New Tools to Assess Carrot Weevil Behavioral Ecology: Still-air Bioassay and Degree-day Activity Model for Ohio

Thesis

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Abstract

Cryptic dispersal, hidden larvae, and long-lived adult stages are among the characteristics of the weevil group that make them difficult to manage. However, it is well known that weevils rely heavily on chemical cues to find hosts and mates, and indeed these cues have been exploited to monitor populations and inform management decisions. A less known and challenging crop-weevil system that would benefit from the investigation of chemical cues is that of the carrot weevil (*Listronotus oregonensis*) and its cultivated hosts: carrot, celery, and parsley. The majority of carrot weevil research has focused on population management with insecticides, while investigation of carrot weevil behavior or chemical ecology has lagged behind significantly. To address these knowledge gaps I (1) adapted a still-air bioassay approach to evaluate the role of short-range volatile cues in carrot weevil host-seeking behavior, and (2) developed a preliminary degree-day model to understand the emergence activity of overwintering adults in north central Ohio.

I found the still-air bioassay to be successful for evaluating carrot weevil response to host volatile odors. Eighty-eight percent of males and 77% of females exhibited attraction to carrot volatiles in the absence of any visual or tactile cues. Similarly, 87% of males were attracted to parsley volatiles in the absence of other cues; however, only 62%

of female weevils responded to the volatiles of parsley. Furthermore, male response to parsley volatiles was significantly stronger than that of females. When weevils were given a choice between carrot root and parsley foliage across three experimental dates, male weevils did not exhibit a preference for either host. Females exhibited the same pattern, with the exception of one experimental date wherein 92% of females preferred carrot volatiles over parsley volatiles.

Results from two seasons of monitoring overwintering adult activity in the field revealed a consistent 2:1 male-female ratio of capture in carrot-baited modified-Boivin traps. My preliminary degree-day models predicted 50% cumulative emergence of overwintering adults at ~167 $\text{CDD}_{7.0\,^{\circ}\text{C}}$ in 2017, while cumulative emergence in 2018 was predicted to occur at ~450 $\text{CDD}_{7.0\,^{\circ}\text{C}}$. Recruitment of overwintering adults to baited traps appeared to be influenced by the presence of overwintered host crops, such that traps placed next to overwintered parsley in 2018 recruited carrot weevils more slowly, but for a longer period of time as compared to traps placed next to empty, previously-infested fields in 2017.

Together, my results suggest that volatile cues are integral to carrot weevil host-finding behavior and that males may have a broader response to host volatiles than females. These results also demonstrate that a still-air bioassay approach can be used successfully to discriminate carrot weevil response to host volatile cues presented in a laboratory setting. This knowledge will help inform the development of new behavioral manipulation strategies for carrot weevil management and reduce insecticide use for more sustainable specialty crop production.

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Publications

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Fields of Study

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Chapter 1: Literature Review

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Abstract

The carrot weevil, *Listronotus oregonensis* (LeConte) (Coleoptera: Curcuclionidae), is a devastating pest of high value Apiaceous crops like carrots (*Daucus carota* subsp. sativus Hoffm.), parsley (*Petroselinum crispum* (Mill.) Fuss), and celery (*Apium graveolens* L.). Although native to North America, it is a serious pest across the Eastern United States and Great Lakes region of the United States and Canada. Females deposit eggs in the petiole of cultivated hosts, and upon hatching, larvae tunnel down through the petiole and into plant roots causing wilting, yellowing, and plant death. Scouting procedures focus mainly on adult activity and require detection of small egg scars on the foliage and crown of the root, or reliance on traps that are only effective at the beginning of the season before the crop emerges. Several avenues of cultural, biological, and chemical control have been explored for this pest, but with limited success. Furthermore, investigation of these management strategies have primarily focused on carrot systems, neglecting other cultivated Apiaceae. Here we present a review of carrot weevil research and highlight key knowledge gaps in the carrot weevil system, which impede our understanding of this insect's biology and behavioral ecology. Future research addressing these key knowledge

gaps will expand our understanding of this pest and contribute to the development and implementation of more effective management strategies.

Introduction

The carrot weevil, *Listronotus oregonensis* (LeConte) (Coleoptera: Curculionidae), is a beetle that attacks cultivated crops in the family Apiaceae, particularly carrots (*Daucus carota* subsp. *sativus* Hoffm.), parsley (*Petroselinum crispum* (Mill.) Fuss), and celery (*Apium graveolens* L.). Damage to these crops is significant, as these specialty crops are valued at $1.3 billion annually in the United States (USDA-NASS 2012). The carrot weevil is native to North America and is found across several regions in the U.S. and Canada. In the U. S., carrot weevil is a historical pest of Apiaceous crops in Texas, Minnesota, Iowa, Wisconsin, southern Illinois, New York, and Massachusetts; however, it is rarely a pest in these regions today (Chittenden 1909, Harris 1926, Boyce 1927, Pepper and Hagmann 1938, Whitcomb 1965, Perron 1971). In contrast, it remains a serious pest in the U. S. and Canadian provinces surrounding the Great Lakes: namely Ohio, New Jersey, Michigan (though sporadic), Ontario, and Quebec, as well as the provinces of Prince Edward Island and Nova Scotia (Simonet and Davenport 1981, Telfer et al. 2018).

Carrot weevil management has largely focused on strategies to monitor adult activity in the field, by scouting for egg scars or using passive traps to synchronize the application of foliar insecticides with peak adult activity. However, despite an awareness of this pest since the 1800's, progress towards effective or novel management strategies remains poor (Capinera 2001). Given its status as a specialty crop pest, carrot weevil has received little attention and research efforts focused on this pest are limited. The majority of published work has focused on management of adults in carrot systems, with significantly less emphasis on key aspects of carrot weevil behavioral ecology that might be exploited for crop protection. Here we present a review of the biology and life cycle of the carrot weevil and describe current integrated pest management (IPM) strategies aimed at reducing crop damage. We also highlight key knowledge gaps in our understanding of this insect's biology that could improve our approach to managing this cryptic and challenging pest.

Description

Adults

Adult carrot weevils have dark brown bodies with light brown or copper colored scales (Fig. 1). These weevils are roughly 6.0 mm in length and 2.2 mm in width (Martel et al. 1976). Males and females can be sexed by comparing the first ventral abdominal segment: it is swollen in the female and slightly depressed in males (Fig. 2) (Whitcomb 1965). Males and females can also be discriminated by comparing the tips of the elytra, which are lobulated beyond the meeting point on females, but not lobulated on males (Torres 2001). Under laboratory conditions, the adult is the longest life stage, surviving up to 392 days at 25 °C (Baudoin and Boivin 1985). Like other weevils, carrot weevil adults often feign death when disturbed.

Eggs

Female carrot weevils begin oviposition (egg laying) in parsley, carrots, and celery when host plants reach the four-leaf stage (Boivin 1999). Eggs are laid in cavities chewed in the petiole or crown of exposed roots. Females typically deposit eggs in the petioles of parsley, while eggs are deposited in both the petioles and crowns of carrot and celery plants. Females lay 2-4 eggs, and then cover the egg cavity with a dark-colored anal secretion (Fig. 3). This dark secretion, known as an oviposition scar, is visible to the naked eye on the crown or petiole of plants, and is used as a diagnostic sign of egg-laying activity (Perron 1971). Eggs are white when first laid, but darken with age, turning black just prior to hatching (Boivin 1999).

Larvae and Pupae

Carrot weevil larvae are legless, cream colored, and have a light brown head capsule (Fig. 4). First instar larvae are roughly 2 mm in length and typically feed internally, tunneling down through the petiole of the plant to the root (Whitcomb 1965, Martel et al. 1976). As the larvae feed, they progress through four instars, inflicting significant damage to the crown and roots of host plants. This damage is particularly devastating to young plants, often resulting in plant death. The final larval instar emerges from the root to pupate, typically within 7 cm of the soil surface. Carrot weevil pupae are exarate, creamy-white, and roughly 5-8 mm long (Fig. 5) (Boivin 1999).

Seasonal Biology

Two temperature-driven biological models have been developed for carrot weevil: one that describes development and duration of each life stage, and another that describes activity or emergence patterns of overwintering adults. The first model developed in the laboratory in Ohio by Simonet and Davenport (1981) describes the developmental time of each life stage using a base temperature of 7 °C. Based on this model, carrot weevils require roughly 630 cumulative degree days (CDD) to complete one generation. This model is useful because it can help predict what developmental stage is likely to be present in the field at a given point in the growing season. Thus, this information can inform a grower's monitoring and management decisions. The second model describes when adult carrot weevils become active again after the overwintering period. This model uses a base temperature of 3.43 °C, which was determined by Rhéaume (2009) to be the lowest temperature at which adult carrot weevils are active in carrot fields. This model is helpful for determining when carrot weevils are initially emerging in the field early in the spring (late March and early April), as well as when they may be actively seeking hosts to feed and lay eggs later in the spring, once crops germinate (May through June).

Mated carrot weevils overwinter in the adult stage, either within host fields or along field margins, and emerge in early spring at 319 $\text{CDD}_{3.43\,°\text{C}}$. Despite having fully-developed wings, carrot weevils are poor fliers and typically migrate into actively growing crop fields each season by walking from overwintering sites (Boyce 1927). Furthermore, soil type can influence carrot weevil dispersal behavior. Bykova and Blatt (2018) demonstrated in the laboratory that when carrot weevils were exposed to arenas with sandy soil, they exhibited more consistent dispersal behavior, walking more quickly over the soil surface. In contrast, when carrot weevils encountered organic or mineral-type soils, they moved less and burrowed more often into the soil. Avoidance of sandy soil is a commonly observed behavior in phytophagous insects, particularly weevils (Björklund et al. 2003, Petersson et al. 2005, Björklund 2008). This sheds light on the abiotic factors that affect carrot weevil movement in the field and may explain higher carrot weevil abundance and pest status in regions where organic soils are found (Bykova and Blatt 2018). Adults begin feeding on plant foliage at 147 ± 9 $CDD_{7\,°C}$, and by 456 ± 47 $CDD_{7\,°C}$ 90% of eggs are laid (Boivin 1988). Female carrot weevils exhibit a reproductive diapause, which is regulated by the interaction of temperature and photoperiod (Stevenson and Boivin). This diapause is induced in reproductively mature females as photoperiod shortens, resulting in reduced oviposition in the fall. As temperature increases in the spring the photoperiod threshold for oviposition decreases. This decrease allows females to oviposit earlier if temperatures are warm enough, so they can take advantage of ideal conditions in early spring (Stevenson and Boivin 1990). On average, a single female lays up to 250 eggs in her lifetime (Baudoin and Boivin 1985).

Eggs require 130 $CDD_{7\,°C}$ to complete development (Simonet and Davenport 1981). Once larvae hatch, they burrow down through the petiole, or drop onto the soil to feed on roots. Larvae proceed through four instars as they feed within the root, requiring 256 $CDD_{7\,°C}$ to complete development (Simonet and Davenport 1981). Once the fourth instar is reached, they stop feeding, exit the root and build a pupation chamber in the soil (Martel et al. 1976, Simonet and Davenport 1981, Collins and Grafius 1984). Pupae normally develop within 244 $CDD_{7\,°C}$. The duration of the pupal stage can last for five days at 18 °C or ten days at 28 °C (Collins and Grafius 1984).

Carrot weevil populations exhibit one to four generations per year; in the northern range one or two generations occur per season, while in the southern range three to four generations have been documented (Pepper and Hagmann 1938, Whitcomb 1965, Simonet and Davenport 1981, Boivin 1985). In addition to cultivated hosts, carrot weevil also feeds and oviposits on weedy hosts, including Queen Anne's lace (*Daucus carota*

L.), pineapple weed (*Matricaria discoidea* DC.), plantain (*Plantago major* L. and *Plantago lanceolata* L.), wild turnip (*Brassica rapa* L.), and parsnip (*Pastinaca sativa* L. and *Sium suave* Walt). However, carrot weevil preference for these weedy hosts, as well as egg and larval development in these hosts, is unknown.

Damage

Adults feed on the foliage and petiole of plants (Boyce 1927); however, this feeding is not significant, nor does it affect yield. In contrast, the larva is the destructive life stage. They tunnel down through the petiole, crown, and root of plants, causing severe damage and even plant death, particularly in young plants (Fig. 6) (Boivin 1999). Larval damage can render carrots unmarketable (Boivin 1999). Damage to parsley is most severe on the crown and roots of the plants, causing chlorosis and necrosis. Additionally, larvae may exit one root to feed on the root of adjacent plants. Carrot weevil damage can result in significant crop losses: up to 70% loss in carrots and parsley (Boivin 1999, Jasinski 2008). Celery is capable of tolerating some feeding damage, as long as plants are not water stressed (Boivin 1999).

