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Title: Assessment of Black Vine Weevil Larval Damage to Cranberries and Development of Alternative Control Strategies

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Vaughn M. Walton

Abstract Black vine weevil (BVW), *Otiorhynchus sulcatus* Fabricius, is a serious pest in cranberry, *Vaccinium macrocarpon*. Larvae feeding below the soil surface cause damage to the roots and underground stems. Knowledge is sparse in regard to the damage potential of BVW in Pacific Northwest cranberry beds. Control with insecticides is limited by inefficacy, environmental contamination of runoff water, and negative effects on pollinators and other non-target organisms. The objectives of this study were two-fold: (1) to determine the relationship between initial BVW egg density and the effect of subsequent larval damage on cranberry plant health and (2) to compare the efficacy and persistence of *Metarhizium anisopliae* (Sorokin), *Steinernema kraussei* (Steiner), and the systemic neonicotinoid imidacloprid to control

BVW in cranberries. Two varieties of potted cranberry vines were infested with six BVW egg densities. Root damage and canopy health was assessed, both continuously and destructively. Greater root damage, lower total shoot length, lower shoot weight and decreased water use were associated with increased egg density. More root damage was observed in 'Stevens' compared to 'McFarlin'. Percent green leaf area was also significantly reduced with increasing BVW egg densities. Results indicate that increased BVW egg densities decrease plant health by increasing subsequent larval damage to the roots. This damage induces drought stress, reduces the amount of photosynthetic tissue in the canopy and limits shoot growth.

To address the second objective, treatments were applied to cranberry beds at two field sites and soil was collected by treatment on five dates during 2009 and 2010. Soil samples were then inoculated with laboratory-reared BVW larvae and incubated at 20˚C to assess potential control by, and persistence of, each treatment in the cranberry soil. *Steinernema kraussei* did not result in significant BVW mortality either by site or sample date. Imidacloprid resulted in significant mortality only in Langlois, Oregon and only on the first sample date. *Metarhizium anisopliae* provided inconsistent results. In Long Beach, Washington, *M. anisopliae* caused significantly higher mortality than any of the other treatments on three of the five sample dates. In Langlois, *M. anisopliae* caused significantly higher mortality than the control only on the last sample date, one year after treatment application. However, average mortality over the entire sampling period indicated that *M. anisopliae* was more effective than either *S. kraussei* or imidacloprid in both locations.

Assessment of Black Vine Weevil Larval Damage to Cranberries and Development of Alternative Control Strategies

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Elizabeth A. Miller, Author

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CONTRIBUTION OF AUTHORS

Dr. Denny J. Bruck assisted in all aspects of Chapters 2 and 3 including experimental design, statistical analysis, interpretation of data and writing of manuscripts.

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DEDICATION

I dedicate this document to those in my life who have taught me.

Your contributions to my journey have been invaluable.

ASSESSMENT OF BLACK VINE WEEVIL LARVAL DAMAGE TO CRANBERRIES AND DEVELOPMENT OF ALTERNATIVE CONTROL STRATEGIES

CHAPTER 1

GENERAL INTRODUCTION
Review of black vine weevil in Pacific Northwest cranberries

Elizabeth A. Miller

1.1 Introduction

Cranberries are one of North America's commercially produced crops and are indigenous to the continent (Pollack and Perez 2001). The United States cranberry industry is valued at around $444 million annually with a total of nearly 38,200 acres in production, 4,000 of which are in the Pacific Northwest (NASS 2009). There are several insect pests threatening cranberry production in the Pacific Northwest (Thomson et al. 1999). One of these is the black vine weevil (Coleoptera: Curculionidae), a prolific beetle with root feeding larvae (Smith 1927). It is a somewhat cryptic species and infestations may go undetected for long periods of time. Management of BVW can be difficult due to the insect's behavior and the lack of suitable control options (Cowles 1995, Kim Patten, unpublished data). This review will address the pest status of BVW in Pacific Northwest cranberries as well as its biology and life cycle and various methods for management of BVW with a focus on biological control options that include the entompathogenic fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin (Clavicipitaceae) and the entomopathogenic nematode *Steinernema kraussei* (Steiner). There will also be a short discussion on the growth and culture of cranberries and the constraints imposed by the unique production practices of the cranberry industry.

1.2 Black Vine Weevil

Black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius), is one of a complex of polyphagous root weevils that attacks plants worldwide and feeds on over

80 plant species, a number of which are commercial crops (Smith 1932). Adult BVW feed on foliage, causing a characteristic notching along the margin of leaves; however, unless on ornamental crops, this damage is rarely of economic importance. The major damage is caused by larvae feeding on the roots and crown of a plant (Smith 1927, Cowles 2001). Damage to the root system reduces vigor and water absorption and subsequently leads to plant desiccation (Schread 1972).

1.2.1 Description of black vine weevil

Smith (1932) described the BVW in detail. The adult has a distinctive head with a long “snout” and geniculate antennae (Fig. 1.1A). Fully sclerotized adults are black and measure 8.5 to 11.5 mm in length. Their elytra have a beaded appearance and small tufts of orange hair that appear as tan spots to the unaided eye. Pupae are milky white and somewhat resemble the adult in form. Eggs have a pearly white appearance when first laid and darken as the embryo within matures; nonviable eggs remain white. The larva is a white, legless grub with a dark brown head capsule (Fig. 1.1B). The body is covered with small hairs and is slightly translucent, which allows the stomach contents to be seen and can give the larva a pinkish or yellowish hue, depending on the host plant. The first instar has a straight body; as the larva grows, its body becomes more curved with each instar, taking on a characteristic C-shape (Smith 1932).

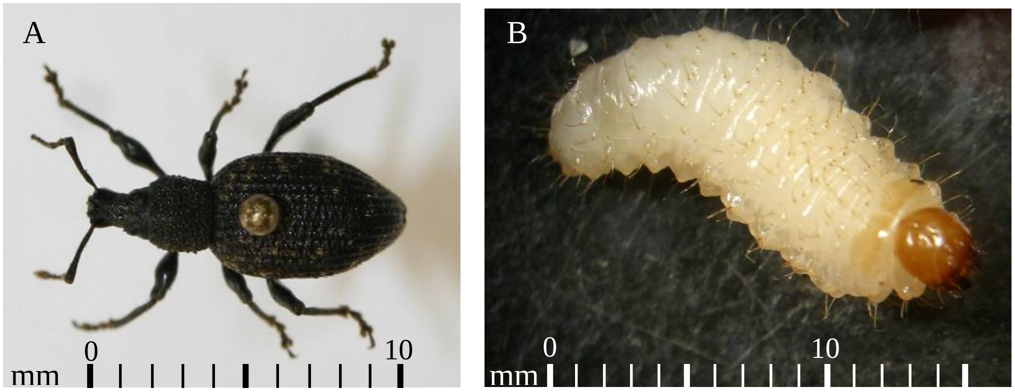

Fig. 1.1. Black vine weevil (A) adult and (B) larva.

1.2.2 Pest status of black vine weevil in Pacific Northwest cranberries

The black vine weevil is found in commercial cranberry beds throughout the Pacific Northwest (Linda White, personal communication) and is known to cause substantial losses to the cranberry industry (Ingraham 1991). Larvae consume fine roots and girdle underground stems of cranberry plants (Crowley 1923). Because larvae are subterranean and adults nocturnal, weevils can exist in the field undetected for a length of time until the plants experience drought stress during warm periods. At this point, weevil damage will become immediately evident as vines wilt, become dry and brittle, or die off (Kim Patten, personal communication). In Washington cranberry beds, it is estimated that infestations of over 12 black vine weevil larvae per square meter create patches of dead or dying vines within two seasons (Booth et al. 2002). Field observations indicate that damaged areas left untreated may quadruple in size within one year (Ingraham 1991). Soil type influences the damage potential of BVW. Lighter soils such as peat and sand are a more suitable habitat for weevil larvae (Wilcox et al. 1934) and efficacy of control treatments is greater in lighter soils

(Haukeland and Lola-Luz 2010, Nielson and Boggs 1985). This is an important consideration for cranberry producers, as cranberry beds in the Pacific Northwest often contain lighter soils (Davenport and DeMoranville 1993).

In addition to BVW, another species of root weevil, *Lepesoma (Dyslobus) bakeri* Van Dyke (Coleoptera: Curculionidae) is commonly found in cranberry beds in southern Oregon (Rosenstiel 1989). The pest status and biology of this species are largely unknown, but personal observations in the field indicate that its life cycle is inconsistent with that of BVW. Specifically, teneral adults of *L. bakeri* are present in September and sclerotized adults are present in December, suggesting that adult emergence of this species occurs during fall and winter. Adult emergence of BVW, however, occurs in spring or early summer and very few survive to overwinter in cranberries (Schread 1972).

1.2.3 Biology and life cycle of black vine weevil

Black vine weevil was introduced from Europe over a century ago through importation of infested plants. Only females are known for this species and they reproduce via thelytokous parthenogenesis. A single fecund individual left unmanaged can result in an infestation (Smith 1927, Schread 1972, Nielson and Dunlap 1981). Adults are flightless, having fused elytra and reduced hind wings (Smith 1932). Black vine weevils typically overwinter as inactive late-instars or pre-pupae in the soil, although a small percentage of the population overwinters as adults (Smith 1932). In the early spring, when soil temperatures are around 5°C, immature larvae become active and resume feeding before entering a pre-pupal stage (Smith 1932). Later instars generally pupate at the same time as overwintered pre-pupae, while smaller larvae take longer to mature and will pupate later in the season (Smith 1932). The pupal stage, which is protected within a pupal cell in the soil, typically begins in May and lasts approximately 20 days (Garth and Shanks 1978, Smith 1932).

