, larvae had the ability to move constantly over time. For 3rd instar larvae/V7 plant stage, we observed more constancy in the distribution on the plant over the evaluation. However, for 5th instar larvae/V7,R1 plant stage, for example, even in the first evaluations we did not detect larval presence in some plant sections and leaf positions. Larval size and plant growth stage affects the behavior and acceptance of a suitable host, and in turn movement patterns, stimulating larvae gradually to move off the plant. This highly active behavior was already observed for other lepidopteran species. Measuring movement of *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), 50% of the larvae had moved off the plant within 6 hours[36].

Larval behavior at a specific instar can contribute to understanding the spatial distribution of a species on a specific host plant. For example, in maize it was reported that small *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) larvae presented an aggregate behavior, while medium and large *S. frugiperda* larvae exhibited a random pattern[37]. The causes of this behavior are not always clear and many of these details can influence IPM and IRM strategies. Even though we did not assess feeding behavior, it is known that timing of movement relative to feeding and the age of the larvae are important[38]. In general, it is relevant to know whether movement consists of discrete steps with each interval of feeding followed by movement or whether movement is mostly one long, continuous step from one to another section in the same plant or the plant of origin to the final host with no feeding between points. We suggest as a future research to characterize the neonate's dispersion on the plant, once the larvae will start to move and find a suitable feeding site[23].

Successful dispersal will depend on the host plant and its architecture, egg load, consequent first instar establishment, and costs of migration; these costs will change with larval age and may have impact on later instars and adult development[39]. Also, larvae may be affected by environmental conditions. A noticeable characteristic during the evaluation was that we observed higher larval movement to the middle and lower plant section and to the abaxial leaf position during the warm hours of the day. Similar

behavior was also reported for *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) in dry beans[40]. At 14 days after hatch (DAH), most 4th instars larvae were found on the soil surface. No larvae were found on the plant or soil surface after 14 DAH. Observations by time of day clearly showed that the majority of 5th instars spent the day on or in the ground, moving up the plant in the dusk and returning to the ground by sunrise. Even in larger crops, such as corn, when *S. frugiperda* infestation was done above the ear zone, larvae moved downward to the ear zone over time[16].

This on-plant information also helps to understand the dispersion dynamic throughout the day and can improve insecticide spraying efficiency. Based in our observed larval movement, results reinforce the theory to apply insecticides and release entomopathogenic enemies early in the morning or at the dusk, when the insects will be exposed to the products. Cannibalism and predation are also causes that could impact population dynamics, and this behavior was already reported for this species and when interacting with other species[41]. Another important mortality factor in the agroecosystem is the presence of natural enemies, which was visually detected in our study and might also explain the number of missing larvae. The larvae position and high movement on and between plants can also in turn expose the insect to predators and parasitoids[26].

Regarding the movement among plants, no significant, but notable differences in the dispersion were observed. Our results showed that long distances were covered by *A. gemmatalis* regardless of the position and orientation chosen, indicating the absence of a homogenous movement. This type of movement is a ubiquitous life-history trait of organisms, with profound consequences on the spatial and temporal dynamics of populations [42]. Thus, movement of this species appears to be nondirectional and independent[28]; however, especially under field conditions, it could be influenced by biotic or abiotic factors. In a similar study with *Spodoptera eridania* Stoll (Lepidoptera: Noctuidae) larvae, it was also observed that the species move in all directions independent of the season or cultivar[14]. Pannuti et al. 2016 assessing *S. frugiperda* and *S. albicosta* movement observed a dispersal governed by nondirectional sensory information of both species; however, *S. frugiperda* remained nearer the release point, presented a more aggregated and symmetrical distribution.

The mean distances found here were approximately 40.00 cm in the infested row, and up to 50.00 cm for the other orientations. The mean of maximum distance varied between 38.00 to 142.52 cm, reaching 202.62 cm to the NE. Similarly, in a greenhouse and in open field trials, larvae of *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) were observed to move from initially infested plants to at least four plants away, with the majority of larvae staying within three plants away from the release plant[34]. *Spodoptera frugiperda* larvae exhibited greater movement to the North within the infested row. However, no significant differences were observed when considering general N and S orientations, and this northern movement may have been influenced by wind or another environmental factor[12].

Despite that in our studies we characterized the movement of *A. gemmatalis* in non-*Bt* soybean, we can use this behavior information to apply to a *Bt* field. Previous research supported the inference that

resistance evolves faster at high larval movement rates because of the increased likelihood of larvae moving from non-*Bt* plants to *Bt* plants and the increased survival rate of heterozygous larvae relative to homozygous susceptible larvae[43]. Thus, in practical terms, the distance traveled by the insects may affect the evolution of resistance to *Bt* crops, in association with configuration and size of the refuge areas[18,44]. Initial high-dose/refuge strategies were initially designed to delay resistance to *Bt* crops. Among them, the use of seed mixtures (refuge in the bag 'RIB') has been suggested to manage resistance to *Bt* toxins[43]. Although RIB can be an option in IRM, insects that exhibit high larval movement across plants generally favor the evolution of resistance in a seed mixture refuge, since such movement exposes insects to sublethal doses of the toxins, especially for single-gene *Bt* events[35,44].