Methods for monitoring carrot weevil populations

Use of passive traps to monitor adults

Several techniques have been evaluated to monitor carrot weevil populations in crop fields. Given their small size and cryptic behavior, actively scouting fields for adult carrot weevils is not recommended. Rather, passive sampling methods, including pan traps and pitfall traps, have been tested as indicators of adult activity in crop fields (Perron 1971, Ryser 1975); however, these methods are unreliable. Pan traps are most useful in capturing flying insects and pollinators (Disney et al. 1982, Vrdoljak and Samways 2012), while pitfall traps capture too few individuals to accurately reflect activity in the field (Boivin 1985). Blacklight traps placed within 0.25 miles of crop fields have also been used to monitor adult carrot weevils; however, this method does not accurately measure activity or level of infestation because they target flying insects attracted to lights, and carrot weevils rarely fly (Perron 1971, Ryser 1975, Boivin 1985). Likewise, active sampling techniques for adults, like suction-sampling and sweep-netting, do not provide reliable indication of adult activity because these methods sample the foliage rather than the base of plants or the soil surface, where adult carrot weevils tend to be found (Ryser 1975).

The most reliable monitoring tool developed to date is known as the “Boivin trap.” A true Boivin trap, or radiator trap, consists of 22 wooden plates, separated by metal washers placed over a carrot (Boivin 1985). A “modified-Boivin trap” has also been developed and is more easily constructed and used (Ghidiu and VanVranken 1995). This trap consists of a wooden base with “teeth” that surround a canal where a whole carrot is placed to serve as bait (Fig. 7). These traps are the most successful in attracting adults carrot weevils, although numbers may still be low. The utility of these traps decreases once crop hosts have emerged (Boivin 1999) possibly because the carrot bait within is unable to compete with volatiles emanating from crop hosts. Thus, growers must rely on other monitoring methods to inform their management strategies once traps become ineffective.

Scouting for egg-laying scars

Detecting egg-laying scars has been explored as a potential monitoring strategy for the carrot weevil. Ryser (1975) placed sentinel parsley plants in the field and monitored the appearance of egg-laying scars. However this strategy was unsuccessful, most likely because the number of sentinel plants was insufficient. Currently, scouting egg-laying scars is the recommended strategy for monitoring adult carrot weevil activity in parsley. Torres and Hoy (2002b) found that sampling 150 parsley plants, in an x-shaped pattern across the field, is most effective for gaining a reliable measure of carrot weevil activity. Following this method, ten plants are examined at equal intervals along an x-shaped transect, such that seven groups of ten plants are evaluated along one “line” of the x-shape and eight groups of ten plants are evaluated along the other “line.” This allows growers to systematically evaluate the presence of egg-laying scars in both the center and edges of a field. Based on this scouting method, action is recommended if 1% of plants exhibit oviposition scars (Torres and Hoy 2002b). This process is tedious, time consuming, requires correct identification of scars and does not provide advance warning. Once egg-laying scars are seen, it is too late to intervene and prevent damage. Additionally, this method of scouting has only been empirically evaluated in parsley cropping systems and the results of this method may differ on other crops. Even with trap monitoring and scouting, carrot weevil damage can go unnoticed until it is severe (Torres and Hoy 2002b).

Carrot weevil IPM strategies

Cultural Control

Several cultural control methods are recommended to reduce carrot weevil infestation. Rotating crop fields to new locations, at least one-quarter mile away from previous fields in the landscape, is assumed to reduce new outbreaks because carrot weevil typically disperse by walking (Pepper and Hagmann 1938). Tilling residual crop material and removing weedy margins where carrot weevils overwinter may also reduce damage (Whitcomb 1965, Grafius and Collins 1986). These strategies aim to limit carrot weevil access to alternative habitat, which may serve as a refuge from management tactics, or harsh environmental conditions during the winter. The use of barrier crops or row covers can interfere with carrot weevil ability to find host resources. For example, Rekika et al. (2008) determined that floating row covers can reduce carrot weevil damage by up to 75% and improves yield in carrots. Delayed planting also reduces damage by allowing carrots to escape the peak ovipositional period (Boivin 1988). However, delayed planting slows crop development, as cool temperatures and spring rains promote germination. Thus, growers who delay planting may struggle to achieve a robust crop stand, resulting in fewer harvests. Taken together, this reduces net income and therefore is not commonly practiced (Boivin 1988, Torres and Hoy 2002a). Therefore, carrot weevil management continues to be a challenge for commercial growers, particularly in the Great Lakes regions of North America.

Chemical Control

Foliar insecticides are the predominate tool used against adult carrot weevil in all cropping systems. The cancellation of the primary insecticide for this pest in the U.S., guthion (azinphos-methyl), due to human health and environmental concerns, removed the most effective tool used by growers (Curl et al. 2002, Ferrari et al. 2007). Other insecticides like phosmet, malathion, diazinon, methomyl, and pyrethroids are available for use against carrot weevil, but these products have not been used with the same level of success (Boivin 1999, Torres and Hoy 2002a). Current chemical recommendations for carrot weevil in carrot, celery and parsley can be found in Table 1 (Arancibia et al. 2019, Egel et al. 2019).These insecticides target young larvae or adults; however, larval exposure to insecticides is limited because they are typically concealed within plant tissue or soil, whereas adults appear to have limited susceptibility to field labeled rates of these insecticides (Pree et al. 1996). The behavioral or physiological mechanisms underlying carrot weevil tolerance to insecticides are not well understood. Furthermore, the canopy becomes dense and complex as these crops grow, making thorough coverage and direct contact with adult weevils difficult to achieve. A recent study by Telfer et al. (2018) investigated the toxicity of neonicotinoid spinosyns, ryanoid, pyrethroid, and organophosphate insecticides to adult carrot weevils in both laboratory and field experiments. In the lab, insecticides were applied directly to adult carrot weevils using a 1/9th scale Potter (miniature) spray tower at two and four times the label rate. Of these insecticides, only the neonicotinoid (clothianidin), and the organophosphate (phosmet) resulted in greater than 50% mortality, and this was only the case when these active ingredients were applied at four times the label rate. Telfer et al. (2018) conducted small plot field trials in 2015 and 2016 with the same insecticides applied as seed treatments, foliar sprays, and in-furrow applications in carrot fields. Although cyantraniliprole was ineffective in laboratory toxicity trials against carrot weevil adults (less than 10% mortality), cyantraniliprole seed and foliar treatments resulted in a 10 ton/hectare increase in yield in 2016 compared to the control (Telfer et al. 2018). Physiological tolerance and limited contact with insecticides, due to carrot weevil biology or host plant characteristics, renders chemical control of carrot weevil ineffective and unsustainable as a long-term population reduction strategy. It should be noted no equivalent study has been conducted to evaluate efficacy of current insecticides for carrot weevil in parsley.

Biological control

Biological control by natural enemies has been explored as a means of carrot weevil population suppression and has been extensively studied in carrot cropping systems. Carrot weevils infected with entomopathogenic fungi (EPF) have been observed in the field; however the application of a commercially available EPF, *Beauveria bassiana* Vuill. (Botanigard ES, BioWorks Inc., Victoria, NY), had no effect on carrot weevil damage in carrots compared to untreated plots (Telfer et al. 2018). Ground beetles (Coleoptera: Carabidae), which forage along the soil surface for prey, in theory, could provide biological control for all carrot weevil life stages. Under laboratory conditions smaller carabids were the best consumers of carrot weevil eggs, while *Pterostichus melanarius* Illiger, a large carabid beetle, was the only species to consume teneral (newly emerged) and overwintered adults. All sizes of carabids consumed fourth instar larvae

(Baines et al. 1990); however, these promising laboratory results may not translate to outcomes in the field, as the majority of the carrot weevil life cycle occurs in the plant or soil, rather than along the soil surface (Baines et al. 1990). Four species of parasitoid wasps, *Anaphes victus* Huber, *Anaphes listronoti* Huber, *Anaphes cotei* Huber, and *Anagrus* spp., all in the family Mymaridae, parasitize carrot weevil eggs (Hopper et al. 1996, Huber et al. 1997). Mortality caused by these parasitoids ranges from 49% to 68% (Collins and Grafius 1986, Hopper et al. 1996, Huber et al. 1997). However, evidence to date suggests that parasitoids are often present in low numbers in commercial fields, limiting their utility as biological control agents. Although several studies have evaluated predators and parasitoids with varying levels of success, intensive use of foliar insecticides has likely limited the effectiveness of above-ground natural enemies in conventionally managed systems (Lemay et al. 2018). In spite of the thorough investigation of parasitoids and predators in carrot systems, no published work has investigated these populations in celery or parsley.

To date, entomopathogenic nematodes (EPNs) have demonstrated the most promise as carrot weevil biological control agents. Several stages of the carrot weevil life cycle are closely associated with the soil, making it a prime candidate for EPN attack. EPNs are soil-dwelling parasites that enter hosts through natural openings in the body cavity. Once inside, they release bacteria that kill the insect and the nematode feeds on the remains (Kaya and Gaugler 1993). EPNs are particularly effective biological control agents of root weevils in general, including black vine weevil and citrus root weevil (Kaya and Gaugler 1993). Miklasiewicz et al. (2002) conducted laboratory and field experiments evaluating the biological control potential of five commercially available EPNs against carrot weevil in Ohio: *Steinernema carpocapsae* Poinar*, S. riobrave* Cabanillas, Poinar &Raulston*, S. feltiae* (Filipjev), *Heterorhabditis megidis* Poinar, and *H. bacteriophora* Poinar. In lab studies focusing on the adult carrot weevil life stage, *S. carpocapsae* was the most effective EPN in sandy soil, causing 88% cumulative mortality over an 8-day period, while *H. bacteriophora* was most effective in muck soil, causing 81% cumulative mortality of adult carrot weevils. Of the five species tested, *H. bacteriophora* exhibited the best biological control potential in the field, causing the highest mortality of carrot weevil larvae (38%), pupae (80%), and adults (40%). Furthermore, field plots of parsley treated with *H. bacteriophora* exhibited the lowest levels of plant mortality. *H. bacteriophora* populations also persisted at greater numbers compared to *S. carpocapsae* (Miklasiewicz et al. 2002). However, in a similar study, comparing the efficacy of both *Heterorhabditis* and *Steinernema* nematodes in carrot fields, conducted in the Holland Marsh region of Quebec, Canada, *Steinernema* nematode species caused higher mortality of carrot weevil larvae (Boivin 1999). A carrot weevil - specific nematode, *Bradynema listronoti* Zeng, has been isolated from the hemocoel of female carrot weevil collected in Ontario, Canada (Zeng et al. 2007). This nematode sterilizes female carrot weevils, most likely by inhibiting the production of juvenile hormone which is required for maturation of the reproductive system (Gagnon et al. 2018). Interestingly, male carrot weevils were not sterilized when infected by *B. listronoti*, but exhibited higher mortality than females (Gagnon et al. 2018). Despite the potential this EPN species holds for reducing carrot weevil reproduction, it is uncommon and has not been evaluated as a biological control agent (Zhao et al. 1991, Boivin et al. 2013).

Because of low economic thresholds (1 % of plants with an oviposition scar) and relatively high costs, inundative use of EPNs is not a common part of carrot weevil IPM strategies. However, the efficacy of EPNs may be enhanced by altering soil management strategies. When tillage was reduced and a cover crop (mixture of barley and clover) was added to carrot fields in Ohio, the persistence of *H. bacteriophora* increased roughly two-fold after two years (Bal et al. 2014). Furthermore, the severity and proportion of damaged carrots was reduced in plots that received *H. bacteriophora*, intercropping, and reduced tillage treatments (Bal et al. 2014). By changing soil management strategies in ways that support EPN populations and are more compatible with inoculative EPN releases, it may be possible to increase the utility of EPNs for management of carrot weevil.

Knowledge Gaps and Future Directions

Carrot weevil management in North America remains a major challenge, particularly in the Great Lakes regions of the U.S. and Canada where concentrated production of carrots, parsley, and celery occur. Interestingly, carrot weevil has continued pest status in some regions, while pest status has declined in others. Although carrot weevil management continues to be a major challenge in several regions, communication of IPM strategies and their outcomes has been very limited, both across U.S. regions and internationally. Thus, a stronger network to share knowledge regarding the relative success of cultural, chemical, and biological control strategies, either alone or in combination, would serve as a valuable resource for producers. By combining knowledge of the successes and failures in carrot weevil management across regions and crops, we can begin to develop informed, crop-specific carrot weevil management strategies.

Limited understanding of carrot weevil host- and mate-finding behavior is a key obstacle in the development of novel tools or strategies for management. Unlike well-studied weevil pests such as the pepper weevil (*Anthonomus eugenii* Cano), boll weevil (*Anthonomus grandis grandis* Boheman), or plum curculio (*Conotrachelus nenuphar* (Herbst)), research efforts to identify attractants, repellents, and sex or aggregation pheromones for carrot weevil are essentially nonexistent. We also lack knowledge of carrot weevil preference for cultivated versus weedy hosts. This information is necessary to develop more effective and sustainable IPM strategies. Gaining such information about the basic biology of carrot weevil would open the door to semiochemical-based approaches, like push-pull, which exploit insect host- and mate-finding behaviors (Cook et al. 2007, Khan et al. 2016). Furthermore, the use of sex pheromones or host-specific attractants, alone or in combination, could dramatically improve population monitoring, or contribute to the development of novel trapping techniques. The limited success of modified-Boivin traps, which are baited with carrots, but look nothing like a carrot, suggests that chemical cues play a role in host location. This aspect of carrot weevil behavior has yet to be formally investigated. Addressing such knowledge gaps will provide critical information that can be combined with understanding of life history traits, dispersal, and predictive models to develop novel monitoring and management strategies for this pest.