Emergence of adults can occur from mid-May to July (Schread 1972). Sclerotized adults found earlier in the spring are assumed to have overwintered (Smith 1932). Upon emergence, adults begin pre-ovipositional feeding on foliage. This is the most extensive feeding period for adult BVW (Smith 1932). BVW adults are nocturnal, feeding at night and hiding during the day under the plant canopy (Cowles 1995); however, if debris on the soil surface is too moist, or if the host plant is large with arboreal architecture, adults may hide within the plant canopy (Smith 1932). BVW are very good crawlers and disperse readily within and between fields. However, they tend to oviposit near the place of emergence (Nielsen and Dunlap 1981). Their pattern of spread is outward from a central location (such as an infested host plant) or from adjacent infested fields (Smith 1932). Wide-scale disturbance of one field (such as mowing) will prompt a mass migration of adults to a new area as they search for more suitable host plants (personal observation, Maier 1978, Wilcox et al. 1934). Oviposition typically begins in late June or July and continues until September (Patten personal communication, Smith 1927, Smith 1932, Garth and Shanks 1978). Overwintered adults will begin ovipositing as early as April (Garth and Shanks 1978). The number of eggs laid in a season can vary depending on temperature and host quality and ranges from 50 to 380 eggs per adult female under field conditions (Son and Lewis 2005, Cram and Pearson 1965). For weevils that overwintered as adults, the number of eggs laid can be closer to 400, and under greenhouse or laboratory conditions as many as 1,000 eggs may be laid by one female in a season (Cram and Pearson 1965, Garth and Shanks 1978). Cram and Pearson (1965) compared differences in oviposition rates between different plant hosts. When cranberry served as the host, the average female laid 160 eggs per season. Females prefer to lay eggs under the canopy of plants and at varying soil depths, likely dependent on soil moisture and temperature (Garth and Shanks 1978). However, females have been observed dropping single eggs on the soil surface as well as forcing eggs into crevices within plant debris (Smith 1932). Eggs hatch within approximately two weeks (Schread 1972).

Smith (1932) described larval development in detail. First instars burrow into the soil immediately upon hatching to begin feeding. If the soil is too dense to accommodate this, the larvae will die. Larvae will complete six instars before pupating. When ready to molt, the larva will stop feeding and form a cell in the soil where it will shed its skin and head capsule. It will remain in the cell until the new head capsule is hardened before emerging to feed again. During this critical time of development, which lasts up to seven days, mortality can be high. Voracious feeding on larger roots commences at the start of the fourth instar and initiates the most damaging stage of the insect for the host plant. Once mature, the larva will void its gut contents to smooth the interior wall of a pupal cell formed in the soil. The weevil will remain in this cell during the transformation from larva to pupa to adult unless the cell is disturbed. Depending on soil temperature, this can take anywhere from three weeks, at an average temperature of 14ºC, to eight or nine months, at an average temperature of 7ºC (Smith 1932).

1.3 Control of black vine weevil

Control of BVW is difficult. This is due in part to the hardy nature and nocturnal habits of the adults as well as the subterranean location of the larvae (Cowles 1995). Larvae often feed undetected in the soil and cause most of the root injury before their presence is noticed (Kim Patten, personal communication).

Cultural practices can be important for maintaining low BVW population levels (Rutgers 2000, Patten and Daniels 2010), but alone are insufficient for economic control. Registered insecticides and biological products are available for control of BVW adults and larvae (Patten and Daniels 2010). The efficacy of each is variable and largely dependent on environmental factors such as soil type (Haukeland and Lola-Luz 2010, Nielson and Boggs 1985, Shah et al. 2007), weather (Booth et al. 2002, Luz et al. 2008) and seasonal timing (Bruck 2007, Booth et al. 2002, Shapiro-Ilan and Gaugler 2006). Due to stringent regulations for chemical insecticides, biological controls are becoming more attractive (ODA 2009). Of particular and recent interest is the entomopathogenic fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Clavicipitaceae), which is commercially available and used in the United

States in ornamental crops (Ansari et al. 2004, 2008, 2010; Booth et al. 1998, 2000; Bruck 2005, 2009; Bruck and Donahue 2007).

1.3.1 Cultural controls

Cultural practices recommended for control of BVW in other crops are impractical for cranberry producers. These include crop rotation and timing of plowing and planting (Wilcox et al. 1934). A new cranberry bed takes from four to seven years to establish and may produce for 50 years or more before it is rotated (WSU 2000). Wilcox et al. (1934) suggested selection of varieties with vigorous root growth to overcome BVW infestation in strawberries, believing that these plants were better able to withstand periodic attack. In cranberries, 'Stevens', 'Early Black', 'Wilcox' and 'Beckwith' are some varieties notable for their vigorous growth (Eck 1990). 'Stevens' has a more vigorous root system and a lower shoot-to-root ratio, making it less susceptible to drought stress (Baumann et al. 2005). This may be an important consideration for cranberry producers when establishing new beds in previously infested areas.

Trap cropping is a technique to lure an insect pest away from crop plants. In the case of BVW, alfalfa (Wilcox et al. 1934) and strawberry are highly desirable hosts (Fisher and Bruck 2008). Smith (1932), however, found that BVW prefers *Rumex* and *Primula* species to strawberry. Cram and Pearson (1965) reported that marginal non-crop vegetation (such as Himalyan blackberry and salal) offered more nutrition for ovipositing BVW than cranberry or blueberry hosts. These desirable plant hosts on field margins may accumulate BVW and, if not destroyed, have potential to contribute to high BVW populations.

Physical removal of BVW adults from the field has been a successful method for reducing populations in strawberries (Wilcox et al. 1934). Pre-ovipositional adults were handpicked or shaken from plants at night while feeding. This method was relatively cost-effective and reduced the population below economic thresholds for nearly ten years (Wilcox et al. 1934). In cases where suitable plant hosts are found among the surrounding vegetation, the labor required to remove a sufficient number of adults may be beyond what is practical. Trapping of adults underneath boards or other debris to collect and destroy weevils is another method of physical removal, which is not practiced in cranberries. Currently, several new prototypes of specially designed traps are being evaluated in strawberry fields (Bruck, unpublished).

Observations indicate that infestations decrease following wet summers and increase following summers with less rain, indicating that BVW larvae prefer unsaturated soil (Smith 1932). Flooding to control larvae has been considered one of the more effective control options used in cranberries (Linda White, personal communication). Populations of BVW can be managed using post-harvest floods lasting seven to ten days or more (Patten and Daniels 2010). This may be unfavorable due to the potentially negative effects of prolonged submergence on the cranberry vines themselves (Eck 1990). In the Pacific Northwest, midwinter floods have not always been effective, possibly due to the protective nature of overwintering larval cells (Patten and Daniels 2010). Also of note, BVW adults that are not killed by flooding may be spread to other beds if floodwater is recycled and moved between beds (Wilcox et al. 1934).

Sanding is a technique that is common in west coast cranberry production. The application of a 2.5- to 4-cm layer of sand to a bed every three or four years has a dual purpose. It is believed to invigorate old vines to produce new growth as well as suppress populations of other soil-dwelling insects such as cranberry girdler *Chrysoteuchia topiaria* (Zeller), green cranberry spanworm, *Itame sulphurea* (Packard) and cranberry tipworm, *Dasineura oxycoccana* (Johnson) (Rutgers 2000). Although there is documented success controlling insects such as cranberry girdler (Davenport and Schiffhauer 2000), sanding has not been demonstrated as an effective method to suppress populations of BVW.

1.3.2 Chemical controls

Most cranberry growers currently make use of standard soil applications of systemic and contact insecticides in order to control BVW in cranberry plantings (DeFrancesco 2010). Chemical control of BVW is targeted toward larvae and pre-ovipositional adults. The timing of applications often coincides with bloom, and so pollinator safety is of great concern (DeFrancesco 2010). The Washington State University Cranberry Pest Management Guide recommends imidacloprid for control of larvae in late summer and during the dormant season (Patten and Daniels 2010). Imidacloprid is a neonicotinoid insecticide that provides 30 to 85 percent control of BVW larval populations in potted plants, depending on the application rate and the soil media (Shah et al. 2007). Patten has observed its efficacy on peat soils to be inconsistent at best (personal communication).

Adults can exhibit a significant level of tolerance to many insecticides and apparently possess the ability to detoxify certain compounds (Cowles 1995). For example, in rhododendrons, pyrethroids (such as bifenthrin) must be applied at the highest labeled rate to be effective, and even then, adult BVW may recover from such a treatment as soon as one day later (Cowles 1995). Organophosphates currently used to control adults (acephate, for example) also have poor efficacy and provide only temporary control (Patten, unpublished data). In addition, these products have a long pre-harvest interval and so must be applied before the entire adult population has emerged. Historically, chlorinated hydrocarbons (aldrin, dieldrin, etc.) gave very good control of BVW due in part to their long residual activity. Use of these compounds, however, was banned in the United States in the 1970's. Currently, indoxacarb (e.g. Avaunt, DuPont, Wilmington, DE, USA) and cryolite bait are recommended in Washington for control of adult BVW (Patten and Daniels 2010). Indoxacarb belongs to a class of insecticides called oxadiazines that are considered to be "reduced-risk" pesticides. It is applied during bloom and must be applied at night to minimize contact with pollinators. It may be necessary to re-apply indoxacarb every 10 to14 days until no adults are detected by night sweep-netting (Patten and Daniels 2010). Patten reports very good control with this product if applied several times during the period of adult emergence (unpublished data). Cryolite bait is sodium aluminofluride and its mode of action is stomach poisoning, requiring ingestion. It can be applied during bloom with no risk to pollinators. Thiamethoxam is another neonicotinoid that is recommended for control of adults. Patten reports poor control with both cryolite and thiamethoxam in Washington cranberry beds (unpublished data).

Increasing concern about the environmental risks of using insecticides has led to changes in regulation of such chemicals and reduced the number and utility of many insecticides nationally. In the Pacific Northwest, measures limiting use of pesticides near waterways have been implemented to protect salmon populations (ODA 2009). Many of the restricted compounds are organophosphate, carbamate and pyrethroid insecticides, which are considered to be more toxic than certain "reduced-risk" insecticides that are now available to growers. However, scrutiny of pesticide use and its effects on the environment are ever-increasing and development of alternative strategies, such as biological control, is becoming increasingly important (Yadav 2010).