Seed mixtures can increase survival of heterozygous larvae relative to homozygous susceptible larvae when individual larvae feed on both *Bt* and non-*Bt* plants[45]. Consequently, when there is a high larval movement rate, it is expected that plant mixtures increase the effective genetic dominance of resistance to *Bt* crops, resulting in increased selection for resistance[46]. There are studies with other pest species and crops that demonstrate RIB is not always appropriate. In a field study conducted with seed mixes of non-*Bt* and *Bt* pyramid maize, *D. saccharalis* larvae were able to move from infested plants, as well as to adjacent rows, so larvae could feed on non-*Bt* maize until they reached a size that allowed their survival on *Bt* maize[34]. This strategy may not be ideal for resistance management of *A. gemmatalis* to the Cry1Ac toxin, and a more appropriate strategy, such the use of strip or block refuge, may be required. However, possible movement across the border between *Bt* and non-*Bt* crops must first be examined, as it may also have an important role in resistance evolution[44].

In our open field study, we recovered a lower number of larvae with high variability among plots; however, this is representative on most real conditions. We found a higher frequency of larvae recovered in the different quadrants compared to the infested or across rows, similar to observed for *S. albicosta* larvae, where about 32% of were recovered within the infested row and 75.3% within a radius of 1.7 m of the infested plant[12]. Mortality in the early larval stages is commonly high in Lepidoptera [24]. The authors reported that almost 50.00% mortality occurs by first instar parasitism. Fanela et al. (2020) observed a higher mean number of recovered larvae at each distance interval for *S. eridania*. However, the study was conducted in cages, with a more controlled environmental. For instance, the crop environment will strongly influence how predation and desiccation, or the impact of rainfall, will affect survival during larval movement from plant-to-plant or to and from hiding/feeding places[38]. In a similar study, larval recovery from central non-*Bt* plants in seed blends was 27.50% less than that from the pure non-*Bt* plantings[47]. However, even with larvae moving less in non-*Bt* plants, a detailed study of *H. armigera* feeding behavior in *Bt* and non-*Bt* treated diet choice bioassays demonstrated larvae could not detect *Bt* and only avoided it post ingestion[48]. Recovery and aversion post *Bt* ingestion is less likely as protein toxicity increases, such as with toxins that approach a high dose. This approach is important since this behavior can impact resistance evolution.

Overall, our results serve as baseline for the support of *A. gemmatalis* probabilistic models and to improve their predictive ability. The present results corroborate other studies, where the findings suggest that each species must be considered independently, and one should not expect a one-size-fits-all IRM plan to be suitable for all pest species[49]. Additional *A. gemmatalis* biological and ecological studies should be conducted in association with fitness costs aiming to elucidate the relationship between larval movement and the evolution of insect resistance. In temperate areas, such as in much of the United States, selected populations of *A. gemmatalis* would not overwinter, but the selection pressure can be high in the southern United States and in tropical areas such as Brazil. As new transgenic events and/or pyramids become available worldwide, the importance of understanding *A. gemmatalis* and other target pest species' behaviors will increase.

Methods

The studies were conducted under field conditions at the University of Nebraska Haskell Agricultural Laboratory, Concord, NE, USA, during the 2019 crop season.

Plants

The research did not involve the collection of plant material in nature. All plants used in the study were grown from commercially available seeds. The non-*Bt* cultivar Channel 2516R2X was used in all studies. The study complies with relevant institutional, national, and international guidelines and legislation.

Insects

Anticarsia gemmatalis eggs and larvae were commercially acquired (Benzon Research Inc., Carlisle, PA, USA) and reared in plastic pots containing 15 mL of artificial diet (based on diet developed by USDA, Stoneville, MS and University of Georgia, Athens, GA, USA). The insects were kept in a rearing chamber [25 ± 2°C, RH 60 ± 10%, 14:10 (L:D)] until the instar required for each assay (on-plant larval movement). For the plant-to-plant assay, eggs were purchased close to hatching in order to standardize infestations of the plants in the field with neonates (24-h old).

On-plant larval movement

The design was as follows: There were 4 plots of 5 non-touching soybean plants. Six velvetbean caterpillar (*A. gemmatalis*) larvae were released the middle section of each plant. Thus, each plot had 30 larvae on the plants at the start of the experiment. The locations of the larvae were recorded in terms of both leaf and plant section position. The plant sections were divided into top, middle, lower, and off the plant, and the leaf locations into abaxial, adaxial, and off the plant. The field infestation occurred at dusk (about 8pm) to reduce larval stress. The larval positions were measured at 10 different time points (8, 10, 12, 14, 16, 18, 20, 32, 36, 44 hours). The 8 to 20 hours are on the first day, while 32, 36, and 44 hours are the observations from the second day (i.e. 32 hours corresponds to day 2 at 8 am). The experiment was

done for 4 different combinations (assays) of larval stage of development (instar), as well as soybean stage of development (vegetative and reproductive)[50], which consisted in the follow combinations: 3rd instar/V7; 4th instar/R3; 5th instar/V7,R1; 5th instar/R3,R4. The assays were all analyzed separately, and time was considered a continuous scale for the analysis.