In general, we know very little about carrot weevil movement patterns as they move from overwintering sites into new fields. It is assumed that dispersal occurs via undirected walking from field to field. Although carrot weevils do fly given particular environmental conditions, observations of flight are exceedingly rare and carrot weevil flight behavior has not been extensively studied (Boyce 1927). Isolated populations of carrot weevil can be found throughout the U.S. and Canada and given that dispersal is limited to walkable distances, it is possible that these populations may be unique in their population genetics and behavior, with implications for the outcomes of carrot weevil management strategies across regions.

In the case of female carrot weevil, it is unclear if eggs are deposited in host plants at random, and what cues signal a viable oviposition site. Efforts to determine carrot weevil dispersal behavior and spatial distribution within and across fields, particularly for female carrot weevils, could inform cultural IPM practices, like the spatial arrangement of trap crops or inter-cropping design, to disrupt egg-laying behavior on the focal crop.

Concluding Remarks

The carrot weevil is a devastating pest of valuable specialty crops. The larvae are the truly damaging stage of this pest, tunneling through the root and causing severe economic damage. Management tactics for this pest include crop rotation as well as foliar application of insecticides. However, these methods are frequently ineffective resulting in significant economic losses. Biological control methods have been investigated against the carrot weevil, but none have yet contributed to consistent population suppression

(Collins and Grafius 1986, Hopper et al. 1996, Huber et al. 1997, Miklasiewicz et al. 2002). A clear majority of published work has focused on the management of carrot weevil in carrot systems. A broader understanding of management strategies in parsley and celery are needed to improve control in these systems. Further research on carrot weevil biology, particularly behavioral ecology, is needed to develop sustainable and effective IPM strategies for this insect in the future.

Figure 1. Dorsal view of the adult carrot weevil (*Listronotus oregonensis*).

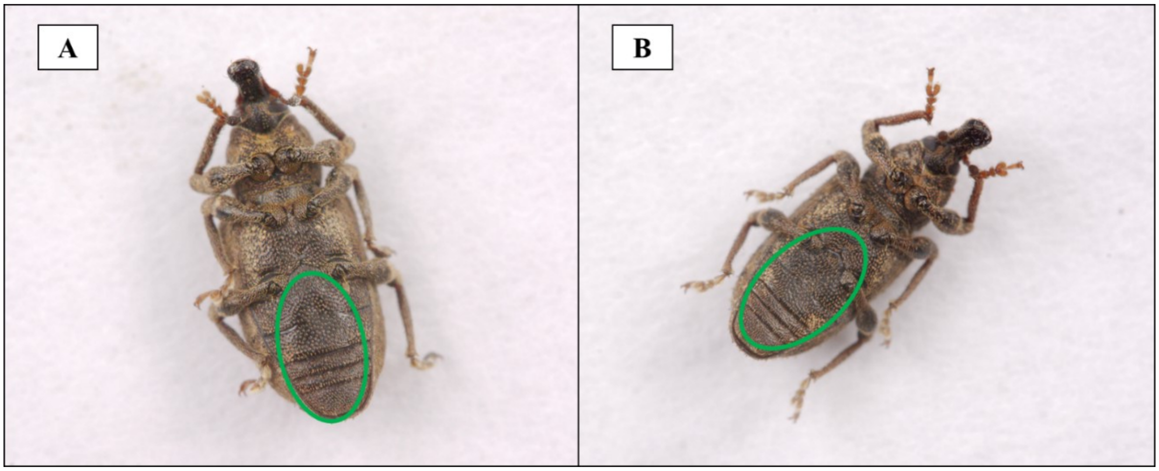

Figure 2. Ventral view of an adult female carrot weevil (A) and adult male carrot weevil (B). The first abdominal segment is swollen in females and depressed in males, indicated by green circles.

Figure 3. Two carrot weevil oviposition (egg-laying) scars on the petiole of a carrot plant, indicated by black arrows.

Figure 4. Fourth-instar larvae of the carrot weevil.

Figure 5. Pupal stage of the carrot weevil (ventral view).

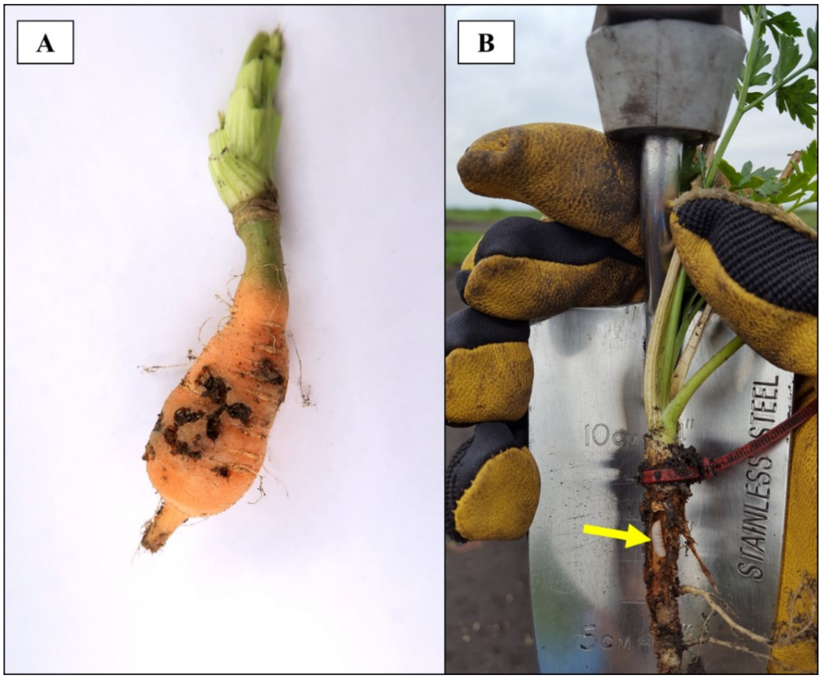

Figure 6. Feeding damage by larval carrot weevil to young carrot (A), and parsley root (B). Carrot weevil larva indicated by the yellow arrow.

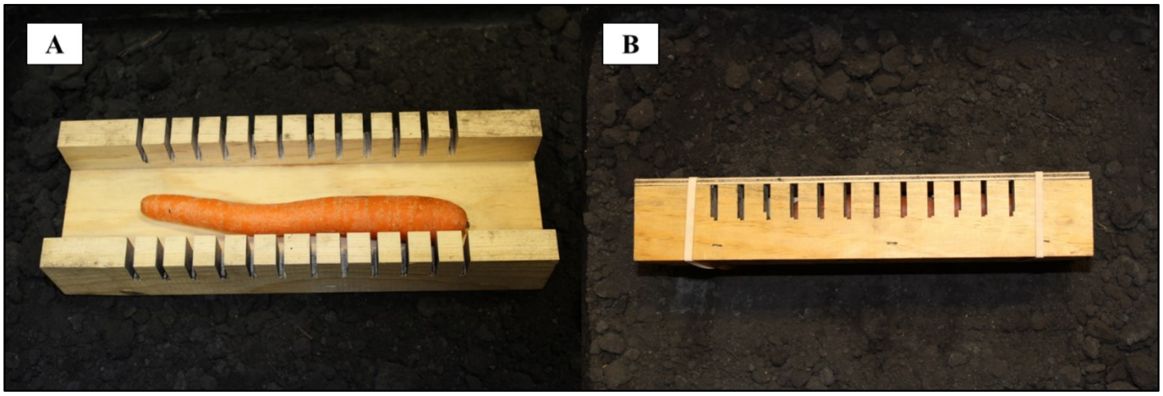

Figure 7. A modified Boivin trap baited with carrot. View of an open trap from the top (A), and closed trap viewed from the side, showing “teeth” of the trap (B).

Table 1. List of insecticides currently recommended for carrot weevil (*Listronotus oregonensis*) in carrot, celery, and parsley.

Active ingredient	**Class**	**Product Name**	**Crop**
Malathion	Organophosphate (1B)	Malathion 57 EC	Parsley
Oxamyl	Organophosphate (1A)	Vydate L	Celery, Carrot
Esfenvalerate	Pyrethroid (3A)	Asana XL	Carrot
Beta-cyfluthrin	Pyrethroid (3A)	Baythroid XL	Parsley, Carrot
Cyfluthrin	Pyrethroid (3A)	Tombstone, others	Carrot
Imidacloprid and beta-cyfluthrin	Neonicotinoid (4A) and Pyrethroid (3A)	Leverage 360	Carrot

Chapter 2: Still -Air Bioassay Approach for the Assessment of Carrot Weevil Response to Host Volatiles

Abstract

Despite a long history of pest status in the Great Lakes regions of North America, the carrot weevil continues to elude population monitoring and management efforts in carrot, parsley, and celery production. One key knowledge gap in carrot weevil ecology is understanding of the cues used by this insect to locate host plants. Although visual cues have been evaluated in adult monitoring traps, it appears that traps offering chemical cues from a whole carrot are most attractive. I developed a still-air bioassay to assess carrot weevil response to the volatiles of carrot and parsley in the absence of visual or tactile cues. I found that carrot weevils were significantly more likely to orient towards volatiles from carrot root, with 88% of males and 77% of female responders choosing carrot root over the control. In contrast, only male weevils oriented significantly towards volatiles from parsley foliage, while females exhibited a weaker response. Eighty-seven percent and 63% of responding males and females, respectively, chose parsley foliage over the control. When given a choice between parsley and carrot, male weevils showed no preference for the volatiles of either host. Although the majority of females exhibited the same response as males, females from one experimental date showed a preference for carrot volatiles, with 92% orienting towards the carrot over the parsley. Moreover, these

findings suggest that chemical cues play an important role in short-range, host-finding behavior of this pest and males may respond more broadly to host volatiles than females. My results demonstrate strong potential for the still-air bioassay to be used reliably as a simple screening tool to evaluate carrot weevil response to host volatiles in the absence of other cues.

Introduction

Many insects use olfaction to locate host plants (Bruce et al. 2005, Vittum 2005, Simard et al. 2007). Several studies have demonstrated that host plant volatiles play an important role in host location for several weevil pests and can be combined with pheromones or visual cues to improve the success of trapping and monitoring strategies (Dickens 1989, Butkewich and Prokopy 1993, Wee et al. 2008, Addesso and McAuslane 2009, McGraw et al. 2011, Ukeh et al. 2012, Smart et al. 2014). For example, the pepper weevil can discriminate host plant volatiles in the absence of other cues (Addesso and McAuslane 2009). This work demonstrated that pepper weevils exhibit both short and long-range behavioral responses to host volatile cues. Host volatiles in combination with pheromones are used to monitor plum curculio and have contributed to the development of new population management techniques, such as trap trees. Trap trees acted essentially as attract-and-kill traps by exploiting both pheromones and host volatiles. These trees reduced insecticide application by 70% and prevented penetration of migrating plum curculio populations into apple orchards (Leskey et al. 2008). Similarly for boll weevil, when green leaf volatiles were combined with Grandlure, a component of the boll weevil aggregation pheromone, the efficacy and longevity of trap capture was significantly improved (Dickens 1989). However, there are also examples where the addition of host volatiles does not improve weevil capture, which is the case for the pea leaf weevil: plant volatiles alone did not attract adult pea leaf weevil, and addition of the plant volatiles to the aggregation pheromone did not improve trap capture. Rather, the aggregation pheromone is most important for adult attraction (Evenden et al. 2016, St Onge et al. 2017). These findings have shaped our understanding of key stimuli that influence the host-finding behavior of weevils and led to the use of semiochemical-based behavioral manipulation strategies to improve weevil management. Knowledge gained from these well-studied and economically-important weevils can guide approaches to understanding carrot weevil behavioral and chemical ecology.

To identify semiochemicals that elicit a behavioral response in weevils, a variety of bioassay methods have been used. The most common methods of assessing weevil response to host volatiles are: Y-tube bioassays, wind tunnels, and still-air bioassays. The Y-tube approach assesses weevil response to volatiles presented in moving air by giving individuals a choice between clean air streams and air streams with volatile cues (Van Tol et al. 2002, Szendrei et al. 2009, Hulcr et al. 2011, McGraw et al. 2011, Evenden et al. 2016, St Onge et al. 2017, Duffy et al. 2018b). These assays can be tedious, as well as limiting, because they require constant observation, the use of light, and conditions like directed air movement, which may alter weevil behavior in ways that result in artificial or no behavioral response (Meyer 1976). Similarly, wind tunnels exploit moving air and allow for the assessment of weevil response to long-range chemical stimuli. These assays are more successful for assessing the response of species that have strong dispersal mechanisms, like the pepper weevil (Addesso and McAuslane 2009). However, the carrot weevil is described as a poor flier that rarely flies (Boivin 1999). In contrast to the first two bioassay methods, the still-air bioassay presents volatile cues in a closed system without the use of moving air (Prokopy et al. 1995, Van Tol and Visser 2002, Edde and Phillips 2006, Wee et al. 2008, Collatz and Dorn 2013). This bioassay has allowed for greater host volatile discrimination for some weevil species. For example, when Prokopy et al. (1995) compared adult plum curculio response to host volatiles using a wind tunnel versus a dual-choice, still-air bioassay they found that plum curculio exhibited greater discrimination between control and host treatments when tested in the still-air bioassay. Given the biology of the carrot weevil and constraints of conducting experiments in the dark, I chose to evaluate the utility of the still-air bioassay to assess carrot weevil response to host volatile cues.