1.3.3 Biological controls

Biological control of insect pests involves importation or enhancement of indigenous natural enemies including predators, parasitoids and pathogens to reduce pest populations, and ideally establish in the environment for long-term pest control (Huffaker and Dahlsten 1999). Black vine weevil has many natural enemies. Vertebrates that have been observed predating on BVW include moles, lizards, toads and certain species of birds (Smith 1932). Due to the habits of nocturnal adult BVW and subterranean larvae, feeding by these natural enemies may be incidental. Macroinvertebrate natural enemies of BVW include carabid and staphylinid beetles, brachonid and tachinid parasitoids and ants (Smith 1932, Maier 1978). The natural enemies of BVW which have received the most attention in recent years are entomopathogenic nematodes and fungi. These two groups of organisms are highly regarded for their potential as biological control agents due to their propensity for mass-culture, ease of application, and efficacy (Kaya and Gaugler 1993, Shah and Pell 2003).

1.3.3.1 Entomopathogenic Nematodes. Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae attack a wide variety of insect hosts (Shapiro-Ilan and Gaugler 2006). Insect death is achieved through a symbiotic relationship between the nematode and gut-dwelling bacteria (Torr et al. 2007). The infective juvenile enters the insect through body cavities or directly through the cuticle (depending on the species) and releases the bacteria (*Xenorhabdus* for steinernematids and *Photorhabdus* for heterohabditids) from its gut to digest the insect (Shapiro-Ilan and Gaugler 2006). The entire process of infection can occur in as few as 24 to 48 hours (Shapiro-Ilan and Gaugler 2006). The nematode then feeds on the decaying insect and bacteria as it matures and reproduces. Once all food resources are consumed, another round of infective juveniles is released to find new hosts (Torr et al. 2007). Entomopathogenic nematodes are obligate pathogens, requiring an insect host to complete their life cycle, so their ability to persist in the field after the insect population has been reduced is limited (McGraw and Koppenhöffer 2009). However, EPNs do occur naturally in the Pacific Northwest. Liu and Berry (1995) sampled a wide variety of Oregon soils and found nematodes of the families Heterhohabditidae and Steinernematidae throughout. The majority of these were found in ocean beach soil.

Several species of EPNs are commercially available for use in many different crops, including cranberries. With certain precautions, they can be applied much like chemical insecticides, although special care must be taken regarding storage and application conditions. Entomopathogenic nematodes are temperature, moisture and UV-light sensitive, preferring low or filtered sunlight and high humidity for application (Booth et al. 2002, Smits 1996). Currently, there are three species of EPNs recommended for use against BVW in cranberries. These are *Heterohabditis megidis* (Nemasys H), *Steinernema carpocapsae* (Nematac C) and *Steinernema kraussei* (Nemasys L, Becker Underwood, Ames, IA, USA). Recently, the availability of these commercial EPNs has been inconsistent and cranberry growers are struggling to get a sufficient supply (Knute Andersson, personal communication).

Entomopathogenic nematodes are effective to control soil pests, especially BVW, in both container plants (Bruck et al. 2005, Ansari et al. 2004) and in the field (Willmott et al. 2002). To date, no field studies have shown adverse effects on non-target organisms (Shapiro-Ilan and Gaugler 2006). Due to temperature sensitivity and timing of application, EPNs are often ineffective during the early spring in the PNW, when late-instar BVW are active (Booth et al. 2002). *Steinernema kraussei* is a cold tolerant species that is indigenous to Scotland (Torr et al. 2007). This species is active at lower temperatures than other commercial EPNs. Willmott et al. (2002) found that

S. kraussei (strain: L137) was able to withstand prolonged exposure to winter field temperatures (2.7 °C on average). In addition, it was responsible for 81 percent control of BVW larvae when compared with other nematode species. It is commonly found in soils underneath conifer forests, suggesting an affinity for acidic environments. Furthermore, it is thought to have a predatory or cruising strategy for locating hosts, as opposed to an ambush or "sit and wait" strategy used by many other commercial EPNs (Torr et al. 2007). For these reasons *S. kraussei* is considered to be the most likely candidate for persistent control of BVW in cranberries.

1.3.3.2 Entomopathogenic Fungi. Entomopathogenic fungi (EPF) are found among the divisions Zygomycota, Ascomycota, Deuteromycota, Chytridiomycota and Oomycota (Shah and Pell 2003). These fungi infect many arthropod hosts, including non-insect arthropods as well as non-pest insects (Shah and Pell 2003). Species, and even isolates within a species, can vary widely in regard to host range, optimal growth conditions and virulence (Shah and Pell 2003). Formerly classified as Hyphomycetes, the most commonly utilized EPF include *Beauveria, Metarhizium, Hirsutella, Paecilomyces, Aspergillus, Verticillium, Tolypocladium, Nomureae* and *Culicinomyces* (Butt et al. 2001). EPF are known to have an epizootic effect on insect populations and have been observed to oscillate between periods of increased and decreased virulence (Tanada and Kaya 1993; Bruck and Donahue 2007). Numerous factors including insect density, susceptibility and behavior, environmental conditions and pathogen behavior may facilitate an epizootic leading to an increase in insect mortality (Tanada and Kaya 1993). Several EPF are registered and commercially available biopesticides. One of these is *Beauveria bassiana* (Balsamo) Vuillemin (Clavicipitaceae) (Mycotrol, Botanigard; Bioworks, Inc, Victor, NY, USA). It is used widely in North America on several different orders of insects on a variety of tree and field crops (Shah and Pell 2003). Cranberry producers currently use Mycotrol for control of root weevils (DeFrancesco 2010).

1.3.3.2.1 Metarhizium anisopliae. Another EPF of recent interest is *Metarhizium anisopliae*, which has been widely studied in various cropping systems. This species is not registered for use in food crops in the United States, but is available for use in ornamental nurseries. *Metarhizium anisopliae* is parasitic in at least seven orders of insects (Zimmerman 2007). Infection begins with the attachment of a spore to the insect cuticle. The spore germinates and forms appressoria ("pressing organs") from which needle-like penetration pegs arise to puncture the cuticle (Zimmerman 2007). The fungus overcomes the defensive immune response of the host insect through inhibition of cytokines or inter-cellular signaling proteins (Butt et al. 2001). It is then able to grow into the cuticle and, using the hemolymph as a nutrient source, spread through the body of the insect via growth of vegetative hyphae (Thomas and Read 2007). As the fungus grows and consumes the hemolymph, the insect is starved. Once the host is dead, the fungus grows out of the cadaver and produces conidial spores for infection of new hosts (Zimmerman 2007). Spores of *M. anisopliae* are hydrophobic and are passively dispersed from the cadaver (Shah and Pell 2003).

Metarhizium anisopliae has been in use as a microbial insecticide since the late 1800's (Butt et al. 2001). It is currently developed and registered as a biological control agent for a variety of insect species including scarab grubs, spittlebugs, cockroaches, termites (Butt et al. 2001), and root weevils (Kepler and Bruck 2006). In Brazil, *M. anisopliae* is used to treat roughly 100,000 hectares of sugar cane for spittlebug, demonstrating the promise of its applicability in large-scale commercial agriculture (Butt et al. 2001). Of particular interest is the discovery by Kepler and Bruck (2006) that plants inoculated with *M. anisopliae* (commercially available strain F52, Met52, Novozymes Biologicals, Inc., Salem, VA) may be attractive to BVW compared to untreated plants. This gives it an advantage over chemical insecticides, which often have repellent effects on BVW (Bruck, personal communication). In addition, *M. anisopliae* has been shown to be rhizosphere competent and persist in the root zone of plants for over one year, both in containers (Bruck 2005) and in field soils (Hu and St. Leger 2002).

The F52 strain of *M. anisopliae* is most virulent at temperatures ranging from 20 to 28°C; temperatures below 20°C retard fungal growth and infectivity (Bruck 2007). Booth et al. (2000) showed a particular strain of *M. anisopliae* (strain 5139) to be effective against BVW at 14°C. They also found that *M. anisopliae* will not grow or germinate at temperatures of 4°C or below. Fernandes et al. (2008) found strains of *M. anisopliae* to be relatively cold sensitive in regard to activity. However, Rath et al. (1995a) found *M. anisopliae* to be virulent at temperatures ranging from 2 to 25°C in a sand-peat based media. The results of this study showed that neither cold nor fluctuations in temperature affected the growth or virulence of DAT F-100 strains of *M. anisopliae*. Moisture levels are also a concern for use of *M. anisopliae* in cranberry production due to the nature of the cranberry harvest, as well as the wet conditions of northwest winters (Agrimet 2011). Little is known about the efficacy of F52 in high moisture environments. A Brazilian strain of *M. anisopliae* (IP 46) has a high level of virulence at humidity over 98 percent when compared with virulence at lower humidity (Luz et al. 2008). However, certain strains of *M. anisopliae* exhibit tolerance to low humidity and water availability (Matawele et al. 1994). *M. anisopliae* virulence is highest at moistures ranging from 6 to 18 percent (Peng et al. 2010). Excessive soil moisture may hinder the performance of *M. anisopliae*. Virulence of naturally occurring populations of the fungus has been demonstrated to be negatively associated with increasing soil water content (Jabbour and Barbercheck 2009). Further investigation is needed to determine the effect of fluctuating moisture levels on the survival capabilities of *M. anisopliae*.

Rath et al. (1995b) investigated the persistence of *M. anisopliae* applied to the soil in Tasmania, Australia. A granular form of *M. anisopliae* was applied with a seed drill at a concentration of $5.1 \pm 0.7 \times 10^4$ spores/g soil. Soil levels of *M. anisopliae* (strain: DAT F-001) after application ranged from close to applied levels to ten times applied levels (when mummified scarab larvae were present). Rath et al. (1995b) found fungus levels to be twice the applied level three years after application. *Metarhizium anisopliae* did not reduce the numbers of non-target organisms. Their research site had an average monthly air temperature of 5.3 to 15.1°C (this is similar to average winter temperatures in Bandon, Oregon) and an annual rainfall ranging from 50.0 to 62.5 cm (this is considerably drier than the Bandon area in winter).