Plant-to-plant larval movement

The experiment consisted of 7 plots arranged in a randomized block design, 3.0-m long rows, totaling 400 plants per plot. Initially, soybean leaves were removed from the field (same cultivar planted to this experiment) and installed inside the plastic cages (21.5 × 14.5 × 3.5 cm). A piece of cotton moistened in water was wrapped around the base of the petioles to maintain leaf turgidity. Individual eggs were cut from organza cloth where the adults oviposited, and then placed on leaves until they hatched. After hatching, 50 larvae (24-h-old) per leaflet were kept and isolated in a Gerbox (11 × 11 × 3.5 cm) until infestation. The excess larvae were removed with a delicate paintbrush. Artificial infestation was performed by releasing the neonates at 4 locations in the middle section of the central plant of each plot, totaling 200 larvae per plant. The infestation was performed by fixing infested leaflets from the rearing cages to the upper surface of a central plant leaflet with a small piece of adhesive tape, taking care so that the neonates did not stick to the tape. This technique reduced stress to the neonates, resulting in efficient transfer of the neonates to the infested plant[14]. The field infestation occurred at dusk to reduce stress to the larvae and maximize the effectiveness of the infestation.

Destructive sampling of all plants per plot was carried out 12 d after infestation. The number/frequency of larvae were established by the position in which the insects were found in relation to the infested central plant of each plot. Larval position was demarcated in the field by small identification flags on wooden sticks placed at the plant base. Larval movement was categorized based on the presence of each insect on each plant in each plot, and the proportion of larvae present on infested plants and in rows. The rows ran north to south, and larval orientation was categorized as north (N) and south (S) in the same row with respect to the infested plant, and east (E) and west (W) when directly across rows from the infested plant. The position of the larvae was designated as zero when on the infested plant. For larvae not directly N, S, E, or W of the infested plant, the position from the release point was categorized by quadrant: northeast (NE), southeast (SE), northwest (NW), and southwest (SW). Larval mean and maximum distance from the infested plant was determined. The most distant larvae in each quadrant and orientation was considered the maximum distance[12].

Statistical analyses

On-plant analyses were conducted using Proc Glimmix in SAS 9.4[51]. For each leaf location and plant location, a series of Generalized Linear Mixed Models following a Binomial distribution with a logit link function were conducted to determine the proportion of larvae within each location (e.g., proportion of larvae in the top part of the plant vs not in the top part of the plant) accounting for repeated measures over time. The response variable in the binomial model was how many larvae were in each location, at a

particular time point, divided by 30 (total larvae in the plot) (i.e., proportion of larvae within each location – 10/30 larvae in the top plant indicates 33% of larvae are on the top part of the plant). Our fixed effect was treating time as a factor since we have measurements for each time period. We imposed a covariance structure of spatial power to account for correlation among responses in plot and time (i.e., repeated measures). Spatial power was selected because we do not have equal distances between observations. In the analysis, leaf and plant locations were run separately.

To compare quadrants, infested rows, and orientations, a linear mixed model was conducted, using the lmer function in the lme4 package from R version 3.6.3 (R Development Core Team 2020). Direction was considered fixed effect and plot was considered as a random effect. The emmeans package was used to calculate the least squared means estimates for each direction. Tukey adjustment was used to control type I error rates. Any analysis to compare data between rows was not possible to be conducted, since there is only 2 and 1 observation in each direction (W/E), respectively.

Declarations

Acknowledgements

Authors' contribution

SO, ELLB and TEH conceived and designed research. SO and NL conducted experiments. KRH and EAR analyzed data. SO, ELLB and TEH wrote the manuscript. All authors read and approved the manuscript.

Competing interests

The authors declare no conflict of interest.

Data availability

The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. USDA. Department of Agriculture–National Agricultural Statistics Service. USDA - National Agricultural Statistics Service - Quick Stats. https://www.nass.usda.gov/Quick_Stats/ (2021).
2. Kogan, M. Dynamics of insect adaptations to soybean: Impact of Integrated Pest Management. *Environ. Entomol.* **10**, 363–371 (1981).

3. Gagné, R. J., Yukawa, J. & Elsayed, A. K. A new pest species of Resseliella (Diptera: Cecidomyiidae) on soybean (Fabaceae) in North America, with a description of the genus. *Proc. Entomol. Soc. Washingt.* **121**, 168 (2019).

4. Puttler, B. & Long, S. H. The velvetbean caterpillar, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) in Missouri. *J. Kansas Entomol. Soc.* **53**, 437–440 (1980).