The carrot weevil, *Listronotus oregonesis* (LeConte), is a serious pest of the cultivated Apiaceae, including carrots (*Daucus carota* subsp. *sativus* Hoffm.), parsley (*Petroselinum crispum* (Mill.) Fuss), and celery (*Apium graveolens* L.) in North America. Awareness of the carrot weevil and its impact on these specialty crops are limited to local or regional producers, generally in the Mid-Atlantic and Great Lakes regions of the United States and Canada, as well as the out-lying Canadian provinces of Nova Scotia and Prince Edward Island. Roughly 200,000 acres of carrots, parsley, and celery are grown in the United States, and with values ranging from $9,000 to $12,000 an acre, these crops contribute significantly to the U. S. economy each year (USDA-NASS 2012). Ohio is the fourth largest producer of fresh parsley in the U. S. (USDA-NASS 2012), and in muck soil regions of the state where fresh parsley is grown, the carrot weevil causes significant yield loss.

Female carrot weevils lay eggs in the petioles of young plants, and upon hatching larvae burrow down to the root system and feed while developing through four instars (Boivin 1999). By the time larvae enter the pupal stage, plant roots can be severely damaged leading to yellowing, stunting, and plant death (Chittenden 1924, Boyce 1927). Because all life stages of this insect are concealed, either in the soil or within the plant, the only reliable means to detect activity in the field is by monitoring adults with carrot-baited traps (Boivin 1985, Ghidiu and VanVranken 1995), or scouting egg scars (Torres and Hoy 2002b). However, these strategies are time intensive, requiring correct identification of tiny egg scars on plants and the repeated servicing of baited traps, which are only effective early in the season (Boivin 1985, Ghidiu and VanVranken 1995). Furthermore, neither of these strategies provides advance warning of damage; once egg scars are detected on plants, it is too late to take preventative action to protect the crop. To date, most carrot weevil research has focused on chemical, cultural, and biological control strategies in carrot systems (Perron 1971, Bélair and Boivin 1985, Boivin 1988, Pree et al. 1996, Capinera 2001, Torres 2001, Miklasiewicz et al. 2002, Rekika et al. 2008, Boivin et al. 2013, Telfer et al. 2018). However, we know much less about carrot weevil host-finding behavior.

Carrot weevils can be captured in a variety of traps when baited with fresh carrot root including pitfall traps, “radiator” traps, and modified-Boivin traps (Boivin 1985, Ghidiu and VanVranken 1995). The construction of “radiator” and modified-Boivin traps is similar; both provide a whole carrot (food) that is surrounded by “teeth” (shelter) where weevils can reside upon reaching the trap. However, compared to the “radiator” trap, the modified trap is more easily assembled, less expensive to construct, and captures an average of 5 more weevils per trap (Ghidiu and VanVranken 1995), perhaps because the modified-Boivin completely covers the carrot, protecting it from desiccation. Interestingly, these modified-Boivin traps are most effective early in the season, before crop fields have germinated. As the season progresses, trap capture diminishes substantially, perhaps because the carrot bait is unable to compete with host volatiles emanating from surrounding crop fields (Boivin 1985). Taken together, these observations suggest that carrot weevils rely on short-range volatile cues to locate potential host plants, but this has not been tested empirically.

The objective of this study was to develop a simple and discriminating bioassay to evaluate carrot weevil response to host volatile odors in the absence of visual or tactile cues. I adapted the still-air bioassay developed for plum curculio (Prokopy et al. 1995) to evaluate the response of male and female carrot weevils to two cultivated host plants, carrot and parsley. Furthermore, I used the bioassay to assess carrot weevil preference for the volatiles of carrot and parsley.

Methods

Insects

All weevils used in this study were field-captured adults, collected from commercial parsley fields in Willard, OH between May 3 and June 15, 2018 using modified-Boivin traps baited with whole carrots. Field-collected weevils were maintained on carrot root in environmental chambers at 27 °C, on a 16:8 L:D cycle, and 70% relative humidity, as described by Martel et al. (1975). Overwintering adults were of unknown age and females were presumed to be mated (Ryser 1975).

Assessing carrot weevil behavioral response to host volatiles

The still-air bioassay developed for plum curculio (Prokopy et al. 1995) was modified to measure carrot weevil response to plant odors. The still-air olfactometer consisted of two small polyethylene treatment chambers (2.6 cm diameter x 2.5 cm height), attached to the bottom of a standard 100 mm plastic petri dish (Fisher Scientific, Hampton, New Hampshire), which served as the introduction chamber (Figure 8). Two holes, 10 mm in diameter and 35 mm apart, were created in the bottom of the petri dish to provide an opening from the introduction chamber into each of the smaller treatment chambers. Next, a 10 mL polyethylene pipette tip (cut to 8 mm base diameter × 6 mm tip diameter × 10 mm length) was inserted through each hole, creating channel for weevils to move from the introduction chamber into each treatment chamber. To prevent weevils from leaving a treatment chamber after making a selection, the exterior surfaces of the pipette tip channels were painted with talc to create a slippery surface weevils could not crawl on. The talc was mixed with isopropyl alcohol (0.6 g/mL) and applied to pipette tip channels using a paintbrush to ensure proper coating of exterior surfaces. The isopropyl alcohol evaporated completely prior to use in experiments. Damp blotting paper was placed in the bottom of the introduction chamber and each treatment chamber to maintain uniformly high humidity throughout the bioassay arena.

I conducted two bioassay experiments in May and June of 2018. In Experiment 1, conducted May 18, 2018, individual carrot weevils were presented with the choice of either 0.25 grams of parsley foliage, or a moist cotton ball (control) (n=119, 60=female and 59=male). In Experiment 2, conducted June 23, 2018, carrot weevils were offered the choice of either 2 grams of carrot root, or a moist cotton ball (n=110, 56=female and 54=male). Experiment 3 was conducted over three dates: September 12, September 20, and October 4, 2019. All individuals used in this experiment originated from the same cohort, and temperature and light conditions were consistent across all three dates. Carrot weevils were offered a choice between 2 grams of carrot or 0.25 grams of parsley foliage (n=119, 59=females and 60=males). The amount of plant tissue used in experiments represented the maximum amount that treatment chambers could accommodate. I scored weevils as making a choice when they left the introductory chamber and entered one of the smaller choice chambers containing either the host treatment or the control. Weevils that did not leave the introduction chamber were recorded as making no choice. All bioassays were conducted in the dark at 21 °C and the placement of each treatment (host or control) was randomized between the left and right treatment chambers to eliminate directional bias. All weevils were starved for 24 hours prior to use in bioassays and were tested only once. Each experiment was initiated by introducing an individual weevil into each still-air olfactometer and allowing them one hour to acclimate. During the acclimation period, the channels into treatment chambers were blocked so that weevils could not enter. After the acclimation period, all channels were opened and the still-air olfactometer was sealed with parafilm to maintain humidity. Each bioassay arena was used only once to avoid contamination. Preliminary observations indicated that most carrot weevils made a choice within a two-hour period, so I recorded carrot weevil choice after two hours. Still-air bioassays, as described above, were replicated until a minimum of 20 weevils of each sex responded in each experiment.

Statistical analysis

Chi-square goodness-of-fit tests were used to test the null hypothesis that responsive carrot weevils showed no preference for host volatiles or the cotton ball control. Weevils that remained in the introduction chamber were excluded from analyses (Table 2), and male and female weevils were analyzed separately. Experiment 3 was also blocked by date to account for any variation across time. When comparing male and female response, male responses were used as expected values. A p-value of 0.05 was used to determine statistical significance. All statistical analyses were conducted in R Studio (R Core Team 2017).

Results

Both male and female carrot weevils exhibited strong positive responses to host volatiles in still-air bioassays within two hours. When given the choice between the volatiles of parsley foliage or a moist cotton ball control, 87% of responding males entered treatment chambers containing flat parsley foliage in the absence of host visual or tactile cues, while 13% of males chose the control ($\chi^2 = 16.13$, df = 1, $P < 0.0001$). In contrast to male weevils, females exhibited no difference in response to chambers containing parsley foliage versus the control ($\chi^2= 1.81$, df = 1, $P = 0.18$), with 62% of females entering treatment chambers containing parsley foliage and 38% choosing the control (Figure 9). Male response to parsley was significantly higher than female response (χ^2= 13.79, df = 1, $P < 0.001$)

When offered the choice between volatiles of carrot root and a moist cotton ball control in the absence of host visual or tactile cues, significantly more females chose the carrot root over the control treatment. Seventy-seven percent of responding females chose chambers containing carrot root (χ^2= 6.55, df = 1, $P = 0.01$), while 23% of females chose the control (Figure 10). Males exhibited a similar response, with 88% of responders entering chambers containing carrot root, and 12% choosing the control (χ^2 = 14.44, df = 1, $P < 0.001$) (Figure 10). Male response was not significantly different from female response (χ^2 = 2.37, df = 1, $P = 0.12$)

When I evaluated carrot weevil preference for volatiles from carrot root and parsley foliage, female weevils did not discriminate between carrot and parsley volatiles in the first two trials (χ^2=3, df=1, $P = 0.08$; χ^2=1.33, df=1, $P = 0.25$). However, on the third date females exhibited a stronger response to carrot over parsley, with 93% of females orienting towards carrot volatiles (χ^2= 10.29, df = 1, $P = 0.001$). When I analyzed female response across all dates, I found that female carrot weevils did not show a preference for either host (χ^2= 0.11, df=1, $P = 0.7456$). Overall, male weevils did not exhibit a preference for volatiles of either host, with 61% and 39% of responders orienting towards carrot and parsley, respectively (χ^2= 2.08, df = 1, *P* = 0.15) (Figure 11). Male response did not change across dates.

Discussion

Results of my still-air bioassays, adapted from Prokopy et al. (1995), demonstrate that carrot weevils use short-range volatile cues to find host resources, and sex-specific differences may exist in their discrimination of host volatile cues. This successful still-air bioassay represents a new and powerful tool that can be used to screen carrot weevil behavioral response to potential attractants and repellents in the laboratory.

We found that carrot weevils exhibited high levels of discrimination between carrot volatiles and the cotton ball control in the absence of visual or tactile cues. This corroborates observations of carrot weevil monitoring efforts in the field, whereby both male and female weevils are attracted to carrot-baited traps that offer only host chemical cues (Boivin 1985, Ghidiu and VanVranken 1995). The level of discrimination demonstrated by male and female weevils towards carrot was notably high, with 88% and 77% of individuals responding, respectively. Male carrot weevils also displayed high levels of discrimination between parsley volatiles (87% of responders) and the control (13% of responders). In contrast, female carrot weevils did not appear to discriminate significantly between volatiles from parsley (62% of responders) and the control (38% of responders) when only chemical cues were present. Furthermore, female response was significantly different compared to male response suggesting that males have a broader response to host volatiles than females. Similarly high levels of discrimination have been observed in bioassays with the vine weevil, wherein ~75% of individuals oriented towards host volatiles from yew and spindle tree versus a clean-air control (Van Tol and Visser 2002). Still-air bioassays have also been used to evaluate responses of the lesser grain borer and apple blossom weevil to host volatiles with success, allowing researchers to identify the most attractive hosts, as well as the most attractive components of the host's volatile profile (Edde and Phillips 2006, Piskorski and Dorn 2010). The high level of discrimination observed in these bioassays indicates carrot weevils perceive and are sensitive to host volatiles.

When carrot weevils were exposed to parsley and carrot volatiles together, males across all three experimental dates and females across two dates did not show a preference for either host. However, on the third date females exhibited a preference for carrot volatiles. Given the experimental design, whereby we presented carrot weevils with the choice of 0.25 grams of parsley foliage or 2 grams of carrot root, it is possible that the amount of tissue offered may have influenced carrot weevil response to either host, such that response to carrot was greater simply because more carrot tissue was offered. Moreover, differing volatile release rates, emerging from differences in the surface area of presented tissues (sliced carrot root versus whole parsley leaves), may have also influenced carrot weevil response to volatile cues. Both carrot and parsley are known to have similar volatile profiles that are dominated by terpenes (MacLeod et al. 1985, Alasalvar et al. 2001). However, the abundance and composition of compounds within a plant's volatile profile can affect insect behavioral response, acting as an attractant or repellent, depending on the abundance and ratios of key compounds (Bruce et al. 2005, Bruce and Pickett 2011). The age of the weevils could also affect carrot weevil preference, which may have contributed to the change in female response to carrot and parsley volatiles. In addition, mating status could have affected carrot weevil response to host volatiles. For example, mating status has been shown to affect host volatile preference of the coffee berry borer. Virgin female coffee berry borers were repelled by red coffee berry odor, while mated females that had not laid eggs were attracted to red berry odor (Mathieu et al. 2001).