Metarhizium anisopliae was recovered from soil at higher than the applied rate for at least four years. The results of the study indicate that this EPF does have the capacity to persist after application in the soil and that soil conditions in Pacific Northwest cranberry production areas may be suitable for survival of *M. anisopliae*. The effect of pH may be a consideration, since cranberries are grown at a pH of between 4 and 5. However, Bruck (2005) showed that *M. anisopliae* will retain virulence in a bark-based media with a pH of approximately 4.5.

Consideration must be given to the compatibility of EPF with the use of fungicides to control for a wide variety of fungal diseases that occur in cranberries (Pscheidt 2010). Numerous studies have evaluated the effect of commercial fungicides on the growth and survival of *M. anisopliae.* Among the fungicides recommended for use in cranberry production, Azoxystrobin, Mancozeb, chlorothalonil, and copper sulfate have been tested for compatibility with *M. anisopliae* (Bruck 2009, Shah et al. 2009, Prabhu et al. 2007, Gupta et al. 2002, Luz et al. 2007). Copper sulfate is minimally toxic and chlorothalonil moderately toxic to *M. anisopliae* (Luz et al. 2007, Gupta et al. 2002). The effects of Mancozeb are more severe and Azoxystrobin, the most studied fungicide, is the least compatible with *M. anisopliae* (Prabhu et al. 2007, Shah et al. 2009, Bruck 2009). Copper hydroxide, a widely recommended fungicide in cranberry production, has not been evaluated for compatibility with *M. anisopliae*.

It has been shown that detection of naturally occurring populations of *M. anisopliae* in field soil is negatively associated with zinc, copper and sulfur concentrations in the soil (Jabbour and Barbercheck 2009). Field sites with insufficient drainage harbor anaerobic bacteria that produce hydrogen sulfide as a byproduct of respiration, and this may negatively affect persistence of *M. anisopliae* in the soil (Swift et al. 1979).

1.4 Cranberry

American cranberry (*Vaccinium macrocarpon*) is native to the east coast of North America and was first brought to the west coast in the late 1800's (Eck 1990). In 2008, there were 38,200 acres of land dedicated to cranberries in the United States, producing 7.6 million barrels (3.45 billion kg) of fruit at a value of nearly $444 million (NASS 2009). Oregon and Washington are the fourth and fifth largest producers of cranberries in the U.S. with 2,700 and 1,700 acres in production, respectively (NASS 2009).

1.4.1 Growth and culture

Belonging to the family Ericaceae, cranberry is a unique crop with regard to how it is grown and harvested. Consequently, management practices can be necessarily unique as well. The cranberry is a woody evergreen vine that spreads vegetatively through the growth of runners. Fruit is set on upright shoots that originate from the runners (Rutgers 2000). A cranberry field consists of a continuous mat of vines; there are no rows in which to walk the field or drive equipment. For this reason, management practices are developed to minimize traffic within the field. For example, chemigation of any fertilizers and pesticides through a sprinkler irrigation system is a widespread practice for cranberry producers (Knute Andersson, personal communication). Cranberries are long-lived perennials; while it may take up to 5 years for a bed to produce a commercial product, it may remain productive for 50 years or more (Rutgers 2000, WSU 2000). New fields are established by scattering cuttings from existing vines onto a newly sanded bed.

Cranberries are self-pollinated, but pollination can be improved by pollinators such as honey bees (Eck 1990). The plant is on a sixteen-month growing cycle, so that in early summer, this year's fruit and next year's fruit buds are developing at the same time (Hart 2000). Fruit is ready for harvest in the fall. Although some fields are dry-picked and the berries sold in the fresh market, the majority is flood-harvested for processing (Rutgers 2000). Fields are constructed as depressed beds surrounded by dikes for this purpose. During this process, the field is flooded with several feet of water and a beater-machine is driven through the field to remove the berries from the vine. Because they are filled with air, the berries float to the surface where they are collected to one corner of the field and lifted into a transport truck using either an elevator or a large suction device (Fig. 1.2). In Oregon, the floodwater is typically re-used by draining it into lower-elevation beds.

1.4.2 Varietal differences

As with most crops, several different varieties of cranberry have been identified or developed in the years since its domestication (Eck 1990). These varieties

Fig 1.2. Cranberry harvest. Cranberries are corralled into a suction-tube, which will draw the water and cranberries out of the bog. The water is diverted while the cranberries are deposited into a truck for transport to the processor.

exhibit differential tolerance to stresses such as drought and insect pressure. For example, a study comparing varieties ‘Ben Lear’ and ‘Stevens’ found that the latter was able to better withstand drought stress due to its higher root-to-shoot ratio and lower leaf area (Baumann et al. 2005). Eck (1990) described the different varieties of cranberry available and their distinctive characteristics of resistance to certain insect pests or fungal diseases. There can be differences within a single variety depending on region or cultural practices (Strik et al. 1999). Currently there is no literature on varietal differences in resistance or susceptibility to BVW.

1.5 Summary

Black vine weevil is a sporadic but serious pest in Pacific Northwest cranberries. Management of BVW in cranberries presents many challenges. The damage potential of BVW in this region's cranberry beds has not been definitively quantified and there is insufficient knowledge of possible tolerance of BVW feeding among cranberry varieties. Although there are several options currently available for control of BVW populations, certain limitations reduce their utility in cranberries. None provide entirely satisfactory control; some pose environmental risks including reduced water quality. The least toxic products (entomopathogenic nematodes, for example) can be expensive to apply, are often not available in the large quantities required to treat entire farms and are inconsistently effective. Entomopathogenic fungus *M. anisopliae* has been shown to persist through time in environmental conditions similar to those found in Pacific Northwest cranberry beds and may have potential for economic control of BVW in cranberries. More knowledge is needed on the performance and persistence of EPNs and EPF at different temperature levels and under field conditions.

The objectives of this research are two-fold: (1) to assess the damage potential of BVW on two common cranberry varieties by looking at root injury caused by larvae and its effects on shoot growth and evapotranspiration and (2) to compare the persistence and efficacy of *M. anisopliae, S. kraussei* and a standard pesticide in cranberry production fields in Oregon and Washington.

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CHAPTER 2

ASSESSMENT OF BLACK VINE WEEVIL (COLEOPTERA: CURCULIONIDAE) LARVAL DAMAGE TO CRANBERRY VINES

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Abstract Black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius), is a serious pest of cranberry, *Vaccinium macrocarpon*. Larvae feeding below the soil surface cause damage to the roots and underground stems. Because larval detection in the field is impractical for growers, we attempted to correlate the damage caused by feeding larvae to adult population levels, with egg density as an indicator of the number of ovipositing adults. In this trial, two varieties of potted cranberry vines, ‘Stevens’ and ‘McFarlin,’ were infested with six BVW egg densities: 0, 5, 10, 20, 40 or 80 eggs per plant. Root damage and canopy health was assessed, both continuously and destructively. Damage increased with increasing egg density and more damage was found in ‘Stevens’ than ‘McFarlin’. In August, water use was significantly higher and total shoot length was lower in plants that were treated with lower egg densities. In October, total shoot length was lower in plants treated with medium to high egg densities. Mean root weight of ‘Stevens’ was significantly heavier than that of ‘McFarlin’ at all egg densities, but there were no differences in root weight among egg densities for either variety. Dry shoot weight and total shoot length were lower for plants treated with 40- to 80-egg densities compared to those with 0- to 20-egg densities. Percent green leaf area was significantly reduced with increasing BVW egg densities. The results of this study indicated that as BVW egg density increased, plant growth and vigor decreased as a result of subsequent larval damage to roots. This damage resulted in drought stress, reduced photosynthetic tissue in the canopy and limited shoot growth.

Key Words: Black vine weevil, cranberry, egg density, root damage, plant health

2.1 Introduction

Cranberries are one of North America's indigenous commercially produced crops (Pollack and Perez 2001). The United States cranberry industry is valued at around $444 million annually with 38,200 acres in production; in the Pacific Northwest region, approximately 4,000 acres are dedicated to cranberries (NASS 2009, Thomson et al. 1999). There are several insect pests threatening cranberry production in the Pacific Northwest. One of these is the black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae); however, losses due to BVW are not clearly quantified (Linda White, personal communication, Ingraham 1991). This prolific species is part of a complex of polyphagous root weevils that attack crops worldwide. Cranberry is one of more than 80 plant species that serve as host for BVW (Smith 1932). Adults feed on foliage, causing a characteristic notching along the margin of leaves; however this damage is rarely of economic importance. The major damage is caused by larvae feeding on belowground plant tissues (Smith 1927). This damage to the root system reduces vigor and water absorption and subsequently leads to plant desiccation (Schread 1972).

Larvae consume fine roots and girdle underground stems of cranberry plants (Crowley 1923). BVW are a cryptic species. They and their damage can exist in the field undetected through most of the year until the plants experience drought stress during warmer and drier periods of summer. At this point, weevil damage becomes immediately evident as vines wilt, become dry and brittle, or die off (Kim Patten, personal communication). Light soils such as peat and sand (typical of West coast cranberry beds) are preferred over heavier soils by weevil larvae (Wilcox et al. 1934). It is estimated that in Washington cranberry beds, infestations exceeding 12 black vine weevil larvae per square meter create patches of dead or dying vines within two seasons (Booth et al. 2002). Field observations indicate that damaged areas left untreated may quadruple in size within one year (Ingraham 1991).

Timing and targeting of control measures are difficult due to the cryptic behavior of BVW and the lack of suitable control options. Monitoring for larvae is tedious and destructive as it involves digging up the cranberry vines and carefully inspecting soil and roots. For these reasons, it is recommended that growers monitor BVW populations by sweep netting for nocturnal adults. Numerous researchers have quantified the fecundity and larval survival of BVW (Cram and Pearson 1965, Smith 1927 and 1932, Nielson and Dunlap 1981, Garth and Shanks 1978, Fisher 2006, Son and Lewis 2005). Of these studies, one has examined the potential fecundity of BVW in cranberries. Cram and Pearson (1965) reported that in British Columbia, BVW fed cranberry foliage laid an average of 163 eggs per female per season with 74 percent viability. This information allows for crude estimation of potential number offspring associated with quantified adult populations. The focus of the current study was to examine the effect of BVW egg density on cranberry plant health with potted plants in simulated field conditions. Information gained from this work will add to a base of knowledge that growers can use to identify pending BVW damage associated with adult populations.