5. Buschman, L. L., Pitre, H. N., Hovermale, C. H. & Edwards, N. C. Occurrence of the velvetbean caterpillar in Mississippi: winter survival or immigration ?. *Environ. Entomol.* **10**, 45–52 (1981).

6. Bortolotto, O. C. *et al.* The use of soybean Integrated Pest Management in Brazil: a review.*Agron. Sci. Biotechnol.* **1**, 25–32 (2015).

7. Formentini, A. C., Sosa-Gómez, D. R., de Paula-Moraes, S. V., de Barros, N. M. & Specht, A. Lepidópteros (Insecta) associados à cultura da soja na Argentina, Brasil, Chile e Uruguai.*Cienc. Rural* **45**, 2113–2120 (2015).

8. Haase, S. *et al.* Development of a recombination system for the generation of occlusion positive genetically modified *Anticarsia gemmatalis* multiple nucleopolyhedrovirus. *Viruses* **7**, 1599–1612 (2015).

9. Huseth, A. S. *et al.* Current distribution and population persistence of five lepidopteran pests in U.S. soybean. *J. Integr. Pest Manag.* **12**, 11–12 (2021).

10. Wilkerson, G. G., Mishoe, J. W. & Stimac, J. L. Modeling velvetbean caterpillar (Lepidoptera: Noctuidae) populations in soybeans. *Environ. Entomol.* **15**, 809–816 (1986).

11. Paula-Moraes, S. V., Hunt, T. E., Wright, R. J., Hein, G. L. & Blankenship, E. E. On-plant movement and feeding of western bean cutworm (Lepidoptera: Noctuidae) early instars on corn. *Environ. Entomol.* **41**, 1494–1500 (2012).

12. Pannuti, L. E. R. *et al.* Plant-to-plant movement of *Striacosta albicosta* (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (*Zea mays*). *J. Econ. Entomol.* **109**, 1125–1131 (2016).

13. Malaquias, J. B., Godoy, W. A. C., Garcia, A. G., Ramalho, F. D. S. & Omoto, C. Larval Dispersal of *Spodoptera frugiperda* strains on bt cotton: a model for understanding resistance evolution and consequences for its management. *Sci. Rep.* **7**, 16109–16109 (2017).

14. Fanela, T. L. M., Baldin, E. L. L., Hunt, T. E. & Faria, R. D. Baseline plant-to-plant larval movement of *Spodoptera eridania* in Bt and non-Bt soybean and its possible impacts on IRM. *J. Econ. Entomol.* **20**, 1–12 (2020).

15. Ramalho, F. S. *et al.* Feeding and dispersal behavior of the cotton leafworm, *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), on Bt and non-Bt cotton: Implications for evolution and resistance management. *PLoS One* **9**, (2014).

16. Pannuti, L. E. R., Baldin, E. L. L., Hunt, T. E. & Paula-Moraes, S. V. On-plant larval movement and feeding behavior of fall armyworm (Lepidoptera: Noctuidae) on reproductive corn stages. *Environ. Entomol.* **45**, 192–200 (2015).

17. Sokame, B. M., Subramanian, S., Kilalo, D. C., Juma, G. & Calatayud, P. A. Larval dispersal of the invasive fall armyworm, *Spodoptera frugiperda*, the exotic stemborer *Chilo partellus*, and indigenous maize stemborers in Africa. *Entomol. Exp. Appl.* **168**, 322–331 (2020).

18. Tabashnik, B. E., Brévault, T. & Carrière, Y. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* **31**, 510–521 (2013).

19. Ross, S. E. & Ostlie, K. R. Dispersal and survival of early instars of european corn borer (Lepidoptera: Pyralidae) in field corn. *J. Econ. Entomol.* **83**, 831–836 (1990).

20. Onstad, D. W. modeling larval survival and movement to evaluate seed mixtures of transgenic corn for control of western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **99**, 1407–1414 (2009).

21. Pannuti, L. E. R. *et al.* External marking and behavior of early instar *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Soybean. *Florida Entomol.* **102**, 90 (2019).

22. Foster, S. P. & Howard, A. J. Adult female and neonate larval plant preferences of the generalist herbivore, *Epiphyas postvittana*. *Entomol. Exp. Appl.* **92**, 53–62 (1999).

23. Perkins, L. E. *et al.* Where to from here? The mechanisms enabling the movement of first instar caterpillars on whole plants using *Helicoverpa armigera*(Hübner). *Arthropod. Plant. Interact.* **2**, 197–207 (2008).

24. Zalucki, M. P., Clarke, A. R. & Malcolm, S. B. Ecology and behavior of first instar larval Lepidoptera. *Annu. Rev. Entomol.* **47**, 361–393 (2002).

25. Zulin, D., Ávila, C. J. & Schlick-Souza, E. C. Population fluctuation and vertical distribution of the soybean looper (*Chrysodeixis includes*) in soybean culture. *Am. J. Plant Sci.* **9**, 1544–1556 (2018).