Male and female response to carrot volatiles was not significantly different. However, female response to parsley volatiles was significantly lower than that of male weevils. These results suggest that female carrot weevils may differ from males in their host-finding behavior. Moreover, a higher ratio of male to female weevils has been observed in carrot-baited traps in the field (E. Long, unpublished data), which further suggests that males may be more responsive to host plant volatiles. These results indicate that carrot weevils orient towards and are attracted to host volatiles; however, males may exhibit a broader response to host volatiles than females. Taken together, results from this study demonstrate that the still-air bioassay is a reliable method for evaluating the behavioral responses of male and female carrot weevils to host volatiles.

Sex-specific differences in host-finding behavior are common among insects, with males and females exhibiting varying levels of response to host volatiles (Edde and Phillips 2006, Sun et al. 2010, Szendrei et al. 2011). For example, only 50% of male annual bluegrass weevils, a close relative of the carrot weevil, were attracted to volatiles from annual bluegrass, while 68% of females chose annual bluegrass over the control (McGraw et al. 2011). The observed differences in male and female carrot weevil response to host volatiles, may suggest that other cues play a role in female host-finding behavior. Both visual and tactile cues can play an important role in host plant discrimination by insects (Prokopy and Owens 1983, Finch and Collier 2000). For example, the Argentine stem weevil does not discriminate between endophyte-free versus endophyte-infected ryegrass when only volatiles are presented, although endophyte infection is known to alter the volatile profile of the host (Qawasmeh et al. 2015). Yet when feeding preference was evaluated, Argentine stem weevils were able to discriminate between endophyte-infected and endophyte-free ryegrass, exhibiting a preference for endophyte-free ryegrass (Pilkington 1987). Thus, a variety of cues can influence the host-finding behaviors and preferences of weevils. Another possible explanation for the sex-specific differences we observed in carrot weevil response to host volatiles is male carrot weevils may colonize host plants first and subsequently attract females to suitable feeding or oviposition sites. Such attraction could occur via plant volatiles released during feeding, or the release of an aggregation pheromone. For example, boll weevil and plum curculio males produce aggregation pheromones, which affect the attractiveness of plant volatiles to both male and female conspecifics (Reddy and Guerrero 2004, Tewari et al. 2014). However, it is unclear if carrot weevils use pheromones for mating or aggregation. Future studies are needed to evaluate whether the presence of conspecifics influences carrot weevil behavioral response to and preference for host plants.

By elucidating carrot weevil host-finding behavior we can move towards the development of monitoring strategies, like those for the pepper weevil and plum curculio, which use host volatiles in combination with other chemical and visual cues to improve detection and inform management decisions (Riley and Schuster 1994, Leskey and Wright 2004). A semiochemical-based approach could improve carrot weevil monitoring in the field by enhancing the level of attraction or the longevity of monitoring traps, making a trap-based monitoring strategy more efficient. Semiochemical-based management strategies have been developed for many insect pests, including the behavioral manipulation of insects for improved population suppression (Reddy and Guerrero 2004, Cook et al. 2007, Smart et al. 2014, Khan et al. 2016, Wallingford et al. 2018). The success of these strategies hinges upon basic understanding of a pest's behavior. Research that examines the combined roles of chemical, physical, and visual cues on carrot weevil host-finding behavior and preference will aid in the development of more effective and sustainable monitoring strategies in the future.

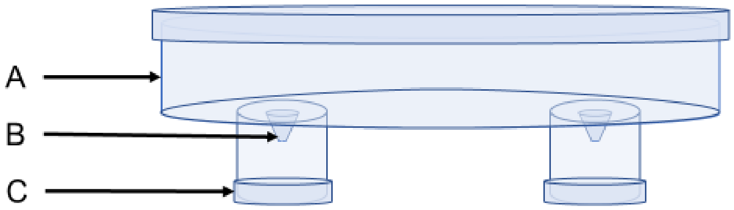

Figure 8. Diagram of the still-air arena used for carrot weevil bioassays. Each arena consisted of an introduction chamber (A) connected by two pipette-tip channels (B) to two small treatment chambers (C). Figure not drawn to scale.

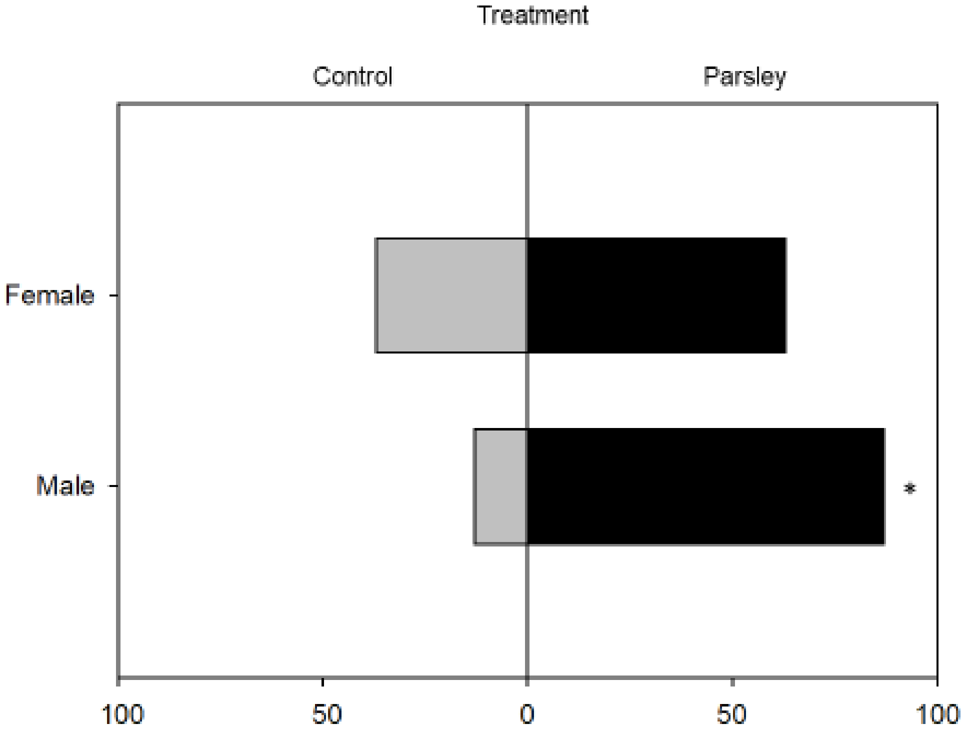

Figure 9. The percentage of female (n=27) and male (n=30) adult carrot weevils responding to volatiles originating from parsley foliage versus a moist cotton ball in still-air bioassays. * denotes $P < 0.001$.

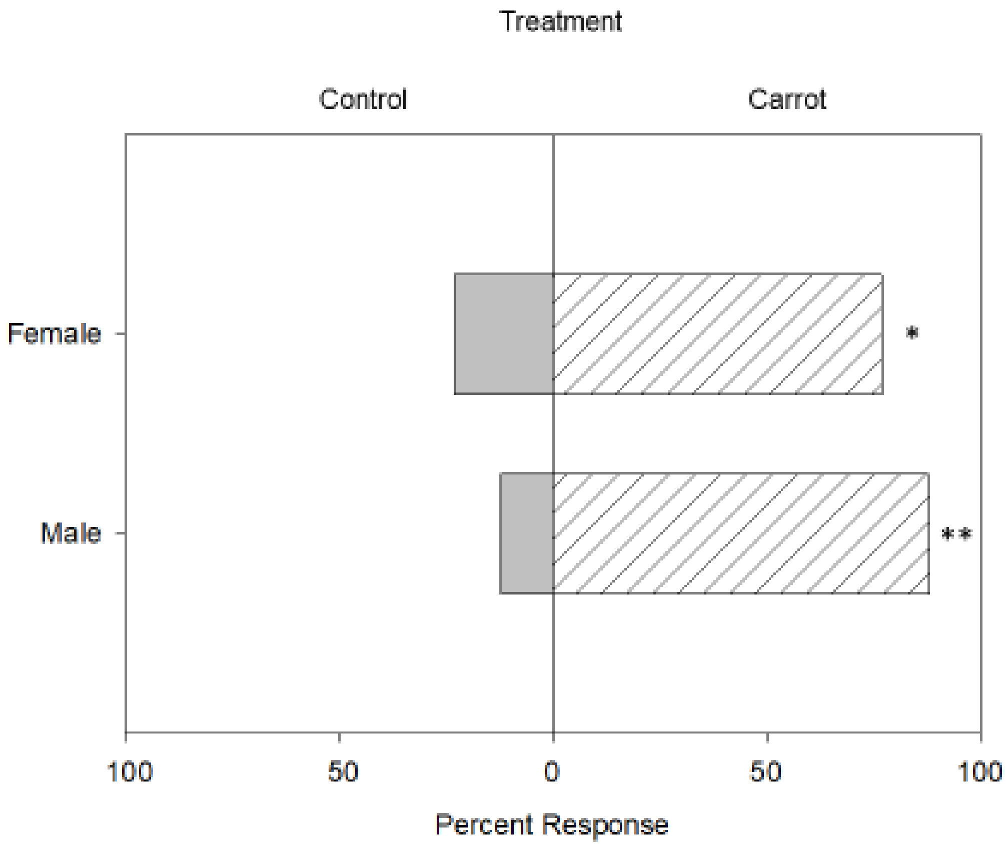

Figure 10. The percentage of female (n=22) and male (n=25) adult carrot weevils responding to volatiles originating from carrot root versus a moist cotton ball in still-air bioassays. * denotes $P < 0.05$, ** denotes $P < 0.01$.

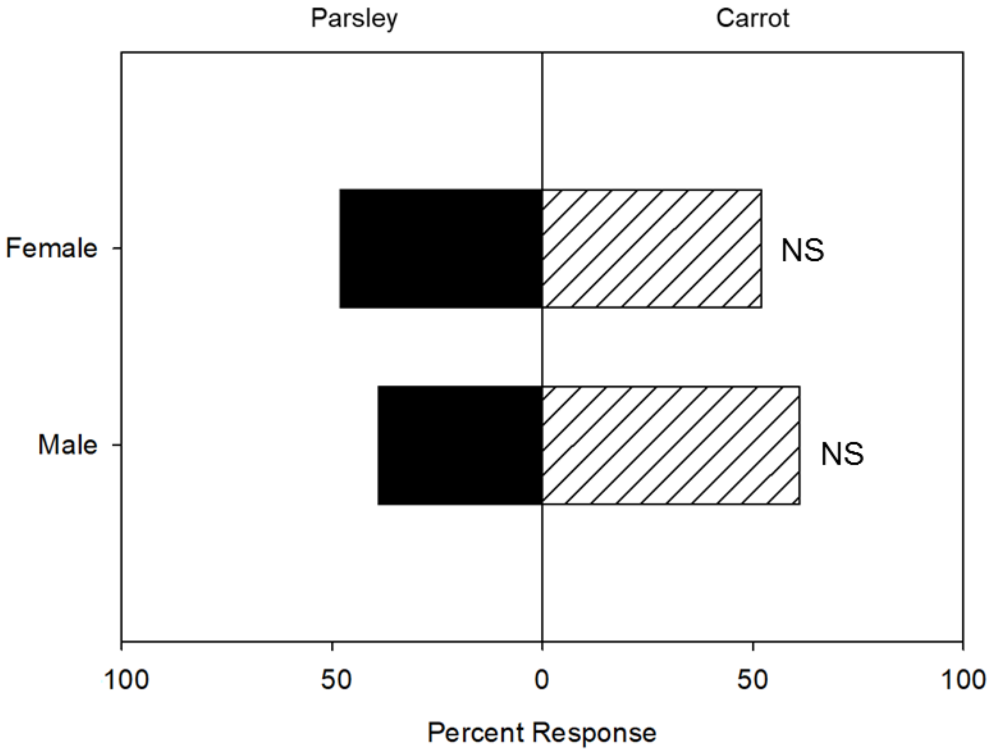

Figure 11. The percentage of female (n=38) and male (n=39) adult carrot weevils responding to volatiles originating from carrot root versus parsley foliage in still-air bioassays. NS denotes no significant difference.

Table 2. Number of male and female carrot weevils that made either no choice, chose the treatment chamber containing a host, or the chamber containing the control.

Experiment	**Sex**	**N/total**[a]	**Choice**	**No. responders**
1. Parsley	Male	30/59	Parsley	26
		(50%)	Control	4
	Female	27/60	Parsley	17
		(45%)	Control	10
2. Carrot	Male	25/54	Carrot	22
		(46%)	Control	3
	Female	22/56	Carrot	17
		(39%)	Control	5
3. Carrot vs. Parsley	Male	39/60	Carrot	24
		(65%)	Parsley	15
	Female	38/59	Carrot	20
		(64%)	Parsley	18

[a] N= total number of weevils responding to a treatment; total= the total number of weevils tested.