2.2 Methods and Materials

2.2.1 Planting and Plant Maintenance

Two varieties of cranberry plant material were collected from two different sources in commercial cranberry beds: ‘McFarlin’ var. from Grayland, Pacific County, Washington and ‘Stevens’ var. from Langlois, Curry County, Oregon. All plant material was collected as mats of vines (similar to sod) in sections that were approximately 60 by 90 cm in area with a root and soil depth of 10 to 15 cm. Foliage was pruned to enable easier handling. Square plugs, sized approximately 10 cm^2, were cut from the mats and planted into pots (10 x 10 x 12 cm). The potting media used was a mixture of 2:1 sand (dredged from the Willamette River, Corvallis, OR, USA) to peat (Sungro Horticulture, Vancouver, BC, Canada). The media (pH: 4.9 - 5.2; EC: 0.5) was hand mixed and formulated to reflect the texture and water holding capacity of the field soils of Langlois, OR, which have a significant amount of coarse sand and slightly decomposed organic matter (Bullards Ferrelo-Hebo complex) (NRCS 2011). This is in contrast to Washington cranberry soils, which have muck-like properties, contain finer sand and have a higher percentage of organic matter that is further decomposed (Seastrand mucky peat and Yaquina loamy fine sand) (NRCS 2011). Vines were planted with approximately 8 cm of headspace from the soil surface to the top edge of the pot, which allowed for top-dressing with soil media following the first fertilization (a technique used to discourage growth of algae and moss). All remaining aboveground lateral stems were pruned back to 2.5 cm above the rim of the pot. The two varieties, 'McFarlin' and 'Stevens,' were potted on 15 February and 20 March, respectively.

All plants were fertilized one month after planting with a granular acidifying organic fertilizer (Down to Earth, Eugene, OR, USA; P:K:N = 4:3:6). The total amount of fertilizer applied to each plant over the course of the study was equivalent to 98 kg/ha in the field and was distributed over four applications (11 March, 11 May, 17 June, 11 August for 'McFarlin'; 20 April, 20 May, 23 June, 28 August for 'Stevens'). Initially, plants were maintained in a greenhouse at a mean daytime temperature of 17 ± 2ºC. On 2 April, all plants were moved to a screen house to harden off before being transferred outdoors.

At this time, 'McFarlin' plants had fully mature leaves and 'Stevens' plants were just beginning to bud. The mean daytime temperature in the screen house was 26 ± 4ºC. At the start of the experiment, growth vigor was maintained by applying a liquid-feed granular fertilizer (Scotts MiracleGro, Marysville, OH, USA; P:K:N = 18:18:21) at a rate of 1.92 mL/liter for four weeks. While indoors, plants were hand-watered as needed.

Trials were conducted at two separate locations in Corvallis, Oregon. In each location, an outdoor raised bed (30 cm deep) was constructed inside of a mesh exclusion cage (1.68 x 1.68 x 1.68m) and filled completely with the same 2:1 sand to peat media that was used for potting. Plants were installed using a "pot-in-pot" technique so the plants could be easily removed throughout the study for data collection. Plants were spaced approximately 5 cm apart and 5 cm from the edge of the bed (Fig. 2.1). Each trial consisted of four blocks of each variety (18 plants per block). Plants were assigned to blocks according to plug vigor (number of aboveground lateral stems at time of planting) to control for variation between plugs. Vigor-assigned blocks were divided into groups as follows: plants containing < 5, 5-9, 10-14, or 15-20 lateral stems. All plants were moved outdoors under shade netting prior to infestation with BVW eggs. Plants were watered as needed throughout the growing season. Mean daily temperature inside the exclusion cages from May to October 2009 was 19.5 ± 0.2ºC.

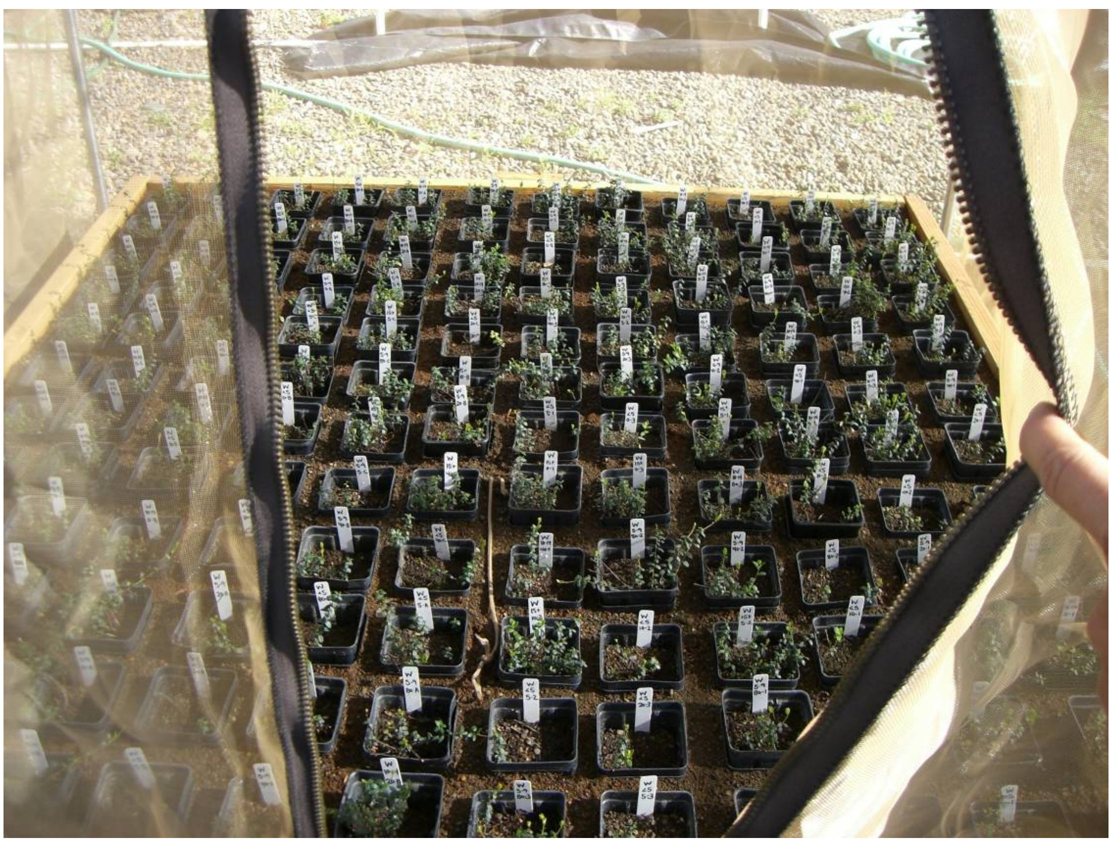

Fig. 2.1. Raised bed inside of mesh exclusion cage. Each potted cranberry is nested inside of an empty pot that is buried so the top of the pot is flush with the soil surface.

2.2.2 Black Vine Weevil Infestation

Because of egg availability as well as to promote uniformity of growth at the time of infestation, ‘Stevens’ plants were infested two weeks later than ‘McFarlin’ plants (29 May for ’McFarlin’; 12 June for ‘Stevens’). Black vine weevil eggs were obtained from colonies at the USDA Horticultural Crops Research Laboratory in Corvallis, OR and collected as described by Fisher and Bruck (2004). Treatment groups included 0, 5, 10, 20, 40 and 80 eggs per pot. Eggs were placed onto Petri dishes fit with a moist filter paper (Whatman no.1) and stored at 4ºC for no more than two weeks until the time of infestation (Fisher and Bruck 2004). In order to increase the probability of larval survival, egg inoculations were performed twice over a 14-day interval, with half of the total egg density applied at each inoculation. For the 5-egg treatment, halving the treatment was not necessary; so all eggs were applied during the first inoculation. All plants were irrigated before infestation. A 15 mm diameter pit was made in the soil next to cranberry stems and eggs were washed from each dish into the pit with deionized water from a laboratory squirt bottle. ‘McFarlin’ plants were infested on 29 May and 12 June. ‘Stevens’ plants were infested on 12 and 25 June. All subsequent plant health measurements were collected first on ‘McFarlin’ plants and two weeks later on ‘Stevens’ plants.

2.2.3 Time-Zero Reference Sampling

In order to get a reference base for cranberry plant health, a sub-sample of 48 plants (three plants from each block of each variety) were measured for length, number of upright shoots, leaf area, above-ground tissue weight and root weight using destructive techniques. Length was measured from the lateral stem (or the soil level, if the shoot came from an underground lateral) to the tip of the most terminal leaf. The length of each upright shoot was measured to the nearest millimeter. Leaf area measurements were conducted by cutting all shoots at the lateral stem or at the soil level. Leaf surface area (cm^2) was measured with a leaf area meter fitted with a conveyor belt (LI-3000, LI-3050A, LI-COR, Lincoln, NE). All plant material was washed and dried in an oven at 73ºC (aboveground tissue for 48 hours; roots for 96 hours) and weighed. Total leaf area and shoot length were summed and correlated using linear regression analysis (STATISTICA 7.1 2010).

2.2.4 Continuous Sampling

The shoot length and water use of each plant was measured on 1 or 16 June, 11 or 28 August, and 7 or 19 October 2009 for ‘McFarlin’ or ‘Stevens,’ respectively. Leaf area measurements and tissue weights were not taken during these data collections because of the destructive nature of the methods.

Water use was measured indirectly as the weight of water lost over 24 hours. All pots were watered to container capacity (the point at which water pools in the head space of the pot) at dawn and allowed to drain for one hour. Each pot was then weighed to the nearest tenth of a gram. Weight measurements were done at 1, 12 and 24 hours after watering. Shoot length and water use at each sampling date were compared between treatments using repeated measures ANOVA (STATISTICA 7.1 2010).

