AN INVESTIGATION OF SUSTAINABLE MANAGEMENT OF CARROT WEEVIL IN THE HOLLAND MARSH, ONTARIO

By

Zachariah Donald Scott Telfer

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ABSTRACT

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Zachariah Donald Scott Telfer | **Advisors**
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The carrot weevil (CW), *Listronotus oregonensis* (LeConte), is a major carrot pest in Canada. Larval CW feeding renders carrot roots unmarketable. The efficacy of the CW IPM program was evaluated. Field trials and analysis of historical data determined CW monitoring did not effectively relate to CW damage and CW has likely developed phosmet resistance. Field trials showed that foliar, seed treatment, or in-furrow insecticide applications generally failed to reduce CW damage, although foliar applications of novaluron and cyantraniliprole showed some efficacy. Throughout field trials, damage from an apparent 2nd CW generation was observed. The Holland Marsh (Ontario) was surveyed for natural enemies of CW. All known natural predators and one parasitoid of CW were found although their impact on the CW population is unknown. Future CW research at the Holland Marsh should focus on improving monitoring techniques, evaluating new insecticides, and examining the feasibility of conservation biological control.

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LIST OF ACRONYMS

CRF – carrot rust fly, *Psila rosae*

CRS – carrot root sections

cv. - cultivar

CW – carrot weevil(s)

DD – degree day

EIL – economic injury level

EIQ – Environmental Impact Quotient

EIQ-FUR – Environmental Impact Quotient, Field Use Rating

IPM – integrated pest management

MCRS – Muck Crops Research Station (University of Guelph)

PBO – piperonyl butoxide

TLS – true leaf stage

TMC – tank mixture concentration

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CHAPTER 1

LITERATURE REVIEW

1.1 Carrot Production in Canada

Carrot (*Daucus carota* L.) production plays a significant role in the Canadian vegetable industry with nearly 9000 ha of carrots grown annually, generating a farmgate value of approximately $95 mill (Statistics Canada 2014). Carrots are a cool season vegetable, typically planted from April to June and harvested in July to October, taking 120 – 180 days to develop (OMAFRA 2010). Ontario and Quebec contribute 75% of Canada's carrot production (Agriculture and Agri-Food Canada 2014). The Holland Marsh region in Ontario is responsible for 59% of Ontario carrot production, supplying the equivalent of 1.8 kg of carrots per Canadian every year (Bartram *et al.* 2007). The Holland Marsh was drained with a canal system in 1930, leaving highly fertile, black, organic soil referred to as muck soil. Most carrots produced in Canada are grown on muck soil, however about 40% of Ontario carrots produced on mineral soil, primarily for the processing industry (Agriculture and Agri-Food Canada 2014).

1.1.1 Distribution and Range of Carrot Weevil Impact

Carrot weevil (CW), *Listronotus oregonenesis* (LeConte) (Coleoptera: Curculionidae), was first detected in the early 1900s in Ohio and Illinois on cultivated Apiaceae species such as parsley (*Petroselinum crispum*), carrot, celery (*Apium graveolens* L.) and dill (*Antheom graveolens* L.) (Chandler 1926; Harris 1926; Pepper and Hagmann 1938). Feeding by CW larvae causes the economic damage to apiaceous crops. Damage to parsley and carrot occurs on the root (Chandler 1926; Harris 1926; Perron 1971; Boivin 1988; Torres *et al.* 2002) with carrot damage occurring on the upper third of the root (Boyce 1927; Boivin 1988). In celery, larvae can feed on any part of the plant but typically feed on the plant crown (Pepper and Hagmann 1938). As the carrot root is the marketable product, CW causes direct yield loss, whereas the foliage is usually the marketable product for celery and parsley and CW feeding results in indirect damage. In addition to cultivated crops, CW has been detected on the weed species: broad leaf plantain

(*Plantago major* L.) and patience dock (*Rumez patientia* L.) (Pepper and Hagmann 1938). Today, CW is present across north-eastern North America (Torres *et al.* 2002, 2005), including US states: Connecticut, Illinois, Iowa, New Jersey, New York, Ohio, Virginia, and Washington D.C. (Chandler 1926; Harris 1926; Boyce 1927; Pepper and Hagmann 1938; Simonet and Davenport 1981), and Canadian provinces: Ontario, Nova Scotia, Prince Edward Island and Quebec (Boivin 1985, Stevenson 1985, Hopper *et al.* 1996; Majka *et al.* 2007). Adult walking is likely the main method of movement as CW has rarely been observed to fly (Boyce 1927; Pepper and Hagmann 1938; Wright and Decker 1957), however some CW have been caught in light traps >2000 ft from the nearest field, suggesting flight is possible (Perron 1971). In Canada, CW is only economically important on carrots (Stevenson and Boivin 1990). The CW was first documented in Ontario in 1908 (Stevenson 1976) and reported again in 1955 (Perron 1971; Stevenson 1985) but did not become economically significant until 1969 (Stevenson 1976). Similarly, CW has been present in Quebec since at least 1908 with CW activity first noticed in commercial carrot production around the late 1960s (Perron 1971). Currently, ~80% and 25% of Quebec and Ontario producers, respectively, report the CW as the primary insect pest in their fields (Agriculture and Agri-Food Canada 2009).

Historically, there has been a single CW generation in Canada (Stevenson and Boivin 1990) compared to multiple CW generations in the US (Harris 1926; Wright and Decker 1957), resulting in different CW pressure. Up to 90% CW damage has been reported in carrots in Illinois and Iowa (Chandler 1926; Harris 1926) and New Jersey has recently experienced 75% damage (Bonham *et al.* 2009). In Canada, CW damage can exceed 40% of carrot yield (Boivin 1985). CW control can be costly, exceeding $300/ha for control measures in parsley with some producers still seeing >20% plant infestation (Torres and Hoy 2005).

1.2 Carrot Weevil Biology

1.2.1 Life Cycle

Adult CW overwinter in plant debris, ditches, and fencerows near the crop and within the infested field (Pepper and Hagmann 1938; Wright and Decker 1957; Grafius and Collins 1986), predominately within the top 5 cm of soil (Grafius and Collins 1986). Adults emerge in the early spring and will feed on carrot leaves; this feeding damage is reported as negligible (Boivin 1988; Bonham *et al.* 2009). Females chew oviposition pits for their eggs at the base of the petiole but can oviposit on exposed carrot crowns (Boivin 1988). As the white egg (~0.8 x 0.5 mm) develops, the colour changes from yellow to brown and then to black shortly before hatching (Boyce 1927; Martel *et al.* 1976). Eggs begin hatching at ≥7ºC and development occurs optimally at 18.3-26.7ºC. It takes 630 $DD_{7^{\circ}C}$ for CW to mature from egg to adult (Simonet and Davenport 1981). Larvae are white to pinkish brown, legless, and have a brown head capsule (Boyce 1927; Martel *et al*. 1976). CW undergoes four larval instars (Fig. 1.1). Immediately after hatching, first instar larvae begin tunnelling through host tissues, moving to the crown of the carrot root, for food (Martel *et al.* 1976). The fourth instar is a non-feeding pre-pupal stage which leaves the host plant to pupate in the soil (Martel *et al.* 1976). Eclosed adults are ~6mm long (Harris 1926; Boyce 1927; Perron 1971; Martel *et al.* 1976) and covered in brown scales which are mechanically removed over their lifetime to reveal the typical grey colouration (Boyce 1927). Post-eclosion, females have a 1-2 week preovipositional period (Wright and Decker 1958; Martel *et al.* 1976; Simonet and Davenport 1981).

Figure 1.1. A) An adult carrot weevil (CW); B) CW eggs; C) 3rd instar CW larvae; and D) CW pupae.

Adult CW oviposit up to 16 eggs daily and >300 throughout their lifetime (Martel *et al.* 1976). They only oviposit on plants that have reached the fourth true leaf stage (TLS) (Boivin 1988). Carrot weevil oviposition begins at 147±9$_{DD7°C}$ with 50% and 90% of oviposition completed by 328±102$_{DD7°C}$ and 456±47$_{DD7°C}$, respectively (Boivin 1988). Multiple generations of CW occur in Iowa and Illinois (Harris 1926; Wright and Decker 1957), whereas one generation has historically occurred in Canada (Stevenson and Boivin 1990). One report has suggested at least a partial second generation of CW could occur in Ontario (Stevenson 1977). This study took CW infested carrots from the field and held them in an insectary, and found that females which emerged in early July could oviposit before the end of the season. However, the climatic conditions of the insectary were not reported. In the field, recently eclosed adult female CW will enter a reproductive diapause based on a temperature-photoperiod interaction; female CW require high temperatures, long photoperiod, or a combination of the two to mature sexually prior to overwintering (Stevenson and Boivin 1990). Therefore, without knowing the climatic conditions of the insectary, it is impossible to evaluate whether recently eclosed female CW could have become sexually mature in the 1970s. Currently, it is believed that a partial second generation of CW has been occurring in Quebec since the mid-1990s (Boivin 2013). There has been no evidence of a second generation occurring in Ontario, but there has been little research examining CW in Ontario since the 1980s.

1.2.2 Monitoring Methods

Non-selective trapping techniques such as coloured plate traps, potted carrot plant traps, and sweeping are ineffective at capturing CW adults (Perron 1971). The first CW trap was a small jar and funnel baited with a carrot, however economic thresholds for this trap type were never established (Boivin 1985). Carrot root sections (CRS) are an alternative form of CW monitoring. Four to five groups of four to five carrot sections, ≥60 mm long by 35-45mm diameter are placed on a transect across a carrot field (Stevenson 1985). CRS need to be replaced every 3-4 days and each section is examined for CW oviposition pits after being replaced (Stevenson 1985). The economic threshold for insecticide application based on CRS is 0.3 oviposition sites/carrot section/day or 0.5 oviposition sites/carrot section/day if under 50% of CRS are infested (Stevenson 1985). The Boivin trap (Boivin 1985) consists of a series of wooden slats held together by bolts, with a space in the center for a carrot root (Fig. 1.2). The slats and carrot root are placed on a board on the ground and covered with a second board on top. The traps attract the carrot weevils because of the carrot volatiles and retain them as a result of the dark, narrow shelter spaces. Traps are placed at the edge of a carrot field and examined 2 to 3 times each week for the presence of adult CW.

Figure 1.2. The Boivin trap, used for monitoring carrot weevil adults.
The carrot bait and tightly spaced wooden slats attract and hold adult weevils.

Carrot root sections and the Boivin trap appear equally effective at assessing CW populations although they may slightly overestimate populations early in the season (Stevenson and Barszcz 1997). The Boivin trap is commonly used in CW integrated pest management (IPM) programs, such as the Muck Crop Research Station (MCRS) (University of Guelph) program in the Holland Marsh, Ontario (Tesfaendrias and McDonald 2010), and a corporate program based predominately in the Montérégie of Quebec (Prisme Consortium 2014). The Boivin trap focuses on cumulative CW trapped throughout the season; 1.5 CW/trap and 5 CW/trap are the thresholds for a first and second spray for control (Boivin 1994). To prevent oviposition, chemical sprays targeting CW should occur when the carrots have reached the second true leaf stage and 7-10 days after, as needed (Pree *et al.* 1996). Additional insecticide applications are not recommended once CW activity has passed the 90% oviposition threshold based on the degree day model ($456 \pm 47_{DD7°C}$) (Boivin 1988). Outside of carrots, established CW monitoring techniques are lacking. Carrots appear to be less attractive to CW in parsley fields, leading to the failure of known trapping methods (Torres and Hoy 2002a) despite no documented host plant preference by CW (Boyce 1927; Pepper and Hagmann 1938).

1.3 Integrated Pest Management Tactics for Carrot Weevil

1.3.1 Cultural Control

It has been noted by several researchers that early sown carrots (early-mid May) are most heavily infested by CW while late sown carrots (early June) avoid most damage (Boyce 1927; Perron 1971; Boivin 1988; Zhao *et al.* 1991). Delaying carrot planting by 40-44 days reduced mean CW egg/plant densities by almost four-fold (Zhao *et al.* 1991). This method is effective as CW only oviposits in carrots past the 4^{th} TLS and will complete 90% of its oviposition by 456 ± 47 $DD_{7°C}$, which typically occurs around mid to late June (Boivin 1988). Changing planting dates may not be an option for CW management in Canada. The harvest of fresh market carrots often begins in July, requiring an early seeding date to ensure sufficient carrot growth, and earlier seeding dates allow for maximal yield in processing carrots (Agriculture and Agri-Food Canada, 2014). Early seeding also assists in reducing the impact of aster yellows (*Phytoplasma* spp.) infection through establishing a healthy crop prior to disease exposure (Agriculture and Agri-Food Canada, 2014). Crop rotation is another potentially effective management technique to reduce CW damage, however the limited land available for vegetable production in CW-infested areas such as the Holland Marsh, Ontario limits the producer’s ability to rotate crops and the effectiveness of crop rortation (Pepper and Hagmann 1938).

1.3.2 Physical Control

Physical control of CW has not been a major focus of research. Floating row covers can reduce CW damage by 65-75% when placed in the field for 40 d after seeding, potentially eliminating the need for pesticide applications in the absence of a heavy CW infestation (Rekika *et al.* 2008). Although this option is promising in principle, the labour and investment costs associated with the use of row covers prohibit its adoption in conventional agriculture, however this could be an effective control strategy in organic or small-scale market garden carrot production operations with CW issues.

1.3.3 Biological Control

Biological control agents could successfully control CW due to several life history traits (Boivin and Belair 1989). Female CW only oviposit in carrot plants past the 4th TLS, providing time early in the season for a biological control agent to target adult CW. In Canada, newly eclosed, adult female CW emerge around July and August, and need to overwinter prior to sexual maturity (Stevenson and Boivin 1990), allowing ample time for predation or parasitism prior to oviposition in crops. Finally, CW live their entire life in close proximity to the soil, allowing predators and nematodes to gain access to CW. These life history traits have resulted in several investigations into biological control of CW.

Parasitoids

The first identified parasitoid of CW was *Anaphes sordidatus* (Giralt) (Hymenoptera: Mymaridae) in Michigan and Ohio (Collins and Grafius 1986a). This species has been separated into two known *Anaphes* spp. attacking CW: *Anaphes listronoti* and *Anaphes victus* (Huber) (Cormier *et al.* 1996). *Anaphes listronoti* is common in regions of Ontario and Quebec with muck soils whereas *A. victus* is only present in the Holland Marsh region of Ontario (Cormier *et al.* 1996). Additionally, a new, undescribed *Anaphes* sp. attacking CW has been found in Nova Scotia (Hopper *et al.* 1996). *Anaphes listronoti* is an egg parasitoid and can produce 4-6 generations over the CW oviposition period (Collins and Grafius 1986a; Boivin 1993), with field parasitism rates against CW reaching 50-90% at times of high CW egg density (Collins and Grafius 1986c; Boivin 1993). In the Holland Marsh, both *Anaphes* spp. emerge before CW oviposition begins and must complete 1-3 generations before migrating to commercial carrot fields (Cormier *et al.* 1996). From 1990 to 1992, *Anaphes* spp. parasitized 16.7 ± 38.9% of CW eggs on carrot plants in commercial field in the Holland Marsh (Cormier *et al.* 1996). The large variability in *Anaphes* spp. parasitism rate in commercial fields is likely related to low resource availability, as only 0.15 ± 0.60 CW eggs / 50 carrot plants observed in the same survey. Additionally, egg parasitism may not be an effective biological control method for CW control as all CW eggs on a carrot plant need to be parasitized to prevent carrot injury (Collins and Grafius 1986c; Cormier *et al.* 1996).

Classical biological control for adult CW has been attempted using *Micronotus hyperodae* Loan (Hymenoptera: Braconidae), a solitary koinobiont endoparasitoid, (Cournoyer and Boivin 2005). *Micronotus hyperodae* is used for control of the Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) and is particularly effective as parasitism sterilizes the pest (McNiell *et al.* 1996) and can transmit bacterial pathogens during oviposition (Jackson and McNiell 1998). Carrot weevil control using *M. hyperodae* appeared promising in the lab, as the wasp was attracted to semiochemicals present on CW whereas other weevil species were unattractive (Cournoyer and Boivin 2004). Unfortunately, after releasing *M. hyperodae* in Quebec in 2004 (Cournoyer and Boivin 2005), the parasitoid has not been recovered over 10 years of sampling (Boivin 2013).

Predators

Ground beetles (Coleoptera: Carabidae) have been the focus of investigations of natural CW predators as multiple species have been found in muck-grown carrots through the CW oviposition period (Baines *et al.* 1990; Zhao *et al.* 1990). Within the detected beetles, smaller carabids (*Bembidion quadrimaculatum oppositum* (Linnaeus) and *Clivina fossor*(Linnaeus)) prefer CW eggs whereas larger carabids (*Anisodactylus santaecrucis* (Fabricius), *Poecilus lucublandus lucublandus* (Say), *and Pterostichus melanarius* (Illiger)) feed preferentially on late instar CW larvae or pupae in the laboratory (Baines *et al.* 1990). When carrot plants were included in laboratory trials, *P. melanarius* was the most effective carabid predator, capable of consuming 5 of 15 presented larvae, pupae or adults in 24 h. Egg consumption by any carabid was negligible (Zhao *et al.* 1990). Additionally, *P. melanarius* was unable to consume CW adults once they had climbed onto the carrot plant (Zhao *et al.* 1990). The current research suggests carabids may not be able to prey upon CW effectively in the field. The muck soil in which carrots are grown also contains a range of other potential predators including the true bug (Hemiptera) families: Anthocoridae, Anthomyiidae, Reduvidae, and Nabidae, and the beetle (Coleoptera) families: Coccinellidae and Staphylinidae, and lacewings (Neuroptera: Chrysopidae) (Chaput 1996).

Entomopathogenic Nematodes

As CW spends much of its life associated closely with soil, entomopathogenic nematodes have been suggested as a good candidate for CW biological control (Boivin and Belair 1989). Both *Steinernema carpocapsae* (Rhabdita: Steinernematidae) and *Heterorhabditis bacteriophora* (Rhabdita: Heterorhabditidae) can cause >80% mortality at the rate of 400 IJ (infective juveniles)/100µl for CW adults and 100 IJ/100µl for CW larvae in laboratory assays (Miklasiewicz *et al.* 2002). Nematodes, modelled using *Steinernema feltiae* (Rhabdita: Steinernematidae) infection, cause CW mortality more rapidly at higher temperatures and when CW are feeding (Boivin and Belair 1989). Field trials suggest *H. bacteriophora* can cause significant mortality on all CW life stages (38, 80, and 50% mortality for larvae, pupae, and adults, respectively) in parsley (Miklasiewicz *et al.* 2002). There are mixed reports of the efficacy of *S. carpocapsae* in the field. A soil treatment of 750,000 *S. carpocapsae* IJ/m^2 produced no effect in parsley (Miklasiewicz *et al.* 2002), however soil applications of 2.2×10^9 *S. carpocapsae* IJ/m^2 or baits consisting of 30 g rasped carrots, 0.3 g streptomycin, and 1.5 g agar treated with 200,000 *S. carpocapsae* IJs significantly reduced CW oviposition and damage in carrots (Belair and Boivin 1995). Both studies noted timing is critical for effective CW control using nematodes (Belair and Boivin 1995; Miklasiewicz *et al.* 2002). Currently, there are no recommendations for using entomopathogenic nematodes in commercial carrot production. Further research is needed to determine the application rates required for economically viable control. In addition to commercially available nematodes, a new strain of nematode, *Bradynema listronoti* (Tylenchida: Allantonematidae) was discovered infesting CW in Quebec (Zeng *et al.* 2007). Female CW can become sterile under *B. listronoti* infection, reducing CW damage, but there is currently no effective mass rearing system to commercialize this species of nematode (Boivin 2013).

1.3.4 Chemical Control

Conventional chemical control for insect pests is commonplace in Canadian carrot fields, with each field receiving an average of 1.8 insecticide applications per year (Agriculture and Agri-Food Canada 2005). Chemical control of CW has always focused on the adult stage (Martel *et al.* 1975b; Boivin 1985). Initially, the recommended control of CW was 4 applications of 1.68 kg / ha DDT over the CW oviposition period (Wright and Decker 1957). The loss of DDT required an effective replacement (Martel *et al.* 1975b; Stevenson 1983, 1985). Phosmet (Imidan 50 WP, Gowan Company, Yuma, Arizona, USA) was registered for CW control in the early 1980s (Stevenson 1983, 1985; Pree et al. 1996) and, based on both weight and area applied, is currently the most used insecticide in Canadian carrot production (Agriculture and Agri-Food Canada 2005). In addition to phosmet, field trials have shown that other organophosphates, including phosalone, chlorpyrifos, and azinphosmethyl, also reduce CW damage (Stevenson 1983). In the USA, several broad-spectrum organophosphorus insecticides (parathion, azinphosmethyl, phosmet) were registered previously for use but were deregistered during the 1990s, despite the absence of alternative chemical controls (Bonham *et al.* 2009). In Canada, phosmet remained the only insecticide available for CW control until the recent label expansions of λ-cyhalothrin (Matador 120 EC) in 2014 (Syngenta Canada Ltd. 2015) and novaluron (Rimon 10 EC) in 2015 (Adama Agricultural Solution Canada Ltd. 2015). There are concerns among Canadian carrot producers and researchers that over 30 years of reliance on phosmet for CW control has selected for phosmet-resistant CW populations. Agriculture and Agri-Food Canada (AAFC) has noted that there is an urgent need for an increased number of insecticide options in Canada (Agriculture and Agri-Food Canada 2014). While two insecticides have recently been registered, λ-cyhalothrin and novaluron, it is important to assess their efficacy in the Holland Marsh, as it is a major Canadian carrot producing region.

The CW is generally insensitive to insecticides (Martel *et al.* 1975b) and can recover from pyrethroid knockdown although cypermethrin can achieve the same level of CW mortality as phosmet in a laboratory assay (Pree *et al.* 1996). Bonham *et al.* (2009) found a seed treatment of fipronil is protects carrots from CW damage more effectively than a thiamethoxam in-furrow treatment or foliar applications of diazinon or spinosad, although damage was still too high for commercial production in years of high infestation. In addition, fipronil is slow to degrade on plants, or in soil or water, may bioaccumulate in some organisms, and the degradation products may be more toxic (Tingle *et al.* 2003), and thus it is unlikely the insecticide will be registered in Canada.

Biopesticides

Research into biopesticides for use against CW has been limited. The only published information on biopesticide activity against CW involves the microbial biopesticide *Bacillus thuringiensis tenebrionis*. It is suggested by Saade *et al.* (1996) that the ingestion of extracted proteins from *B. t. tenebrionis* (*Bt*) spores, with a LC_{50} of 118 µg protein/ml towards CW, could reduce CW populations but this has not been examined in field studies.

A congener of CW, the annual bluegrass weevil (*Listronotus maculicollis*), has been evaluated for susceptibility to the entomopathogenic fungi, *Beauveria bassiana* strain GHA (Hypocreales: Cavicipitaceae) using the commercial product BotaniGard (Laverlam International, Butte, MT, USA). In the field, turfgrass plugs treated with this product caused 78% mortality of *L. maculicollis* after 14 days (Clavet *et al.* 2013). It is possible that entomopathogenic fungi could be effective for CW mitigation in the field, particularly since the weevils spend the majority of their lives within or in close proximity to the soil.

1.3.5 Genetic Control

To date, there has been no research evaluating genetic control options, such as sterile insect release, for CW control.

1.4 Summary

Carrot weevil is an economically important pest of apiaceous crops in northeastern North America. In Canada, CW is primarily a pest of carrots. Larval CW feed on carrot roots, rendering them unmarketable. Although some biological, cultural, and physical controls are available, they are either ineffective or lack uptake by producers, leaving chemical control as the primary method of CW management. Effective monitoring techniques have established thresholds for chemical control, however the effectiveness of trapping techniques varies among different crops and few chemicals are available for effective control.

1.5 Research Objectives

The main objective of this research was to improve CW management particularly in the Holland Marsh, Ontario where the field research was conducted. The established CW IPM program is primarily based on research from the 1980s and may require revision. Over the past 30 years, CW control in Canada has solely relied on foliar phosmet applications. There are concerns that the CW has developed resistance to this chemical, creating a need for alternative insecticides. Optimally, having multiple effective insecticides for CW mitigation should allow for the implementation of a resistance management program to ensure the success of the IPM program. Finally, the natural enemies of CW within the Holland Marsh has not been evaluated. It is possible that there are contains several carabid beetles or mymarid wasps which are known CW natural enemies. Therefore, this research focused on the following three main objectives intended to identify new management options for CW while furthering understanding of CW biology:

1) Evaluation of the efficacy of the established CW IPM program at the Holland Marsh, Ontario;

2) Evaluation of new and registered insecticides available for CW control; and

3) Evaluation of the arthropod biodiversity in the Holland Marsh to identify potential natural enemies of CW.

The corresponding null hypotheses were tested:

I. The CW IPM program does not consistently relate CW monitoring, and economic thresholds derived from monitoring, to observed CW damage in carrots;

II. Insecticide applications administered following the current CW IPM program do not effectively reduce CW damage;

III. Foliar, seed treatment, and in-furrow insecticides do not reduce CW damage;

IV. No CW natural enemies are present in the Holland Marsh.

CHAPTER 2

EVALUATION OF THE ESTABLISHED CARROT WEEVIL INTEGRATED PEST MANAGEMENT PROGRAM IN THE HOLLAND MARSH, ONTARIO

2.1 Abstract

There is an established IPM program for CW in Canada, with economic thresholds based on monitoring. Since the 1980's, CW control has relied on phosmet applications during May and June. Currently, there are concerns among producers and researchers that CW may have developed resistance to phosmet. In addition, CW may be producing a second generation in Canada. Historical data from a CW IPM program at the Holland Marsh was examined, the efficacy of the existing IPM programs was evaluated in field trials, and the difference in CW damage as a result of carrot planting date was assessed. The current CW IPM program monitoring did not relate to CW damage in the field accurately. Field trials found limited control based on recommended IPM insecticide applications, particularly under regular CW pressure. Carrot weevil oviposition was detected in the field prior to the 4th TLS of carrots and occurred earlier in the season than predicted based on the established degree day model. Late May and early June carrot planting dates had reduced CW damage in small plot trials.