26. Vinson, S. B. Host selection by insect parasitoids. *Annu. Rev. Entomol.* **21**, 109–133 (1976).

27. Price P. W. *Insect Ecology*(1997).

28. Bell, W. J. Searching behavior patterns in insects. *Annu. Rev. Entomol.* **35**, 447–467 (1990).

29. ISAAA. Global Status of Commercialized Biotech/GM Crops. https://www.isaaa.org/resources/publications/briefs/55/default.asp (2019).

30. Bel, Y., Zack, M., Narva, K. & Escriche, B. Specific binding of *Bacillus thuringiensis* Cry1Ea toxin, and Cry1Ac and Cry1Fa competition analyses in *Anticarsia gemmatalis* and *Chrysodeixis includens*. *Sci. Rep.* **9**, 1–7 (2019).

31. Bernardi, O. *et al.* Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean against *Anticarsia gemmatalis* and *Pseudoplusia includens* (Lepidoptera: Noctuidae) in Brazil. *Pest Manag. Sci.* **68**, 1083–1091 (2012).

32. Bacalhau, F. B. *et al.* Performance of Genetically Modified Soybean Expressing the Cry1A.105, Cry2Ab2, and Cry1Ac Proteins against Key Lepidopteran Pests in Brazil. *J. Econ. Entomol.* **113**, 2883–2889 (2020).

33. Bueno, R. C. O. de F., Bueno, A. de F., Moscardi, F., Parra, J. R. P. & Hoffmann-Campo, C. B. Lepidopteran larva consumption of soybean foliage: basis for developing multiple-species economic

thresholds for pest management decisions. *Pest Manag. Sci.* **67**, 170–174 (2011).

34. Wangila, D. S. *et al.* Occurrence and larval movement of *Diatraea saccharalis* (Lepidoptera: Crambidae) in seed mixes of non- *Bt* and *Bt* pyramid corn. *Pest Manag. Sci.* **69**, 1163–1172 (2013).

35. Head, G. *et al.* Movement and survival of corn rootworm in seed mixtures of SmartStax® insect-protected corn. *Crop Prot.* **58**, 14–24 (2014).

36. Razze, J. M., Mason, C. E. & Pizzolato, T. D. Feeding behavior of neonate *Ostrinia nubilalis*(Lepidoptera: Crambidae) on Cry1Ab Bt corn: Implications for resistance management.*J. Econ. Entomol.* **104**, 806–813 (2011).

37. Trindade, R. B., Fernandes, M. G., Oliveira, A. C. & Martins, P. H. A. Distribuição espacial de *Spodoptera frugiperda*(J. E. Smith) (Lepidoptera, noctuidae) em milho convencional e Bt.*EntomoBrasilis* **10**, 89–93 (2017).

38. Onstad, D. W. *et al.* Blended refuge and insect resistance management for insecticidal corn. *Environ. Entomol.* **47**, 210–219 (2018).

39. Thompson, J. N. & Pellmyr, O. Evolution of oviposition behavior and host preference in Lepidoptera (1990).

40. Difonzo, C. D., Chludzinski, M. M., Jewett, M. R. & Springborn, F. Impact of western bean cutworm (Lepidoptera: Noctuidae) infestation and insecticide treatments on damage and marketable yield of Michigan dry beans. *J. Econ. Entomol.* **108**, 583–591 (2015).

41. Ongaratto, S. *et al.* Effects of intraguild interactions on *Anticarsia gemmatalis* and *Chrysodeixis includens* larval fitness and behavior in soybean. *Pest Manag. Sci.* **77**, 2939–2947 (2021).

42. Mazzi, D. & Dorn, S. Movement of insect pests in agricultural landscapes. *Ann. Appl. Biol.* **160**, 97–113 (2012).

43. Carroll, M. W., Head, G., Caprio, M. & Stork, L. A. Theoretical and empirical assessment of a seed mix refuge in corn for southwestern corn borer. *Crop Prot.* **49**, 58–65 (2013).

44. Garcia, A. G., Ferreira, C. P., Cônsoli, F. L. & Godoy, W. A. C. Predicting evolution of insect resistance to transgenic crops in within-field refuge configurations, based on larval movement. *Ecol. Complex.* **28**, 94–103 (2016).

45. Mallet, J. & Porter, P. Preventing insect adaptation to insect-resistant crops: Are seed mixtures or refugia the best strategy? *Proc. R. Soc. B Biol. Sci.* **250**, 165–169 (1992).

46. Brévault, T., Tabashnik, B. E. & Carrière, Y. A seed mixture increases dominance of resistance to Bt cotton in *Helicoverpa zea*. *Sci. Reports 2015 51* **5**, 1–7 (2015).

47. Dimase, M. *et al.* Larval movement and survival of *Helicoverpa zea* (Boddie) in seed blends of non-Bt and Bt maize containing Agrisure Viptera® trait: Implications for resistance management. *Crop Prot.* **138**, 105339 (2020).