2.2.5 Destructive Sampling

During the October 2009 sampling, plants were entering dormancy (as indicated by the senescence of leaves). Plants that had already died were removed and measured at this time. Plants that remained alive were destructively sampled during February 2010. Tissues were sampled in the same manner as described above. In this case, leaf area was measured by separating green, purple and dead leaf areas. These measurements were transformed into percentages of total leaf area for analysis. After processing the aboveground plant material, the root mass of each plant was searched for BVW larvae. The head capsule width of each recovered larva was measured to determine developmental stage (La Lone and Clark 1981).

Root material was air-dried for a minimum of 48 hours before determining the number of shoots with larval feeding damage. Shoots longer than 2 cm were separated from the root mass and feeding damage was recorded to determine a percentage of total shoots with damage.

Average shoot length and number, green leaf area, larval survival, root and shoot tissue weights and percent damaged stems were compared between treatment groups using GLM ANOVA (STATISTICA 7.1 2010).

2.3 Results

2.3.1 Time-zero Reference Sampling

Multiple regression of leaf area and shoot length showed highly significant correlation with leaf surface area increasing with increased shoot length (R^2 = 0.9487; df = 2, 45; F = 435.67; *P* <0.001). Variety did not have a significant effect on the relationship between leaf area and shoot length (*P* = 0.9961). Due to this strong association and the destructive nature of measuring leaf area, shoot length was used as an indicator of leaf area for all but the final measurements.

2.2.2 Continuous Sampling

Initial analysis indicated that some treatments, such as 0-5, 10-20, and 40-80 eggs per plant resulted in similar plant responses (Table 2.1). For this reason, egg densities were grouped into three density categories: low (0-5 eggs), medium (10-20 eggs), and high (40-80 eggs). There was a similar effect over time for both shoot growth and water use (Fig. 2.2). In June, there were no differences in mean total shoot length between the three egg density categories. In August, all groups had shoot growth, but the mean total shoot length of the high-density group was significantly lower than that of the low-density group. (Tukey's HSD, df = 855; *P* = 0.0005). By October, low-density plants had a significantly higher mean total shoot length than medium- and high-density plants (Tukey's HSD, df = 855, *P* < 0.01).

Table 2.1 Parameters from t-tests for similarity of means[a] between BVW egg densities

Comparisons	24 hr water loss (g/24 hr)				Total shoot length (cm)			
No. BVW eggs/plant	Mean ± SE	t-value	df	*P* value	Mean ± SE	t-value	df	*P* value
0	18.28 ± 0.82	0.58600	286	0.55834	21.20 ± 1.24	-0.68333	286	0.49495
5	17.63 ± 0.77				22.53 ± 1.49			
10	15.92 ± 0.65	0.26634	286	0.79017	19.05 ± 1.24	0.38521	286	0.70037
20	15.66 ± 0.69				19.38 ± 1.22			
40	14.92 ± 0.72	0.59128	286	0.55480	15.93 ± 1.03	0.25433	286	0.79942
80	14.36 ± 0.64				15.56 ± 1.06			
low (0-5)	17.96 ± 0.56	2.94799	574	0.00333*	21.87 ± 0.97	2.42392	574	0.01566*
medium (10-20)	15.79 ± 0.48				18.71 ± 0.87			
medium (10-20)	15.79 ± 0.48	1.70056	574	0.08957	18.71 ± 0.87	2.60499	574	0.00943*
high (40-80)	14.64 ± 0.48				15.75 ± 0.74			

P values marked with an asterisk indicate significance at the 95% confidence level.
[a]Means are an average over all three sampling events.

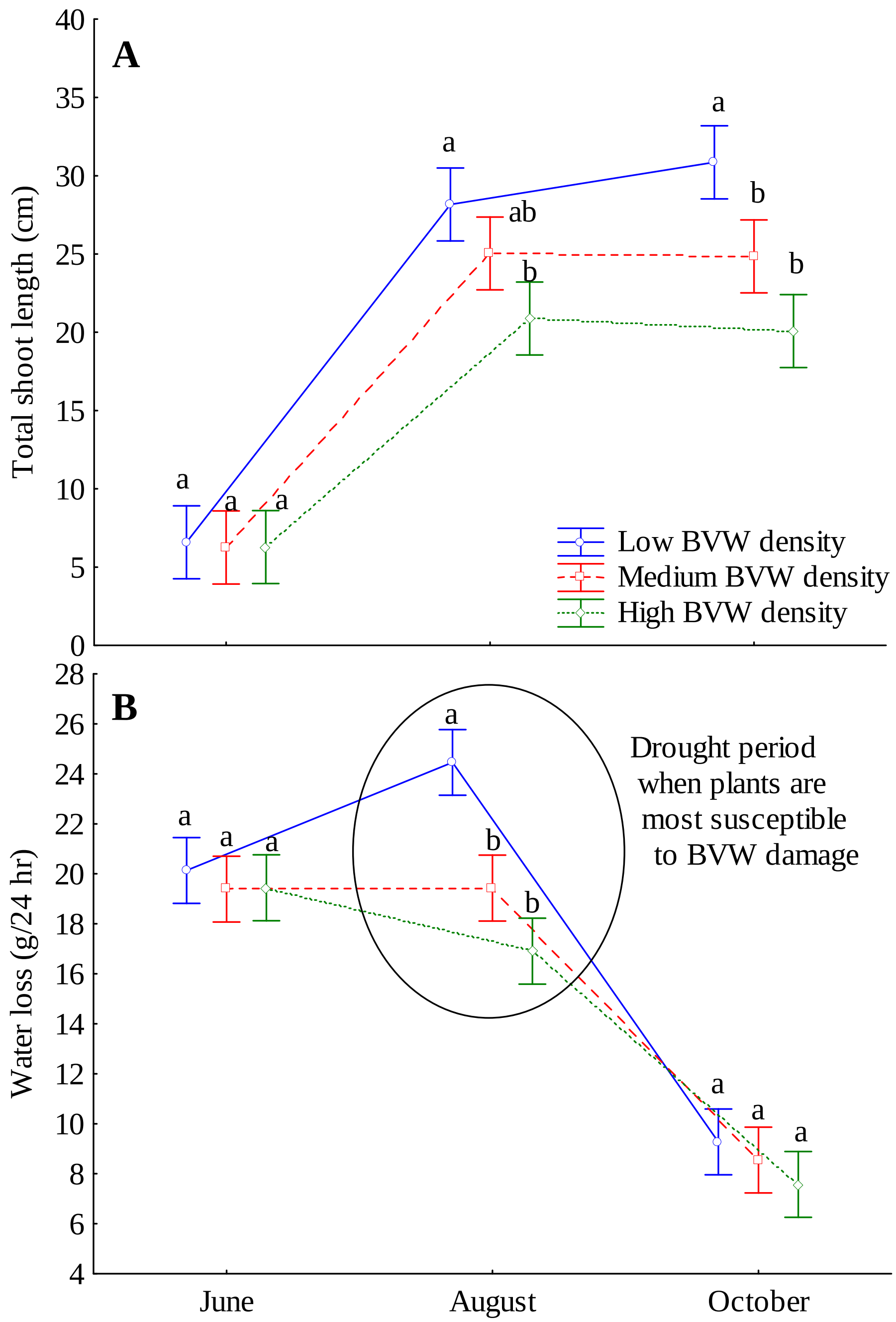

Fig. 2.2. Effect of three BVW egg densities on (A) shoot growth and (B) water use from June to October 2010. Low BVW density equals 0-5 eggs per plant, medium BVW density equals 10-20 eggs per plant and high BVW density equals 40-80 eggs per plant. Varieties and vigor blocks are pooled. Means accompanied by the same letter on any given date are not significantly different (P<0.05, Tukey's HSD). Bars denote 95% confidence intervals.

Water loss was uniform across all treatment groups during June. In August, a period of high heat, and consequently, high stress, the medium and high density groups displayed no increase in mean water weight lost, while the low density group exhibited a significant increase in water loss ($F = 7.30$; $df = 8, 1708$; $P = 0.00001$). In October, overall water loss decreased and was again uniform in all treatment groups.

2.2.3 Destructive Sampling

No variety effects were found and therefore these data were pooled for analysis. Dry shoot weight, total shoot length and percent green leaf area differed significantly between high and low BVW egg densities (Fig. 2.3). Greater shoot weight and a longer shoot length corresponded with lower egg densities. Plants treated with 40-80 eggs had lower shoot weight and shorter length than plants treated with 0-5 eggs. There were significant differences in mean percent green leaf area. Plants treated with 0 eggs had a higher mean percent green leaf area than any of the other densities. The 20-, 40- and 80-egg treatment groups had significantly lower means, but did not differ significantly from each other.

Varietal differences occurred in response to BVW feeding for certain parameters (Table 2.2). A comparison of control groups indicated that mean root weight of ‘Stevens’ was 1.72 times that of ‘McFarlin’ ($F = 41.72$; $df = 1, 32$; $P < 0.0001$). Mean root weight across all treatments was 1.82 times greater in ‘Stevens’ ($F = 219.57$; $df = 1,281$; $P < 0.00001$). The interaction between variety and treatment was not significant, and therefore data were pooled for analysis of variance. There were no