2.2 Introduction

Integrated pest management (IPM) is an established methodology that aims to manage pest infestations while balancing economic impact, environmental concerns, and maintenance of a resilient agroecosystem (Higley and Pedigo 1993). IPM programs integrate multiple management approaches including cultural, physical, biological, genetic and chemical methods and generally attempt to exhaust all non-chemical control options before applying pesticides (Peshin *et al.* 2009). In addition, any chemical applications are supposed to be justified using the Economic Injury Level concept (EIL), where the cost of control is equal or less than the economic loss caused by the pest damage (Higley and Pedigo 1993). The EIL Concept is used to identify economic thresholds based on pest population or damage monitoring, corresponding to the level at which pesticide applications become economically justified since the cost of pesticide application is lower than the cost of the plant injury and loss of yield.

IPM programs can be classified into four levels (Kogan 1998):

Level 0 - involves little or no effort to monitor pests and relies on calendar-based pesticide applications.

Level 1 - uses the EIL concept: pesticides are applied based on field monitoring results that exceed pre-determined economic thresholds. In addition, some cultural control options such as crop rotation may be used, but in general Level 1 uses reactionary pest control tactics.

Level 2 – primarily relies on preventative pest control tactics, using cultural and physical control options, and incorporates interactions of multiple pests into management decisions.

Level 3 - works at the level of the agro-ecosystem, assessing impacts of all pests and diseases and their respective control measures.

Management of CW is predominately focused on insecticide applications. In Canada, phosmet (Imidan 50 WP/Imidan 70 WP; Gowan Company, Yuma, Arizona, USA), λ-cyhalothrin (Matador 120 EC; Syngenta Canada, Guelph, ON), and novaluron (Rimon; Adama Agricultural Solutions Canada, Winnipeg, MB) are the available registered products. Phosmet was proven to be effective for CW damage mitigation in the early 1980's (Stevenson 1983) and the insecticide has been considered the primary product for CW control since registration (Agriculture and Agri-Food Canada 2009). Λ-cyhalothrin and novaluron have only been registered for CW control since 2014 and 2015, respectively. There is an established degree day model to predict the oviposition period of emergent overwintered adults and insecticide applications are based upon this model. Oviposition is expected to begin at 147 ± 9 $DD_{7°C}$ and 90% of oviposition is completed around 456 ± 47 $DD_{7°C}$ (Boivin 1988). Once the 90% oviposition threshold has been reached, insecticide applications are not recommended. In the Holland Marsh, where this field research occurred, oviposition generally occurs between May and June.

Although CW management is dependent on insecticide applications, there are established thresholds based on CW monitoring, thus CW IPM can be classified as a Level 1 program. The monitoring techniques have established thresholds for the application of chemical controls based on an economic injury level of 2% CW damage (Stevenson and Barscsz 1997). The most common monitoring method uses the Boivin trap (Fig. 1.2; Boivin 1985). The trap consists of wooden plates, separated by metal washers and surrounding a bait consisting of a full carrot root. Multiple traps (4-6) are placed around the field border or near sheltered areas and overwintering sites. The number of CW captured per trap is assessed 2-3 times per week. Two economic thresholds, 1.5 and 5 cumulative CW per trap, justify the first and second insecticide application (Boivin 1994).

Instead of tracking CW captured with the Boivin trap, CW oviposition activity can also be monitored using carrot root section traps (CRS) (Fig 2.1, Stevenson 1985). This technique requires small sections of carrots (60 mm long by 35-35 mm diameter) in groups of 4-5 sections along a transect going into the field. This trapping technique evaluates the number of oviposition sites in each CRS. Thresholds for CRS are not cumulative; finding 0.3 oviposition sites per CRS per day justifies an insecticide application, however if <50% of the CRS are infested, the threshold is increased to 0.5 oviposition sites per CRS per day (Stevenson 1985). The Boivin traps and CRS monitor CW with similar efficacy although both tend to overestimate the size of early populations (Stevenson and Barszcz 1997). For both traps, insecticide applications justified by the economic thresholds are best timed at the 2nd and 4th TLS of the developing crop in order to kill the adult CW prior to oviposition into the crop which begins at the 4th TLS (Boivin 1998). Both traps are only useful in monitoring CW activity at the beginning of the season. The Boivin trap and CRS rely on carrots as attractants or baits so as the carrot crop matures, the traps and developing crop compete for CW (Boivin 1985).

Alternative CW control options are not currently available in the established CW IPM program. Although crop rotation can be effective, CW infestation problems are prevalent in muck soil areas in which the limited land available for vegetable production in CW infested areas limits the possibility of effective crop rotation (Pepper and Hagmann 1938). Floating row covers that protect the crop for 40 days past seeding can also reduce CW damage by 65-75% (Rekika *et al.* 2008) but they are expensive in terms of purchase price, time and labour, which means they are unlikely to be adopted in commercial production scenarios.

Figure 2.1. Carrot root sections placed in a carrot field to monitor carrot weevil oviposition.

Established IPM programs require continual research and investigation to ascertain if they still provide adequate pest control (Peshin *et al.* 2009). Various aspects, such as degree day models and economic injury thresholds, may need modification as a result of changes in degree day accumulation or pest behaviour and activity. In addition, resistance management (predominantly for pesticide application) is very important to ensure the sustainability and efficacy of an IPM program. Resistance management requires continuous evaluation of pest susceptibility to the pesticide in use.

In the 1980's, there was a substantial research effort to establish control options for CW (Simonet and Davenport 1981; Stevenson 1983; Boivin 1985; Stevenson 1985) however there has been minimal research and no IPM program evaluation since then. Recently, there have been growing concerns among producers and researchers at the Holland Marsh that CW pressure has been increasing. The research reported in this thesis evaluated the efficacy of the established CW IPM program in the Holland Marsh. In this study, trends in CW activity were assessed using recent data collected in conjunction with the MCRS IPM program at the Holland Marsh. In addition, field trials performed at MCRS evaluated the efficacy of the IPM program and examination of potential changes in CW activity including oviposition period, preferred host stage, and the possible occurrence of a second generation.

2.3 Methods

2.3.1 Assessment of Historical Data from the Carrot Weevil IPM Program at the Holland Marsh

In order to assess the efficacy of the CW IPM program in the Holland Marsh, historical CW monitoring and damage data were collected from the University of Guelph – Muck Crops Research Station (MCRS) IPM program. The MCRS staff monitor CW using two pairs of Boivin traps, placed around participating producers' fields. Scouts, trained by staff at MCRS, count the number of CW trapped in all participating fields twice per week. Occasionally, traps cannot be monitored based on re-entry intervals of pesticides applied in monitored fields. The Boivin traps are generally monitored from early May until July – the typical oviposition period of CW in the region based on the established degree day model. Prior to harvest in the fall, MCRS staff take ten random samples of ten carrots each from each field. These samples are washed in a small vegetable drum washer and assessed for CW damage. In this program, CW captures and damage from each participating producers' fields were collected each year from 2009 to 2015. Using the cumulative number of CW from the monitoring data, CW IPM thresholds (No insecticide spray recommended at >1.5 CW / trap, one insecticide spray recommended at 1.5 to 5 CW/trap, two insecticide sprays recommended at ≥5 CW / trap) were calculated for each field for all years.

2.3.2 Field Trials

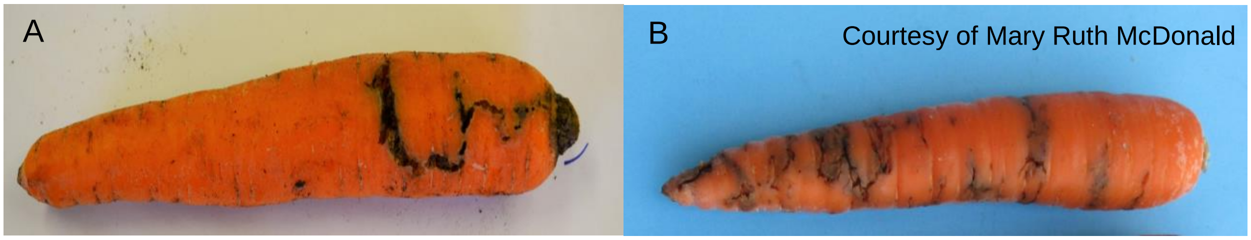
Carrot Weevil IPM Program Evaluation - 2015

A field trial was conducted in 2015 to assess the efficacy of the IPM recommendations for the primary carrot insect pests in the region, CW and carrot rust fly (CRF) (*Psila rosae* (Fabricius) (Diptera: Psilidae)). Nine plots, 25 X 14 m large, were established in a commercial field near the MCRS with the outermost and centre plots seeded with soybean (*Glycine max*) (cv. S04-D3) on June 18 for a different project examining a potential interaction with CRF. The remaining six plots were triple-seeded (70 seeds / m) carrots (cv. Enterprise) directly onto raised beds with a precision seeder (Stanhay Webb Ltd., Bourne, UK) on May 28 2015. with three replications per treatment and planted in a linear sequence with alternating treatments.

Treatments consisted of control (i.e. no insecticide applications) or currently prescribed IPM protocols for both CW and CRF. Three pairs of Boivin traps and five yellow sticky traps were used to monitor CW and CRF, respectively, and were examined twice per week. Plots managed using the IPM protocol received insecticide applications based on established CW and CRF thresholds: 1.5 and 5 cumulative adult CW per trap to justify the first and second insecticide applications for CW, timed at the 2^{nd} and 4^{th} TLS respectively, and CRF levels of 0.01 flies per trap per day triggered insecticide applications for CRF control. Insecticide applications targeting CW are only justified according to the IPM program if the 90% oviposition threshold, based on the CW oviposition degree day model, has not been reached. A single application of phosmet (Imidan 70 WP) at a rate of 1.6 kg/ha was applied to the plots receiving the IPM protocols on 19 June, as the carrots had reached the 2^{nd} TLS. Despite reaching the second economic threshold, the 90% CW oviposition threshold was reached before the carrots developed to the 4^{th} TLS, meaning the second insecticide application is not recommended according to the IPM program. Based on trapping rates of CRF exceeding threshold, cypermethrin (Ripcord, BASF Canada, Mississauga, ON) was applied to the IPM plots at 175 ml/ha on 19 June, 4 August, and 25 August. All insecticides were applied using a tractor-mounted sprayer calibrated to deliver 500 L water/ha. Fertilizer, fungicide, and herbicide applications are provided in Appendix 1, Table A7.2.

On 14 August and 15 October, three 1.5 m carrot row sections were sampled randomly from each plot to assess for CW and CRF damage. Between 19-21 August and 15-16 October, carrot samples were washed in a small vegetable drum washer and inspected visually for CW and CRF damage. The number of damaged and marketable carrots (marketable was defined as no insect damage) was recorded. Insect damage was differentiated primarily on the basis of size, form, and location. CW tunneling is larger than CRF tunneling and moves downward on the carrot root starting around the carrot crown the carrot root, and is typically located in the upper third of the root (Fig. 2.2A), whereas CRF tunnelling moves horizontally across the carrot root, and is usually concentrated near the bottom of the root (Fig. 2.2B). The weight of all damaged and marketable carrots was also recorded during the second sampling period.

Figure 2.2. A) Carrot weevil damage on a carrot root. The tunnels often exceed 1 cm in width



and depth and move downward from near the crown of the carrot root. B). Carrot rust fly damage. The tunnels are mostly near the bottom 2/3rds of the carrot root, and tunnels do not exceed 1 cm in width and depth.

Carrot Weevil IPM Program Evaluation - 2016

The CW IPM Program Evaluation conducted in 2016 replicated the methods of the 2015 trial. Carrots (var. Enterprise) were direct seeded (70 seeds / m) onto raised beds with a Stanhay precision seeder on 24 May at the MCRS and in the same commercial field used in the 2015 Carrot Weevil IPM Program Evaluation. Plot size was reduced to 15 x 14 m.

As in 2015, plots were planted in a linear sequence with alternating treatments. The plots at the MCRS did not contain soybean plots whereas the plots in the nearby commercial field had soybean plots planted in the centre and outermost plots of the trial, as described in the 2015 CW IPM Program Evaluation trial. Three pairs of Boivin traps and five yellow sticky traps were monitored twice per week for CW and CRF, respectively, at each field. Following the established CRF and CW IPM recommendations, phosmet was applied on 21 June for CW control in both fields. Similar to 2015, both CW economic thresholds were met although the crop had not developed to the 4th TLS by the time the 90% CW oviposition threshold had been reached. Cypermethrin was applied on 4 August, 16 August for CRF control at the MCRS. The commercial field never surpassed any CRF monitoring thresholds. All insecticides were applied at the recommended field rate using a tractor mounted sprayer calibrated to deliver 500 L water/ha. Details of the fertilizer, fungicide, and herbicide applications are provided in Appendix

1, Tables A7.3 and A7.4 for the commercial field trial and the MCRS trial, respectively. On 25 July and 3 October, five 1.5 m carrot row sections were sampled randomly from each plot to assess for CW and CRF damage. Between 1-3 August and 5-8 October, carrot samples were washed in a small drum washer and inspected visually for CW and CRF damage, using the same methods and criteria employed in 2015. The weight of all damaged and marketable carrots was also recorded during the second sampling period.

Planting Date Trial - 2016

Damage observed in field trials during the 2015 field season suggested that CW may not adhere strictly to the established degree day model for oviposition. To assess this possibility, a planting date trial was conducted in 2016. Carrots (cv. Enterprise) were direct-seeded (70 seeds / m) using a Stanhay precision seeder at the MCRS in six separate age cohorts. Planting dates were spaced approximately 10 days apart, started on 2 May and ended on 21 June. The dates selected were based on the CW oviposition period in the Holland Marsh, with the intention that the last age cohort should escape all CW oviposition from overwintered adults based on the established degree day model for oviposition.

Each plot consisted of three 7 m rows triple-seeded with carrots, using 66 cm wide rows. Plots were arranged using a randomized complete block design with five replicates per treatment. In this trial, no insecticides were applied to avoid any interference with CW attack. Each planting date received an application of linuron (750 ml/ha of Lorox DF, E. I. du Pont Canada Company, Mississauga, ON) and an adjuvant (1.0 L/ha of Assist Oil, BASF Canada, Mississauga, ON) at the 2nd TLS for weed control. The additional fertilizers, fungicides, and herbicides applied in this trial can are reported in Appendix 1. On 25 July and 4 October, two 1.5 m sections of carrot row were sampled randomly from all plots to assess for CW and CRF damage, with the exception of the 20 June planting date during the first sampling period. This cohort was sampled two weeks later, on 8 August, as the carrots had not grown to a suitable size to sample during the initial sampling period. Between 1-3 August and 5-7 October, carrot samples were washed in a small drum washer and inspected visually for CW and CRF damage, using the same methods and criteria employed in 2015. The weight of all damaged and marketable carrots was also recorded during the second sampling period.

Carrot Root Section Monitoring - 2016

To improve the information available concerning the timing and duration of the CW oviposition period in the Holland Marsh, CRS were used to monitor CW activity at the MCRS in 2016. Carrots were cut into sections measuring 35 x 60 mm and placed in four groups of five in a transect across the MCRS, following Stevenson (1985). Every 3-4 days, these sections were collected and replaced, from 22 May to 21 August. After each collection, root sections were brought back to the University of Guelph and the number of CW oviposition cavities and total CW eggs present in each root section were counted using a compound microscope (OptiTech Scientific Instruments, Pickering, ON) at 10x magnification.

2.3.3 Statistical Analysis

Assessment of Historical Data from the Carrot Weevil IPM Program at the Holland Marsh

Using the historical data collected from the MCRS between 2009 and 2015, a Spearman's Rank Correlation was performed using *Proc Freq* in SAS 9.4 University Edition (SAS Institute, Cary, NC) to examine relationships between number of CW trapped in all fields, CW IPM threshold, CW damage, and year. Spearman's Rank Correlation was used as some variables were not normally distributed. An Analysis of Variance (ANOVA) was performed using *Proc Mixed* in SAS 9.4 University Edition. This analysis examined the fixed effect of year, the number of recommended insecticide applications based on CW IPM threshold, and their interaction with observed CW damage. The producer who owned the field was incorporated as a random effect. Residual plots were examined to ensure the assumptions of the ANOVA were met. This ANOVA used the fixed effect of the number of recommended insecticide applications instead of the number of CW trapped because the number of recommended insecticide applications allowed for a normal distribution of errors. Another ANOVA was conducted in SAS 9.4 University Edition to examine the fixed effect of year on the cumulative number of trapped

CW, incorporating the random effect of the producer who owned the field. For all ANOVAs, Tukey's HSD was used for mean separation. All analyses set α=0.05.

Assessment of Historical Data from the Carrot Weevil IPM Program at the Holland Marsh

Data were analysed using SAS 9.4 University Edition. Due to differences in average CW damage, the MCRS trial and the commercial field trials were analysed separately. Using *Proc Mixed*, a repeated-measures ANOVA was performed to examine the fixed effect of treatment, harvest date, and the interactions between these variables on CW damage. Individual plots were assessed as repeated measures. Block was included as a random effect. Residual plots were created to ensure the assumptions of all ANOVAs were met. Tukey's HSD was used for mean separation.

Planting Date Trial

Data were analysed using SAS 9.4 University Edition. Using *Proc Mixed*, a repeated-measures ANOVA was performed to examine the fixed effect of planting date, sample date, and the interactions between these variables on CW damage. Individual plots were assessed as repeated measures. Block was included as a random effect. *Proc Mixed* was also used to perform an ANOVA to assess the fixed effect of planting date on yield, including the block as a random effect. Residual plots were created to ensure the assumptions of all ANOVAs were met. Tukey's HSD was used for mean separation.

2.4 Results

2.4.1 Assessment of Historical Data from the Carrot Weevil IPM Program at the Holland Marsh

Several significant correlations were found (Table 2.1). Observed CW damage was positively correlated with the number of CW trapped in a field. There was a positive correlation between year and the number of CW trapped as well as CW monitoring threshold. Overall, correlation co-efficients were low (between 0.20 to 0.45). Fields that reached the second CW monitoring threshold, justifying two insecticide applications, had significantly higher CW damage than fields that did not reach either CW economic threshold (Fig. 2.3, df=2, F=3.72, p=0.027). Observed CW damage differed significantly between years (Fig. 2.4, df=6, F=3.75, p=0.0018), with 2011 having significantly higher CW damage than 2009, 2012, and 2013. Producer had no significant effect on CW damage (df=26, Z=1.49, p=0.068). The year by threshold interaction had no significant effect (df=12, F=0.77, p=0.679), indicating thresholds had a consistent effect across years. From 2009 to 2015, the cumulative number of CW per trap captured in the Holland Marsh has significantly increased (Fig. 2.4., df=6, F=37.48, p=<0.001).

Table 2.1. Correlations of carrot weevil (CW) damage and trap counts collected from the Muck Crops Research Station (University of Guelph) from 2009-2015 based on Spearman's Rank Test. Correlation coefficients are presented in plain text and p-values are presented in italics.

	CW Economic Threshold	Cumulative CW per trap	Observed CW Damage
Year	0.423	0.437	0.143
	<0.001	*<0.001*	*0.062*
Observed CW Damage	0.228	0.247	
	0.0028	*0.0012*	
Cumulative CW per trap	0.942		
	<0.001		

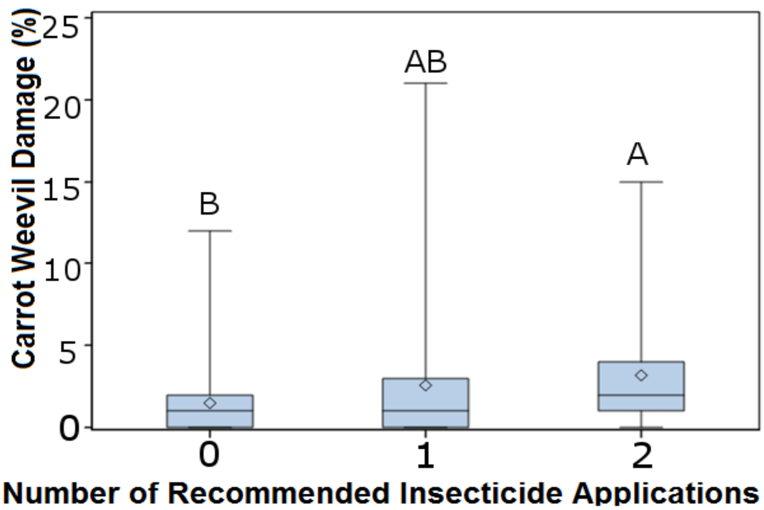


Figure 2.3. The relationship between carrot weevil (CW) economic thresholds for insecticide application based on monitoring and observed CW damage in carrots from 2009 to 2015 at the Holland Marsh, Ontario. Data obtained from the Muck Crops Research Station IPM program (University of Guelph) records. Different letters denote significantly different groups (α=0.05). The diamond denotes the average, the bold horizontal line denotes the median, and the whiskers denote the minimum and maximum.

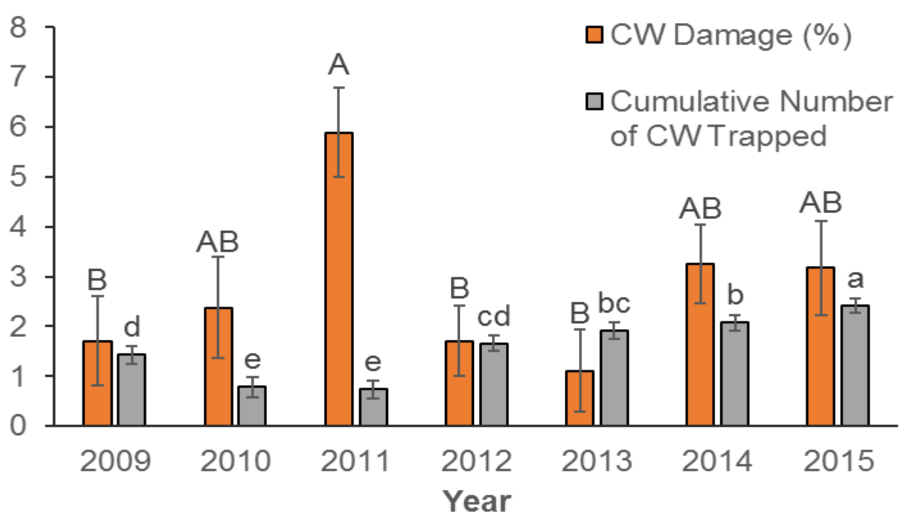


Figure 2.4. Mean carrot weevil (CW) damage and cumulative number of CW trapped in carrot fields participating in the Muck Crops Research Station (University of Guelph) Integrated Pest Management Program from 2009 to 2015 at the Holland Marsh, Ontario. Different letters denote significantly different groups, with upper case letters comparing CW damage and lower case letters comparing cumulative number of CW trapped (α=0.05).

2.4.2 Field Trials

Carrot Weevil IPM Program Evaluation

In 2015, applying insecticides according to the existing IPM program recommendations did not significantly reduce CW damage (Fig. 2.4.2, df=1, F=3.27, p=0.121) nor improve yield (df=1, F=5.94, p=0.051) compared to the untreated control. Between the August and October sampling efforts in 2015, there were no differences in observed CW damage (df=1, F=1.18, F=0.319) and yield significantly increased (df=1, F=1235.19, p=<0.001). There was no significant interaction between harvest date and treatment with respect to CW damage (df=1, F=0.14, p=0.719) or yield (df=1, F=2.06, p=0.201), demonstrating that there was no difference in treatment effects across harvest dates. Carrot weevil damage was low across the trial, with a trial average of 3.8 ± 2.1 %.

The 2016 trial at the MCRS received an average of 29.5 ± 2.2% CW damage at the second sampling date, compared to the commercial field trial with 6.4 ± 0.5% CW damage, which resulted in different treatment effects (Table 2.2). At the MCRS, the IPM program significantly reduced CW damage compared to the control (df=1, F=6.83, p=0.040), however the IPM program had no effect on CW damage compared to the control at the commercial field (df=1, 1.36, p=0.287). CW damage increased significantly between the first and second sampling dates in both the MCRS trial (df=1, F=47.04, p=<0.001) and commercial field trial (df=1, F=21.16, p=0.004). There was no interaction between sampling date and treatment effects in the MCRS trial (df=1, F=0.31, p=0.596) or commercial field trial (df=1, F=0.76, p=0.418), therefore different treatments did not change CW damage between the sampling efforts. With higher CW damage at the MCRS, the IPM program significantly increased yield compared to the untreated control (df=1, F=42.92, p=<0.001), however this effect was not seen in the commercial field (df=1, F=0.26, p=0.623).

Table 2.2. Mean carrot weevil (CW) percent damage and yield in the 2015 and 2016 IPM evaluation trials in a commercial field and at Muck Crops Research Station (MCRS) (University of Guelph) in the Holland Marsh, Ontario. Each trial and year were analysed separately, and an asterisk beside a number indicates a significant difference compared to the control for that location and year (α=0.05).