48. Luong, T. T. A., Zalucki, M. P., Perkins, L. E. & Downes, S. J. Feeding behaviour and survival of *Bacillus thuringiensis*-resistant and *Bacillus thuringiensis*-susceptible larvae of *Helicoverpa armigera*

(Lepidoptera: Noctuidae) exposed to a diet with *Bacillus thuringiensis* toxin. *Austral Entomol.* **57**, 1–8 (2018).

49. Onstad, D. W. *et al.* Seeds of change: corn seed mixtures for Resistance Management and Integrated Pest Management. *J. Econ. Entomol.* **104**, 343–352 (2011).

50. Fehr, W. R. & Caviness, C. E. Stages of soybean development. Iowa State University (1977).

51. SAS Institute. SAS/STAT: user's guide, version 9.4. *SAS Inst.* **Cary, NC,** (2001).

Tables

Table 1. Estimates for the proportion of *Anticarsia gemmatalis* larvae (3rd instar/V7) in each leaf location by hour. Concord, NE, U.S, 2019 crop season.

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.39 ± 0.05	0.25 ± 0.06	0.35 ± 0.06
10	0.27 ± 0.04	0.36 ± 0.07	0.36 ± 0.06
12	0.08 ± 0.03	0.54 ± 0.07	0.37 ± 0.06
14	0.11 ± 0.03	0.45 ± 0.07	0.44 ± 0.06
16	0.17 ± 0.03	0.45 ± 0.07	0.38 ± 0.06
18	0.18 ± 0.04	0.30 ± 0.06	0.51 ± 0.07
20	0.32 ± 0.04	0.24 ± 0.06	0.43 ± 0.06
32	0.32 ± 0.04	0.19 ± 0.05	0.49 ± 0.07
38	0.05 ± 0.02	0.36 ± 0.07	0.59 ± 0.06
44	0.19 ± 0.04	0.20 ± 0.05	0.60 ± 0.06
F	7.64	6.61	3.01
df	9	9	9
P	< 0.0001	< 0.0001	0.0111

P-values < 0.05 indicate statistical significance at a 95% confidence interval

Table 2. Estimates for the proportion of *Anticarsia gemmatalis* larvae (4th instar/R3) in each leaf location by hour. Concord, NE, U.S, 2019 crop season.

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.53 ± 0.07	0.28 ± 0.07	0.17 ± 0.06
10	0.10 ± 0.03	0.64 ± 0.08	0.24 ± 0.08
12	0.05 ± 0.02	0.62 ± 0.08	0.32 ± 0.09
14	0.08 ± 0.03	0.58 ± 0.08	0.33 ± 0.09
16	0.04 ± 0.02	0.56 ± 0.09	0.39 ± 0.10
18	0.08 ± 0.03	0.32 ± 0.08	0.60 ± 0.10
20	0.04 ± 0.02	0.26 ± 0.07	0.70 ± 0.09
32	0.08 ± 0.03	0.16 ± 0.05	0.76 ± 0.08
38	0.01 ± 0.01	0.18 ± 0.06	0.82 ± 0.06
44	0.11 ± 0.04	0.02 ± 0.01	0.87 ± 0.05
F	18.38	10.74	15.70
df	9	9	9
P	< 0.0001	< 0.0001	< 0.0001

P-values < 0.05 indicate statistical significance at a 95% confidence interval

Table 3. Estimates for the proportion of *Anticarsia gemmatalis* larvae (5th instar/V7,R1) in each leaf location by hour. Concord, NE, U.S, 2019 crop season.

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	.	0.16 ± 0.07	0.73 ± 0.10
10	.	0.11 ± 0.05	0.88 ± 0.06
12	.	0.12 ± 0.06	0.88 ± 0.06
14	.	0.11 ± 0.05	0.89 ± 0.05
16	.	0.10 ± 0.05	0.90 ± 0.05
18	.	.	.
20	.	.	.
32	.	.	.
38	.	.	.
44	.	.	.
F	.	0.37	2.22
df	.	4	4
P	.	0.8288	0.1156

P-values < 0.05 indicate statistical significance at a 95% confidence interval

Table 4. Estimates for the proportion of *Anticarsia gemmatalis* larvae (5th instar/R3,R4) in each leaf location by hour. Concord, NE, U.S, 2019 crop season.

Hour	Leaf location		
	Adaxial	Abaxial	Off plant
8	0.30 ± 0.06	0.25 ± 0.06	0.44 ± 0.08
10	0.21 ± 0.05	0.24 ± 0.06	0.53 ± 0.08
12	0.16 ± 0.04	0.25 ± 0.06	0.58 ± 0.08
14	0.07 ± 0.03	0.20 ± 0.05	0.72 ± 0.07
16	0.07 ± 0.03	0.07 ± 0.03	0.86 ± 0.04
18	0.03 ± 0.02	0.03 ± 0.02	0.94 ± 0.03
20	0.02 ± 0.01	0.02 ± 0.01	0.97 ± 0.02
32	.	.	.
38	.	.	.
44	.	.	.
F	4.97	4.95	21.18
df	6	6	6
P	0.0057	0.0027	< 0.0001

P-values < 0.05 indicate statistical significance at a 95% confidence interval

Table 5. Mean and maximum distance (± SE) covered, larvae number, and frequency (%) of 24-h-old *Anticarsia gemmatalis* in different positions and orientations relative to the infested plant in non-*Bt* soybean 12 days after release. Concord, NE, U.S, 2019 crop season.