Chapter 3: Monitoring Overwintering Carrot Weevil Activity to Develop an Activity Model in Ohio

Abstract

Degree-day models are powerful tools that can be used to inform pest management decisions. For the carrot weevil, degree-day models have been developed to predict developmental time and emergence from overwintering. However, the only emergence degree-day model currently available was developed in Canada and may not apply to carrot weevil activity in Ohio. My goals were to 1) assess the utility of modified-Boivin traps as early season (prior to crop emergence) monitoring tools for overwintering carrot weevils, and 2) generate an emergence degree-day model specific to Ohio carrot weevil populations. Towards these goals, modified-Boivin traps were placed on the edge of previously infested parsley fields in 2017 and overwintered parsley fields in 2018. In both years, we observed that male carrot weevils consistently outnumbered females, with twice as many males as females at any given time. Interestingly, the degree-day models describing overwintering adult activity varied between years. In 2017, adult emergence was earlier as well as shorter, with 50% cumulative emergence of overwintering adults estimated to occur at 167 $CDD_{7.0\,°C}$. In contrast, in 2018 overwintering adult activity began later and lasted longer, with 50% of adult emergence estimated to occur at approximately 450 $CDD_{7.0\,°C}$. These results suggest that the presence of an overwintered

host crop can influence carrot weevil dispersal and therefore the utility of monitoring tools placed in proximity to these fields. Furthermore, monitoring previously infested sites could be used to inform management decisions.

Introduction

Understanding the emergence and dispersal patterns of pest insects is a critical step in development of a successful integrated pest management program. Degree-day modeling is a tool that has been used to predict pest emergence and dispersal patterns in multiple crop-weevil systems (Parajulee et al. 1996, Merrill et al. 2010, Akotsen-Mensah et al. 2011, Aghaee and Godfrey 2014). For example, a degree-day model for the emergence of rice water weevil was developed from trap catch (Zou et al. 2004). This degree-day model informed growers of the period of peak activity and led to earlier planting dates to avoid infestations. As a result, growers that planted earlier had higher yields than those who planted later and treated with insecticides (Thompson et al. 1994, Aghaee and Godfrey 2014).

Previous research has characterized temperature-based carrot weevil development through life stages in the lab and found that it takes 630 cumulative degree days (CDD) to complete one generation (Simonet and Davenport 1981). Additionally, an activity model based on cumulative degree days has also been described for the carrot weevil in Quebec, with a base temperature of 3.8 °C (Rhéaume 2009). However, emergence and activity patterns may vary across the carrot weevil's geographic range due to differences in climate, or agricultural practices such as crop rotation or tillage. For example, parsley plants may be left to overwinter in the field after final harvest, which could alter dispersal behavior to overwintering sites (Torres and Hoy 2002a). Furthermore, it is suspected that carrot weevil may be exhibiting an additional generation in the Canadian providence of Quebec (Boivin et al. 2013). Therefore, models developed in Canada may not accurately predict the activity of overwintering weevils in Ohio.

The carrot weevil is the most important pest of fresh parsley in muck soil regions of Ohio. Growers in these regions have struggled to manage carrot weevils for the reasons described previously in Chapter 1. Historically, baited traps have been used in north central Ohio to monitor adult carrot weevils after crop germination. However, these traps have proven unreliable in attracting adults during the growing season. Meanwhile, the presence of egg scars on plants indicates weevils are active in the crop, despite their absence in baited traps. The limited success attracting adults to traps in the presence of crop hosts suggests that baited traps must compete with crop odors to attract carrot weevils. It is unclear if modified-Boivin traps may prove more successful for monitoring the activity of overwintering adults, which are active prior to the germination of crop hosts.

The goals of this study were to 1) evaluate the utility of modified-Boivin traps as monitoring tools when placed out early in the season (prior to crop emergence), and 2) develop a predictive degree-day model for the emergence of overwintering adult carrot weevils in Ohio.

Methods

Field Sites and Collections

In collaboration with producers, we identified fields that were infested with carrot weevils the previous year and returned to these sites to monitor the emergence of overwintering adults. In 2017, we placed twelve modified-Boivin traps on the edges of three fields that contained parsley and were infested in 2016. We placed traps when the fields were empty of any crop (host or non-host) and checked them twice each week to record the number and sex of the weevils present in each trap. We monitored traps from April 7 - June 12, 2017. In 2018 we followed the same approach, except that we placed traps on the margin of a previously infested field where parsley was left to overwinter. In May, overwintered parsley was tilled out and green onion was seeded into the field. We continued to monitor carrot weevil emergence from this previously infested field in the presence of green onion, a non-host crop. Traps were monitored from April 10 - July 5, 2018.

Degree-day Models

To develop predictive models for the activity of overwintering carrot weevil adults, cumulative degree days (CDD) were calculated from a biofix date of March 1, with a single sine calculation method in DegDay v. 1.01 (Snyder 2002). We used the daily maximum/minimum temperature data collected from the nearest weather station located at the Muck Crops Research Station in Willard, OH. The lower developmental threshold was set at 7.0 °C (Simonet and Davenport 1981). The relationships between degree-day accumulations and the cumulative proportion of adults collected in the modified-Boivin traps were analyzed using a two- [$y=1/[1+\exp(a+b*x)]$] or three-parameter [$y=1/[1+\exp(a+b*x^c)]$] Weibull cumulative distribution function, where y is the cumulative proportion of adults collected, x is cumulative degree-days (base 7.0 °C), and a, b, and c are shape, scale, and location parameters, which describe the slope, spread, and x-intercept of the predicted curve, respectively. These parameters were estimated in the least squares non-linear estimation module of Statistica 13.0 (Dell 2015) using the Levenberg-Marquardt method and starting values of 0.001 for all parameters (Duffy et al. 2018a). For both years, the start date for overwintering adult activity was determined as the first date on which adult weevils were detected in any of the modified Boivin traps whereas the end date was determined as the date at which fewer than 20 total adults were captured in all traps for two consecutive trapping periods.

Results and Discussion

We collected 2,474 adult weevils in modified-Boivin traps in 2017, and 2,074 individuals in 2018. In both years, we observed that male carrot weevils consistently outnumbered females in baited traps. For example, at any given time during the monitoring period, there were nearly twice the number of males as females. A similar pattern has been observed with cabbage seedpod weevils, whereby more male than females were caught in emergence traps at the beginning of the season, but during peak emergence the sex ratio was 1:1, and after peak emergence the sex ratio switched in favor of females (Ulmer and Dosdall 2006). One possible explanation for the male-biased sex ratio observed in carrot weevil monitoring traps is that males have a broader response to host volatiles and recruit to baited traps more than females. However, it is also possible that the population of weevils we monitored simply contained more males than females, or that females became active later than males.

The degree-day models describing overwintering adult activity varied between years. In 2017, a two parameter Weibull cumulative distribution function (Table 3) provided the best fit to the data, describing 98.1% of variation in the relationship between cumulative degree-days (base 7.0 °C) ($CDD_{7.0\ °C}$) and the cumulative proportion of total adults caught in the modified-Boivin traps (Figure 12). Fifty percent cumulative emergence of overwintering adults was estimated at approximately 167 $CDD_{7.0\ °C}$ that year, when the crop host was absent from the field, with nearly 99% emergence being estimated by 500 $CDD_{7.0\ °C.}$

In contrast, the degree-day model describing overwintering adult activity in 2018 was best fit by a three parameter Weibull cumulative distribution function (Table 3), which described 98.8% of variation in the relationship between $CDD_{7.0\ °C}$ and the cumulative proportion of total adults caught in the modified-Boivin traps (Figure 13). In 2018, the year when overwintered parsley was present in the field during carrot weevil emergence from overwintering sites, 50% of adult emergence was estimated at approximately 450 $CDD_{7.0\ °C}$ with 99% emergence being estimated at greater than 1000 $CDD_{7.0\ °C.}$ Interestingly the emergence model developed by Rhéaume (2009) had four parameters which is different from both models presented here. This difference could be caused by the length of the monitoring period, which extended into September.

Although unreplicated, these findings suggest that cropping practices, like leaving hosts in the field to overwinter, may influence the timing and dispersal patterns of carrot weevils caught in modified-Boivin traps from previously infested fields to surrounding fields in the landscape. For example, in 2017 when no host plants were present in monitored fields, the period of overwintering carrot weevil emergence was estimated to be both earlier and shorter in duration than in 2018, when overwintered parsley was present. This suggests that carrot weevils readily dispersed from empty fields, presumably in search of hosts, where they were intercepted by modified-Boivin traps. In contrast, when a crop host was present in 2018, the period of overwintering adult emergence was estimated to begin later and last twice as long. This suggests that carrot weevils exhibited a delay in dispersal activity and trickled more steadily from the field containing overwintered parsley into adjacent fields in the landscape. Torres and Hoy (2002) observed a greater number of adults and oviposition scars in overwintered parsley in the same region of Ohio, further supporting the idea that the presence of this host resource alters carrot weevil dispersal behavior. Taken together, these results suggest that crop hosts left to overwinter in the field may serve to retain overwintering adults, such that their dispersal from these sites is delayed in the spring. These results may also suggest that the presence of hosts alters carrot weevil perception of, or attraction to, carrot bait used in modified-Boivin traps. This could explain why the period of carrot weevil capture was relatively quick in the absence of hosts (no competition with trap bait), but prolonged when a crop host was present. Future research evaluating the role of host-plant volatile competition on carrot weevil response to baited traps in the presence of hosts, non-hosts, and intercropped species of both could reveal potential strategies to exploit plant semiochemicals for improved carrot weevil management.

Furthermore, these results suggest that monitoring previously-infested sites, even those that are planted with non-hosts the following year, might be useful in estimating the abundance and activity of overwintering carrot weevils and the potential risk of re-infestation posed by these sources of carrot weevils in the landscape. The emergence patterns predicted by these models can help growers synchronize crop protection activities against carrot weevils based on CDD, rather than based on trap monitoring or scouting egg-laying scars.

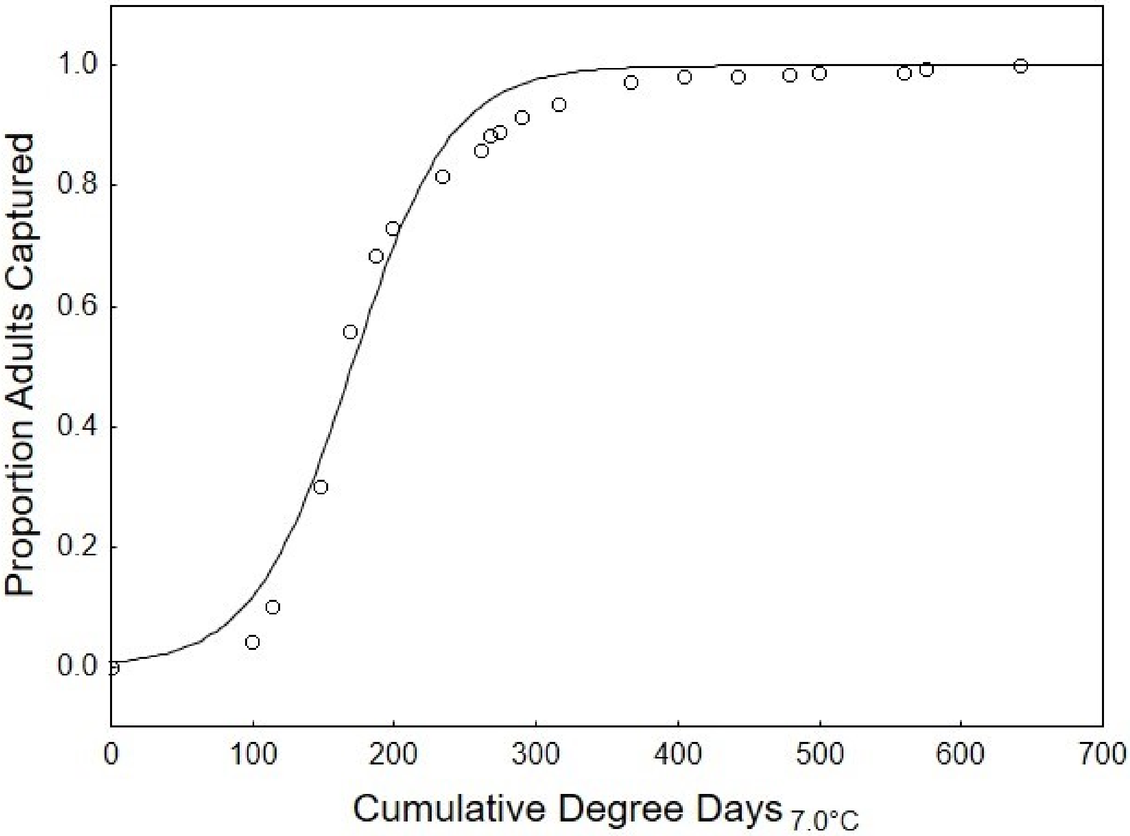

Figure 12. Degree day model (base 7.0 °C) describing the activity of overwintering adult carrot weevil adults in Ohio in 2017, when no crops were present.