		Mean CW Damage (%)[1]			
Location and Year	Treatment	Mid-Season Sample	Late-Season Sample	Sample Average	Yield (t/ha)
Commercial 2015	Control	3.9	5.5	4.7	107.4
Commercial 2015	IPM	2.4	3.2	2.8	98.7
Commercial 2016	Control	3.1	7.2	5.1	65.2
Commercial 2016	IPM	2.9	5.6	4.3	68.8
MCRS 2016	Control	14.1	33.4	23.4	69.1
MCRS 2016	IPM	8.7	25.2	17.0 *	82.3 *

[1] Samples within the same location and year were assessed together using repeated measures.
[2] Percent damaged is based on carrot number.

Planting Date Trial

Overall, the planting date trial in 2016 experienced a trial average of 36.9 ± 2.8% CW damage. Later planting dates resulted in significantly lower CW damage (Table 2.3, df=5, F=97.46, p=<0.001). Across the entire trial, CW damage increased by 14.4 ± 1.9% between the July and October assessments (df=5, F=56.64, p=<0.001) and there was no significant interaction between planting date and sampling date (df=5, F=1.23, p=0.311), indicating that effects due to planting date did not change among sampling dates. Examination of the net CW damage increases among sampling dates revealed that the final three planting dates experienced 10-15% more CW damage between sampling dates than the first planting date (df=5, F=4.96, p=0.001). Overall, yield was significantly affected by planting date, with the plots planted on 30 May and 10 June producing the greatest yield (df=5, F=40.01, p=<0.001).

Table 2.3. Mean carrot weevil (CW) percent damage and carrot yield across several planting dates in a trial at the Muck Crops Research Station (University of Guelph) at the Holland Marsh, Ontario.

Planting Date	Mean CW Damage (%)[1, 2]			Yield (t/ha)[3]
	25 July Sample	3 Oct Sample	Combined Average	
02-May	59.5 ab[4]	65.5 a	62.5 a	31.4 e
09-May	42.0 c	58.4 ab	50.2 b	44.4 d
20-May	25.0 d	43.9 bc	34.5 c	65.5 bc
30-May	4.8 e	24.7 d	14.8 d	72.7 ab
10-Jun	1.6 e	15.2 de	8.4 d	85.1 a
21-Jun	2.4 e	13.7 de	8.0 d	55.5 cd

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent damaged is based on carrot number.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 3.
[4] Different letters within columns Combined Average and Yield, and between both columns for sampling date denote significantly different groups according to Tukey's HSD (α=0.05).

Carrot Root Section Monitoring

CW oviposition was detected during the first sampling period using CRS, 19-24 May 2016 (Fig. 2.4). In 2016, the degree day model of CW oviposition predicted oviposition to begin between 25-26 May 2016 ($145\pm9_{DD7^{\circ}C}$) and the 90% oviposition threshold of $456\pm47_{DD7^{\circ}C}$ was reached between 17-24 June 2016. Therefore, CW oviposition began earlier than expected based on this model. Using CRS, the established economic threshold of 0.03 egg cavities / CRS / day was above the threshold until the predicted oviposition period reached the 90% threshold.

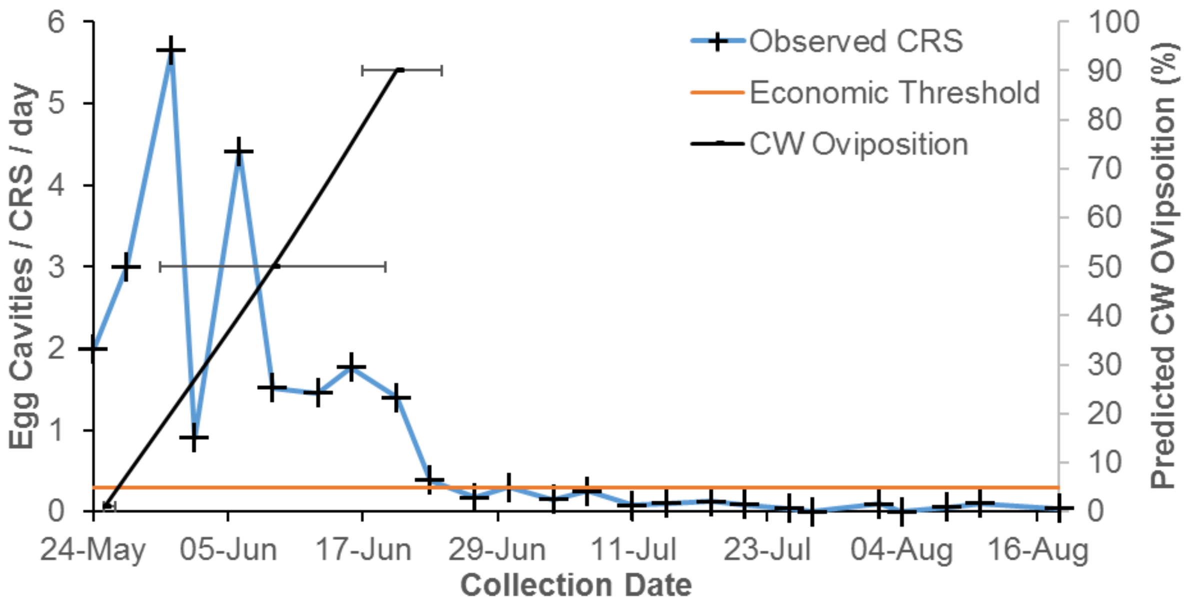


Figure 2.5. Carrot weevil (CW) oviposition on carrot root sections (CRS) compared to predicted CW oviposition according to the established degree day model at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario, 2016. Collection dates are denoted with a +.

2.5 Discussion

Overall, the results of this study suggest the current recommendations of the CW IPM program do not effectively and consistently reduce CW damage and increase carrot yield, at least in the Holland Marsh regions. When historical data from the MCRS CW IPM program were examined, there was a large variation in the number of insecticide applications recommended and observed CW damage, as fields recommended to apply no insecticides for CW control received >10% damage. The results from all CW IPM evaluation trials suggest that the current recommended insecticide applications fail to reduce CW damage compared to an untreated control consistently. These results, when taken together, reduce confidence in both the monitoring and insecticide applications recommended by the CW IPM program.

The lack of efficacy of the CW IPM program is likely based on two issues: current monitoring/economic thresholds no longer accurately relate to observed CW damage, and novel activity the CW has exhibited, resulting in earlier CW oviposition by both degree day and crop life stage. These issues are based in evidence collected during and as a result of field trials and the assessment of the historical data of the MCRS IPM program, and will be discussed below.

The historical CW data from the MCRS IPM program shows that increasing CW captures and CW thresholds are related to increases in CW damage. Although there is a positive correlation between CW presence and CW damage, the results show that CW damage was extremely variable in the Holland Marsh by both time and location, which highlights the need for more accurate monitoring methods. Some fields did not reach any CW economic thresholds yet still had over 10% damage at harvest (Table 2.2). Therefore, this is a clear case of CW IPM program failure, as the EIL is set at 2% (Stevenson and Barscsz 1997) and insecticide applications should have been justified for this level of CW damage. Since economic thresholds require the monitoring methods to accurately relate to crop injury (Higley and Pedigo 1993), current CW monitoring fails to produce accurate economic thresholds for the protection of carrot fields. A more precise and accurate monitoring method should improve the reliability and efficacy of economic thresholds for CW.

Few monitoring methods have been examined or discussed using attractants other than carrot roots. Current monitoring methods do not work in parsley fields, instead scouts must physically examine 145 plants per field for CW oviposition which is time and labour consuming (Torres and Hoy 2002). This suggests a better CW attractant than a carrot root is needed, since trapping methods relying on carrot root baits fail to attract CW in parsley fields. CW does not have a known preferred host plant beyond the Apiaceae (Pepper and Hagmann 1938) so identifying alternative attractants may be difficult. Due to the lack of available information of CW attractants, improving CW monitoring methods may be difficult.

It is possible that CRS may be a more effective CW monitoring technique than the Boivin trap for the CW IPM program. In 2016, monitoring with CRS produced the same economic thresholds as the Boivin trap at the MCRS, with both monitoring methods recommending two insecticide applications at the 2nd and 4th TLS. The ability to monitor oviposition (which directly results in damage to carrots) rather than CW presence could potentially increase the utility of CW monitoring technique for the sake of crop protection. This increase in accuracy could result from monitoring the activity that results in damage on the carrots, oviposition, whereas the Boivin trap monitors the presence of female and male adult CW indiscriminately. Past trials have found the monitoring techniques to produce similar results (Stevenson and Barszcz 1997), although CW activity may have changed since these trials occurred. Additionally, an action threshold for insecticide applications could also be determined using CRS, ensuring insecticides are applied while the CW is active within the field. In contrast, the current monitoring methods recommend an economic threshold, applying insecticide based on crop life stage.

There is evidence that CW activity has changed in the Holland Marsh region, as new oviposition behaviour was detected during field trials. In 2016, monitoring with CRS found high rates of CW oviposition during the first monitoring period from May 19-24, roughly 2 oviposition pits / CRS / day were found (Fig. 2.4). The established degree day threshold did not predict CW oviposition to begin until May 25-26, and the rates of oviposition were well above the economic threshold set for CRS, at 0.3 oviposition pits / CRS / day (Stevenson 1985). While this is only one occurrence, and more CRS monitoring should be performed in more fields over several years, there is a possibility that CW oviposition is no longer following the established degree day model in the Holland Marsh which has been utilized for CW management for several decades.

In addition to earlier oviposition than predicted according to the established degree day model, CW oviposition in carrot plants prior to the 4th TLS was also detected (Fig. 2.5). Carrot mortality due to CW attack is unreported in the scientific literature, although young celery plants have been observed to wilt and die due to larval CW feeding (Swift and Davis 1963). Feeding from adult CW is reported to cause no mortality to carrots (Boivin 1988) though one publication has noted CW feeding can cause some carrot seedling death (Pree *et al.* 1996). Pree *et al.* (1996) stated, “Carrot weevil feeding killed some of the young seedlings, and consequently, there were fewer carrots per meter in untreated plots than in plots treated with phosmet or chlorpyrifos”. This statement makes it unclear whether adult or larval CW feeding was the cause of carrot death. Carrot weevil oviposition in carrots prior to the 4th TLS also appeared to result in the death of the carrot. Throughout 2015 and 2016, dead carrots were observed throughout most field trials, typically early in the season in late May and June. Often, the foliage of the carrots began to wilt and when the carrot root was pulled out, there was significant tunneling damage directly down the middle of the root, which contains the vascular tissues, which occasionally broke the root (Fig. 2.6A). Carrots with similar damage have been observed throughout the Holland Marsh over the past 4-5 years (Mary Ruth McDonald, personal communication, Jan. 4, 2017). It is possible that CW oviposition prior to the 4th TLS results in larval feeding prior to significant root development, leading to the tunnelling through critical root tissues. Occasionally, broken roots still contained CW larvae (Fig. 2.6B). Although CW causes direct damage, and the dead carrot would be rendered unmarketable even if it survived, CW larvae can potentially move between carrots through the soil (Pepper 1942). Therefore, carrot death caused by CW attack may result in the CW larvae moving to another food source, increasing the overall number of carrots affected by a single CW larvae.

Figure 2.6. A carrot weevil oviposition pit (yellow circle) on a second true leaf stage carrot.

Figure 2.7. A) The tunneling damage on this carrot was severe enough to separate this section of root from the remainder of the root; and B) the same carrot present in A. A carrot weevil larvae present within the tunnel of this carrot root.

These new issues occurring with the CW IPM program have resulted in a reduced efficacy of CW control measurable in field trials. In both 2015 and 2016, the IPM program evaluation trial failed to mitigate CW damage effectively compared to the untreated control (Table 2.2). The 2015 and 2016 IPM program evaluation trial performed in a commercial field found that the CW IPM program did not significantly reduce CW damage. In the 2016 trial at the MCRS, there was a significant reduction in CW damage compared to the control and yield was increased by ~10 t/ha, however total CW damage at the second sampling was over 20% and as such may not have been harvested based on the cost of sorting out the damaged carrots. The failure of the IPM program, through recommending insecticide applications without a substantial reduction in CW damage, occurred while both economic thresholds (5 cumulative adult CW/trap) were reached in all managed sites in both years. Therefore, some combination of the economic thresholds, the spray timings associated with the insecticide applications, and the insecticide applied require alteration for effective CW mitigation. These concerns about the economic thresholds agree with the results obtained from the assessment of the historical performance of the CW IPM program, where fields reaching both economic thresholds based on CW monitoring only slightly increased CW damage compared to reaching no thresholds (Fig. 3.2).

The declining efficacy of the IPM program may also be an indicator that CW populations in the Holland Marsh are developing resistance to phosmet (Imidan 50 WP/Imidan 70 WP), the primary insecticide used for chemical control. The development of CW resistance to phosmet in the Holland Marsh is of great concern to carrot producers, as more than 50% of carrot-producing hectares in Canada receive phosmet applications (Agriculture and Agri-Food Canada 2009). Pree *et al.* (1996) conducted a field trial from 1991-1994 and found significant reductions in CW damage as a result of phosmet application, but control was variable with damage still exceeding 10% of yield in some cases. Improving the efficacy of CW chemical control within the IPM program may require changes to the monitoring methods, economic thresholds, and registered insecticides.

The planting date trial results are consistent with previous results that planting later in the season reduces CW damage (Boyce 1927; Perron 1971; Boivin 1988; Zhao *et al.* 1991). In the current trial, the final three planting dates (30 May, 10 June, 21 June) all resulted in similarly reduced levels of CW damage (Table 2.3). Planting on 21 June resulted in significantly lower marketable yield compared to 30 May and 10 June. Consequently, delaying planting can reduce damage but there is a trade off with overall yield. Therefore, an optimal planting date should be sufficiently late to reduce CW damage while providing the highest possible marketable yield. This trial should be repeated over several years to establish an optimal time frame for planting date during which CW damage is reduced while total carrot yield remains high. It is possible than planning planting dates in association with degree days, in order to avoid CW oviposition period, may allow for an easily measurable time frame for carrot planting that is applicable across different years and weather conditions. Alternatively, establishing separate planting dates within the same field, likely with the early seeded carrots surrounding the field borders for ease of management, may allow the creation of a trap crop or CW oviposition sink within fields. Based on the results of the planting date study, planting a limited number carrots within the first week of May and the rest of the crop 3-4 weeks later could result in the CW predominately attacking the earlier seeded carrots. This could make management of the pest more efficient/practical by focusing control efforts on limited areas of the field and reducing overall CW damage to the majority of the crop. Alternatively, the first cohort of carrots could be killed in an attempt to kill the CW larvae resulting from early oviposition. Future research is still needed to assess the practicality and efficacy of using early seeded carrots as a trap crop or sink for CW eggs.

In conclusion, the null hypothesis that the CW IPM does not relate CW monitoring to observed CW damage is accepted. Though there is a correlation between increased CW number and increased CW damage, this relationship is weak and fails to accurately predict high CW damage. The null hypothesis that insecticide applications administered following the current CW IPM do not effective reduce CW damage is also accepted. In field trials, the IPM program overall failed to provide effective CW control, reducing CW damage in only one of three trials. The trial that showed the reduction in CW damage still received over 25% CW damage in the treated plots, which is too high to describe as effective control as the direct damage cause by the pest renders all damaged yield unmarketable.

The current study has established that there are significant shortfalls in CW control using the current IPM protocols in the Holland Marsh. This shortfall is demonstrated by the failure to achieve effective CW damage reduction in field trails comparing plots treated with CW IPM protocols with untreated plots. This failure may be rooted in several causes, including monitoring efforts not accurately relating to observed CW damage, and earlier CW oviposition with relation to both degree days and crop stage. One possible approach to improving CW management could be improved monitoring of oviposition activity in the field. In addition, the current CW oviposition degree-day model should be re-examined because oviposition outside of the modelled period has been detected. Later planting dates should also be investigated as a potential cultural control approach for avoiding CW oviposition activity and maintaining yield. An extension of this approach could involve a limited early planting of some carrots as a trap crop to help reduce overall CW damage throughout the entire field later in the season.

CHAPTER 3

INSECTICIDE EFFICACY FOR CARROT WEEVIL MITIGATION

3.1 Abstract

Chemical control has historically been the primary method to mitigate carrot weevil (CW) damage. In Ontario, concerns about CW resistance to the industry standard insecticide, phosmet, have emerged. In the current study, insecticides were screened for efficacy on CW adults using a 1/9th scale model of a Potter spray tower. Only phosmet, clothianidin, and λ-cyhalothrin caused significant mortality to adult CW at rates based on the recommended field rates in carrots. In 2015 and 2016 field trials, the efficacies of seed treatment, foliar, and in-furrow applications for CW control in carrots were examined. In both years, most insecticides failed to provide significant control at recommended rates in carrots. Foliar applications of cyantraniliprole and novaluron had the greatest efficacy while phosmet was only effective when paired with 1.0% piperonyl butoxide. Field trials revealed novel CW behaviour, including oviposition at the carrot 2nd TLS and CW larval feeding resulting in carrot mortality. Results in both years provided evidence of a second CW generation, which should not be controlled using current IPM program recommendations.

3.2 Introduction

Apart from crop rotation, insecticide application is the primary strategy for CW mitigation in the Holland Marsh. In the Holland Marsh, insecticide applications have been relied upon heavily, as crop rotation is difficult due to the limited land available for crop production (Pepper and Hagmann 1938, Semel 1957). Historically, CW management has depended on high rates of organophosphorus or organochlorine insecticides. Early CW control focused in parsley employed arsenic-based baits and dusts that were applied when 5% of examined crop plants contained a CW oviposition pit (Pepper and Hagmann 1938). Organochlorines were initially effective for CW control on parsley. For example, three applications of dieldrin and heptachlor in granulated (1.68 and 2. 24 kg ai/ha, respectively) or emulisifed (0.42 and 1.12 kg ai/ha, respectively) form during the growing season achieved a ~90% reduction in CW damaged

(Semel 1957). DDT, applied as a foliar spray at a rate of 1.0 lb AI / ac four times at 7-10 day intervals starting at the first sign of CW oviposition, controlled CW successfully in carrots and performed better than arsenic-based baits (Wright and Decker 1957). Similarly, dieldrin (0.56 kg ai/ha) and parathion (1.12 kg ai/ha) also reduced CW damage by 40-80% (Wright and Decker 1957). In contrast in carrots, soil amendments of 11.21 kg ai/ha dieldrin and 1.12 kg ai/ha parathion that targeted developing CW larvae resulted in no significant reduction in damage (Wright and Decker 1957).

With the banning of DDT, a replacement for the organochlorines was required to ensure CW control (Martel *et al.* 1975a; Stevenson 1983). In-furrow applications of the carbamate insecticide carbofuran (2.2 kg ai/ha) reduced CW damage by >85% in some field trials (Stevenson 1977), although producers indicated it was ineffective in the Holland Marsh, possibly due to degradation resulting from the high microbiological activity in muck soil (Stevenson 1983). The organophosphorus insecticide phosmet caused 100% CW mortality when applied as a 0.1% solution of technical insecticide in a laboratory experiment using a Potter spray tower and another laboratory experiment showed residual activity (~50% CW mortality) in organic soil when applied at 0.56 kg ai/ha (Martel *et al.* 1975b). In the field, two applications of 1.1 kg ai/ha phosmet sprayed during the oviposition period of the CW early in the growing season controlled CW damage successfully (Stevenson 1983). Based on these results, phosmet was subsequently was registered in Canada for CW control in the early 1980s (Stevenson 1983).

Phosmet was also used extensively in the US for CW control in the 1980s. Deregistration of phosmet in the early 1990s left producers with no chemical control options (Bonham *et al.* 2009). In Canada, phosmet continued to be used in carrot production and control options for CW were expanded to include λ-cyhalothrin (Matador 120 EC) in 2014 and novaluron (Rimon 10 EC) in 2015. In the Holland Marsh, phosmet (Imidan 50WP or Imidan 70WP) remains the industry standard for CW control, but there are increasing concerns among producers and researchers that CW has developed resistance to this insecticide. Studies by Martel *et al.* (1975b) suggested that Ontario CW have already developed resistance to the cyclodienes.

Effective resistance management requires the use of insecticides with different modes of action. For that reason, novel insecticides that are effective in mitigating CW damage and have a different mode of action to phosmet need to be identified and registered so that an effective integrated resistance management program can be introduced at the Holland Marsh. Limited research on chemical management of CW has been conducted since the 1980s. In the intervening period, an abundance of new insecticide formulations have been developed that warrant investigation for CW control. One important avenue of research is the investigation of systemic insecticides to control CW. Since CW eggs and larvae develop within the carrot, systemic insecticides, applied as a seed treatment or foliar application, can target CW eggs and early instar larvae eliminating or complementing attempts to kill free-living adults prior to oviposition (Martel *et al.* 1975a). Fipronil has been shown by Bonham *et al.* (2009) to be an effective systemic seed treatment for controlling CW damage. Although, fipronil is unlikely to be registered in Canada due to concerns about high persistence, toxicity of its degradation products, and bioaccumulation (Tingle *et al.* 2003).

The deployment of novel insecticides is expected to improve CW control, but it is important to consider the environmental and health effects of these insecticides. Phosmet is applied at very high rates of 1.1 kg ai/ha. In 2005, approximately 5,000 kg of phosmet were applied to Canadian carrot fields, making it the most used insecticide by weight in Canadian carrot production (Agriculture and Agri-Food Canada 2009). The Environmental Impact Quotient (EIQ) and EIQ-Field Use Rating (EIQ-FUR), developed by Kovach *et al*. (1992), allows for a relative comparison of potential impacts of all pesticides. The system converts various measures of toxicities and exposure routes into rankings from 1-5, creating a minimum value of 6.7 and a maximum value of 225, and can be broken into three major components: worker safety, consumer safety, and environmental safety. The field use rating component adjusts EIQ values based on the amount of AI introduced into the environment, increasing the relevance for field use comparisons. For example, the EIQ of phosmet is 32.8, but when adjusted to the EIQ-FUR, following the industry standard of two applications in carrots at the label rate of 1.1 kg AI / ha, the EIQ-FUR is 65.6 and the environmental safety component of the EIQ-FUR is extremely high, 178.1 (Eshenaur *et al*. 1992-2015).

While the EIQ has been used to compare risk, several criticisms have been made regarding the calculation of the EIQ and the EIQ-FUR. The EIQ calculation also may be biased towards certain variables within the equation. An analysis based on simulated EIQ calculations found that of the 10 variables used in the calculation, plant surface half-life is a primary driver of EIQ values, accounting for nearly 30% of the variation (Kniss and Coburn 2015). Dermal and chronic toxicity each explained >20% of the variation and no other variables accounted for more than 10% of the variation in EIQ values (Kniss and Coburn 2015). Fish, bird, and bee toxicity, as well as soil half-life, were not major drivers of EIQ values. This suggests the relative rankings may be biased towards mammalian safety and exposure rather than overall environmental safety. The EIQ-FUR calculation uses a multiplication of the EIQ by the rate applied, relying on an assumption that double the insecticide applied should result in double the risk. This assumption does not concur with the classical sigmoidal dose-response curve, where toxicity is not linearly related to dose, exhibiting plateaus at low and high concentrations (Dushoff *et al.* 1994). Despite these criticisms, the EIQ still has merit as an approachable, communicable number to convey a relative risk to a wide array of audiences, as shown through its continued use throughout the scientific literature (see Bues *et al.* 2004, Cross and Edwards-Jones 2006, Biddinger *et al.* 2014, and Beckie *et al.* 2014) and pest management programs (see Cornell Cooperative Extension 1993). Optimally, a novel insecticide for CW mitigation will be associated with lower detrimental environmental impacts than currently employed agents.

Laboratory and field trials were performed to assess novel options for chemical control of CW. The laboratory trials focused on contact insecticides and provided a basis for selecting insecticides in field trials. Field trials were performed from May to October in 2015 and 2016 and examined foliar and seed treatment insecticide applications to mitigate CW damage in the Holland Marsh. In addition, a 2016 field trial assessed the ability for the insecticide synergist piperonyl butoxide (PBO) to improve the CW control achieved with phosmet applications. For all field trials, EIQ and EIQ-FUR were calculated.

3.3 Methodology

3.3.1 Carrot Weevil Rearing

Carrot weevil rearing methods were partially based on Martel *et al.* (1975a) and partially on CW rearing methods developed during the course of this research (Standard Operating Procedure; Appendix 3). A culture of CW adults was obtained from Dr. Guy Boivin (Agriculture and Agri-Food Canada, St-Jean-Sur-Richelieu, QC). This laboratory colony has been in culture for approximately 300 generations, with no previous exposure to insecticides. Wild-type genes have not been introduced into this colony for >10 years to avoid contaminating the colony with a nematode, *Bradynema listronoti* (Tylenchida: Allantonematidae), that occurs naturally in Quebec fields (Zeng *et al.* 2007) Carrot weevils were reared at 24 ± 1 °C, 70 ± 10% RH and 18:6 L:D photoperiod. Approximately 100-200 adult CW were placed in 3.8 L glass jars containing a single carrot (min. 3 cm diam.) for feeding and oviposition. The bottom of each jar was lined with 15 cm filter paper (Whatman Grade 1 Qualitative, GE Healthcare Life Sciences, Mississauga, ON) and the top opening was covered with a cleaning tissue (Kimberly-Clark Professional, Roswell, GA, USA) underneath 1 mm mesh and sealed with an elastic band. Carrots, filter paper, and cleaning tissues were replaced every two to three days. For larval development, the replaced carrots from jars containing ovipositing CW were held in a sealed 25 X 18 X 10 cm plastic container and transferred two weeks later to 20.2 X 27.9 cm mesh trays held in 25 X 18 X 10 cm plastic containers with a lid containing a 5 x 10 cm hole covered in fine mesh and lined with ~1 cm of sterilized muck soil for CW pupation. Emerging adults were collected in small jars attached to the colony boxes using a slice of carrot as bait and placed into 1 gallon jars. If 6-8 jars of CW were ovipositing actively, new adults were held in the colony room for 1 week and then moved to 15 ± 1 °C, 16:8 L:D photoperiod to induce a female reproductive diapause. Diapausing females were maintained until needed for sustaining colony production or laboratory trials. Jars of ovipositing CW were used for 4-6 weeks, after which oviposition declined and any surviving CW were killed by placing the jar in a -20°C freezer.