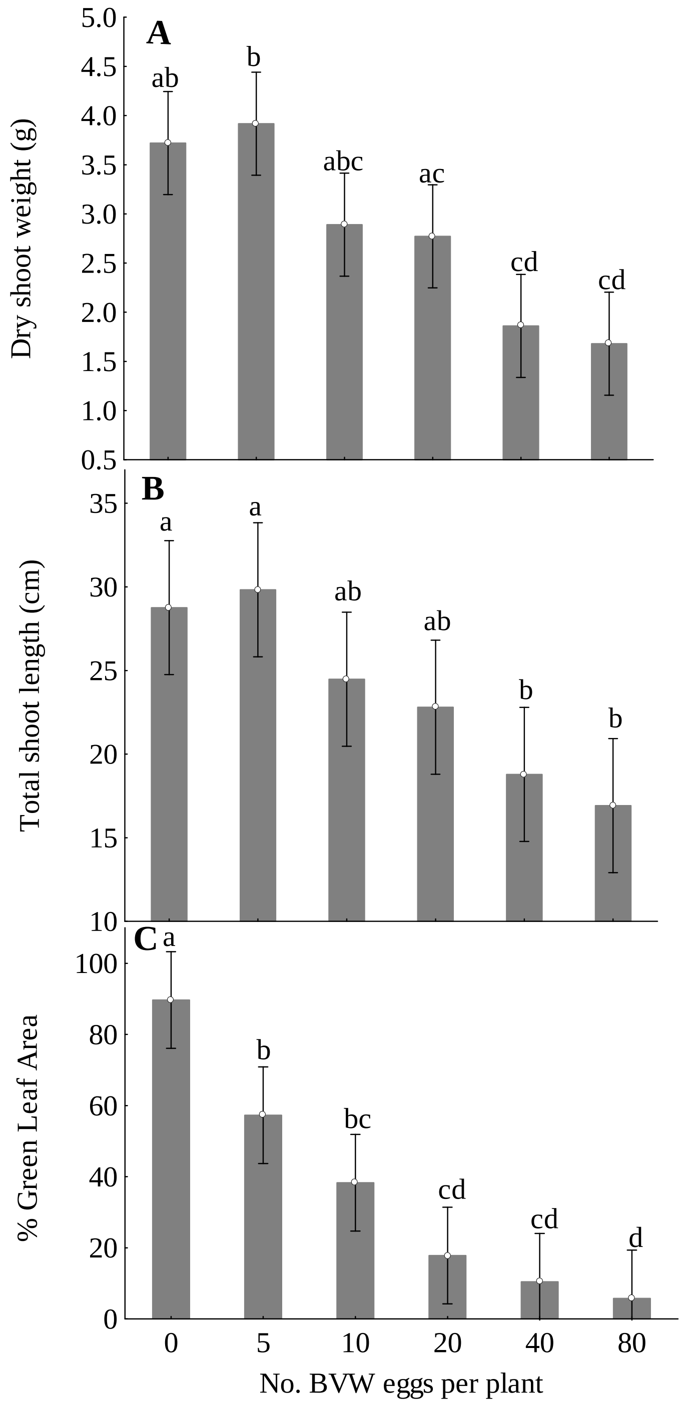

Fig. 2.3 Effect of six BVW egg densities on (A) dry shoot weight, (B) total shoot length and (C) percent green leaf area after one season of growth. Means accompanied by the same letter are not significantly different (*P*<0.05, Tukey's HSD). Bars denote 95% confidence intervals.

Table 2.2 Mean ± SE of parameters with a varietal effect.

Variety	BVW egg density	% Damaged stems	Dry root weight (g)	Larvae recovered
Stevens	0	4.57 ± 1.33a	10.29 ± 0.60a	0.42 ± 0.19a
	5	20.86 ± 3.22b	8.53 ± 0.52a	1.0 ± 0.22ab
	10	21.45 ± 2.87b	8.66 ± 0.43a	0.96 ± 0.19ab
	20	32.4 ± 3.88bc	9.16 ± 0.48a	1.67 ± 0.29b
	40	43.55 ± 3.05cd	9.22 ± 0.66a	1.21 ± 0.28ab
	80	52.28 ± 3.10d	8.84 ± 0.60a	0.63 ± 0.22a
McFarlane	0	11.45 ± 2.41a	5.97 ± 0.44a	0.21 ± 0.06a
	5	15.66 ± 2.05ab	5.63 ± 0.43ab	0.33 ± 0.18a
	10	22.94 ± 3.02bd	4.83 ± 0.38ab	0.21 ± 0.11a
	20	25.77 ± 2.56bc	4.38 ± 0.34b	0.25 ± 0.11a
	40	34.59 ± 2.60c	4.34 ± 0.32b	0.21 ± 0.07a
	80	31.06 ± 2.94cd	4.87 ± 0.43ab	0.17 ± 0.11a

Means followed by the same letter do not differ significantly (Tukey's HSD, $P < 0.05$)

significant differences in mean dry root weight between treatments (F = 1.41; df = 5,282; P =0.21986). Varietal differences also occurred with percent damaged underground stems and the number of larvae recovered. For these parameters, the interaction between variety and treatment group was significant ($P < 0.05$), and each variety was analyzed separately.

For 'Stevens', the control group had a significantly lower mean percent-damaged stems than 5- through 80-egg density levels. Plants treated with the 20-egg density had a significantly higher mean number of larvae recovered, and the 0- and 80-egg groups had a significantly lower mean number of larvae recovered.

For 'McFarlin', the differences in percent damage were less distinct, but the control group had a significantly lower mean percent-damaged stems than 10- and higher egg density levels. There were no significant differences in mean larval recovery between treatments.

2.4 Discussion

Time-zero reference sampling indicated a relationship between shoot length and leaf area, making shoot length an accurate indicator of leaf surface area in cranberries. In young plants, photosynthesis is proportional to leaf area (Koyama and Kikuzawa 2009). Taking into account the impact of plant architecture on light transmittance to individual leaves, it can be assumed that the effects of BVW damage on total shoot length have an impact on the photosynthetic capacity of a plant.

During continuous sampling we examined water use as an indicator of root health and saw seasonal influences in the effect of BVW feeding damage on the cranberry plant. As temperature and water demands increase in mid-summer, higher BVW populations compromise the ability of the root system to translocate water from the soil to the foliage. This is evident by the marked differences in weight loss between the high and low BVW egg density treatments. Plants can exhibit reduced rates of growth and stomatal conductance and reduced photosynthesis as a response to root herbivory (Gange and Brown 1989, Hou et al. 1997). These responses are most pronounced under low moisture conditions. In the case of BVW in Oregon, such drought stress coincides with a period of increased larval feeding intensity. The damaged root system and subsequent desiccation of the canopy reduces the growth capacity of the plant, and this effect is still evident following the period of high drought stress. In the current study, water use was relatively uniform in October in all treatment groups, but differences in total shoot length were apparent. Plants subjected to the highest egg density treatments displayed lower total shoot length, presumably due to increased shoot mortality.

Destructive sampling revealed varietal and treatment differences. Mean root weights of control plants ‘Stevens’ and ‘McFarlin’ indicated that ‘Stevens’ had larger root systems in this experiment, despite having a higher mean larval recovery and higher mean percentage of damaged stems. Our data supports data presented by Baumann et al. (2005), who demonstrated that ‘Stevens’ has a deeper rooting system, less foliar growth and a lower shoot-to-root ratio compared to other varieties, making it less susceptible to drought stress. Although plant metrics in our study suggested that ‘Stevens’ is a more vigorous variety overall, there were no significant varietal differences in shoot weight, shoot length or green leaf area. The differences in green leaf area between BVW egg density treatments indicate that a plant stressed by larval feeding may enter a senescent stage at an earlier date, which could have consequences for fruit ripening and bud set during the following season. Although no such work has been done on cranberries, reduced photosynthesis has been shown to have a negative effect on fruit quality in grapes, especially on sugar levels (Keller et al. 1998).

In the 5-egg density treatment, we observed greater mean shoot length and shoot dry weight compared to all other egg density treatments, including the 0-egg density treatment. Although statistically non-significant, this result may be due to compensatory growth of minimally damaged plants that are still sufficiently robust to overcome the BVW injury. Studies have shown that plants can respond to low levels of root herbivory with increased growth (Ridenour and Callaway 2003, Weed et al. 2011), suggesting existence of a beneficial damage threshold.

At the conclusion of the experiment, we were unable to recover many viable larvae. The mean number of recovered larvae never exceeded two specimens from any treatment group. Such results could indicate a certain carrying capacity of the plants for the insect population that has been exceeded by the study design (Blossey and Schat 1997). Although other factors could be involved, including inadequate methods for larval recovery, uncontrolled migration of larvae, and predation, these would likely result in more extreme variability than we observed. In the case of BVW, it is important to correlate damage caused by feeding larvae to the number of adults because larval detection in the field is impractical for growers. Further work is needed to define this correlation, including a careful study of oviposition behavior, fecundity, and larval survival of BVW in cranberries.

Increased BVW egg density has a negative effect on host cranberry plant health. The severity of the effect depends on varietal and seasonal differences. Future studies should include screening of cranberry varieties to assess tolerance to BVW root damage as well as an assessment of the effect of root damage on actual yield. In addition, studies of short-term influences on perennial crops do not always give the most accurate or applicable results and therefore suggest a more long-term study of BVW infestation in cranberries. A more detailed investigation of the mechanisms of plant decline as a result of BVW damage, such as translocation of water and nutrients from the roots to the plant canopy, should also be included in future work.

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CHAPTER 3

ALTERNATIVE STRATEGIES FOR MANAGEMENT OF BLACK VINE WEEVIL (COLEOPTERA: CURCULIONIDAE) IN CRANBERRIES

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Abstract Field and laboratory trials were conducted to compare *Metarhizium anisopliae* (Sorokin) and *Steinernema kraussei* (Steiner) to the systemic pesticide imidacloprid for control of black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius) in cranberries. Treatments were applied at two field sites in fall of 2009 and soil was collected from plots on five dates during 2009 and 2010. Soil samples were taken to the laboratory and incubated with laboratory-reared BVW larvae to assess potential control by, and persistence of, each treatment in the cranberry bed soil. *Steinernema kraussei* did not result in significant BVW mortality at either site on any sample date. Larval reduction by *M. anisopliae* was inconsistent. In Long Beach, Washington, *M. anisopliae* caused significantly higher mortality than any of the other treatments on three of the five sample dates. In Langlois, Oregon, *M. anisopliae* caused significantly higher larval mortality than the control on the last sample date, one year after treatment application. Average mortality over the entire sampling period and cumulative BVW survival for the duration of the experiment indicated that *M. anisopliae* was a more suitable control agent compared to *S. kraussei* and imidacloprid in both locations. Based on these results and the literature, these biological control agents appear to be at least as effective as standard pesticide applications. Application temperatures and soil moisture were potential factors associated with the inconsistent performance of *M. anisopliae*.