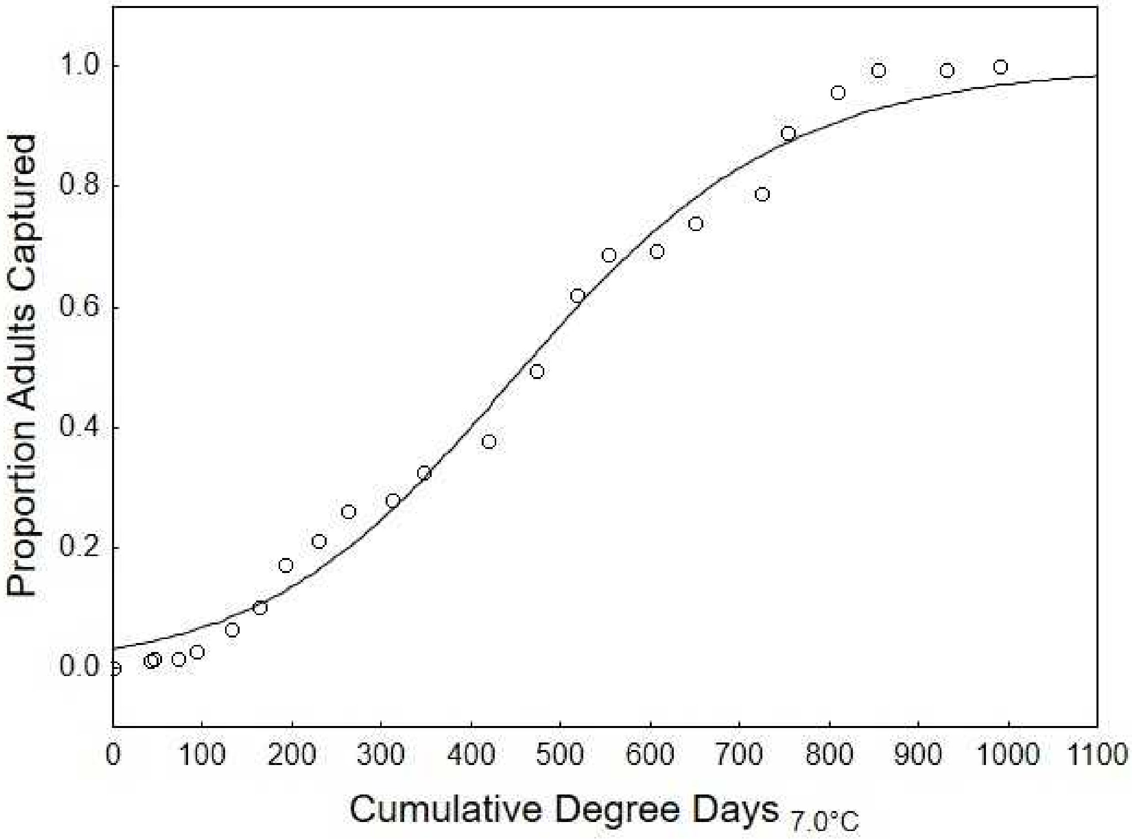

Figure 13. Degree day model (base 7.0 °C) describing the activity of overwintering adult carrot weevil adults in Ohio in 2018, when overwintered parsley was present.

Table 3. Parameter estimates and regression statistics for two- and three-parameter Weibull-cumulative distribution functions describing the relationship between cumulative degree days (base 7.0 °C) and activity of overwintering adult carrot weevils (*Listronotus oregonensis*) in Ohio from April 7 - June 12 in 2017 and April 10 - July 5 in 2018.

Year	**R^2**	**Mean Square Error**	**df**	**Parameter**	**Estimate (±SE)**	**t-value**	**P-value**
2017	0.981	0.0023	2, 19	a	4.900 ± 0.452	10.8	< 0.0001
2017	0.981	0.0023	2, 19	b	-0.029± 0.003	-11.3	< 0.0001
2017	---	---	---	c	NS	---	---
2018	0.988	0.0017	3, 21	a	2.468 ± 0.964	2.6	0.0138
2018	0.988	0.00017	3, 21	b	-0.006 ± 0.001	-7.5	< 0.0001
2018	0.988	0.00017	3, 21	c	1.363 ± 0.597	2.3	0.0329

Bibliography

Addesso, K. M., and H. J. McAuslane. 2009. Pepper weevil attraction to volatiles from host and nonhost plants. Environmental entomology 38: 216-224.

Aghaee, M.-A., and L. D. Godfrey. 2014. A Century of Rice Water Weevil (Coleoptera: Curculionidae): A History of Research and Management With an Emphasis on the United States. Journal of Integrated Pest Management 5: D1-D14.

Akotsen-Mensah, C., R. T. Boozer, A. G. Appel, and H. Y. Fadamiro. 2011. Seasonal Occurrence and Development of Degree-Day Models for Predicting Activity of *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in Alabama Peaches. Annals of the Entomological Society of America 104: 192-201.

Alasalvar, C., J. M. Grigor, D. Zhang, P. C. Quantick, and F. Shahidi. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. Journal of Agricultural and Food Chemistry 49: 1410-1416.

Arancibia, R. A., Mark S. Reiter, Steve L. Rideout, Tom P. Kuhar, L. K. Strawn, David B. Langston, J. S. Jr., James Wilson, and H. Doughty. 2019. Mid-Atlantic Commercial Vegetable Production Recommendations Virginia Cooperative Extension.

Baines, D., R. Stewart, and G. Boivin. 1990. Consumption of carrot weevil (Coleoptera: Curculionidae) by five species of carabids (Coleoptera: Carabidae) abundant in carrot fields in southwestern Quebec. Environmental entomology 19: 1146-1149.

Bal, H. K., N. Acosta, Z. Cheng, H. Whitehead, P. S. Grewal, and C. W. Hoy. 2014. Effect of soil management on *Heterorhabditis bacteriophora* GPS11 persistence and biological control in a vegetable production system. Biological Control 79: 75-83.

Baudoin, G., and G. Boivin. 1985. Effets d'accouplements répétés sur l'oviposition du charançon de la carotte, *Listronotus oregonensis* (Coleoptera: Curculionidae) en laboratoire. Ann. Soc. Entomol. Québec 30: 23-27.

Bélair, G., and G. Boivin. 1985. Susceptibility of the carrot weevil (Coleoptera: Curculionidae) to *Steinernema feltiae*, *S. bibionis, and Heterorhabditis heliothidis*. Journal of Nematology 17: 363.

Björklund, N. 2008. Cues for shelter use in a phytophagous insect. Journal of insect behavior 21: 9-23.

Björklund, N., G. Nordlander, and H. Bylund. 2003. Host-plant acceptance on mineral soil and humus by the pine weevil *Hylobius abietis* (L.). Agricultural and Forest Entomology 5: 61-66.

Boivin, G. 1985. Evaluation of Monitoring techniques for the carrot weevil, *Listronotus oregonensis* (Coleotera:Curculionidae). The Canadian Entomologist 117: 927-933.

Boivin, G. 1988. Effects of carrot developmental stages on feeding and oviposition of carrot weevil, *Listronotus oregonensis* (Le Conte)(Coleoptera: Curculionidae). Environmental entomology 17: 330-336.

Boivin, G. 1999. Integrated management for carrot weevil. Integrated Pest Management Reviews 4: 21-37.

Boivin, G., P. G. Parker, and D. R. Gillespie. 2013. Biological control programmes in Canada 2001-2012, pp. 214-220. CABI, Wallingford, United Kingdom.

Boyce, A. M. 1927. A Study of the Biology of the Parsley Stalk-Weevil *Listronotus Latiusculus* Boheman Coleoptera: Curculionidae. Journal of Economic Entomology 20: 814-821.

Bruce, T. J., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: a volatile situation. Trends in plant science 10: 269-274.

Bruce, T. J. A., and J. A. Pickett. 2011. Perception of plant volatile blends by herbivorous insects - Finding the right mix. Phytochemistry 72: 1605-1611.

Butkewich, S. L., and R. J. Prokopy. 1993. The effect of short-range host odor stimuli on host fruit finding and feeding behavior of plum curculio adults (Coleoptera: Curculionidae). Journal of chemical ecology 19: 825-835.

Bykova, O., and S. Blatt. 2018. Effect of soil type on carrot weevil movement behavior. Journal of insect behavior 31: 321-333.

Capinera, J. 2001. Handbook of vegetable pests, pp. 121-122. Academic Press, London, United Kingdom.

Chittenden, F. 1909. The parsley stalk weevil. US Dept. Agric. Bull 82: 14-19.

Chittenden, F. 1924. The parsley stalk weevil, *Listronotus latiusculus* Boheman, a potential pest. Bull. Brooklyn Entomol. Soc 19: 84-86.

Collatz, J., and S. Dorn. 2013. A host-plant-derived volatile blend to attract the apple blossom weevil *Anthonomus pomorum* – the essential volatiles include a repellent constituent. Pest management science 69: 1092-1098.

Collins, R., and E. Grafius. 1984. A literature review of the carrot weevil, *Listronotus oregonensis*. Dept. Entomology, Michigan State University, East lansing, Michigan, Report 14.

Collins, R., and E. Grafius. 1986. Biology and life cycle of *Anaphes sordidatus* (Hymenoptera: Mymaridae), an egg parasitoid of the carrot weevil (Coleoptera: Curculionidae). Environmental entomology 15: 100-105.

Cook, S. M., Z. R. Khan, and J. A. Pickett. 2007. The use of push-pull strategies in integrated pest management. Annual review of entomology 52: 375-400.

Curl, C. L., R. A. Fenske, J. C. Kissel, J. H. Shirai, T. F. Moate, W. Griffith, G. Coronado, and B. Thompson. 2002. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. Environmental Health Perspectives 110: A787-A792.

Dell Inc. 2015. Dell Statistica (data analysis software system), version 13. software. dell.com.

Dickens, J. C. 1989. Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. Entomologia Experimentalis et Applicata 52: 191-203.

Disney, R., Y. Erzinclioglu, D. d. C. Henshaw, D. Howse, D. Unwin, P. Withers, and A. Woods. 1982. Collecting methods and the adequacy of attempted fauna surveys, with reference to the Diptera. Field studies. London 5: 607-621.

Duffy, A. G., G. S. Powell, J. M. Zaspel, and D. S. Richmond. 2018a. Billbug (Coleoptera: Dryophthoridae: *Sphenophorus spp.*) Seasonal Biology and DNA-Based Life Stage Association in Indiana Turfgrass. Journal of Economic Entomology 111: 304-313.

Duffy, A. G., G. P. Hughes, M. D. Ginzel, and D. S. Richmond. 2018b. Volatile and Contact Chemical Cues Associated with Host and Mate Recognition Behavior of *Sphenophorus venatus* and *Sphenophorus parvulus* (Coleoptera: Dryophthoridae). Journal of chemical ecology 44: 556-564.

Edde, P. A., and T. W. Phillips. 2006. Potential host affinities for the lesser grain borer, *Rhyzopertha dominica*: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts. Entomologia Experimentalis et Applicata 119: 255-263.

Egel, D. S., Rick Foster, Elizabeth Maynard, Mohammad Babadoost, C. R. Ajay Nair, Megan Kennelly, Mary Hausbeck, Zsofia Szendra, Bill Hutchison, Touria Eaton, Celeste Welty, and S. Miller. 2019. Midwest Vegetable Production Guide for Commercial Gorwers. Purdue University Extension Publication.

Evenden, M. L., C. M. Whitehouse, A. St. Onge, L. Vanderark, J. P. Lafontaine, S. Meers, and H. A. Cárcamo. 2016. Potential for semiochemical-based monitoring of the pea leaf weevil (Coleoptera: Curculionidae) on field pea (Fabaceae) in the Canadian Prairie Provinces. The Canadian Entomologist 148: 595-602.

Ferrari, A., A. Venturino, and A. M. P. de D'Angelo. 2007. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. Pesticide Biochemistry and Physiology 88: 134-142.

Finch, S., and R. Collier. 2000. Host-plant selection by insects–a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata 96: 91-102.

Gagnon, A.-È., G. Boivin, G. Bélair, and B. Mimee. 2018. Prevalence of a nematode castrator of the carrot weevil and impact on fecundity and survival. Parasitology: 1-6.

Ghidiu, G. M., and R. W. VanVranken. 1995. A modified carrot weevil (Coleoptera: Curculionidae) monitoring trap. The Florida Entomologist 78: 627-630.

Grafius, E., and R. Collins. 1986. Overwintering sites and survival of the carrot weevil, Listronotus oregonensis (Coleoptera: Curculionidae). Environmental entomology 15: 113-117.

Harris, H. M. 1926. A new carrot pest, with notes on its life history. Journal of Economic Entomology 19: 494-496.

Hopper, L., J.-P. Le Blanc, and G. Boivin. 1996. The detection of *Anaphes sp*. nov.[Hymenoptera: Mymaridae], an egg parasitoid of the carrot weevil in Nova Scotia. Phytoprotection 77: 79-82.

Huber, J. T., S. Côté, and G. Boivin. 1997. Description of three new *Anaphes* species (Hymenoptera: Mymaridae), egg parasitoids of the carrot weevil, *Listronotus oregonensis* (LeConte)(Coleoptera: Curculionidae), and redescription of *Anaphes sordidatus* Girault. The Canadian Entomologist 129: 959-977.