3.3.2 Laboratory Trials – Spray Tower

Laboratory Assay 1: Assessment of Relative Insecticide Toxicity to Carrot Weevil

Cyantraniliprole, chlorantraniliprole, clothianidin, imidacloprid, λ-cyhalothrin, phosmet, spinosad, and spinetoram were assessed for their efficacy using formulated products on the susceptible Boivin CW colony described in the previous section (Table 3.1). Carrot weevils, 2-4 weeks old, were taken from the overwintering portion of the colony and sorted into groups of 10 adults of the same age and gender, following the sexing protocol described by Whitcomb (1965). Groups of CW were held in 30 ml plastic cups (Solo Cup Company, Lake Forest, IL) with a fresh carrot slice at 15±1ºC, 16:8 L:D for 1-3 days prior to testing. On the day of testing, weevils were gently cleaned using a Kimwipe™ delicate task wiper (Kimberly-Clark Corporation, Irving, TX) to remove any frass or carrot material which typically accumulates on their bodies during their time in the colony.

Application rates for each pesticide were calculated using the following equation (Cutler et al. 2006):

$$\text{TMC} = \text{recommended label rate} \times (1\ \text{ha} \times 500\ \text{L ha}^{-1}) \times (1\ \text{L} \times 1000\ \text{ml}^{-1}) \times (1\ \text{L} \times 1000\ \text{g}^{-1}) \times 1000000\ \text{mg L}^{-1} \times \text{g active ingredient L}^{-1}$$

Where TMC (tank mixture concentration) is the concentration of insecticide in solution that should be in a tank mixture prepared for a foliar application in a carrot field. A standard spray rate of 500 L / ha was used to calculate all insecticide TMC. The recommended application rate for use in carrots according to the most recent Canadian label as of January 2015 was used. Clothianidin was the only insecticide with no registration on carrots and the recommended label rate for clothianidin to control Colorado Potato beetle in potatoes was used (Valent Canada, Inc., 2015). Initially, 1x TMC was going to be an additional treatment, but preliminary assays showed negligible mortality for almost all treatments at this dose. Multiple testing dates were required for adequate replication. For each testing date, fresh stock solutions of each insecticide were prepared within 48 h prior to the trial and each test solution was prepared within 12 h prior to the trial. All test solutions consisted of formulated insecticide dissolved in distilled water. The concentrations of all insecticide treatments and stocks are listed in Table 3.1. For each testing date, an additional treatment consisting of distilled water was used as a control for mortality.

Table 3.1. Formulated insecticides and their respective tank mix concentration (TMC) used in laboratory trials examining carrot weevil (CW) susceptibility to contact application of formulated insecticides dissolved in distilled water using a 1/9th scale Potter spray tower.

Active Ingredient	Formulation	Manufacturer	Stock (ppm AI)	TMC (ppm AI)		
				1x	2x	4x
Chlorantraniliprole	Coragen	E.I. Du Pont, Canada	1000	-	150	300
Cyantraniliprole	Exirel	E.I. Du Pont Canada	1000	-	150	300
Phosmet	Imidan 50 WP	Gowan Company LLC.	5000	1125	2250	4450
λ-cyhalothrin	Matador 120 EC	Syngenta Canada Inc.	500	-	19.92	39.84
Imidacloprid	Admire 240 SC	Bayer Cropscience Inc.	1000	-	96	192
Clothianidin	Clutch	Valent Canada, Inc.	1000	-	105	210
Spinosad	Success	Dow AgroSciences Canada Inc.	1000	-	174.72	348.84
Spinetoram	Delegate	Dow AgroSciences Canada Inc.	1000	-	100	200

Insecticide treatments were applied using a custom-built miniature (1/9th scale) Potter spray tower placed in a fumehood. The 1/9th scale Potter spray tower uses an Iwata® Eclipse HP-BCS airbrush (Wyndham Art Supplies, Guelph, Canada) with an air compressor delivering 15 PSI to apply the insecticides. Each insecticide application consisted of a single 1 ml spray lasting 13-14 sec. To ensure the spray tower was distributing treatments effectively, the spray pattern was examined using water-sensitive paper (Syngenta Canada, Guelph, Ontario) using 1 ml of deionized water. For the applications, 10 male or 10 female CW were distributed randomly ventral-side up in the bottom of a 50 mm glass petri dish line with filter paper (Sartorius AG, Bohemia, NY). In order to reduce CW mobility during treatments, each group of 10 CW were held in a fridge at 6 ± 2 ºC for 3-5 h and then shaken gently prior to application to limit movement, as CW feign death when disturbed (Harris 1926; Boyce 1927; Pepper and Hagmann 1938). Post-application, each group of CW were transferred immediately to a clean 30 ml plastic cup containing a fresh slice of carrot and held at 24 ± 2 ºC for 24 h. As a control treatment, 1 ml of distilled water was applied to groups of CW (2-4 replications for each testing date) in the same manner as the insecticide applications. Between changes in treatment or TMC rate, the spray tower was flushed with three alternating sprays of 1 ml of 1% Liquinox® (Alconox, INC., White Plains, NY) solution then 1 ml of acetone followed by a flush of distilled H_20 to ensure no insecticide residue remained in the tower. At 24 h post-application, CW were assessed for mortality. To assess for mortality, each CW was squeezed gently with forceps. CW that exhibited no response (no movement) were considered dead.

Laboratory Assay 2: Assessment of Holland Marsh Carrot Weevil Resistance to Phosmet

Adult CW were collected throughout the Holland Marsh from May to July, 2015. The CW were returned to the University of Guelph (Guelph, ON) and reared following the methods described previously for 6-8 months prior to testing. As these CW were taken directly from commercial fields, they are considered to be a wild population that had experienced insecticide exposure. The Holland Marsh CW strain was segregated from the Quebec strain. Using the methods described previously, each CW strain was assessed for susceptibility to phosmet at 1x and 2x TMC using the 1/9th scale Potter spray tower, following the same experimental procedure outlined in the previous section. Treatments of distilled water were applied on each testing date to control for mortality. During Laboratory Assay 1 assessment at 24 h, phosmet applications often produced moribund CW that appeared to have difficulty walking or standing but were still capable of movement. In order to allow for the insecticide to exert its toxic effects fully, the time of assessment was moved to 48 h for Laboratory Assay 2.

3.3.3 Field Trials

A series of field trials described below were performed at MCRS on muck soil (soil: pH ≈ 6.8, organic matter ≈ 64.8%). After observing high levels of CW damage in 2015 at the MCRS, field trials in 2016 were performed in the locations as 2015 field trials in an attempt to receive high CW damage in both field seasons. Field trials were performed in conjunction with another graduate student (Jason Lemay) investigating improvements to carrot rust fly (CRF) (*Psila rosae*, (Fabricius) (Diptera: Psilidae) control and as such all trials were assessed for insect damage by both pests. Fungicide, herbicide, and fertilizer applications for 2015 and 2016 trials can be found in Appendix 2, Table A7.5 and A7.6, respectively. All field trials were direct seeded using precision seeder (Stanhay Webb Ltd., Bourne, UK) except for trials containing insecticide seed treatments, which used a custom-built push-cone seeder. All field trials were seeded at a rate of 70 carrot seeds / m.

Sampling Methods and Damage Assessment for all Field Trials in 2015 and 2016

In 2015, two 1.5 m row sections were sampled randomly by hand, taking all carrots in each 1.5 m section, from each plot to assess CW and CRF damage in mid August and mid October. Between 10-13 August and 14 October 2015, two random 1.5 m row sections were sampled from each plot to assess CW and CRF damage unless otherwise noted. Within eight days of sampling, all carrot samples were washed in a small drum washer and inspected visually for CW and CRF damage. The number of CW damaged, CRF damaged, and marketable carrots (marketable was defined as no insect damage) were recorded. Insect damage was differentiated as described in Chapter 2.

In 2016, two random 1.5 m row sections were sampled by hand, taking all carrots in each 1.5 m section, from each plot to assess CW and CRF damage in late July and mid October. Early oviposition by CW into carrot plants resulted in up to 20 dead carrots in a single 1.5 m row section. The total amount of dead carrots in both 1.5 m row sections per plot were recorded during the first sampling date and included in the number of carrots with CW damage. By the time the second sampling occurred, the dead carrots had decomposed and could not be counted.

Within nine days of sampling, carrot samples were washed in a small drum washer and inspected visually for CW and CRF damage, and the number of damaged and marketable carrots was recorded. Insect damage was differentiated as described in Chapter 2.

The weight of all damaged and marketable carrots was determined and recorded for the second set of samples for both years.

2015 Comparison of Seed and Foliar Treatments

A variety of seed treatments were compared to three foliar treatments. Carrots (cv. Bolero) seeded on May 28 2015 at the MCRS. Each plot consisted of two rows, 66 cm apart and 6 m in length. A randomized complete block arrangement with four replications of eight treatments was used. Each block, containing a replicate of each treatment, was separated by 1.5 m of unseeded space. A total of 32 plots were established for a total trial area of ~315 m^2.

Seed treatments were applied as seed film coatings by Dr. Alan Taylor (Cornell University, Geneva, NY) at a constant rate of 4.51 g AI/100 g seed. Seed treatments consisted of: clothianidin + imidacloprid (Sepresto 75 WS, Bayer CropScience Inc., Mississauga, ON), flupyradifurone (Sivanto Prime FS480, Bayer CropScience Inc., Mississauga, ON), cyromazine (Trigard, Syngenta Canada, Guelph, ON) and cyantraniliprole (HGW86, Dow AgroSciences, Calgary, AB). Three foliar treatments consisted of phosmet (Imidan 70 WP, 1.6 kg/ha; Gowan Canada LLC., Yuma AZ), and cypermethrin (Ripcord, 175 ml/ha; BASF Canada Inc., Mississauga, ON).

Three foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles (TeeJet Technologies, Springfield, IL, USA) calibrated to deliver 400 L/ha at 240 kPa. Phosmet and cypermethrin were applied to the same plots and served as a positive control for CW and CRF control, respectively. All foliar products were applied on 21 June for CW and 1st generation CRF control, and cypermethrin was applied again on 4 and 27 August for 2nd generation CRF control, according to the existing IPM recommendations.

Within each block, one plot received no seed treatment or foliar insecticide to serve as an untreated control. All seeds were treated with thiram (Thiram 42 S (0.21 g AI/100 g seed; Bayer CropScience Inc., Mississauga, ON) for fungal disease control. Each plot was sampled on August 13 and October 15 as described previously to assess the effect of treatments.

2016 Comparison of Seed and Foliar Treatments

A variety of seed treatments were compared to three foliar treatments. Carrots (cv. Bolero) seeded on May 20 2016 at the MCRS. Each plot consisted of four rows, 86 cm apart and 6 m in length. A randomized complete block arrangement with five replications per of five treatments was used. Each block, containing a replicate of each treatment, was separated by 1.5 m of unseeded space. A total of 25 plots were established for a total trial area of ~560 m^2.

Seed treatments were applied as seed film coatings by Dr. Alan Taylor (Cornell University, Geneva, NY) at a constant rate of 7.29 g AI/100 g seed. The rate was increased compared to the 2015 comparison of seed treatment and foliar insecticide trial, as that trial failed to mitigate CW damage effectively. Seed treatments consisted of: flupyradifurone (Sivanto Prime FS480, Bayer CropScience Inc., Mississauga, ON), cyantraniliprole (Fortenza, Syngenta Canada, Guelph, ON), and tefluthrin (Force 3G, Syngenta Canada, Guelph, ON). A different formulation of cyantraniliprole was used as Dr. Taylor indicated the producer of HGW86, used in 2015, was not interested in horticultural applications of the product (personal communication, Dr. Al Taylor).

Two foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. Phosmet and cypermethrin were applied to the same plots and served as a positive control applications for CW and CRF control, respectively. All foliar products were applied on 30 June for CW and 1st generation CRF control and cypermethrin was applied again on 11 August for CRF control, according to the existing IPM recommendations.

Within each block, one plot received no seed treatment or foliar insecticide to serve as an untreated control. All seeds were treated with thiram (Thiram 42 S (0.21 g AI/100 g seed; Bayer CropScience Inc., Mississauga, ON) for fungal disease control. Each plot was sampled on July 25 and October 3 as described previously to assess the effect of treatments.

2015 Comparison of Foliar Treatments

Five foliar insecticides were assessed for their efficacy in reducing CW damage in 2015. Carrots (cv. Enterprise) were seeded on June 4 2015 at the MCRS. Each plot consisted of three rows, 66 cm apart and 5 m in length. A randomized complete block arrangement containing four replications per treatment was used. A total of 24 plots were established for a total area of ~240 m^2.

Treatments consisted of phosmet (Imidan 70 WP, 1.6 kg/ha), λ-cyhalothrin (Matador 120 EC, 83 ml/ha), spinetoram (Delegate, 200 g/ha), cyantraniliprole (Exirel, 750 ml/ha), and clothianidin (Clutch, 105 g/ha). Foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. All treatments were applied on 25 June for CW control, according to existing IPM recommendations. Within each block, one plot received no foliar sprays to serve as an untreated control. Each plot was sampled on August 13 and October 15 as described previously to assess the effect of treatments.

2016 Comparison of Foliar Treatments

Seven foliar insecticides were assessed for their efficacy in reducing CW damage in 2015. Carrots (cv. Enterprise) were seeded on May 28 2016 at the MCRS. Each plot consisted of four rows, 86 cm apart and 5 m in length. A randomized complete block arrangement containing five replications per treatment was used. Each block contained two rows of 4 plots and each row of plots were separated by 1.5 m of unseeded space. A total of 40 plots were established for a total area of ~870 m^2.

Treatments consisted of phosmet (Imidan 70 WP, 1.6 kg/ha) and cypermethrin (Ripcord, 175 ml/ha), novaluron (Rimon 10 EC, 83 ml/ha; Adama Agricultural Solutions Canada, Winnipeg, MB), spinetoram (Delegate, 200 g/ha), cyantraniliprole (Exirel, 1500 ml/ha), λ-cyhalothrin (Matador 120 EC, 83 ml/ha), *Steinernema feltiae* (Rhabdita: Steinernematidae) (Nemasys, 500,000 IJ/m; BASF Canada Inc., Mississauga, ON), and *Beauveria bassiana* (Botanigard ES, 2 L/ha; Lam International Corporation, Butte, MT, USA). Clothianidin was not examined in 2016 as 2015 results found an increase in CW damage in clothianidin-treated plots. In 2016 as laboratory colonies of CW at the University of Guelph and Agriculture and Agri-Food Canada became infested with a fungal pathogen, which inspired the inclusion of *B. bassiana* in order to assess the ability of an entomopathogenic fungus to control CW. The nematode *S. feltiae* was included as a new nematode product became available and applications of nematodes have shown some success for CW control (Belair and Boivin 1995; Miklasiewicz *et al.* 2002). All insecticides and *B. bassiana* were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. *Steinernema feltiae* were applied as a drench at 555 L/ha.

Treatments were applied on June 23, July 7, and August 11. The first two applications were timed to protect the developing crop at the 2nd and 4th TLS, and the August 11 application was intended to mitigate CW damage from potential 2nd generation CW. As the registration of phosmet limits the product to two applications per season in carrots, phosmet was not applied on August 11. Within each block, a single plot received no foliar sprays to serve as an untreated control. Each plot was sampled on July 26 and October 4 as described previously to assess the effect of treatments.

2016 Insecticide Synergist Assessment

In an attempt to increase the efficacy of the primary product for CW and CRF control, a trial compared the efficacy of phosmet and cypermethrin with and without the addition of a synergist (piperonyl butoxide (PBO)) (Acros Organics, NJ, USA) for controlling carrot insect pests. Carrots (cv. Enterprise) were seeded on May 28 2016 at the MCRS. Plots consisted of four rows, 85 cm wide and 5 m long. A randomized complete block arrangement containing four replications per treatment was used. Each block, containing a replicate of each treatment, was separated by 1.5 m of unseeded space. A total of 20 plots were established for a total trial area of ~400 m^2.

Treatments consisted of a cypermethrin (Ripcord, 175 ml/ha), cypermethrin + 1.0% PBO, phosmet (Imidan 70 WP, 1.6 kg/ha), and phosmet + 1.0% PBO. Prior to mixing field treatments, 90% PBO was mixed with 95% ethanol to form a 10% PBO solution to improve solubility. Foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. Foliar applications were applied on 23 June, to control CW and 1st generation CRF, and 11 August, to control 2nd generation CRF and a potential 2nd generation CW. Each plot was sampled on July 26 and October 4 as described previously to assess the effect of treatments.

2015 Comparison of In-Furrow and Foliar Treatments

In-furrow applications followed by foliar applications were assessed for their ability to reduce CW and CRF damage. Carrots (cv. Enterprise) seeded on June 4 2015 at the MCRS. Plots consisted of three rows, 66 cm apart and 10 m in length. A randomized complete block arrangement with four replications was used. Each block, containing a replicate of each treatment, was separated by 1.5 m of unseeded space. A total of 24 plots were established for a total trial area of ~225 m^2. A randomized complete block arrangement with four replications was used. Each block, containing a replicate of each treatment, was separated by 1.5 m of unseeded space. A total of 24 plots were established for a total trial area of ~225 m^2.

Treatments consisted of an untreated control, an industry standard application of cypermethrin (Ripcord, 175 ml/ha) or four different combinations of an in-furrow application and foliar spray. The two in-furrow applications consisted of imidacloprid (Admire 240, 1.0 L/ha; Bayer CropScience Inc., Calgary, AB) or thiamethoxam + cyantraniliprole (Minecto-Duo 40 WG, 5 g/100 m row; Syngenta Canada, Guelph, ON). Each in-furrow application was applied to two plots per block, which then received three foliar applications of flupyradifurone (Sivanto Prime SL 200, 1.0 L/ha) or two applications of cyantraniliprole (Exirel, 1.0 L/ha) then cypermethrin (Ripcord, 175 ml/ha) to make a total of four treatments. In-furrow were applied at seeding using a tractor equipped with four TeeJet 8005 XRC (10 ml/s) fan nozzles and calibrated to deliver 250 L water/ha. Foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. Foliar applications were made on 23 June, 4 August, and 27 August. This trial was designed to control CRF, so these foliar sprays were timed to control 1st and 2nd CRF damage. The in-furrow application and June 23 foliar applications were timed appropriately to control CW during the early season oviposition period. Each plot was sampled on August 14 and October 15 as described previously to assess the effect of treatments.

2016 Comparison of In-Furrow and Foliar Treatments

In-furrow applications followed by foliar applications were assessed for their ability to reduce CW and CRF damage again in 2016. Carrots (cv. Enterprise) were seeded on 4 June 2015 at the MCRS. Plots consisting of three rows, 66 cm apart and 10 m in length. A randomized complete block arrangement with four replications was created with.

Treatments consisted of an untreated control, an industry standard application of cypermethrin (Ripcord, 175 ml/ha) or four different combinations of an in-furrow application and foliar spray. The two in-furrow applications consisted of bifenthrin (Capture LFR, 14.1 ml/100 m row; FMC Corporation, Philadelphia, PA, USA) or cyantraniliprole (Verimark, 1.0 L/ha; E.I. Du Pont Canada Company, Mississauga, ON). Each in-furrow application was applied to two plots per block, which then received three foliar applications of flupyradifurone (Sivanto Prime SL 200, 1.0 L/ha) or three applications of cypermethrin (Ripcord, 175 ml/ha) to make a total of four treatments. In-furrow were applied at seeding using a tractor equipped with four TeeJet 8005 XRC (10 ml/s) fan nozzles and calibrated to deliver 250 L water/ha. Foliar treatments were applied using a CO_2 backpack sprayer equipped with 4 TeeJet 8004 fan nozzles calibrated to deliver 400 L/ha at 240 kPa. Foliar applications were made on 23 June, 4 August, and 11 August. This trial was designed to control CRF, so these foliar spays were timed to control 1st and 2nd CRF damage. The in-furrow and June 23 foliar application were timed appropriately to control CW during the early season oviposition period. Each plot was sampled on August July 25 and October 3 as described previously to assess the effect of treatments.

EIQ Calculation

EIQ and EIQ-FUR were calculated for all insecticides used in the seed treatment and foliar trials using the equations presented in Kovach *et al.* (1992). For all insecticides, except flupyradifurone and cyantraniliprole, the Field Use EIQ Calculator provided by the Cornell Cooperative Extension (Eshenaur *et al*. 1992-2015) was used. Flupyradifurone and cyantraniliprole values were calculated based on toxicology information provided in safety data sheets, following the instructions of Kovach *et al*. (1992).

3.3.4 Statistical Analysis

Laboratory Trials – Spray Tower

Laboratory Assay 1 was analysed using an ANOVA in the *Proc Glimmix* procedure in SAS University Edition 9.4 (SAS Institute, Cary, NC) to assess differences in proportional CW survival 24 h post-application. Mortality in the control was below 10%, and mortality in treatments was adjusted according to Abbott's formula (Abbott 1925) prior to analysis. The fixed effects were insecticide formulation, rate of application, sex, and the interactions of these effects. Trial date was a random effect. The CW age and CW emergence date were included initially as random effects in the model, but were removed as they had a co-variance parameter of 0. To ensure the assumptions of the analysis of variance were met, scatter plots of studentized residuals were examined. Due to heterogeneity of error, the model was transformed using a Gaussian-Hermite quadrature, which helps to normalizes error with a dataset following a Beta distribution and dominated by values of 0 and 1. Least significant means were calculated for each insecticide and compared using Tukey's HSD.

Laboratory Assay 2 was analysed using an ANOVA in *Proc Mixed* using SAS University Edition 9.4. Mortality in the control was below 15%, and mortality in treatments was adjusted according to Abbott's formula (Abbott 1925). The fixed effects of the model included CW strain, sex, rate, and the interactions of these effects. Trial date was a random effect. To ensure that the assumptions of the analysis of variance were met, scatter plots of studentized residuals were examined. Least significant means were calculated for all variables included in the fixed effects and compared using Tukey's adjustment.

Field Trials

All field trials were assessed using *Proc Mixed* in SAS University Edition 9.4. A repeated-measures ANOVA was performed for all trials except for the 2016 insecticide synergist trial, examining the fixed effect of treatment, sampling date, and the interaction of these factors on carrot weevil damage. Block included as a random effect. Sampling date was included as a repeated measure. For 2016 trials, dead carrots counted during the first sampling were added to the total number of carrots with CW damage. Differences in marketable yield were assessed using an ANOVA, assessing the fixed effect of treatment with the random effect of block. To ensure the assumptions of all analyses of variance were met, studentized residuals were plotted and examined. In the 2015 seed treatment analysis, a location factor examining plot distance to the field edge was included. In 2015, due to low replication, Dunnett's Test was used to compare means against the control. In 2016, replication increased and least significant means were calculated for all variables included in the fixed effects and compared using Tukey's adjustment. For all analyses, estimate statements comparing CW damage between the first and second sampling effort were performed.

The insecticide synergist trial was assessed as a factorial experiment using *Proc Mixed* in SAS University Edition 9.4. Differences in CW damage were assessed using an ANOVA, examining the fixed effect of the insecticide, PBO application, sampling date, and the interaction of the factors. Block was included as a random effect. Differences in marketable yield were assessed using an ANOVA, assessing the fixed effects of insecticide, PBO application, and the interaction of these factors. Block was included as a random effect. For both analyses, studentized residual were plotted and examined to ensure the assumptions of the ANOVA were met and Tukey's adjustment was used for means separation. Three estimate statements were performed to measure the difference in CW damage due to sampling date, the insecticide used, and PBO application. Estimate statements measuring the difference in yield due to the insecticide used and PBO application were also performed.

3.4 Results

3.4.1 Laboratory Trials – Spray Tower

Laboratory Assay 1: Assessment of Relative Insecticide Toxicity to Carrot Weevil

In Laboratory Assay 1, significant differences in mortality were found due to the insecticide formulation (df=7, F=188.92, $p<0.001$) and application rate (df=1, F=22.00, $p<0.001$) but not CW sex (df=1, F=3.21, F=0.075) (Fig. 3.1). There were interactions between the insecticide applied and rate of application (df=7, F=2.5, p=0.019), and CW sex and insecticide applied (df=7, F=8.04, $p<0.001$), although there was no interaction between CW sex and rate of insecticide applied (df=1, F=2.39, p=0.124) nor among all factors (insecticide applied, rate of application, and CW sex) (df=7, F=0.50, p=0.833) (Fig. 3.2). Therefore the simple effects of combinations of insecticide, and rate of application and CW sex should be examined. Overall, most treatments produced limited mortality. Mortality in the control, distilled water, was under 10% in all instances.

During assessment of mortality for Laboratory Assay 1, experiment units occasionally contained dead CW that had burrowed into the carrot slice. Unfortunately, no records of the frequency of this behaviour were kept. Anecdotally, insecticide treatments that produced greater mortality were associated with a higher frequency of dead CW burrowed into the slice of carrot. Each experimental unit received a fresh carrot slice post-application, so it is not likely that this behaviour altered the insecticide exposure or dose.