Quadrants	Mean distance (cm)	Maximum distance (cm)	Larvae number	Frequency (%)
NW	90.02 ± 16.71 a	124.10 ± 27.27 a	6	17.14
	(53.23 − 126.81)	(64.07 − 184.12)		
SW	68.14 ± 14.31 a	69.95 ± 23.33 a	7	20.00
	(36.58 − 99.70	(18.51 − 121.39)		
NE	104.72 ± 16.73 a	142.52 ± 27.30 a	9	25.72
	(67.89 − 141.54)	(82.42 − 202.62)		
SE	69.29 ± 12.67 a	78.54 ± 20.65 a	13	37.14
	(41.20 − 97.38)	(32.80 − 124.29)		
F	1.77	2.62		
df	3	3		
P	0.24	0.12		
Orientation				
N	77.25 ± 9.60 a	102.30 ± 14.88 a	24	48.98
	(56.11 − 98.38)	(69.55 − 135.05)		
S	66.43 ± 8.99 a	78.23 ± 13.92 a	25	51.02
	(46.81 − 86.05)	(47.83 − 108.63)		
F	0.76	1.56		
df	1	1		
P	0.39	0.23		
Infested Row				
N	51.54 ± 14.06 a	62.25 ± 15.79 a	9	64.29
	(12.49 − 90.59)	(18.41 − 106.09)		
S	56.16 ± 26.69 a	87.50 ± 29.96 a	5	35.71
	(17.97 − 130.28)	(4.32 − 170.68)		
F	0.06	1.42		
df	1	1		
P	0.82	0.3		
Across rows				

W	37.75	38.00	2	66.67
E	40.00	40.00	1	33.33
F	-	-	-	
df	-	-	-	
P	-	-	-	

LS-means adjusted by Tukey test, followed by the same lower case letter in each column are not significantly different (*P* > 0.05)
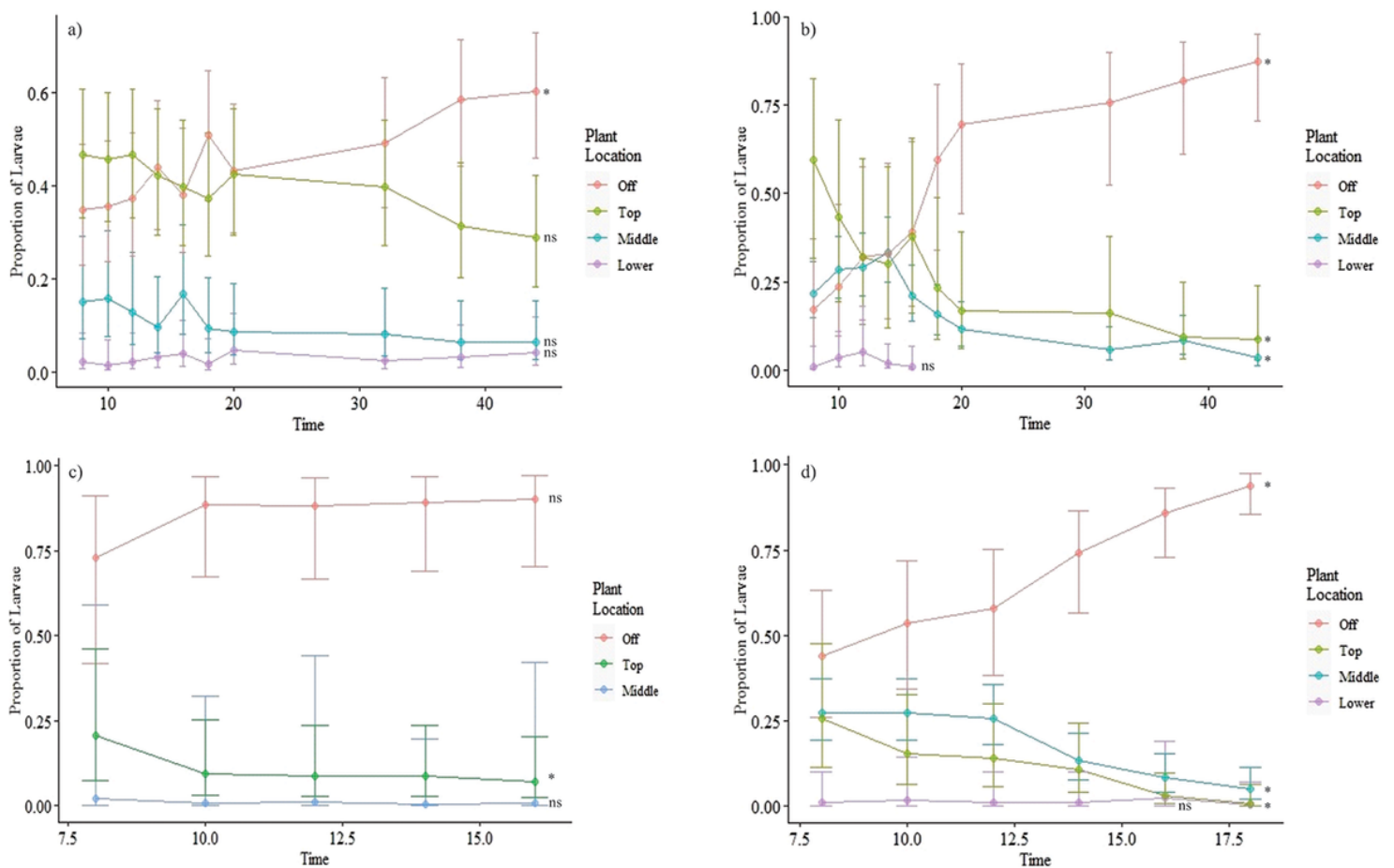
Figures