Hulcr, J., R. Mann, and L. L. Stelinski. 2011. The Scent of a Partner: Ambrosia Beetles Are Attracted to Volatiles from Their Fungal Symbionts. Journal of chemical ecology 37: 1374-1377.

Jasinski, J. 2008. Parsley pest management strategic plan for Ohio. *In* U. S. D. o. Agriculture [ed.].

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annual review of entomology 38: 181-206.

Khan, Z., C. A. Midega, A. Hooper, and J. Pickett. 2016. Push-pull: chemical ecology-based integrated pest management technology. Journal of chemical ecology 42: 689-697.

Lemay, J., Z. Telfer, C. Scott-Dupree, and M. R. McDonald. 2018. The Impact of the Carrot Rust Fly and Carrot Weevil Integrated Pest Management Program on the Ground-Dwelling Beetle Complex in Commercial Carrot Fields at the Holland Marsh, Ontario, Canada. Environmental entomology.

Leskey, T. C., and S. E. Wright. 2004. Monitoring plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), populations in apple and peach orchards in the mid-Atlantic. Journal of Economic Entomology 97: 79-88.

Leskey, T. C., J. C. Piñero, and R. J. Prokopy. 2008. Odor-baited trap trees: a novel management tool for plum curculio (Coleoptera: Curculionidae). Journal of Economic Entomology 101: 1302-1309.

MacLeod, A. J., C. H. Snyder, and G. Subramanian. 1985. Volatile aroma constituents of parsley leaves. Phytochemistry 24: 2623-2627.

Martel, P., H. J. Svec, and C. R. Harris. 1975. Mass rearing of the carrot weevil, *Listronotus Oregonensis* (Coleoptera: Curculionidae), under controlled environmental conditions. The Canadian Entomologist 107: 95-98.

Martel, P., H. Svec, and C. Harris. 1976. The life history of the carrot weevil, *Listronotus oregonensis* (Coleoptera: Curculionidae) under controlled conditions. The Canadian Entomologist 108: 931-934.

Mathieu, F., V. Gaudichon, L. O. Brun, and B. Frérot. 2001. Effect of physiological status on olfactory and visual responses of female *Hypothenemus hampei* during host plant colonization. Physiological Entomology 26: 189-193.

Meyer, J. R. 1976. Positive Phototaxis of Adult Alfalfa Weevils1 to Visible and near-Infrared Radiation. Annals of the Entomological Society of America 69: 21-25.

McGraw, B. A., C. Rodriguez-Saona, R. Holdcraft, Z. Szendrei, and A. M. Koppenhöfer. 2011. Behavioral and electrophysiological responses of *Listronotus maculicollis* (Coleoptera: Curculionidae) to volatiles from intact and mechanically damaged annual bluegrass. Environmental entomology 40: 412-419.

Merrill, S. C., A. Gebre-Amlak, J. Scott Armstrong, and F. B. Peairs. 2010. Nonlinear Degree-Day Models for Postdiapause Development of the Sunflower Stem Weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 103: 302-307.

Miklasiewicz, T., P. S. Grewal, C. W. Hoy, and V. Malik. 2002. Evaluation of entomopathogenic nematodes for suppression of carrot weevil. BioControl 47: 545-561.

Parajulee, M. N., L. T. Wilson, D. R. Rummel, S. C. Carroll, and P. J. Trichilo. 1996. Climatic Data-Based Analysis of Boll Weevil (Coleoptera: Curculionidae) Overwintering Survival and Spring Emergence. Environmental entomology 25: 882-894.

Pepper, B. B., and L. E. Hagmann. 1938. The carrot weevil, *Listronotus latiusculus* (Boh.), a new pest on celery. Journal of Economic Entomology 31: 262-266.

Perron, J. 1971. Insect pests of carrots in organic soils of southwestern Quebec with special reference to the carrot weevil, *Listronotus oregonensis* (Coleoptera: Curculionidae). The Canadian Entomologist 103: 1441-1448.

Petersson, M., G. Örlander, and G. Nordlander. 2005. Soil features affecting damage to conifer seedlings by the pine weevil *Hylobius abietis*. Forestry: An International Journal of Forest Research 78: 83-92.

Phillips, T., X.-L. Jiang, W. Burkholder, J. Phillips, and H. Tran. 1993. Behavioral responses to food volatiles by two species of stored-product coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). Journal of chemical ecology 19: 723-734.

Pilkington, S. 1987. The behavioural biology of Argentine stem weevil in relation to host-plant characters. Master of Science (M. Sc.) Masters, Massey University Palmerston North, New Zealand.

Piskorski, R., and S. Dorn. 2010. Early-Season Headspace Volatiles from Apple and Their Effect on the Apple Blossom Weevil *Anthonomus pomorum*. Chemistry & Biodiversity 7: 2254-2260.

Pree, D., A. Stevenson, and E. Barszcz. 1996. Toxicity of pyrethroid insecticides to carrot weevils: enhancement by synergists and oils. Journal of Economic Entomology 89: 1254-1261.

Prokopy, R. J., and E. D. Owens. 1983. Visual detection of plants by herbivorous insects. Annual review of entomology 28: 337-364.

Prokopy, R. J., S. S. Cooley, and P. L. Phelan. 1995. Bioassay approaches to assessing behavioral responses of plum curculio adults (Coleoptera: Curculionidae) to host fruit odor. Journal of chemical ecology 21: 1073-1084.

Qawasmeh, A., A. Raman, and W. Wheatley. 2015. Volatiles in perennial ryegrass infected with strains of endophytic fungus: impact on African black beetle host selection. Journal of applied Entomology 139: 94-104.

R Core Team 2017. R: A language and environment for statistical computing, version. R Foundation for Statistical Computing, Vienna, Austria.

Reddy, G. V., and A. Guerrero. 2004. Interactions of insect pheromones and plant semiochemicals. Trends in plant science 9: 253-261.

Rekika, D., K. A. Stewart, G. Boivin, and S. Jenni. 2008. Floating rowcovers improve germination and reduce carrot weevil infestations in carrot. HortScience 43: 1619-1622.

Rhéaume, A.-J. 2009. Modélisation d'un écosystème agricole tritrophique: la carotte cultivée, le charançon de la carotte (*Listronotus oregonensis*) et Anaphes victus, un parasitoïde des oeufs. Masters thesis, Université Laval Québec City, Québec.

Riley, D., and D. Schuster. 1994. Pepper weevil adult response to colored sticky traps in pepper fields. Southwestern Entomologist 19: 93-107.

Ryser, B. 1975. Investigations regarding the biology and control of the carrot weevil, *Listronotus oregonensis* (LeConte). New Jersey. MS thesis, Rutgers University, New Brunswick, NJ.

Simard, L., J. Brodeur, and J. Dionne. 2007. Distribution, abundance, and seasonal ecology of *Listronotus maculicollis* (Coleoptera: Curculionidae) on golf courses in Quebec, Canada. Journal of Economic Entomology 100: 1344-1352.

Simonet, D., and B. Davenport. 1981. Temperature requirements for development and oviposition of the carrot weevil. Annals of the Entomological Society of America 74: 312-315.

Smart, L. E., G. I. Aradottir, and T. J. A. Bruce. 2014. Role of semiochemicals in Integrated Pest Management, pp. 93-109. *In* D. P. Abrol (ed.), Integrated Pest Management. Academic Press, San Diego.

Snyder, R. L. 2002. DegDay, version 1.01. Department of Land, Air, and Water Resources: University of California, Davis, CA, USA.

St Onge, A., H. A. Cárcamo, and M. L. Evenden. 2017. Evaluation of Semiochemical-Baited Traps for Monitoring the Pea Leaf Weevil, *Sitona lineatus* (Coleoptera: Curculionidae) in Field Pea Crops. Environmental entomology 47: 93-106.

Stevenson, A., and G. Boivin. 1990. Interaction of temperature and photoperiod in control of reproductive diapause in the carrot weevil (Coleoptera: Curculionidae). Environmental entomology 19: 836-841.

Sun, X.-L., G.-C. Wang, X.-M. Cai, S. Jin, Y. Gao, and Z.-M. Chen. 2010. The tea weevil, *Myllocerinus aurolineatus*, is attracted to volatiles induced by conspecifics. Journal of chemical ecology 36: 388-395.

Szendrei, Z., E. Malo, L. Stelinski, and C. Rodriguez-Saona. 2009. Response of Cranberry Weevil (Coleoptera: Curculionidae) to Host Plant Volatiles. enve Environmental Entomology 38: 861-869.

Szendrei, Z., A. Averill, H. Alborn, and C. Rodriguez-Saona. 2011. Identification and field evaluation of attractants for the cranberry weevil, *Anthonomus musculus* Say. Journal of chemical ecology 37: 387-397.

Telfer, Z., J. Lemay, M. R. McDonald, and C. Scott-Dupree. 2018. Assessing new chemical control options for the carrot weevil (*Listronotus oregonensis*) and carrot rust fly (*Psila rosae*) in Ontario. Crop Protection 109: 86-94.

Tewari, S., T. C. Leskey, A. L. Nielsen, J. C. Piñero, and C. R. Rodriguez-Saona. 2014. Use of Pheromones in Insect Pest Management, with Special Attention to Weevil Pheromones, pp. 141-168. Academic Press, Cambridge, Massachusetts.

Thompson, R. A., S. S. Quisenberry, F. K. N'guessan, A. M. Heagler, and G. Giesler. 1994. Planting Date as a Potential Cultural Method for Managing the Rice Water Weevil (Coleoptera: Curculionidae) in Water-Seeded Rice in Southwest Louisiana. Journal of Economic Entomology 87: 1318-1324.

Torres, A. N. 2001. Biology, ecology, and decision rules for carrot weevil, *Listronotus oregonensis* (LeConte)(Coleoptera: Curculionidae) management in parsley in the Great Lakes region. Doctorate, The Ohio State University.

Torres, A. N., and C. W. Hoy. 2002a. Relationship between carrot weevil (Coleoptera: Curculionidae) infestation, damage, and planting dates of parsley (Apiaceae). The Canadian Entomologist 134: 125-135.

Torres, A. N., and C. W. Hoy. 2002b. Sampling scheme for carrot weevil (Coleoptera: Curculionidae) in parsley. Environmental entomology 31: 1251-1258.

Ukeh, D. A., C. M. Woodcock, J. A. Pickett, and M. A. Birkett. 2012. Identification of Host Kairomones from Maize, *Zea mays*, for the Maize Weevil, *Sitophilus zeamais*. Journal of chemical ecology 38: 1402-1409.

Ulmer, B. J., and L. M. Dosdall. 2006. Spring Emergence Biology of the Cabbage Seedpod Weevil (Coleoptera: Curculionidae). Annals of the Entomological Society of America 99: 64-69.

USDA-NASS. 2012. 2012 Census of Agriculture United States Summary and State Data, pp. 34. *In* U. S. D. o. Agriculture [ed.].

Van Tol, R., and J. Visser. 2002. Olfactory antennal responses of the vine weevil *Otiorhynchus sulcatus* to plant volatiles. Entomologia Experimentalis et Applicata 102: 49-64.

Van Tol, R., J. Visser, and M. Sabelis. 2002. Olfactory responses of the vine weevil, Otiorhynchus sulcatus, to tree odours. Physiological Entomology 27: 213-222.

Vittum, P. J. 2005. Annual bluegrass weevil: a metropolitan pest on the move. Golf Course Manag 73: 105-108.

Vrdoljak, S. M., and M. J. Samways. 2012. Optimising coloured pan traps to survey flower visiting insects. Journal of Insect Conservation 16: 345-354.

Wallingford, A. K., D. H. Cha, and G. M. Loeb. 2018. Evaluating a push–pull strategy for management of *Drosophila suzukii* Matsumura in red raspberry. Pest management science 74: 120-125.

Wee, S. L., A. M. El-Sayed, A. R. Gibb, V. Mitchell, and D. M. Suckling. 2008. Behavioural and electrophysiological responses of *Pantomorus cervinus* (Boheman)(Coleoptera: Curculionidae) to host plant volatiles. Australian journal of entomology 47: 24-31.

Whitcomb, W. D. 1965. carrot weevil in Massachusetts.

Zeng, Y., R. M. Giblin-Davis, Y. Weimin, G. Bélair, G. Boivin, and K. W. Thomas. 2007. *Bradynema listronoti* n. sp.(Nematoda: Allantonematidae), a parasite of the carrot weevil *Listronotus oregonensis* (Coleoptera: Curculionidae) in Quebec, Canada. Nematology 9: 608-622.

Zhao, D. X., G. Boivin, and R. K. Stewart. 1991. Simulation model for the population dynamics of the carrot weevil, *Listronotus oregonensis* (LeConte) (Coleoptera: Curculionidae). The Canadian Entomologist 123: 63-76.

Zou, L., M. J. Stout, and D. R. Ring. 2004. Degree-Day Models for Emergence and Development of the Rice Water Weevil (Coleoptera: Curculionidae) in Southwestern Louisiana. Environmental entomology 33: 1541-1548.