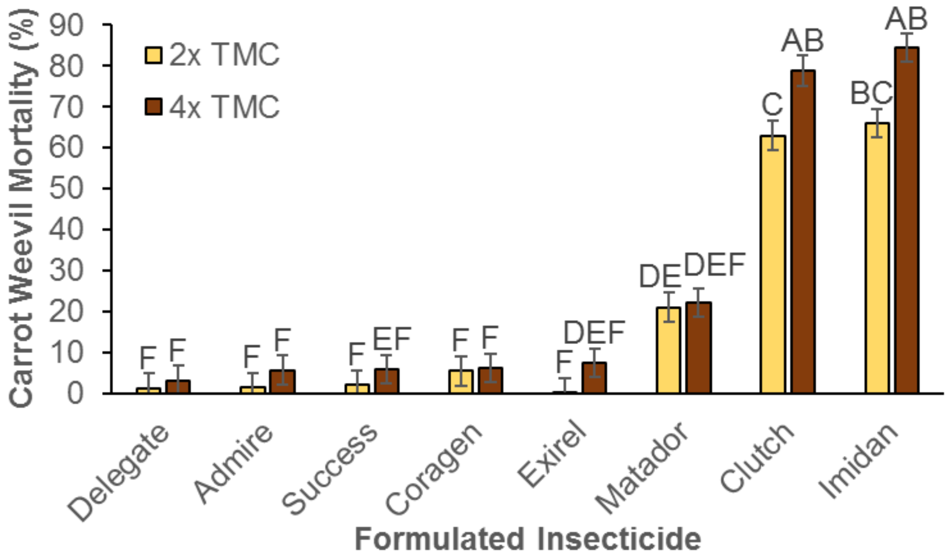


Figure 3.1. Mean percent mortality of a susceptible Quebec strain of adult carrot weevils (CW) to formulated insecticides 24 h post-application using a 1/9th scale Potter spray tower. Applications were performed at 2x and 4x tank mixture concentration (TMC) and mortality was adjusted using Abbott's formula. Bars with a different letter are significantly different according to Tukey's HSD (α=0.05).

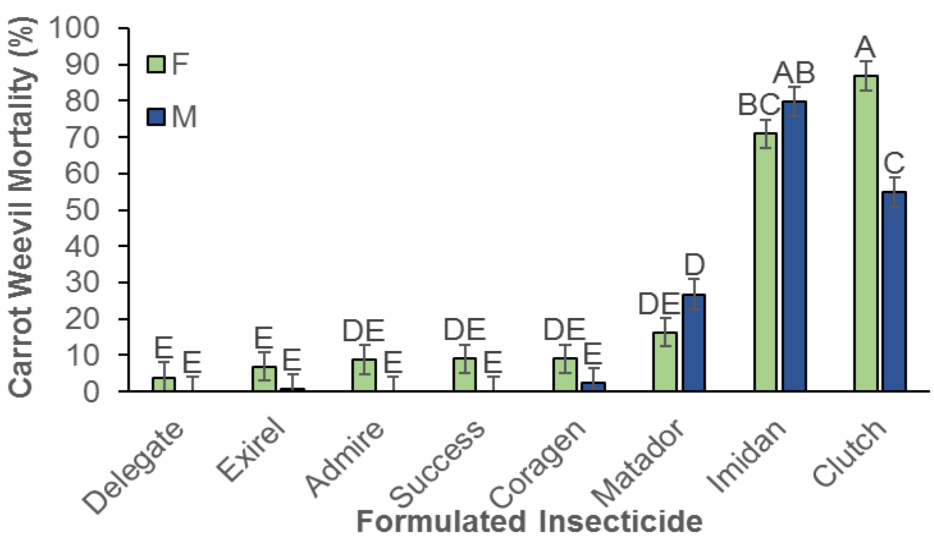


Figure 3.2. Mean percent mortality a susceptible Quebec strain of male (M) and female (F) adult carrot weevils (CW) to insecticides 24 h post-application using a 1/9th scale Potter spray tower. Mortality was adjusted using Abbott's formula. Different letters denote significantly different groups according to Tukey's HSD (α=0.05).

Laboratory Assay 2: Assessment of Holland Marsh Carrot Weevil Resistance to Phosmet

In Laboratory Assay 2, the Quebec strain showed significantly greater mortality at 48 h when exposed to phosmet compared to the Holland Marsh strain (Fig. 3.3, df=1, F=209.33, p<0.001). Male CW exhibited significantly greater mortality than females (df=1, F=25.80, p<0.001). Increasing the phosmet concentration significantly increased mortality (df=2, F=87.19, p<0.001) and the rate of phosmet had a significant interaction with strain (df=1, F=91.46, p<0.001). There was also a significant interaction between the strain and sex (df=2, F=14.62, p=0.001) although the rate of phosmet and sex had no interaction (df=1, F=0.31, p=0.582). Overall, there was a significant interaction between rate of Imidan, sex, and strain (Fig. 3.3, df=2, F=4.54, p=0.040) therefore these factors must be examined in relation to each other. Mortality in the control, distilled water, was under 15% in all instances.

During rearing prior to Laboratory Assay 2, both laboratory colonies had an infestation of an unidentified fungal disease causing adult mortality. Affected CW were observed with the fungus growing out of gaps in the exoskeleton. During the assessment of mortality for Laboratory Assay 2, CW were examined for potential fungal growth. Fungal growth was recorded on under 5% of all individuals tested and occurred on both CW strains.

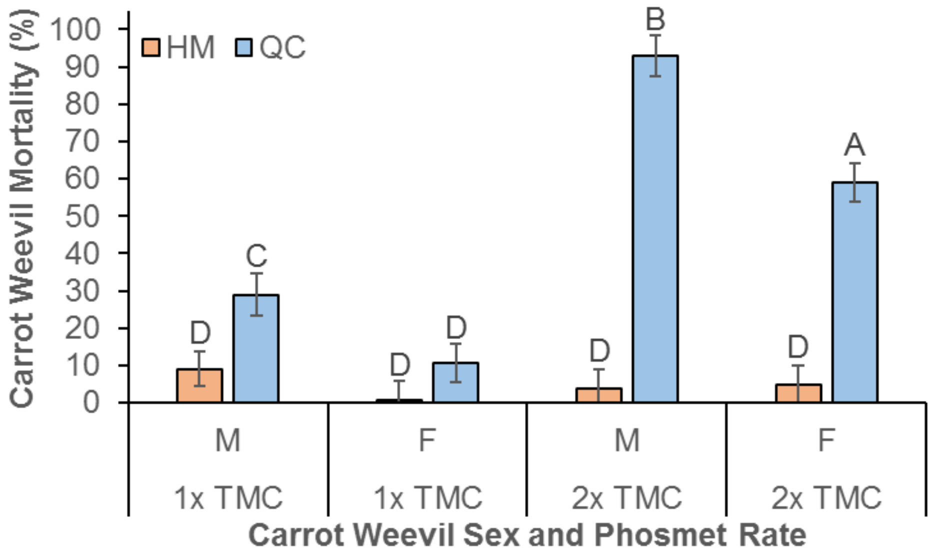


Figure 3.3. Mean percent mortality of adult male (M) and female (F) carrot weevils from a laboratory colony Quebec strain (QC) and a field-collected Holland Marsh strain (HM) after exposure to phosmet using the formulation Imidan 50 WP at 1x and 2x field tank mixture concentration (TMC) (1125 and 2250 ppm phosmet, respectively). Mortality was adjusted using Abbott's formula. Bars with different letters are significantly different according to Tukey's test (α=0.05).

3.4.2 Field Trials

As discussed in Chapter 2, personal observations in 2015 and 2016 field trials detected novel CW activity. In 2016, carrots prior to the 4^{th} TLS were observed with CW oviposition pits. Unfortunately, the frequency of this occurrence was not recorded. In both years, trials contained dead carrots with tunneling damage throughout the vascular tissue of the carrot root. In 2016, every trial had some number of dead carrots and this number was recorded during the first sampling period. In 140 samples, there was an average of 5.5 (standard deviation of 4.06) dead carrots per sampling effort with the number of dead carrots ranging from 0-25.

2015 Comparison of Seed and Foliar Treatments

In the 2015 comparison of seed and foliar treatments, only carrots receiving cyantraniliprole as a seed treatment had significantly reduced CW damage compared to the control (HGW86) (Table 3.2, df=7, F=3.19, p=0.0093). Despite the observed reduction in CW damage with cyantraniliprole, none of the treatments significantly increased yield compared to the untreated control (df=7, F=1.60, p=0.17). Across the entire trial, average CW damage exceeded 40% and significantly increased between sampling efforts, from 43.1 ± 3.7 to 52.3 ± 3.7, (df=1, F=58.88, p<0.001), suggesting CW feeding and damage was occurring late in the growing season. There was a significant edge effect in the trial, where CW damage was higher in plots closer to an adjacent field in which carrots had been grown in the previous season (df=7, Z=1.71, p=0.044). There was no interaction between treatment and sampling date for CW damage (df=7, F=1.16, p=0.35) nor yield (df=7, F=1.17, p=0.34).

Table 3.2. Effects of seed and foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2015 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		App'n Method	Mean CW Damage by sampling date (%)[1,2]			Mean Yield[3] (t/ha)
Active Ingredient	Formulation		Aug. 13	Oct. 15	Combined Average	
Control	N/A		41.6 a[6]	55.7 a	48.6 a	31.5 a
Clothianidin + Imidacloprid	Sepresto 75S	ST[4]	46.0 a	46.3 a	46.2 a	37.7 a
Cryomazine	Trigard	ST	44.9 a	46.9 a	44.9 a	40.8 a
Cyantraniliprole	HGW86	ST	33.5 a	40.0 a	36.7 b	42.1 a
Flupyradifurone	Sivanto Prime FS480	ST	47.2 a	54.7 a	51.0 a	24.9 a
Phosmet	Imidan 70 WP	F[5]	42.2 a	57.5 a	49.9 a	32.5 a
Cypermethrin	Ripcord	F	43.4 a	57.9 a	50.7 a	29.5 a

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 15.
[4] ST = seed treatment
[5] F = foliar treatment
[6] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different to the control according to Dunnett's test at α = 0.05.

2016 Comparison of Seed and Foliar Treatments

In the 2016 comparison of seed and foliar treatments, only carrots receiving cyantraniliprole as a seed treatment had significantly less CW damage than the control (Table 3.3, df=4, F=3.01, p=0.030). There was an average of 33.52 ± 1.42% CW damage across the entire trial. The seed treatment of cyantraniliprole significantly increased yield compared to the untreated control and all other treatments except for the combined foliar applications of phosmet and cypermethrin (df=4, F=5.63, p=0.001). Carrot weevil damage significantly increased by 17.92 ± 2.42% between the first and second sampling date (df=1, F=55.05, p<0.001). There was no interaction between treatment and sampling date (df=4, F=0.28, p=0.880).

Table 3.3. Effects of seed and foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2016 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		App'n Method	Mean CW Damage by sampling date (%)[1,2]			Mean Yield (t/ha)[3]
Active Ingredient	Formulation		July 25	Oct. 3	Combined Average	
Control	N/A		20.2 a[6]	39.0 a	29.6 a	35.8 b
Tefluthrin	Force 3G	ST[4]	17.4 a	36.0 a	26.7 ab	44.1 b
Cyantraniliprole	Fortenza	ST	8.8 a	27.2 a	18.0 b	57.9 a
Flupyradifurone	Sivanto Prime FS480	ST	21.0 a	34.1 a	27.6 ab	43.0 b
Phosmet + Cypermethrin	Imidan 70 WP Ripcord	F[5]	11.7 a	32.4 a	22.1 ab	46.6 ab

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 3.
[4] ST = seed treatment
[5] F = foliar treatment
[6] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different according to Tukey's test at $\alpha = 0.05$.

2015 Comparison of Foliar Treatments

No foliar insecticides reduced CW damage compared to the control in 2015. Significantly higher CW damage was observed in the clothianidin-treated plots (Table 3.4, df=5, F=3.76, p=0.008). This increase in CW damage in the clothianidin-treated plots also resulted in significantly lower marketable yield compared to the control (df=5, F=3.15, p=0.020). Across the entire trial, CW damage significantly increased from 8.3 ± 2.0 to 11.7 ± 2.0 between the August and October harvest dates (df=1, F=8.82, p=0.006). There was no significant interaction between CW damage and harvest date (df=5, F=0.49, p=0.78) nor CW damage and yield (df=5, F=0.74, p=0.60).

Table 3.4. Effects of foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2015 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		Mean CW Damage by sampling date (%)[1,2]			Mean Yield (t/ha)[3]
Active Ingredient	Formulation	Aug. 13	Oct. 15	Combined Average	
Control	N/A	7.7 a[4]	9.3 a	8.5 b	62.2 a
Phosmet	Imidan 70 WP	11.5 a	13.5 a	12.5 b	59.2 a
λ-cyhalothrin	Matador 120 EC	6.6 a	11.5 a	9.0 b	56.1 a
Cyantraniliprole	Exirel	6.1 a	12.5 a	9.3 b	57.0 a
Spinetoram	Delegate	4.4 a	9.1 a	6.8 b	60.0 a
Clothianidin	Clutch	13.3 a	14.9 a	14.1 a	54.5 b

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 15.
[4] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different compared to the control according to Dunnett's test at α = 0.05.

2016 Comparison of Foliar Treatments

An average of 35.9 ± 1.6% CW damage was observed across all plots in the 2016 assessment of foliar treatments at the second sampling date. Despite this level of damage, there was a significant treatment effect (Table 3.5, df=7, F=9.30, p<0.001). Between the two sampling dates, CW damage increased by an average of 15.1 ± 2.1% across the entire trial (df=1, F=52.67, p<0.001) and there was no significant interaction between sampling date and treatment (df=7, F=0.67, p=0.693). Despite significant reductions in CW damage, yield was not significantly different between treatments (df=7, F=1.69, p=0.126).

Table 3.5. Effects of foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2016 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		Mean CW Damage by sampling date (%)[1,2]			Mean Yield[3] (t/ha)
Active Ingredient	Formulation	July 26	Oct. 4	Combined Average	
Control	N/A	28.1 a[4]	42.5 a	35.3 a	63.8 a
Phosmet + Cypermethrin	Imidan 70 WP Ripcord	16.0 a	37.2 a	26.6 ab	70.2 a
λ-cyhalothrin	Matador 120 EC	25.0 a	37.8 a	31.4 a	60.4 a
Novaluron	Rimon 10 EC	9.4 a	16.0 a	12.7 c	78.5 a
Cyantraniliprole	Exirel	11.3 a	23.1 a	17.2 bc	72.2 a
Spinetoram	Delegate	28.6 a	43.8 a	36.2 a	61.3 a
Beauveria bassiana	Botanigard	22.4 a	41.2 a	31.8 a	62.1 a
Steinernema feltiae	Nemasys	26.1 a	46.0 a	36.0 a	59.9 a

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 4.
[4] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different according to Dunnett's test at $\alpha = 0.05$.

2016 Insecticide Synergist Assessment

In the insecticide synergist trial, plots within the trial experienced an average of 54.9 ± 1.9 % CW damage at the second sampling. Insecticide treatment significantly reduced CW damage (Table 3.6, df=4, F=15.06, p<0.001) with phosmet-treated plots exhibiting an average of 10.7 ± 2.7% lower CW damage compared to cypermethrin-treated plots (df=21, t=4.02, p=0.001). The addition of PBO to insecticide application significantly reduced CW damage (df=1, F=21.77, p<0.0001) by an average of 12.2 ± 2.7% compared to insecticide-treated plots that did not receive the addition of PBO (df=21, t=4.56, p<0.001). There was no interaction between the insecticide applied and the addition of PBO with respect to CW damage (df=1, F=1.08, p=0.307). Carrot weevil damage significantly increased between sampling dates (df=1, F=109.01, p<0.001) by an average of 25.3 ± 2.67% across all treated plots (df=21, t=9.64, p<0.001). There was no interaction between sampling date and insecticide treatment (df=1, F=0.68, p=0.418) or sampling date and addition of PBO with insecticide treatment (df=1, F=0.18, p=0.675) with respect to CW damage. There was no interaction between sampling date, insecticide treatment, and addition of PBO with insecticide treatment with respect to CW damage (df=1, F=1.20, p=0.264).

In the insecticide synergist trial, significant differences in CW damage related to significant differences in marketable yield. The insecticide treatment had a significant effect on yield (df=1, F=18.16, p=<0.001) with phosmet-treated plots yielding 12.47 t carrots/ha more than cypermethrin-treated plots (df=25, t=3.79, p=0.001). The addition of PBO significantly improved yield (df=1, F=41.23, p=<0.001), where plots treated with insecticide and PBO 18.79 t carrots/ha compared to insecticide-treated plots that did not receive the addition of PBO (df=25, t=5.71, p=<0.001). There was a significant interaction between insecticide treatment and the addition of PBO (Table 3.6, df=1, F=6.51, p=0.016), therefore all factors must be examined with respect to differences in yield.

2015 Comparison of In-Furrow and Foliar Treatments

In 2015, no treatment had a significant impact on CW damage (Table 3.7., df=5, F=1.28, p=0.294). At the second sampling date, the plots averaged 15.5 ± 1.1% CW damage across all treatment and control groups There was no significant interaction between sampling date and treatment (df=5, F=1.13, p=0.364). Between the first and second sampling dates, CW damage did not significantly increase (df=1, F=2.38, p=0.133). Although there were no significant treatment effects on CW damage, several treatments significantly reduced marketable yield compared to the control (df=5, F=7.65, p<0.001).

Table 3.6 Effects of insecticide application with and without piperonyl butoxide (PBO) on carrot weevil (CW) damage and marketable yield in trials conducted in 2016 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		Mean CW Damage by sampling date (%)[1,2,3]			Mean Yield[1,3] (t/ha)
Active Ingredient	Formulation	July 26	Oct. 4	Combined Average	
Control	N/A	41.2 bcd[4]	64.6 a	52.9 a	32.7 b
Phosmet	Imidan 70 WP	25.8 de	57.8 ab	41.8 b	35.3 b
Phosmet & 1.0% PBO[4]	Imidan 70 WP	15.0 e	38.8 cd	26.9 c	61.5 a
Cypermethrin	Ripcord	38.9 cd	60.6 a	49.8 ab	30.3 b
Cypermethrin & 1.0% PBO[4]	Ripcord	27.6 de	53.1 abc	40.3 b	41.6 b

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 4.
[4] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different according to Tukey's test at $\alpha = 0.05$.

2016 Comparison of In-Furrow and Foliar Treatments

In 2016, the plots in the in-furrow trial received an average of 32.8 ± 1.3% CW damage at the second harvest. In-furrow chemical treatments did not differ significantly from each other or the control group with respect to CW damage (Table 3.8., df=5, F=0.69, p=0.636) or yield (df=5, F=2.24, p=0.065) on either sampling date. CW damage significantly increased by 23.1 ± 1.9% (df=1, F=147.24, p=<0.001) between the first and second sampling dates. There was no significant interaction between sampling date and treatment (df=5, F=0.35, p=0.880).

Table 3.7. Effects of in-furrow and foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2015 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		Mean CW Damage by sampling date (%)[1,2,3]			MeanYield[1,4]
Active Ingredient	Formulation	Aug. 14	Oct. 15	Combined Average	(t/ha)
Control	N/A	11.5 a[4]	14.3 a	12.9 a	57.8 a
Imidacloprid [z] Cyantraniliprole[y] Cypermethrin [y]	Admire 240 Exirel Ripcord	13.2 a	14.0 a	13.6 a	54.4 ab
Imidacloprid [z] Flupyradifurone[y]	Admire 240 Sivanto Prime FS 200	12.8 a	9.7 a	11.3 a	49.3 bc
Thiamethoxam[z] & Cyantraniliprole [z] Cyantraniliprole[y] Cypermethrin [y]	Minecto Duo 40 WG Exirel Ripcord	14.2 a	19.9 a	17.1 a	53.0 ab
Thiamethoxam[z] & Cyantraniliprole [z] Flupyradifurone[y]	Minecto Duo 40 WG Sivanto Prime FS 200	12.5 a	12.6 a	12.6 a	54.8 ab
Cypermethrin[y]	Ripcord	12.2 a	22.5 a	17.4 a	45.8 c

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 15.
[4] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different according to Tukey's test at $\alpha = 0.05$.

Table 3.8. Effects of in-furrow and foliar treatments on carrot weevil (CW) damage and marketable yield in trials conducted in 2016 at the Muck Crops Research Station (University of Guelph), Holland Marsh, Ontario.

Treatment		Mean CW Damage (%)[1,2,3]			Mean Yield[1,3] (t/ha)
Active Ingredient	Formulation	July 25	Oct. 3	Combined Average	
Control	N/A	11.5 a[4]	30.4 a	21.0 a	38.2 a
Bifenthrin [z] Cypermethrin[y]	Capture LFR Ripcord	9.0 a	33.4 a	21.2 a	42.2 a
Bifenthrin [z] Flupyradifurone[y]	Capture LFR Sivanto Prime FS 200	10.4 a	37.1 a	23.8 a	37.7 a
Cyantraniliprole [z] Cypermethrin [y]	Verimark Ripcord	7.4 a	28.3 a	17.8 a	49.6 a
Cyantraniliprole [z] Flupyradifurone[y]	Verimark Sivanto Prime FS 200	10.0 a	34.0 a	22.0 a	46.9 a
Cypermethrin[y]	Ripcord	9.7 a	33.4 a	21.6 a	39.6 a

[1] Data from both sampling dates for carrot weevil damage were assessed using repeated-measures.
[2] Percent CW damage is calculated from the number of carrots with CW damage over the total number of sampled carrots.
[3] Yield in t/ha was extrapolated from the average marketable yield of two 1.5 m carrot row section samples on Oct. 3.
[4] Values within the columns Combined Average and Yield, and between both columns for sampling date with different letters are significantly different according to Tukey's test at $\alpha = 0.05$.
[y] Foliar applications
[z] In-furrow applications

EIQ Assessment, 2015

Calculated EIQ and EIQ-FUR values for the insecticides used in all trials in 2015 and 2016 are reported in Table 3.9 and 3.10. Phosmet had the highest EIQ-FUR (32.8, 65.6 according to 1 and 2 applications, respectively) while λ-cyhalothrin had the highest EIQ (44.2).

Table 3.9. Environmental Impact Quotient (EIQ) and EIQ-Field Use Rating (FUR) values for all insecticides used in carrot weevil field trials at the Muck Crops Research Station (University of Guelph), Holland Marsh, 2015 and 2016.

						Components		
Chemical	Formulation	Rate	Applications	EIQ	EIQ-FUR	Consumer	Worker	Ecological
Foliar								
Phosmet	Imidan 70 WP	1.6 kg / ha	1	32.8	32.8	2.5	6.9	89.1
Phosmet	Imidan 70 WP	1.6 kg / ha	2	32.8	65.6	4.9	13.8	178.1
Cypermethrin	Ripcord 400 EC	175 ml / ha	1	36.4	3.4	0.6	1.3	8.5
Cypermethrin	Ripcord 400 EC	175 ml / ha	3	36.4	6.6	1.1	2.5	16.3
λ-cyhalothrin	Matador 120 EC	83 ml / ha	1	44.2	0.4	0.0	0.2	1.0
λ-cyhalothrin	Matador 120 EC	83 ml / ha	3	47.2	1.2	0.1	1.0	2.5
Clothianidin	Clutch 50 WG	105 g / ha	1	32.1	1.5	0.4	0.5	2.6
Imidacloprid	Admire 240	1.0 L / ha	1	36.7	7.5	2.1	1.4	19.1
Spinetoram	Delegate	200 g / ha	1	27.8	1.2	0.1	0.3	3.3
Spinetoram	Delegate	200 g / ha	3	27.8	3.7	0.3	0.9	9.9
Cyantraniliprole	Exirel	750 ml / ha	1	23.7	1.8	1.4	0.8	3.4
Cyantraniliprole	Exirel	750 ml / ha	2	23.7	3.6	2.7	1.5	6.6
Cyantraniliprole	Exirel	750 ml / ha	3	23.7	10.7	8.1	4.5	19.8
Flupyradifurone	Sivanto Prime SL 200	1.0 L / ha	2	25.3	4.3	3.1	3.9	7.0
Flupyradifurone	Sivanto Prime SL 200	1.0 L / ha	3	25.3	13.0	9.2	11.8	21.0
Novaluron	Rimon 10 EC	820 ml / ha	3	14.3	3.0	0.6	1.3	7.2
In-Furrow								
Bifenthrin	Capture LFR	14.1 ml / 100 m row	1	44.4	9.2	1.6	2.9	23.0
Cyantraniliprole	Verimark	1.0 L / ha	1	23.7	4.7	3.6	2.0	8.8
Cyantraniliprole	Minecto-Duo 40 WG	5 g / 100 m row	1	23.7	2.4	1.8	1.0	4.4
Thiamethoxam	Minecto-Duo 40 WG	5 g / 100 m row	1	33.3	3.0	1.1	0.9	6.9
	Combined			57.0	5.4	2.9	1.9	11.3
Seed Treatment								
Cyromazine	Trigard	4.51 g AI / 100 g seed	1	18.3	0.8	0.6	0.3	1.5
Cyantraniliprole	HGW86	4.51 g AI / 100 g seed	1	23.7	1.1	0.9	0.5	2.1
Flupyradifurone	Sivanto Prime FS 480	4.51 g AI / 100 g seed	1	25.3	1.2	0.9	1.1	1.9
Clothianidin	Sepresto 75 WS	4.51 g AI / 100 g seed	1	32.1	1	0.3	0.3	2.5
Imidacloprid	Sepresto 75 WS	4.51 g AI / 100 g seed	1	36.7	0.4	0.1	0.1	1
	Combined		1	68.8	1.4	0.4	0.4	3.5
Tefluthrin	Force 3 G	7.29 g AI / 100 g seed	1	25.3	0.1	0.0	0.0	0.1
Flupyradifurone	Sivanto Prime FS 480	7.29 g AI / 100 g seed	1	25.2	2.0	1.4	1.8	3.1
Cyantraniliprole	Fortenza	7.29 g AI / 100 g seed	1	23.7	5.5	3.1	1.2	6.9
Clothianidin	Sepresto 75 WS	7.29 g AI / 100 g seed	1	32.1	1.2	0.3	0.4	3.0
Imidacloprid	Sepresto 75 WS	7.29 g AI / 100 g seed	1	36.7	0.5	0.1	0.1	1.2
	Combined		1		1.7	0.4	0.5	4.2

3.5 Discussion

Insecticide applications in general did not provide control of the CW, failing to reduce CW damage, in the Holland Marsh region. This failure to control CW may be due to the lack of efficacy of the insecticides. In Laboratory Assay 1, imidacloprid, spinetoram, spinosyn, and cyantraniliprole caused less than 20% mortality at two rates. Only clothianidin and phosmet caused >60% mortality at either rate and λ-cyhalothrin caused 20-30% mortality at both rates (Fig. 3.1). Previous studies have concluded that adult CW are generally tolerant of insecticide exposure (Martel *et al.* 1975b) suggesting the low mortality generally found in Laboratory Assay 1 could have been expected. There was a significant CW sex by treatment interaction in Laboratory Assay 1, where clothianidin killed significantly more females than males (Fig. 3.2). The cause of higher female mortality with clothianidin is not known but likely relates to differences in biology between the two sexes.