Figure 1

Graphical distribution of estimated proportion *Anticarsia gemmatalis* larvae in different combinations involving instars (insect development) and soybean stages (vegetative and reproductive). **a)** 3rd instar/V7; **b)** 4th instar/R3; **c)** 5th instar/V7,R1 and **d)** 5th instar/R3,R4. * = significant; ns = non-significant.
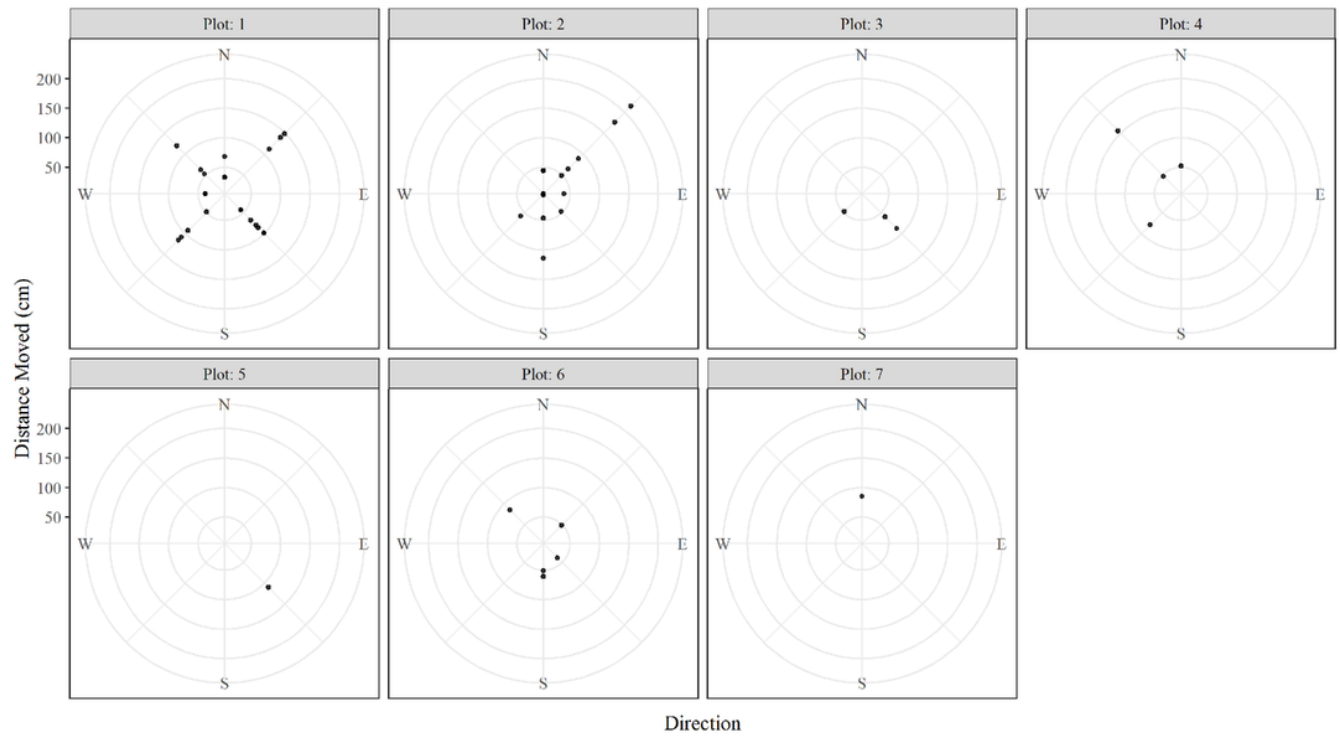
Figure 2

Illustrative plots of distance moved by 24-h-old *Anticarsia gemmatalis* larvae relative to different positions and orientations, 12 days after release.