Trials using the 1/9th scale Potter spray tower only examined adult contact exposure, and in some case insecticides will be more toxic via ingestion. For example, imidacloprid and spinosad are more toxic to the gnat, *Liohippelates collusor* (Diptera: Chloropidae), when exposed via ingestion compared to contact (Jiang and Mulla 2006). Similarly, cyantraniliprole is >400x more toxic to corn earworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) via ingestion compared to topical applications (Bird 2016). Given more time and resources, alternative routes of exposure could have been examined here. Further research into the oral toxicity of the compounds examined would be worthwhile to establish the effect of the route of exposure. The total deposition of insecticide, or the proportion of spray volume that reaches the test surface, using the 1/9th scale Potter spray tower may have also altered exposure as some of the test solution is lost during application. A full-scale Potter spray tower will deposit ~14% of the spray volume (Liu and Stansly 1995), whereas the tower used in this trial deposits only 1.6-2.8% of the spray volume (Tomascik 2015). Laboratory Assays 1 and 2 only addressed the relative efficacy of the insecticides tested in relation to recommended field application concentrations as opposed to toxicity according to a dose per unit body mass. As both laboratory assays used aqueous preparations of these insecticides, it is reasonable to assume that the amount of solution deposited on the beetles was consistent for the different products. Therefore, the observed relative potencies of the field rate concentrations are probably valid.

In Laboratory Assay 2, a significant interaction between CW sex and strain was observed with males from the Quebec strain exhibiting significantly greater mortality following phosmet exposure compared to the Quebec females. This effect was not observed in Laboratory Assay 1 however this trial used lower rates (1x and 2x TMC in the Laboratory Assay 2 compared to 2x and 4x TMC in Laboratory Assay 1). It is possible Quebec strain males could be more affected by a lower phosmet dose compared to Quebec strain females.

At both concentrations of phosmet tested (1x and 2x TMC), the Quebec strain was significantly more susceptible than the strain collected from the Holland Marsh (Fig. 3.3). This suggests that over thirty years of phosmet use in the Holland Marsh has resulted in a reduction of susceptibility to phosmet in the CW population. This reduced phosmet susceptibility represents a major concern for producers if it translates into limited phosmet efficacy in the field. Personal discussions with producers in the Holland Marsh have indicated that the producers in the region have a reduced confidence in the efficacy of phosmet and are considering, if not already applying, off-label insecticides to attempt to mitigate CW infestations. Throughout the 2015 and 2016 field trials presented here (Tables 3.2, 3.3, 3.4, 3.5, and 3.6), phosmet was used as a positive control, or commercial standard, for CW control. In all of these trials, except for the synergist assessment (Table 3.6), phosmet applications had no effect on CW damage or marketable yield when compared to an untreated control. In Chapter 2, phosmet applications following the CW IPM program also failed to provide any significant improvement in yield or reduction in CW damage in two of three trials (Table 2.1, 2.2, 2.3). Previous research in the Holland Marsh has shown phosmet to be effective in reducing CW damage (Stevenson 1983, Stevenson 1985, Pree *et al.* 1996), using similar application methods and timings as used in the trials reported here. With the Holland Marsh strain exhibiting an insusceptibility to phosmet in Laboratory Assay 2, a failure of phosmet to control CW in 2015 and 2016 field trials, and past research indicating the efficacy of phosmet in the Holland Marsh, it is likely that the Holland Marsh CW population is developing resistance to phosmet.

The insecticide synergist trial found a significant reduction in CW damage when cypermethrin or phosmet were applied with the addition of 1.0% PBO (Table 3.6), with PBO affecting the efficacy of the insecticide regardless of the insecticide applied. Phosmet also reduced CW damage significantly better than cypermethrin and marketable yield nearly doubled when phosmet and PBO were applied together (Table 3.6). Although PBO improves chemical control of CW, it may not be cost effective for producers. The insecticide synergist trial was ~400m^2 and PBO was applied to half of the treated plots. Using technical grade PBO diluted to

1.0%, the application cost to the ~200m^2 of treated plots was roughly $100. Therefore, a single PBO application at this rate costs ~$5000/ha. Future research should assess the efficacy of lower PBO rates and, if commercial applications are pursued, examine opportunities with chemical industries to lower the cost of application.

Although PBO effectively reduced CW damage when paired with insecticide applications, the route through which PBO resulted in improved insecticide efficacy is not clear. Cytochrome P_{450}s are a family of enzymes partially responsible for the degradation of xenobiotics and these enzymes are inhibited by PBO (NPIC 2000). Therefore, the two likely routes through which insecticide efficacy could be improved using PBO are decreased insecticide degradation via the target organism or decreased degradation within the soil to improve residual activity of the insecticide. These options are not mutually exclusive and both may be important to the efficacy of insecticides paired with PBO applications. Decreased degradation by the target organism is often the desired effect of PBO applications (NPIC 2000).

The organic muck soil at the Holland Marsh can support an incredibly diverse microbiome. Producers and researchers have previously expressed concerns of increased insecticide degradation due to this soil microbiome while examining in-furrow treatments of carbofuran (Stevenson 1983). Soil microflora can thrive in the presence of insecticides (Sarnaik et al. 20014) and have adapted to degrade them. Some bacteria are even being developed for use in bioremediation due to their ability to degrade hazardous compounds (Cycon and Piotrowska-Seget 2016). Reduced insecticide efficacy due to soil biodegradation has previously been documented in carrot fields in the UK, with phorate (organophosphorus insecticide) degradation occurring so rapidly in over 30% of tested fields that the phorate applications likely did not confer any level of mitigation to the targeted CRF infestations (Suett and Jukes 1997). Reduced pesticide degradation due to the addition of PBO has been shown to occur in laboratory studies using the soil-dwelling fungi *Cunninghamella elegans* (Lendner) in culture (Zhu *et al.* 2010) and a combination of *Pestalotiopsis* sp. NG007 and several basidiomycetes in soil (Yanto and Tachibana 2014). PBO has also been documented to reduce carbofuran degradation using soil collected from fields receiving in-furrow applications of carbofuran (Talebi and Walker 1994). The potential for the microbiome in the soil to reduce the residual activity of phosmet could be a great concern to producers in the Holland Marsh, particularly considering the phosmet insensitivity exhibited by the Holland Marsh CW population in Fig. 3.3. If soil degradation is a significant factor in the reduced efficacy of phosmet, insecticides registered for CW control should be evaluated for their persistence in the soil present in the Holland Marsh to ensure the CW population is receiving accurate insecticide doses during foliar applications.

In 2015 and 2016, no seed treatment insecticide application effectively mitigated CW damage. Cyantraniliprole as a seed treatment did significantly reduce CW damage in both years, but even in this case CW damage still exceeded 25% (Table 3.2, Table 3.3). The reduction in CW damage in plots with cyantraniliprole-treated seeds only significantly related to yield in 2016, with a ~10 t/ha increase compared to the control (Table 3.3). This increase is important to growers, however the >25% CW damage may be too high to cost-effectively harvest the field. The lack of efficacy of seed treatment insecticides could be due to CW tolerance of insecticide exposure or the rates used here are too low to provide lethal insecticide concentrations in the carrot root at the time of CW feeding. It is not expected that seed treatments could control a second generation of CW although pairing seed treatment insecticides with subsequent foliar insecticide applications was not examined in field trials reported here. If cyantraniliprole-treated seeds fail to effectively control CW damage due to the decreased concentration of cyantraniliprole within carrots later into CW oviposition period, additional foliar applications may produce effective control. Further research is needed to confirm this hypothesis.

The only other published report to examine the efficacy of seed treatments for CW control (Bonham *et al.* 2009) found seed treatments of fipronil, at a rate of approximately 3 mg AI / 100 g seed, effectively reduced CW damage by 60-80% compared to an untreated check. Even with this large percent reduction, CW damage still reached economically unacceptable levels, from roughly 10-30%, although this trial was performed in New Jersey which experiences multiple generations of CW. Bonham *et al.* (2009) also found variable results with respect to yield, as their 2004 trial found fipronil-treated seeds produced ~80 t/ha more marketable carrots than the untreated control while their 2005 trial found no difference in yield between untreated plots and plots with fipronil-treated seeds. The discrepancies in yield observed in field trial reported here or by Bonham *et al.* (2009) may be an artifact of sampling methods. As yield is based on weight, small differences in the amount of carrots sampled can become extreme when extrapolated to field-level yield whereas CW damage is based proportion of affected carrots and should be less affected by slight variations in sample size.

In 2015 and 2016, trials comparing the efficacy of foliar insecticide applications found only cyantraniliprole and novaluron significantly reduced CW damage. Novaluron is currently registered for use against CW in Canada. Other insecticides registered for CW control in Canada, phosmet and lambda-cyhalothrin, did not provide any significant CW mitigation as foliar applications. Cyantraniliprole is currently not registered for CW control but is registered for some minor insect pests in carrots in Canada. In 2015, a single foliar application resulted in no significant CW mitigation with respect to yield or CW damage (Table 3.4). In the 2016 foliar trial, the application rate of cyantraniliprole was doubled and the number of foliar applications increased to three. With this application method of cyantraniliprole, CW damage was reduced by ~20% compared to the control (Table 3.5). Similarly, three applications of novaluron also resulted in a ~20% decrease in CW damage compared to the control although neither novaluron or cyantraniliprole applications significantly improved marketable yield. Novaluron and cyantraniliprole treated plots exhibited an average of 16 and 23.1% CW damage by the second sampling date, respectively, and CW damage increased by an average of 15% across all plots within the trial. If a second generation of CW is occurring, it is possible novaluron and cyantraniliprole may be providing effective control of the first generation while control methods for the second generation still need to be established.

In both 2015 and 2016, in-furrow applications followed by foliar applications had no significant effect in reducing CW damage or improving marketable yield (Table 3.6, Table 3.7). A significant difference in yield was noted in the 2015 trial although CW damage was not significantly different between any trials suggesting CW are not responsible for the observed differences in marketable yield. As discussed previously, extrapolating these small plot trials to yield across a field can be variable and percent CW damage is a more confident measure of control. Overall, in-furrow applications appear ineffective for reducing CW damage.

In 2015 and 2016, CW activity in the Holland Marsh did not conform to predictions based on the assertion that CW do not oviposit into carrots until the 4th TLS and the oviposition degree day model derived by Boivin (1988). Based on temperature observations in 2015, this degree day model predicted that CW oviposition in the Holland Marsh should have begun between 10-14 May and 90% oviposition should have been completed between 9-17 June. In 2015 field trials, foliar applications were applied between June 21 and 25 and carrots within these plots were at the 2-3 TLS. Since this was past the 90% CW oviposition degree day threshold, no more foliar sprays were applied for CW control. Based on these facts, minimal CW damage was expected in both of these trials. Despite the 90% oviposition threshold reached prior to the development of carrots to the 4th TLS, a mean of 52.3 ± 3.7%, 11.7 ± 2.0%, and 15.5 ± 1.1% CW damage was observed in the seed treatment and foliar, foliar, and in-furrow and foliar trial, respectively.

These field-observed inconsistencies with previous CW activity were also observed in 2016. Across all trials in 2016, carrots at the 2nd TLS were also observed to contain CW oviposition pits (Fig. 2.5). In 2016, the model proposed by Boivin (1988) predicted 90% CW oviposition to be completed between June 21-27. Across all 2016 field trials, the first foliar application occurring during the period in which 90% CW oviposition was supposed to be completed. During this first foliar application in 2016, carrots plants were at the 2nd TLS. In 2016, all field trials received at least 30% CW damage and some plots exhibited over 60% CW damage. Therefore, there appear to be inconsistencies with the current degree day model for CW oviposition and CW now oviposits into carrot plants prior to the 4th TLS. Likely, insecticide applications need to occur earlier than currently recommended to respond to these changes.

In the 2015 foliar application and seed and foliar treatment trials, CW damage increased by 9.2 ± 1.9% and 3.4 ± 1.2%, respectively, between the two sampling dates. In 2016, all field trials at the MCRS showed an increase in CW damage of at least 15% between the first and second sampling date. The first sampling effort in both years was timed to ensure 90% eggs from overwintered CW could have developed into adults, based on the oviposition degree day model (Boivin 1988) and the degree day model for CW development from egg to adult presented in Simonet and Davenport (1981). As such all CW damage from this first CW generation should have been documented at the first sampling date. In Chapter 2, the IPM Evaluation trial at the MCRS and the planting date trial in 2016 also had significant increase in CW damage between sampling dates. This large increase in damage between sampling dates in the 2015 and 2016 field trials provides evidence that a second CW generation in the Holland Marsh.

A second CW generation causes additional problems with CW monitoring efforts. Both established methods, the Boivin trap and CRS, rely on using actual carrots as an attractant or bait. Whereas this is effective in the early season, by the time second generation oviposition should begin, carrots fields are well established. This creates a situation in which the developing crop is more attractive to the CW than the monitoring traps. In addition, there are no effective methods to recommend for the control of a second CW generation in Canada. These methods are needed for Canadian producers, particularly if large increases in CW damage, such as in 2016, continue.

Similar to results reported in Chapter 2, there was additional evidence that CW were killing young carrot plants in several trials at the MCRS. In 2015, several dead carrot plants were found early in the season with the vascular tissue within root tunnelled through completely. These symptoms were also found in the 2016 trials, where dead carrots were present in nearly all plots. Often, the tunneling damage on these dead carrots was visible and occasionally CW larvae were still present in these dead carrots. With plots receiving up to 25 dead carrots per two 1.5 m row section sampled, and 70 seeds/m planted, this new CW attack could result in over 10% carrot death in addition to the observed CW damage in harvested carrots.

With the change in CW activity and the potential development of phosmet resistance within Holland Marsh CW, a new chemical control plan for CW is needed at least within the Holland Marsh. One of the challenges to developing a revised IPM program for CW is the need to balance efficacy and environmental safety. A tool for evaluating the latter is the EIQ-FUR that can be calculated for candidate chemical control products. As shown in the table of EIQ-FUR values (Table 3.9), phosmet, the primary product used currently for CW control, is the least safe of all the compounds evaluated. The most important factor contributing to the ranking of phosmet is the high application rate of phosmet: 1.1 kg ai / ha per application, which is 1-2 orders of magnitude higher than that of any other tested insecticide. In general, seed treatment insecticides had the lowest EIQ-FUR ranking. These results are unsurprising as seed treatments can reduce overall insecticide input across the field while obtaining similar control since all insecticide is applied directly to the plant (Bonham et al. 2009). Despite their low rankings, most of the compounds, including seed treatments, evaluated in these field trials showed limited efficacy in terms of mitigating CW damage, and therefore are not plausible candidates for a revised IPM program.

Cyantraniliprole showed some efficacy in CW damage mitigation as both a seed treatment and foliar application. Effective control as a foliar application resulted from three foliar applications, which had an EIQ-FUR of 10.7. In comparison, cyantraniliprole as a seed treatment had an EIQ-FUR of 5.5. At both harvest dates, these treatments produced similar CW damage, with 11.3% and 8.8% CW damage at the first sampling date and 23.1 and 27.2% CW damage for the foliar and seed treatment, respectively. The other foliar treatment to reduce CW damage significantly, novaluron, had an EIQ-FUR of 3.0 with three foliar applications. The combination of a cyantraniliprole seed treatment combined with three foliar applications of novaluron provide an EIQ-FUR of 8.5, a value nearly 7x smaller than the EIQ-FUR of two phosmet applications (65.6), while providing multiple modes of action for CW control in the same growing season. Although, this combination of treatments still needs to be examined for its efficacy.

In conclusion, CW activity is changing in the Holland Marsh, and effective chemical control options still need to be identified. Results from all trials suggest foliar applications of novaluron and seed or foliar treatments of cyantraniliprole can be effective for mitigating CW damage. Other seed, foliar, and in-furrow insecticides examined did not mitigate CW damage effectively throughout the growing season. Future research should pair seed treatments with foliar applications to allow for a longer period of protection during the carrot production season. Piperonyl butoxide shows promise for improving the reduction in CW damage with current chemical control protocols although lower rates than the 1.0% PBO used here should be examined to establish a cost-effective treatment. The established degree day model of CW oviposition is no longer reliable in the Holland Marsh and insecticide applications past the 90% oviposition degree day threshold may be justified. The discovery of CW larvae and increasing damage late in the growing season add to the evidence that a second generation of CW is likely occurring and additional sprays could be needed to protect carrot crops adequately. The results of both laboratory tests and field trials indicate that chemical control of CW should move away from repeated phosmet applications, as the chemical fails to mitigate damage adequately in our field trials. This appears, at least in part, to reflect the reduced phosmet susceptibility observed in laboratory trials using Holland Marsh CW. Furthermore, this insecticide has a high EIQ and EIQ-FUR compared to other insecticides. When the results of field trials presented here are compared to other published reports of the efficacy of phosmet for CW control in the Holland Marsh, it is likely that the Holland Marsh population is developing resistance to phosmet. In addition to examining the susceptibility of Holland Marsh CW to phosmet over time, to evaluate the concerns of phosmet resistance, the soil in the Holland Marsh should be assessed for its ability to degrade phosmet compared to other soils. Overall, the null hypothesis that foliar, seed treatment, and in-furrow insecticides do not reduce CW damage is rejected as some seed and foliar insecticides significantly reduced CW damage. However, most foliar, seed, and in-furrow insecticides failed to mitigate CW damage.

CHAPTER 4

SURVEY OF CARROT WEEVIL NATURAL ENEMIES IN THE HOLLAND MARSH

4.1 Abstract

Natural enemies can provide, or help improve, control of certain insect pests, and can be incorporated into IPM programs through conservation biological control, inundative releases, insecticide selection, or through dynamic action thresholds. In order to take advantage of natural enemies in an IPM program, they need to be identified. There are a number of potential natural enemies for the CW, including several carabid and mymarid species. In this study, the CW natural enemy community in the Holland Marsh, Ontario was sampled using pitfall traps and parasitoid baits. All known carabid (predator) natural enemies of the CW were found, but only one CW parasitoid was found over two years of sampling. Future research should examine the efficacy of these natural enemies at reducing CW populations and damage, while also looking to improve the abundance and diversity of the natural enemy complex.

4.2 Introduction

Agricultural fields can house a wide diversity of natural enemies – primarily predators and parasitoids. Natural enemies can be an important aspect of IPM programs due to their potential to reduce pest densities. In the United States alone, natural enemies are estimated to save farmers $4.5 billion annually due to this reduction in pest pressure (Losey and Vaughn 2006). In some cases, such as with soybean aphid (*Aphis glycines* Matsumura), monitoring natural enemy presence can estimate a pest reduction, creating a dynamic action threshold based on pest and natural enemy presence, resulting in a decrease of some insecticide applications without sacrificing marketable yield (Hallett *et al.* 2014). Conservation biological control, or manipulating the agro-ecosystem in order to promote natural enemies, is another method through which natural enemies can be incorporated into IPM programs. The weaver ant *Oecophylla smaragdina* (Hymenoptera: Formicidae) is an effective predator of several insect pests of citrus crops in Vietnam (Van Mele and Cuc 2000). Producers in Vietnam practice several conservation biological control methods, including providing food for the weaver ant colonies, placing bridges between trees to increase ant colony range, and protecting colonies from other ant species to promote the natural biological control this species provides, and there is a clear benefit – producers promoting weaver ant colonies apply roughly half the insecticide and fungicide applications compared to other producers with no observed difference in yield (Van Mele and Cuc 2000). Having an established natural enemy community can also impact pesticide selection for IPM plans. In cotton grown in the southern United States, *Bemisia tabaci* (Gennadius) is a significant pest that the natural enemy community cannot reduce below economically damaging levels, yet by moving towards selective insecticides, predators and parasitoids can be conserved to improve pest suppression and reduce insecticide application (Naranjo and Ellsworth 2009). In order to incorporate conservation biological control in an IPM program the natural enemy community needs to be identified and evaluated.

There are several carabid (predators) species that have been identified as CW natural enemies, all of which have been found in carrots grown in organic soil throughout Quebec (Baines *et al.* 1990; Zhao *et al.* 1990). *Anisodactylus sanctaecrucis* (Fabricius), *Pterostichus melanarius* (Illiger), *Poecilus lucublandus lucublandus* (Say), *Bembidion quadrimaculatum oppositum* (Linnaeus), and *Clivina fossor* (Linnaeus) have all been observed to prey upon various life stages in petri dish assays (Baines *et al.* 1990). Laboratory trials including carrots plants (e.g., 'field-realistic') found these natural enemies may struggle to prey upon CW effectively in field situations, including the inability of *P. melanarius* to consume CW adults once the CW had move onto the carrot plant (Zhao *et al.* 1990). Despite minimal evidence for high CW predation due to these natural enemies, CW damage can be extremely variable depending on the field and year and it is possible that even limited predation could significantly impact CW damage. To date, the carabid community present in the Holland Marsh has not been assessed.

In the Holland Marsh, there are two established carrot weevil (CW) parasitoids, *Anaphes victus* and *Anaphes listronoti* (Hymenoptera: Mymaridae) (Cormier *et al.* 1996). These parasitoids are closely related, and were described as the same species (*Anaphes sordidatus*) until recently (Cormier *et al.* 1996). Over the degree day modeled CW oviposition period, these parasitoids can produce between 4-6 generations (Collins and Grafius 1986a; Boivin 1993). *Anaphes* spp. parasitism rates against CW eggs can reach up to 50-90% at peak CW egg density (Collings and Grafius 1986c; Boivin 1993) although parasitism of all CW eggs on a carrot plant is required to avoid carrot injury (Collins and Grafius 1986c; Cormier *et al.* 1996).

In this chapter, monitoring efforts designed to evaluate the CW natural enemy community present in the Holland Marsh region of Ontario, a significant region for carrot production in Canada, will be discussed. Commercial fields and the carrot insect IPM Evaluation trial were monitored for their carabid communities using pitfall traps, while CW parasitoids (*Anaphes* spp.) were monitored using carrot baits containing CW eggs. Carabids were the focus of all pitfall trapping, as the group encompasses all known CW natural enemies.

4.3 Methodology

4.3.1 Pitfall Trapping

Commercial Fields

In both 2015 and 2016, four commercial carrot fields (different fields in each year) included in the University of Guelph – MCRS IPM program at the Holland Marsh, Ontario were selected to be monitored for known CW natural enemies using pitfall traps. Each year consisted of a different group of four fields and the fields were selected to encompass the geographical distribution of the Holland Marsh with a north, south, east, and west region field monitored each year. Pitfall traps were installed in each field on May 28 2015 and May 30 2016. Each pitfall trap consisted of two 500 ml clear plastic deli cups (ShortReed Paper Inc., Guelph, ON) stacked together with the top deli cup filled with ~300 ml of 70% ethanol (Fig. 4.1). Traps were replaced weekly until the last week in August, though occasionally some traps were not collected due to re-entry intervals of pesticide application in particular fields. Two deli cups per pitfall trap allowed for quick replacement of the top deli cup during the weekly trap collection. Each pitfall trap also had a Styrofoam plate (12 cm in diameter) secured directly above the trap using wooden skewers to protect the traps from rain and sunlight.

After collection, traps were returned to the University of Guelph and stored at 4 ± 2ºC until identification could take place. If needed, insects were removed from traps using a 1 mm sieve. Bousquet (2010) and Goulet and Bousquet (2014) taxonomic keys were used for identification of carabids.

Figure 4.1. A pitfall trap used for monitoring Carabidae in carrot fields in the Holland Marsh, Ontario.

IPM Program Evaluation Trials

Pitfall traps, following in the same method of establishment and replacement as the commercial fields, were placed into each plot of the carrot insect IPM Program Evaluation trials (Chapter 1). Each plot contained two pitfall traps, placed equidistant from each other and the plot borders in the middle of each plot. Traps were collected and replaced weekly, returned to the University of Guelph and stored at 4 ± 2ºC until identification could take place. If needed, insects were removed from traps using a 1 mm sieve. Bousquet (2010) and Goulet and Bousquet (2014) taxonomic keys were used for identification of carabids.

4.3.2 Parasitoid Egg Baits

Following Cormier *et al.* (1996), CW *Anaphes* parasitoids were monitored in the Holland Marsh region of Ontario using CW egg baits. These egg baits consisted of carrots containing ≥25 CW oviposition pits. To achieve this, large carrots were placed within CW colony jars (see Chapter 3.3.1 and Appendix 2 for more details) at 24 ± 1ºC for 24 h, 0-3 days prior to use. In 2015, most egg baits were stored in a fridge (4 ± 2ºC) for 2 days prior to use in the field. In 2016, due to a failure in the development of CW eggs in 2015, carrot baits were inoculated with CW eggs starting the day previous to deployment into the field, eliminating the need to refrigerate baits.

Each week, from approximately June 1 – August 30, three CW egg baits were placed near a field edge, secured using metal wire, and left for three days. Upon collection, CW oviposition cavities in were examined for CW eggs, and 25 CW eggs from 25 different oviposition cavities from each CW egg bait were removed and placed into a Beem® embedding capsule (Soquelec, Montreal, QC). When removing CW eggs, the egg nearest to the opening of the oviposition cavity was taken as the most accessible egg had the highest chance of being parasitized (Cormier *et al.* 1996). In 2015, CW parasitoids were monitored at the MCRS and a commercial field at the Holland Marsh, though in 2016 the CW laboratory colony could only support monitoring at the MCRS. The MCRS was chosen as it supports higher *Anaphes* parasitism rates than commercial fields in the Holland Marsh (Cormier *et al.* 1996). In addition, the CW colony used to produce CW egg baits in 2015 originated from a long-term laboratory colony in Quebec whereas in 2016 the CW colony consisted of CW collected from the Holland Marsh. Beem capsule size was reduced from 2015 to 2016, from size 00 to the smaller size 3.

After CW eggs were added to Beem capsules, the CW eggs were held for three weeks to allow for egg or CW parasitoid development. In 2015, CW eggs held at room temperature (23 ± 3ºC, 40 ± 20%RH) while in 2016 CW eggs were held at 24 ± 1ºC, 70 ± 10%RH in the CW laboratory colony rearing chamber. After three weeks, each capsule was examined to determine the status of the CW egg: no development, CW larvae production, or CW parasitoid production.

When CW eggs did not develop, they were examined for potential causes of mortality, such as physical damage, and most eggs showed no obvious cause of mortality.

4.3.3 Statistical Analysis

To assess carabid diversity collected from pitfall traps, Simpson’s Diversity Index for each location was calculated using the package *vegan* in R, version 3.2.3 (The R Foundation, Vienna, Austria). Rarefaction curves were developed using the packages *picantes, SPECIES, rich,* and *BiodiversityR* in R, version 3.2.3. The successful development of CW eggs used in the CW parasitoid baits was compared between the two years using *Proc ttest* in SAS 9.4 University Edition (SAS Institute Inc., Cary, NC).

4.4 Results

4.4.1 Pitfall Trapping

Overall, 2958 individual carabids were caught and identified across both sampling years (Table 4.1). With all pitfall traps combined, the dominant species (making up >95% of abundance) were: *Pterostichus melanarius* (24.6%), *Anisodactylus sanctaecrucis* (21.7%), *Amara lunicollis* (14.2%), *Stenolophus comma* (13.5%), *Bembidion quadrimaculatum oppositum* (8.2%), *Bembidion* spp. (4.7%), *Omophoron americanus* (3.2%), *Clivina fossor* (2.4%), *Amara patreulis* (2.0%), and *Dychirius montanus* (1.2%). The pitfall traps also contained potential predators outside of the Carabidae. Although these specimens were not the focus of the research, and as such were not quantified, arthropods within the Arachnae and Staphylinidae were common within pitfall traps.

In 2015, 1021 carabids were caught and identified across all pitfall traps. The dominant species were *Anisodactylus santaecrucis* (27.9%), *Pterostichus melanarius* (25.3%), *Amara* spp. (9.9%), *Stenolophus comma* (19.4%) *Omophoron americanus* (8.8%), *Clivina fossor* (2.4%), and *Cicindela duodecimguttata* (2.0%).

In 2016, 1132 carabids were caught and identified in commercial fields, resulting in *Anisodactylus santaecrucis* (27.4%), *Pterostichus melanarius* (20.6%), *Amara lunicollis* (18.3%), *Bembidion quadrimaculatum oppositum* (10.6%), *Stenolophus comma* (10.0%) *Bembidion* spp. (3.7%), *Amara patreulis* (3.0%), and *Clivina fossor* (2.4%) making up the dominant species.

In the commercial carrot fields sampled, fields sampled in 2016 provided greater species diversity and abundance compared to 2015 (Fig. 4.2). Within years, there were differences in the size and diversity of the carabid community among fields. In the three carrot insect IPM Evaluation trials, rarefaction analysis found that all plots were very similar, containing between 15-19 carabid species (Fig. 4.3).

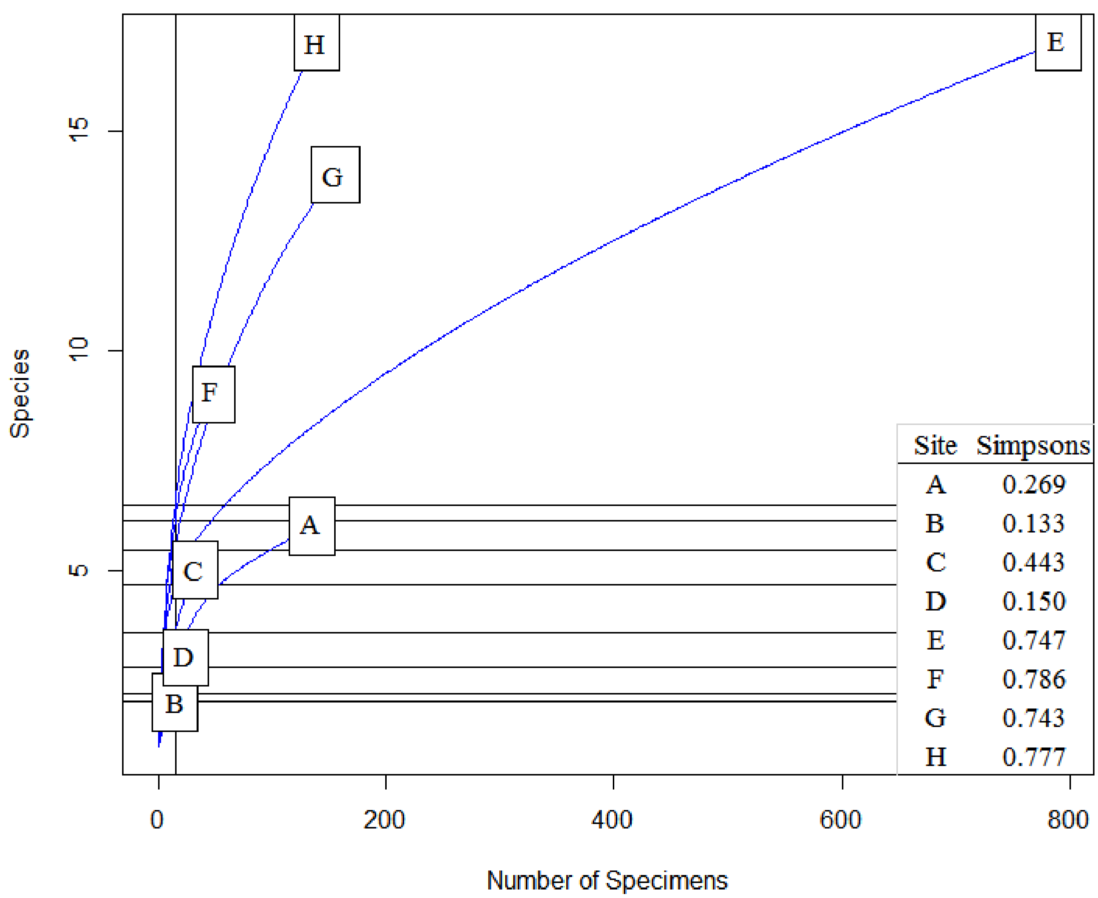


Figure 4.2. Rarefaction analysis and Simpson's Diversity Index of the carabid community from June to August 2015 and 2016 in eight commercial carrot fields in the Holland Marsh, Ontario. Fields A through D were sampled in 2015 and fields E through G were sampled in 2016. The

solid vertical line denotes the number of specimens used for rarefaction and horizontal lines indicate the number of species found in each field at the first rarefied sampling.

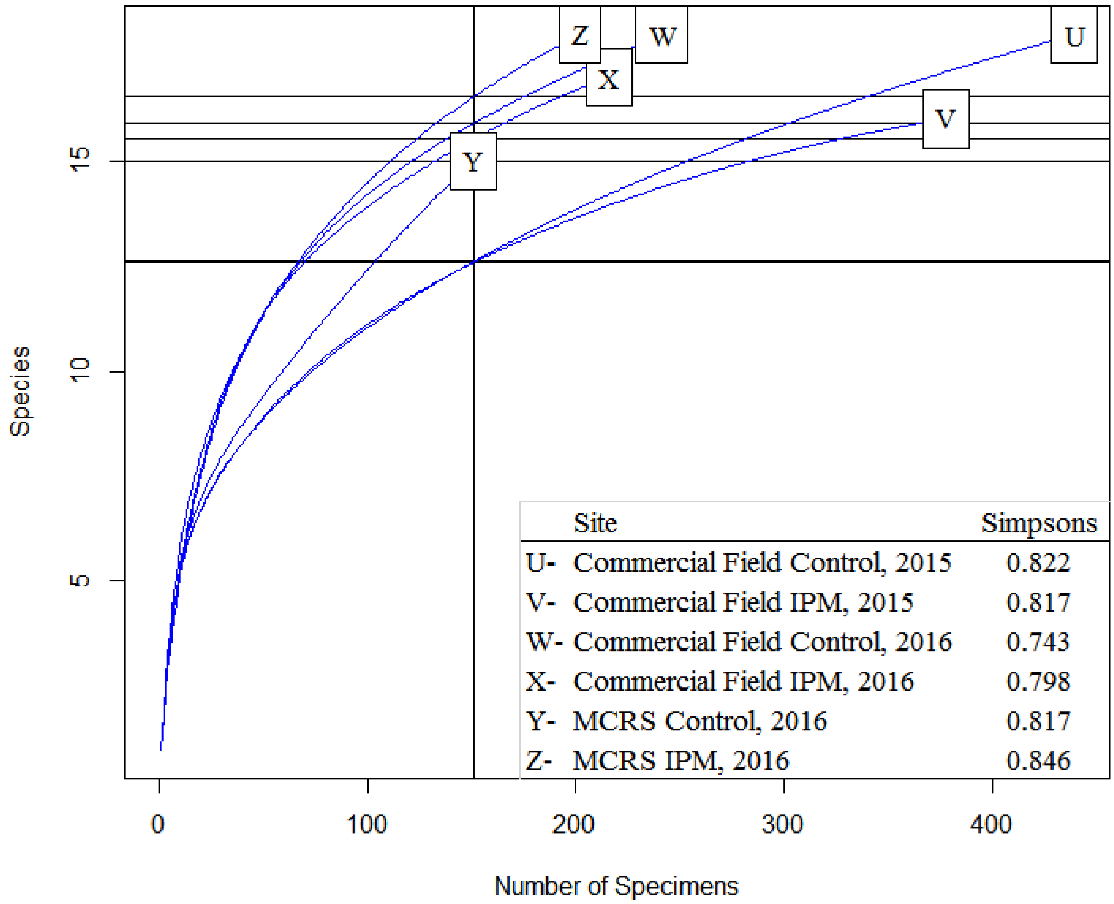


Figure 4.3. Rarefaction analysis and Simpson's Diversity Index of the carabid community from June to August in the carrot insect IPM evaluation trials performed at the Muck Crops Research Station (University of Guelph) and a commercial field in the Holland Marsh, Ontario, 2015 and 2016. The solid vertical line denotes the number of specimens used for rarefaction the samples and horizontal lines indicate the number of species found in each field at the first rarefied sampling.

Table 4.1. Carabids captured in commercial carrot fields and carrot insect IPM evaluation trials using pitfall traps in the Holland Marsh, Ontario, 2015 and 2016.

Taxa	Combined Traps		Grower Fields		IPM Trials	
	Total	%Abundance	Total	%Abundance	Total	%Abundance
Pterostichus melanarius	727	0.246	301	0.225	426	0.263
Anisodactylus sanctaecrucis	642	0.217	429	0.321	213	0.131
Amara lunicollis	421	0.142	208	0.155	213	0.131
Stenolophus comma	400	0.135	121	0.090	279	0.172
Bembidion quadrimaculatum oppositi	242	0.082	120	0.090	122	0.075
Bembidion spp	140	0.047	42	0.031	98	0.060
Omophron americanus	95	0.032	4	0.003	91	0.056
Clivinia fossor	72	0.024	30	0.022	42	0.026
Amara patruelis	60	0.020	34	0.025	26	0.016
Dyschirius montanus	35	0.012	13	0.010	22	0.014
Cicindela duodecimguttata	31	0.010	5	0.004	26	0.016
Poecilus lucublandus	28	0.009	10	0.007	18	0.011
Bradycellus atrimedeus	12	0.004	6	0.004	6	0.004
Harpalus somnulentus	9	0.003	3	0.002	6	0.004
Loricera pilicornis pilicornis	9	0.003	1	0.001	8	0.005
Atranus sp.	8	0.003	1	0.001	7	0.004
Pterostichus permundus	5	0.002	2	0.001	3	0.002
Amara avida	2	0.001	2	0.001	0	-
Dyschirius pallipennis	2	0.001	0	-	2	0.001
Patrobus longicornus	2	0.001	0	-	2	0.001
Porotachys bisulcatus	2	0.001	1	0.001	1	0.001
Schizogenus lineolatus	2	0.001	2	0.001	0	0.000
Sterocerus fessor	2	0.001	0	-	2	0.001
Acupalpus partiarius	1	-	0	-	1	0.001
Amara obesa	1	-	0	-	1	0.001
Anisodactylus verticalis	1	-	1	0.001	0	-
Carabus spp.	1	-	1	0.001	0	-
Chlaenius cordicollis	1	-	0	-	1	0.001
Dicaelus teter	1	-	0	-	1	0.001
Poecilus chalcites	1	-	0	-	1	0.001
Polyderis laevus	1	-	0	-	1	0.001
Pseudamaria arenaria	1	-	1	0.001	0	-
Tachyta inornata	1	-	0	-	1	0.001

4.4.2 Parasitoid Egg Baits

In both years, there were issues with eggs successfully developing within the embedding capsules, with only three eggs producing parasitoids. All of these parasitoids were collected during the first sampling date in 2016 and all emerged parasitoids identified as *A. listronoti*.

There were differences in egg survival, with significantly more CW eggs developing into larvae in 2016 compared to 2015 (Fig. 4.4, df=24, t=19763.4, p=<0.001).

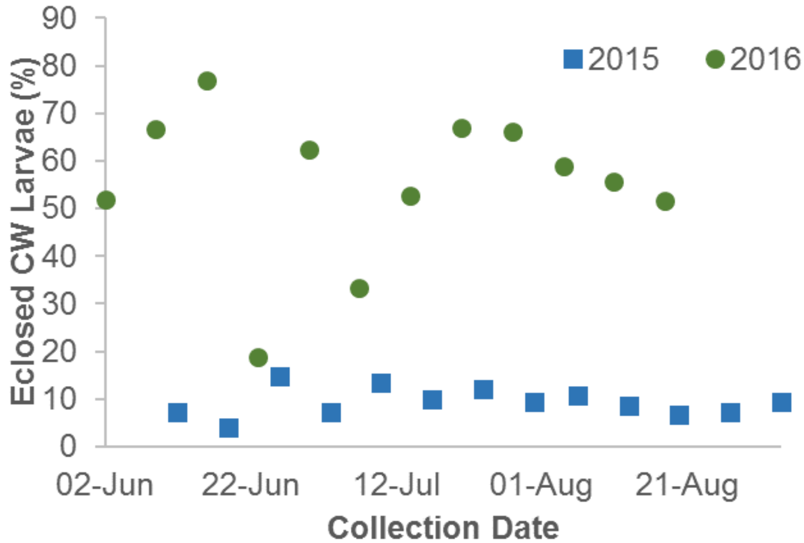


Figure 4.4. Successful carrot weevil (CW) egg development from CW eggs held in embedding capsules after a three-day field exposure within a developed carrot root in the Holland Marsh, Ontario to monitor for potential CW parasitoids, 2015 and 2016.

4.5 Discussion

In both years of pitfall trapping, all known carabid natural enemies of CW were found in the Holland Marsh. Although, abundance varied greatly among species and among locations. *Pterostichus melanarius* was found in all fields, and *A. sanctaecrucis* was also commonly found, with both species each making up >20% of the carabid abundance. Adult *P. melanarius* and *A. sanctaecrucis* often exceed 10 mm in size, making them among the larger carabids captured during the study. *Clivina fossor* and *B. quadrimaculatum oppositum* are smaller carabids, usually 2-6 mm in size, and were only found in a few fields. This observed result may not accurately relate to the carabid species evenness within the Holland Marsh as larger carabids are more likely to be found in pitfall traps (Spence and Niemela 1994). The higher capture rates of larger carabids are potentially due to overall higher mobility and larger ranges compared to smaller carabids or smaller carabids may escape more readily from pitfall traps (Spence and Niemela 1994). Unidentifiable remains of carabids were observed within our pitfall traps. It is possible that the trapped arthropods preyed upon smaller carabid species within the pitfall traps, consequently reducing the number of identifiable small carabids. Occasionally, weather interfered with the efficacy of pitfall trapping – precipitation resulted in traps being filled with water or muck soil, and occasionally high temperatures caused most of the ethanol within the trap to evaporate.

It is currently unclear if the carabid CW natural enemies can directly reduce CW damage within a season. All five Carabidae known to prey upon CW can effectively prey upon 4th instar and pupal stage CW (Baines *et al.* 1990; Zhao *et al.* 1990), but these stages do not exist until after CW larvae should have already damaged the carrot (Chandler 1926; Harris 1926; Perron 1971; Boivin 1988; Torres *et al.* 2002). With the likely occurrence of a 2nd CW generation in Canada, as discussed in Chapter 2 and 3 and suggested by Stevenson (1977) and Boivin (2013), the ability for carabid natural enemies to reduce CW damage increases.

The only carabid known to be capable of preying upon adult CW is *P. melanarius* (Baines *et al.* 1990; Zhao *et al.* 1990), which was abundant in pitfall traps across the Holland Marsh in 2015 and 2016. By preying upon adults early in the growing season, CW damage could be prevented by reducing the number of ovipositing CW females. Currently, the ability for *P. melanarius* to prey upon CW in field-relevant conditions is questionable, as the carabid is unable to feed on CW while the prey is on a carrot plant, creating an inherent refuge for CW within fields (Zhao *et al.* 1990). Despite this refuge, *P. melanarius* was able to eat roughly 4-5 of 15 CW larvae, pupae, or adults when placed in a 21 cm plastic pot containing five carrots (Zhao *et al.* 1990). In contrast, the next best predators, *P. lucublandus* and *A. sanctaecrucis*, fed upon around 1-2 larvae or pupae out of 15 in the same experimental set up (Zhao *et al.* 1990).

Egg predation is another route through which CW damage can be prevented. Both *B. quadrimaculatum oppositum* and *C. fossor* are effective predators of CW eggs when tested in a petri dish (Baines *et al.* 1990). However, when *B. quadrimaculatum oppositum* was tested for CW egg predation using a real carrot containing CW oviposition pits, there was no evidence of CW egg predation when compared to carrots containing CW oviposition pits without exposure to the carabid (Zhao *et al.* 1990), and *C. fossor* was only examined for CW egg predation in a petri dish assay. A black exudate is typically produced by the ovipositing CW to cover their oviposition pits (Harris 1926; Boivin 1988). This exudate likely confers some level of protection against egg predation and may be the reason *B. quadrimaculatum oppositum* failed to prey upon any CW eggs in a field-realistic setting (Zhao *et al.* 1990). Carabid CW natural enemies likely have some impact on the overall population of CW residing in a field, but it is difficult to predict any amount of CW damage mitigation they may provide.

During sampling, other potential predators of CW were observed. Various Aleocharinae (Coleoptera: Staphylinidae) beetles were frequently found, which are suspected to prey upon another carrot insect pest, the carrot rust fly (*Psila rosae)* (Sivasubramaniam *et al.* 1997). Several arachnids, predominately Aranae and Opiliones, were also recovered in all locations. The spider *Tenuiphantes tenuis* (Aranae: Linyphiidae) has been identified as a significant predator of a CW congeneric, *Listronotus bonariensis*, in pastureland in New Zealand, with *T. tenuis* estimated to prey upon up to 3.9 *L. bonariensis*/m^2 per day (Vink and Kean 2013). Other potential natural enemies present in the Holland Marsh have been suggested by Chaput (1996), including the hemipteran families Anthocoridae, Reduviidae, and Nabidae, as well as lacewings (Neuroptera: Chrysopidae) and ladybirds (Coleoptera: Coccinellidae), and these groups of insects were observed in carrot fields during sampling periods and during maintenance of field trials reported in Chapter 2 and 3 although these insects were not captured in the pitfall traps. It is possible that these natural enemies may prey upon CW adults although future research is needed to confirm this.

The CW parasitoid monitoring efforts in 2015 and 2016 were mostly unsuccessful. In 2015, nearly all CW eggs failed to develop. There were often >85% of eggs that failed to hatch with no discernable cause of mortality in 2015, while the 1990-1992 samplings the trial was based on experienced only 13% unexplained mortality (Cormier *et al.* 1996). Egg development improved in 2016, with 60-70% of eggs developing, yet that still leaves a difference of 20-30% in unexplained mortality compared to the original study. Differences in methodology may explain the increase in egg survival between 2015 and 2016, as well as the detection of parasitoids in 2016. First, in 2015 eggs were stored for up to 3 days at 4 ± 2ºC until needed in the field, which is reported to not affect *Anaphes* parasitism (Cormier *et al.* 1996) while in 2016 eggs were placed into the field immediately after the 24 h oviposition period within the colony. The *Anaphes* spp. which parasitize CW eggs have an optimal host age of 2-4 d (Picard *et al.* 1991) and it is possible that in 2015 eggs we collected had developed more while being cooled than the cooled eggs used in Cormier *et al.* (1996), potentially reducing the acceptability of the eggs used in this report. It is also possible that the time spent during cooling affected CW egg development negatively, producing unviable eggs which would not allow for parasitoid development (Picard *et al.* 1991).

In 2015, a laboratory strain of CW originating from a Quebec was used for egg production, but in 2016 a colony established from CW collected from the Holland Marsh was used. The two colony populations could have had different rates of sterile egg production, which ultimately could affect parasitism rates and overall CW egg development during the trials. Finally, different size embedding capsules were used in 2015 and 2016. The size of embedding capsule was not described in Cormier *et al.* (1996), but a reference to Boivin (1988b) indicated the correct size to use, Beem™ embedding capsules size 3, which was used for the 2016 trial, whereas the larger size 0 was used in 2015.

Despite challenges in monitoring CW parasitoids effectively, it is disappointing that only one species of CW parasitoid, *A. listronoti*, was detected over the two years. In the Holland Marsh, *A. listronoti* was responsible for 90% of detected CW egg parasitism, with *A. victus* comprising the final 10% (Cormier *et al.* 1996). Using the same CW egg baits, Cormier *et al.* (1996), achieved nearly 80% parasitism during some sampling dates at the MCRS. Currently, it is unclear whether some differences in methodology or the presence of parasitoids is the cause of these results. Further research should move to an alternate form of monitoring, such as sampling carrot plants that have CW oviposition pits directly, to see if CW parasitoids are successfully finding hosts in a field setting. Field-grown carrots with oviposition pits were also examined by Cormier *et al.* (1996), who found parasitoids in 80% of fields examined in this way, with overall parasitism rates reaching 17%.

Overall, there is an established CW natural enemy community within the Holland Marsh region of Ontario, but the predator and parasitoid populations each have their own unique issues.

It is currently not known how effective the carabids, or other potential predators, are at consuming and reducing overall CW populations. Further research is needed to both evaluate other potential predators, such as spiders and staphylinids, as well as known CW natural enemies for their efficacy in preying upon CW in a field realistic setting. The research here failed to find a substantial CW parasitoid population in the Holland Marsh. Further research is needed to determine whether the parasitoid population has reduced dramatically or that monitoring efforts were not effective. Additional research could also focus on ways to conserve and promote the natural enemies present, including both predators and parasitoids. The Holland Marsh is established on an extensive canal system, in which the berms could provide non-agricultural areas to create plant communities to promote these natural enemies without negatively impacting agricultural production. Despite the potential challenges with the Holland Marsh natural enemy community, the null hypothesis that there are no natural enemies within the Holland Marsh is rejected. This rejection of the null hypothesis is based on the abundance of known CW predators discovered through pitfall trapping and the presence of a CW parasitoid.

CHAPTER 5

GENERAL CONCLUSIONS

Overall, the results of the field and laboratory trials suggest the existing CW IPM program has several issues that require further investigation so that modifications can be made to improve its efficacy at the Holland Marsh and potentially in Quebec. The foremost issue is the reduced efficacy of phosmet in the Holland Marsh region for CW control. A laboratory trial showed that Holland Marsh CW is significantly less susceptible to phosmet exposure compared to a susceptible Quebec (laboratory) strain with no previous exposure to insecticides (Fig. 3.2). In eight separate field trials performed in 2015 and 2016, only two trials resulted in a significant reduction in CW damage when phosmet was applied compared to an untreated control. In one of these trials, CW damage still reached >50% at final harvest, which in a commercial field could result in the producer abandoning the harvest for that season. Fields could be left unharvested as the cost of separating marketable carrots from those with CW damage would be too great to justify the cost of harvest. Phosmet trials for CW mitigation in 1991-1994 found that insecticide was providing excellent control (Pree *et al.* 1996). In the ensuing 22 years, the continued use of phosmet on an annual basis is likely selecting for CW with reduced susceptibility to the product. Additionally, it is possible that the microbiome within Holland Marsh soil has adapted to the frequent applications of phosmet and the rate of phosmet degradation due to the soil microbiome has increased.

Another insecticide registered for CW control, λ-cyhalothrin, failed to provide any CW control in current field trials. In discussions with producers in the Holland Marsh, it seems that this pyrethroid insecticide is rarely used for CW control. Only one potentially effective insecticide is currently registered for CW control for the Holland Marsh – novaluron (a chitin synthesis inhibitor). Since the CW may have already developed a resistance to phosmet, and is known to have a general tolerance to contact insecticide exposure (Martel *et al.* 1975a), additional effective chemistries with alternate modes of action are needed for resistance management. Plots treated with cyantraniliprole, either as a seed or foliar treatment, significantly reduced CW damage in 2016 and should be investigated further to help in resistance management. Further research should assess CW susceptibility to insecticides not examined in Chapter 3, as well as the susceptibility of different CW life stages to insecticide exposure, including alternate routes of exposure. Both effective chemistries in the 2016 Foliar Insecticide Field Trial, novaluron and cyantraniliprole, have some trans-laminar and ovicidal/larvicidal properties and it is possible that effective CW control may depend on those properties. Determining the mechanism of resistance for phosmet by CW is another knowledge gap that requires further research and will shape further research on CW chemical control, particularly if cross-resistance is examined. Results of one field trial (Chapter 3) suggest that the addition of 1% PBO to phosmet significantly improved its efficacy in CW control. It is important to see if this effect translates into increased CW mortality in a laboratory study, as it is unclear if PBO is impacting the CW, the soil microbiome, or both to increase insecticide efficacy.

Another important finding of this research is strong evidence of a 2nd generation of CW occurring in Ontario. In 2016, every field trial had a significant increase in CW damage between the first and second sampling/harvest (in late July and early October, respectively), often ranging from 15-20%. Currently, the IPM program has no recommendations for a 2nd CW generation. If producers are experiencing 2nd generation damage as seen in the field trials, the IPM program must be revised to include recommendations for management of this additional generation. Currently, we do not have the scientific basis for any recommendations. Further research needs to address how this 2nd generation is occurring in the Holland Marsh, and potentially in Quebec as reported by Boivin (2013), and to examine potential solutions. Currently, that is difficult as all CW monitoring traps depend on using carrot roots as an attractant, which become increasingly ineffective as the carrot crop being monitored grows and competes with the carrot root baits in the Boivin trap. Improving monitoring to assess 2nd generation CW activity effectively is the most important step in establishing recommendations for a revised IPM program for CW. Modifications in monitoring may also increase the efficacy of 1st generation CW control, by improving the accuracy and precision of action thresholds, as an analysis of CW damage compared to the action thresholds in commercial fields in this study found damage to be extremely variable across all thresholds (Fig. 2.2).

Any proposed modifications of the IPM program also need to take into account the novel CW behaviour noted in both field seasons. Early in the growing season, carrots at the 2^{nd} TLS were subject to CW oviposition. Historically, CW oviposition only occurred on plants past the 4^{th} TLS (Boivin 1988). In addition, carrots are now dying due to CW larval feeding, made apparent by young carrots with dead foliage and roots tunneled completely through, resulting in death of the meristematic tissue. It is unclear what impact this has on overall CW damage and the IPM program may need to recommend insecticide applications earlier based on crop phenology to provide acceptable protection.

Finally, an abundant community of CW natural enemies was identified within the Holland Marsh, including five carabids: *Pterostichus melanarius, Anisodactylus sanctaecrucis, Poecilus lucublandus, Bembidion quadrimaculatum oppositum,* and *Clivina fossor,* and a parasitoid, *Anaphes listronoti*. While the overall impact these natural enemies may have on CW populations within the Holland Marsh has not been evaluated, it is promising to have such diversity and potential for conservation biological control within an intensively cultivated agroecosystem. Future research should examine causes for differing species diversity among commercial fields and opportunities to promote populations of CW natural enemies in the Holland Marsh, potentially through rejuvenation of the canal berms surrounding the marsh with plants that provide forage and shelter for these natural enemies. Additionally, a nematode, *Bradynema listronoti* is present in some Quebec fields and infect female CW, rendering the CW sterile. It is possible infected CW could be transported to Ontario to inoculate Holland Marsh fields with this nematode. Future research could examine the non-target impact of this nematode as well as it's efficacy at reducing CW oviposition to investigate whether nematode introduction is a viable option for CW management in the Holland Marsh.

Overall, the existing CW IPM program requires revision in southern Ontario. Novaluron may currently be the only effective insecticide for CW control, at least in the Holland Marsh, but future trials are needed to confirm this result. Novel CW behaviour and an apparent 2^{nd} generation appear to be increasing CW pressure in the Holland Marsh, furthering the need for effective recommendations for CW control. Both cyantraniliprole and the addition of 1% PBO to phosmet appear to be potential new control options, but more research is still needed as these are based on trials from a single field season. Carrot weevil natural enemies are present within the Holland Marsh, and further research should focus on assessing their ability to reduce CW populations and promoting natural enemy populations through conservation biological control.

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APPENDICES

Appendix 1. Raw historical Data of the University of Guelph – Muck Crops Research Station IPM Program

Table A7.1. Historical data of the University of Guelph – Muck Crop Research Station's IPM program for carrot weevil.

Producer	Year	Cumulative CW Captured	Threshold	CW Damage (%)
B	2009	0	0	0
I	2009	0	0	0
E	2009	0	0	3
H	2009	0.33	0	1
W	2009	0.5	0	0
C	2009	0.75	0	0
Y	2009	1.5	1	0
M	2009	2	1	2
N	2009	2.5	1	5
F	2009	2.6	1	0
T	2009	5.1	2	5
E	2009	5.5	2	0
C	2009	10	2	0
X	2009	19	2	4
O	2009	25	2	3
O	2010	0	0	1
W	2010	0	0	0
B	2010	0	0	0
L	2010	0	0	2
E	2010	0	0	4
M	2010	0	0	2
H	2010	0.25	0	0
H	2010	0.25	0	1
X	2010	0.66	0	10
H	2010	0.75	0	0
T	2010	1	0	3
I	2010	1	0	0
C	2010	1.5	1	0
W	2010	1.75	1	0
F	2010	4.5	1	2
I	2010	4.75	1	0
R	2010	12	2	6

Y	2010	15	2	1
J	2011	0	0	1
J	2011	0	0	2
O	2011	**0**	0	0
H	2011	0	0	1
H	2011	0	0	2
V	2011	0	0	5
F	2011	0.25	0	1
R	2011	**0.25**	0	0
Y	2011	0.25	0	1
S	2011	0.25	0	0
I	2011	0.5	0	1
H	2011	0.5	0	0
D	2011	0.5	0	12
N	2011	0.75	0	2
I	2011	1	0	0
P	2011	1.25	0	7
W	2011	1.5	1	7
B	2011	2	1	0
E	2011	2	1	1
K	2011	2	1	6
H	2011	2	1	3
M	2011	2.75	1	12
H	2011	3.5	1	0
Q	2011	3.5	1	6
W	2011	3.75	1	1
X	2011	4	1	21
T	2011	4.25	1	2
C	2011	4.5	1	4
L	2011	4.5	1	8
B	2011	8.75	2	9
L	2011	10.5	2	11
F	2012	0	0	0
H	2012	0	0	0
H	2012	0.5	0	0
H	2012	0.5	0	1
J	2012	0.75	0	1
O	2012	1	0	3
W	2012	1.5	1	0
J	2012	1.5	1	0
Y	2012	2.25	1	0

L	2012	2.25	1	1
P	2012	2.25	1	1
S	2012	2.25	1	3
N	2012	3	1	2
C	2012	3.75	1	0
H	2012	3.75	1	1
I	2012	4.5	1	0
L	2012	5	2	12
Q	2012	5	2	1
U	2012	5	2	0
B	2012	5.75	2	1
X	2012	5.75	2	1
H	2012	6	2	8
I	2012	11.25	2	2
W	2012	13	2	0
E	2012	23.35	2	0
T	2012	33.5	2	0
H	2013	0.5	0	0
W	2013	1	0	0
I	2013	1.25	0	0
W	2013	1.5	1	0
X	2013	2	1	0
E	2013	2	1	2
F	2013	2.25	1	1
S	2013	2.5	1	0
N	2013	3.25	1	0
R	2013	4	1	0
L	2013	4	1	1
U	2013	4.2	1	4
J	2013	4.5	1	0
O	2013	4.5	1	2
I	2013	5.5	2	0
H	2013	6.25	2	1
Q	2013	7	2	2
B	2013	7.5	2	0
Y	2013	8.25	2	3
M	2013	9.5	2	3
H	2013	13.75	2	0
P	2013	15.25	2	0
I	2013	24.25	2	0
L	2013	37	2	4

B	2014	0	0	0
H	2014	0	0	3
P	2014	0	0	2
A	2014	0.25	0	1
A	2014	0.25	0	2
W	2014	0.75	0	2
W	2014	1	0	1
I	2014	1.5	1	4
I	2014	1.75	1	5
A	2014	2.75	1	3
F	2014	3.5	1	1
N	2014	5	2	2
H	2014	5	2	0
Q	2014	5.25	2	3
S	2014	7	2	0
I	2014	7.75	2	14
R	2014	8.25	2	2
I	2014	8.5	2	0
X	2014	9.25	2	1
M	2014	11.25	2	1
I	2014	11.25	2	0
O	2014	11.25	2	5
L	2014	11.75	2	14
J	2014	12.5	2	1
C	2014	15	2	2
C	2014	15.75	2	2
Z	2014	19.25	2	12
Y	2014	24.75	2	1
I	2014	26.5	2	2
U	2014	29.5	2	2
Z	2014	56.5	2	4
W	2015	0	0	0
F	2015	1.25	0	0
T	2015	1.75	1	15
I	2015	2	1	2
G	2015	2	1	2
W	2015	2.06	1	0
I	2015	3	1	0
B	2015	3	1	4
B	2015	3.25	1	3
B	2015	5.25	2	4

A	2015	6.75	2	1
J	2015	8	2	1
J	2015	9	2	1
H	2015	9.25	2	1
H	2015	9.5	2	1
O	2015	13.5	2	7
U	2015	13.75	2	4
I	2015	14	2	2
C	2015	20.5	2	4
A	2015	27	2	3
E	2015	28.25	2	15
Y	2015	32.5	2	6
Q	2015	34.5	2	6
Z	2015	36	2	1
Z	2015	73.25	2	7

Appendix 2. Fertilizer, fungicide, and herbicide applications used in 2015 and 2016 field trials.

Table A7.2. Fertilizer, fungicide, and herbicide applications in the carrot insect Integrated Pest Management Program Evaluation trial in a commercial field at the Holland Marsh Ontario. 2015.

Active I+A1:G13ngredient	Formulation	Activity	Producer	Rate	Application	Application Dates
Prometryn	Gesagard 480 SC	Herbicide	Syngenta Canada Inc.	6.0 L / ha	Foliar	25-May-15
Linuron	Lorox	Herbicide	E. I. du Pont Canada Co.	500 mL / ha	Foliar	17-Jun-15
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	Foliar	17-Jun-15
Linuron	Lorox	Herbicide	E. I. du Pont Canada Co.	750 mL / ha	Foliar	22, 26 June 2015
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	Foliar	22, 26 June 2015
Pyraclostrobin	Cabrio EG	Fungicide	BASF Canada	1.1 kg / ha	Foliar	06-Aug-15
Penthiopyrad	Fontelis	Fungicide	E. I. du Pont Canada Co.	1.2 L / ha	Foliar	21-Aug-15
Calcium, Magnesium, Boron, Potassium	Alexin	Fertilizer	Gouws and Scheepers Ltd.	3.0 L / ha	Foliar	06-Aug-15
Calcium	CalciMax	Fertilizer	Gouws and Scheepers Ltd.	3.0 L / ha	Foliar	06-Aug-15
Calcium	CalciMax	Fertilizer	Gouws and Scheepers Ltd.	2.5 L / ha	Foliar	21-Aug-15
Nitrogen, Phosphorous, Potassium	20-20-20	Fertilizer	Plant Products	3.0 L / ha	Foliar	06-Aug-15
Nitrogen, Phosphorous, Potassium	SupraFeed 19/19/19	Fertilizer	TechnoGreen	2.0 L / ha	Foliar	21-Aug-15

Table A7.3. Fertilizer, fungicide, and herbicide applications in the 2016 carrot insect Integrated Pest Management Program Evaluation trial in a commercial field, Holland Marsh region of Ontario.

Active Ingredient	Formulation	Activity	Producer	Rate	Application Date
Prometryn	Gesagard 480 SC	Herbicide	Syngenta Canada Inc.	6.0 L / ha	26-May-16
Glyphosate	R/T 540	Herbicide	Monsanto Canada Inc.	1.0 L / ha	14-Jun-16
Linuron	Lorox	Herbicide	E.I. duPont Canada Co.	300 ml / ha	15-Jun-16
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	15-Jun-16
Linuron	Lorox	Herbicide	E.I. duPont Canada Co.	400 ml / ha	18-Jun-16
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	18-Jun-16
Linuron	Lorox	Herbicide	E.I. duPont Canada Co.	400 ml / ha	23-Jun-16
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	23-Jun-16
Azoxystrobin, Difenoconazole	Quadris Top	Fungicide	Syngenta Canada Inc.	600 ml / ha	7-Sep-16
Calcium	Phosyn Stopit	Fertilizer	Yara Canada Inc.	1.0 L / ha	7-Sep-16
Magneisum, Sulfur	Epsom Salts	Fertilizer	PQ Corp.	1.0 kg / ha	7-Sep-16
Azoxystrobin, Difenoconazole	Quadris Top	Fungicide	Syngenta Canada Inc.	1.0 L / ha	13-Sep-16
Calcium, Boron	CalciMax	Fertilizer	Gouws and Scheepers Ltd.	2.0 L / ha	13-Sep-16

Table A7.4. Fertilizer, fungicide, and herbicide applications in the 2016 Carrot Insect Integrated Pest Management Program Evaluation trial at the University of Guelph – Muck Crops Research Station, Holland Marsh region of Ontario.

Active Ingredient	Formulation	Activity	Producer	Rate	Application Date
Linuron	Lorox	Herbicide	E.I. duPont Canada Co.	400 ml / ha	18-Jun-16
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	18-Jun-16
Azoxystrobin, Difenoconazole	Quadris Top	Fungicide	Syngenta Canada Inc.	600 ml / ha	7-Sep-16
Calcium	Phosyn Stopit	Fertilizer	Yara Canada Inc.	1.0 L / ha	7-Sep-16
Magneisum, Sulfur	Epsom Salts	Fertilizer	PQ Corp.	1.0 kg / ha	7-Sep-16

Table A7.5. Fertilizer, fungicide, and herbicide applications in the 2015 Foliar, Seed Treatment, and In-Furrow Insecticides Carrot Insect trials at the University of Guelph – Muck Crops Research Station, Holland Marsh region of Ontario.

Active Ingredient	Formulation	Activity	Producer	Rate	Application Dates
Prometryn	Gesagard 480 SC	Herbicide	Syngenta Canada Inc.	6.0 L / ha	02-Jun-15
Linuron	Lorox	Herbicide	E. I. du Pont Canada Co.	500 mL / ha	26-Jun-15
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	26-Jun-15
Linuron	Lorox	Herbicide	E. I. du Pont Canada Co.	750 mL / ha	03-Jul-15
Paraffin Base Mineral Oil, Surficant blend	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	03-Jul-15

Table A7.6. Fertilizer, fungicide, and herbicide applications in the 2015 Planting Date, Insecticide Synergist, Foliar, Seed Treatment, and In-Furrow Insecticides Carrot Insect trials at the University of Guelph – Muck Crops Research Station, Holland Marsh region of Ontario. Applications with * denote an application only occurring on the Planting Date trial, and applications with ** denote an application only occurring on the In-Furrow trial.

Active Ingredient	Product	Purpose	Producer	Rate	Date
Bromoxynil	Pardner	Herbicide	Bayer CropScienc	210 ml / ha	12-May-16 *
Oxyfluorfen, n-methyl pyrrolidone	Goal	Herbicide	Dow Agrosciences	210 ml / ha	12-May-16 *
Prometryn	Gesagard 480 SC	Herbicide	Syngenta Canada	6.0 L / ha	25-May-16
Linuron	Lorox	Herbicide	E. I. du Pont Cana	400 ml / ha	18-Jun-16
Paraffin Base Mineral Oil, Surficant	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	18-Jun-16
Linuron	Lorox	Herbicide	E. I. du Pont Cana	400 ml / ha	23-Jun-16
Paraffin Base Mineral Oil, Surficant	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	23-Jun-16
Linuron	Lorox	Herbicide	E. I. du Pont Cana	500 ml / ha	30-Jun-16
Paraffin Base Mineral Oil, Surficant	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	30-Jun-16
Linuron	Lorox	Herbicide	E. I. du Pont Cana	750 ml / ha	5-Jul-16 **
Paraffin Base Mineral Oil, Surficant	Assist Oil	Adjuvant	BASF Canada	1.25 L / ha	5-Jul-16
Linuron	Lorox	Herbicide	E. I. du Pont Cana	1.0 L / ha	29-Jul-16 *
Paraffin Base Mineral Oil, Surficant	Assist Oil	Adjuvant	BASF Canada	1.0 L / ha	29-Jul-16
Boscalid, Pyaclostrobin	Pristine	Fungicide	BASF Canada Inc	700 g / ha	2-Sep-16
Calcium	Phosyn Stopit	Fertilizer	Yara Canada Inc.	3.0 L / ha	2-Sep-16
Magneisum, Sulfur	Epsom Salts	Fertilizer	PQ Corp.	1.0 kg / ha	2-Sep-16
Boron	Solubur	Fertilizer	U.S. Borax Inc.	1.0 kg / ha	2-Sep-16

Appendix 3. Carrot Weevil Rearing – Standard Operating Procedure

Materials Required

1. 20 Plastic containers (42 x 29 x 17 cm), with lids (referred to of Type A Colony Boxes)
2. 20.3 x 27.9 x 5.1 " mesh letter tray, 1 per Colony Box A
3. 20 additional plastic containers (38 x 24 x 15 cm, with lids (hereafter referred to as Colony Box Type B)
4. 20 3.8 gallon glass jars
5. Elastic bands, 1 per glass jar
6. 1 mm mesh, 6 m^2
7. Glue gun
8. Scissors
9. Soft forceps
10. Carrots without foliage (minimum 3 cm in diam. and 18 cm in length)
11. 12.5 cm diameter filter paper, Whatman Grade 1
12. 29.9 cm^2 Kimwipes
13. Labelling tape
14. Ink pen
15. Bleach
16. Spray bottle filled with 2% bleach solution
17. Spray bottle filled with tap water
18. Sterilized muck soil from the University of Guelph – Muck Crops Research Station
19. Two 18.9 L seed pails
20. Dehumidifier
21. Humidifier
22. Growth room maintained at 24±1°C, 70-80% RH, and 16:8 h light:dark photoperiod (Colony Growth Room)
23. Overwintering room maintained at 15±1°C and 14:10 h light:dark photoperiod
24. Walk in cooler (temperature between 3±3°C)
25. Walk in freezer (-20°C)
26. Oven capable of reaching 100°C

27. Metal tray (45 x 20 x 10 cm)

Procedure

1. Colony Set-Up

1. Construct Type A Colony Boxes (Addendum 1).
2. Ensure colony growth room is set up at 24±1°C, 70-80% RH, and 16:8 h light:dark photoperiod.
3. All colony boxes and jars should be washed with soapy water, wiped down with a 50% bleach:water solution, rinsed with water then allow to dry.
4. Place ~200 CW adults in a 1 gallon glass jar lined with filter paper and containing a single carrot; these are the initial ovipositing CW. Mark each jar with the date they were created on labelling tape. Six to eight ovipositing jars provide ample colony growth.
5. Cover each jar mouth with a small square of Kimwipe underneath a fine mesh square, sealed with an elastic band (Fig. A7.1). The 11.8” x 11.8” Kimwipes can be separated into four even sections - each section adequately cover the mouth of a single 1 gallon glass jar.

2. Maintenance

Colony maintenance must be performed every 2 to 3 days. When performing maintenance, check the water level of the humidifier (needs water) and dehumidifier (needs to be emptied) to ensure they can work properly.

Colony Box Preparation

1. Prior to any and all use of colony boxes and jars, colony boxes and jars should be washed in soapy water, wiped down with a 50% bleach:water solution, and rinsed with water.
2. Type A Colony Boxes are filled with sterilized muck soil, ~2 cm depth on bottom of the colony box, and water is added using the spray bottle filled with tap water until the soil

becomes moist but not saturated. A mesh letter tray is placed inside the colony box, sitting above the soil (Fig. A7.2).

Colony Jars

1. Use tap water to wash a carrot for each jar of CW presently in the colony + 2 extras. Carrots should be stored in the walk in cooler.
2. Each jar of CW needs the carrot replaced with one recently washed carrot. While replacing carrots, wipe each jar down with a Kimwipe to remove excess frass and moisture and replace the filter paper. Re-cover the jar with a new piece of Kimwipe underneath the mesh cover secured with an elastic band (Fig. A7.1).
3. Carrots in jars containing ovipositing CW will contain CW eggs. These carrots should be placed into a clean Colony Box Type B. Mark this colony box with the date on a piece of labelling tape. These carrots should be lightly sprayed using a spray bottle with the 2% bleach solution to prevent fungal growth.
4. While replacing carrots in ovipositing CW jars, examine the ages of the jars and the carrots being replaced. If an ovipositing CW jar has been actively ovipositing for >4 weeks or the carrots appear to have minimal oviposition pits (Oviposition pits are generally covered in a black exudate. If unsure, carrots can be examined under a microscope. Eggs will be visible after removing the black exudate with forceps.), bring a jar of overwintering CW back into the colony. Allow the overwintered CW 1 week to re-acclimate to the colony room, then begin using the overwintered CW jar as an oviposition jar and kill the CW in the old or ineffective oviposition jar by placing them in a freezer for 24 hours.
5. Jars containing CW recently emerged from the colony should have their replaced carrots immediately placed in the garbage. Typically, carrots are brought directly to the dumpster as the colony's needs can make garbage bags too heavy for the caretakers and create a smell. Any of these jars ≥7 days old should be moved to the overwintering room.

Colony Boxes

6. Examine the Type B Colony Boxes in use. Any boxes >7 days old should have their carrots placed into a Type A Colony Box (Fig. A7.3). The date marked on the labelling tape of Type B Colony Box should be moved to the Type A Colony Box.
7. Examine the Type A Colony Boxes in use. Spray any mold on the carrots or soil with 2% bleach solution. Once the carrots within these boxes are approximately 4 weeks old and rotten/withered, remove the old carrots and mesh letter tray they are placed on in the colony box. Weevil larvae or pupae should be visible in the soil once carrots are removed from Type A Colony Boxes (Fig. A7.4).
8. Use the soft forceps to sift through the soil of each Type B Colony Box to look for and collect new CW adults and place them into a new, clean 1 gallon jar lined with filter paper and containing a single carrot. Label the jar with date. Examine the jars attached to the end of each Type A Colony box for more adults. Once adults are present in the Type A Colony box, cut a ~1 cm slice of carrot to place in the jar attached to the Type A colony box to attract these adults. Replace this slice of carrot each time you do colony maintenance.
9. If the soil in any Type A Colony box is dry, moisten the soil using the spray bottle filled with tap water.
10. Any Type A Colony Boxes containing fewer than 10 CW pupae should be removed from the colony room and placed in a walk in freezer for 24 hours. All other Type A Colony Boxes in the colony room should have their soil moistened using the water spray bottle.

Soil Recycling

11. Remove the Type A Colony Box from the freezer at 24 h. Once the soil has thawed, store the soil in a 5 gallon pail covered with the lid. Label this pail 'Used Soil'
12. After several Type A Colony Boxes worth of used soil has accumulated, transfer the soil into a large metal tray and bake the soil at 100°C for 24 h for sterilization. Allow 2-3 h outside of the oven for the soil to cool before use in the colony. This soil should be stored in a 5 gallon pail covered with the lid labelled 'Clean Soil'.

Overwintering Maintenance

13. Newly emerged adult CW from the colony should be placed in the overwintering room (15±1°C and 14:10 h light:dark photoperiod) ≥7 days post-emergence.
14. Examine CW jars in the overwintering room every 4-8 days. Carrots, filter paper, and piece of Kimwipe for the jar should be replaced whenever a noticeable amount of frass has built up in the jar. While replacing these items, wipe down the interior of the jar with a Kimwipe to remove excess frass and moisture.

Figures

Figure A7.1. Carrot weevil colony jar used in rearing procedure.

Figure A7.2. Colony Box Type B containing carrots after CW oviposition.

Figure A7.3. Complete Colony Box Type A, containing carrots. The CW eggs in these carrots have hatched and CW larvae are currently feeding on the carrot. These larvae will drop into the soil when ready to pupate.

Figure A7.4. Carrot weevil larvae (left) and pupae (right); should be visible in Type A colony boxes when carrots are removed.

Addendum 1: Type A Colony Box Preparation

1. Cut or burn a large (~5cm X 10cm) hole in the lid of the colony box. Hot glue a piece of fine mesh overtop of the hole.
2. Burn a hole, using a metal pipe warmed with a Bunsen burner, approximately the size of the 4 oz specimen jar lid, into one short side of the colony box. The center of the specimen jar lid also requires a hole burned inside of it to allow the funnel to pass through.
3. Hot glue a funnel onto the inside of the new hole and the 4 oz specimen jar lid onto the outside of the new hole in such a fashion that the funnel will lead into the specimen jar when the specimen jar is screwed onto the lid (Fig. A7.5A).
4. Burn the bottom of the specimen jar off. Hot glue a small square of screen mesh over the hole to seal the end of the jar (Fig. A7.5B).

Figure A7.5. A – Funnel – lid colony box set up. B – Specimen jar for colony box.