Key Words – *Metarhizium anisopliae*, *Steinernema kraussei*, entomopathogens, biological control, imidacloprid

3.1 Introduction

American cranberry (*Vaccinium macrocarpon*) is one of a small number of native commercially produced crops, supporting a national industry valued at around $444 million annually (NASS 2009). Black vine weevil, *Otiorhynchus sulcatus* (Fabricius), is one of several insect pests that have potential for economic impact on cranberry production. While not a major concern for growers in the eastern states, black vine weevil (BVW) is an important pest for cranberry growers in the Pacific Northwest, where nearly 500,000 barrels (22.68 million kg) of cranberries are grown (Rutgers 2000, NASS 2009).

Black vine weevil is one of a complex of polyphagous root weevils that attack ornamental and small fruit crops worldwide; it feeds on more than 80 plant species including cranberry (Smith 1932). Adults feed on foliage, causing characteristic notching along the margin of leaves; however, unless on an ornamental crop valued for its foliage, this damage is rarely of economic importance. The major damage is caused by larval feeding on fine roots and girdling of underground stems (Crowley 1923). This damage to the root system reduces vigor and water absorption and subsequently leads to plant desiccation (Schread 1972). Because they are soil-dwelling, BVW larvae can exist in the field undetected through most of the year until the injured plants experience drought stress during warm periods. At this point, symptoms of weevil damage become evident as vines wilt, weaken, brown and eventually die (Shawa et al. 1984). It has been estimated that in Washington cranberry beds, infestations of 12 or more BVW larvae per square meter create patches of dead or dying vines within two years (Booth et al. 2002). Field observations indicate that damaged areas left untreated may quadruple in size within one year (Ingraham 1991). Production losses may extend for several years, as damaged vines must be replanted and take up to seven years to reach full production (Ingraham 1991).

Control of BVW can be difficult due to its cryptic behavior and a shortage of suitable control options. Infestations of crops may occur as a result of migration from neighboring host plants including blackberry, salal and weeds common to cranberry production areas (Smith 1932, Cram and Pearson 1965). Larvae are well protected within the soil profile where organic matter adsorbs many insecticides before they can come into contact with the insects (Fisher, personal communication). Adults exhibit tolerance to many organophosphate insecticides (Cowles 1995) and timing of applications are restricted by long pre-harvest intervals and compatibility with pollinators (Fisher, personal communication). As a result, chemical pesticides are ineffective or inconsistent at best (Kim Patten, unpublished data). Sanding and flooding are two cultural control methods used by cranberry growers to mitigate BVW populations (Rutgers 2000, Patten and Daniels 2010). Sanding is done by applying 2.5 to 4 cm of sand every three to four years and is believed to invigorate vine growth and prevent emergence of several insect pests including BVW (Rutgers 2000). However, heavy sanding may reduce yield and thus be detrimental (Strik and Poole 1995). Flooding is not considered an effective control option for BVW in the Northwest (Patten and Daniels 2010).

The afore-mentioned factors make biological control an attractive option for pest management in cranberries. Entomopathogenic nematodes (EPNs) are one biological option that is commercially available. Currently, three species of EPNs are marketed for use against BVW in cranberries: (1) *Heterohabditis megidis* (Nemasys H), (2) *Steinernema carpocapsae* (Nematac C) and *Steinernema kraussei* (Nemasys L) (Becker Underwood, Ames, IA, USA). Entomopathogenic nematodes can be very effective against soil-dwelling insect pests, including BVW (Bruck et al. 2005, Ansari et al. 2004, Willmott et al. 2002, Shanks and Agudelo-Silva 1990). In addition, with certain precautions, they can be applied like chemical insecticides, increasing the likelihood of grower-adoption. However, EPNs are temperature, moisture and UV-light sensitive, preferring low or filtered sunlight and high humidity for application (Booth et al. 2002, Smits 1996). Temperature can also affect the virulence of EPNs. *Steinernema kraussei*, a species that is considered to be cold-active, has an optimum temperature range for infectivity of 5º to 20ºC (Ricci et al. 2004). High cost and inconsistent availability further encumber the successful utilization of EPNs by cranberry producers (Knute Andersson, personal communication). In addition, EPNs are obligate pathogens, requiring an insect host to complete their life cycle, so their ability to persist in the field after the insect population has been reduced is limited (McGraw and Koppenhöffer 2009).

Another potential biological control option for BVW in cranberries are entomopathogenic fungi (EPF). These fungi infect a wide variety of insect hosts, typically by growth of a germination tube through the insect cuticle and subsequent vegetative growth within the insect body (Shah and Pell 2003). *Beauveria bassiana* is one EPF registered on cranberry to control root weevils (Botaniguard, Laverlam International Corporation, Butte, MT, USA). *Beauveria bassiana* is known to infect both larvae and adults and can cause an average of forty percent mortality (Barratt et al. 1989, Bruck 2004). Research has shown that persistence of *B. bassiana* is limited in crop production environments, especially in comparison to *Metarhizium anisopliae* (Storey et al. 1989, Vänninen et al. 2000). *Metarhizium anisopliae* is used in nursery crops in the United States and is effective against scarab grubs, spittlebugs, cockroaches, termites and BVW (Butt et al. 2001, Kepler and Bruck 2006, Bruck and Donahue 2007). In Brazil, *M. anisopliae* is used to treat roughly 100,000 hectares of sugar cane for spittlebug, demonstrating the promise of its applicability in large-scale commercial agriculture (Butt et al. 2001). Like EPNs, EPF have preferred conditions for application. In the case of *M. anisopliae,* moderate temperatures and high humidity are favorable application conditions (Ment et al. 2010). This species is rhizosphere competent, meaning it can establish in the narrow zone of soil surrounding developing roots, utilizing root exudates and decaying organic matter for sustenance (Bruck 2005, Hu and St. Leger 2002). This enables the fungi to persist in environments where no insect host is available, giving them a possible advantage over EPNs as a long-term biological control agent. In fact, Bruck and Donahue (2007) reported that a commercially available strain persisted in soilless potting media kept outdoors for two growing seasons.

Pacific Northwest cranberry beds have seasonal low soil temperatures (below 20ºC), irregular irrigation and occasional flooding, factors we were concerned with during this study. Virulence of *M. anisopliae* is optimal at temperatures ranging from 20º to 30ºC (Dimbi et al. 2004) and moistures ranging from 6 to 18 percent (Peng et al. 2010). Certain strains of *M. anisopliae* are known to have virulence at temperatures as low as 5ºC (Rath et al. 1995b). Commercial strain F52 (Met52, Novozymes Biologicals, Inc., Salem, VA, USA) exhibits decreased fungal growth and speed of infectivity at temperatures below 20ºC (Bruck 2007). Excessive soil moisture may also hamper the performance of *M. anisopliae*. Virulence of naturally occurring populations of the fungus has been demonstrated to be negatively associated with increasing soil water content (Jabbour and Barbercheck 2009).

Consideration must be given to the compatibility of EPF with the use of fungicides to control for a wide variety of fungal diseases that occur in cranberries (Pscheidt 2010). Numerous studies have evaluated the effect of commercial fungicides on the growth and survival of *M. anisopliae.* Among the fungicides recommended for use in cranberry production, Azoxystrobin, Mancozeb, chlorothalonil, and copper sulfate have been tested for compatibility with *M. anisopliae* (Bruck 2009, Shah et al. 2009, Prabhu et al. 2007, Gupta et al. 2002, Luz et al. 2007). Copper sulfate is minimally toxic and chlorothalonil moderately toxic to *M. anisopliae* (Luz et al. 2007, Gupta et al. 2002). The effects of Mancozeb are more severe and Azoxystrobin, the most studied fungicide, is the least compatible with *M. anisopliae* (Prabhu et al. 2007, Shah et al. 2009, Bruck 2009). Copper hydroxide, a widely recommended fungicide in cranberry production, has not been evaluated for compatibility with *M. anisopliae*.

The purpose of this study was to compare the efficacy of biological control agents *M. anosopliae* (Met52, wettable powder) and *S. kraussei* (Nemasys L, Becker Underwood, Ames, IA, USA) to a standard pesticide treatment, imidacloprid (Admire 2 Flowable, Bayer Crop Sciences, Research Triangle Park, NC, USA) for control of BVW in cranberries. In addition, we wanted to determine if *M. anisopliae* would persist in cranberry bed soils and provide BVW control for up to one year following application.

3.2 Methods and Materials

Field trials were conducted from 2009 to 2010 on cranberry beds in two separate locations: a commercial farm in Langlois, Oregon and a university research station in Long Beach, Washington.

Both field sites received applications of chlorothalonil in June or July 2009, two to three months prior to the application of the treatments in our study. In July 2010, the Long Beach site received applications of chlorothalonil and the fungicide ferbam. The Langlois site was not treated with fungicides during the course of our study.

3.2.1 Treatment Applications

Treatments were applied on 16 September 2009 at the Long Beach site (5 replicates) and 21 September 2009 at the Langlois site (8 replicates). Applications were made as a soil drench covering square meter plots at the following label-recommended rates (the volume of water used to apply each treatment was also per label instructions): (1) *S. kraussei* - 5.0×10^5 nematodes per square meter diluted in 5.29 kL/ha of water; (2) *M. anisopliae* – 5.0×10^9 colony forming units (CFU) per square meter diluted in 5.29 kL/ha of water; (3) imidacloprid – 1.75 L/ha diluted in 5.61 kL/ha of water; (4) control – 5.29 kL/ha of water only. All treatments were followed with one liter of water to each plot to rinse the products from the foliage into the soil. At Long Beach, treatments were watered in by hand with 14.57 L/m^2 of water. At Langlois, treatments were watered in with 11.36 L/m^2 of water using the in-field irrigation system.

At Long Beach, conditions at the time of application were overcast with 0.56 cm of precipitation, mean humidity 88 percent and mean air temperature of 17°C. At the Langlois site, conditions were full sun and an air temperature of 29ºC, which are unfavorable conditions for application of *S. kraussei* and *M. anisopliae*. Because rescheduling of treatment applications was not possible, precautions were taken to mitigate the impact on the study of undesirable application conditions. At the Langlois site, each treatment was watered in as soon as it was applied and the field plots were irrigated for 30 minutes each day for four days following application, while temperatures remained high.

3.2.2 Soil Sampling and Inoculation with BVW

Data from this trial is based on laboratory mortality under controlled temperatures. The purpose of using these controlled conditions was two-fold. First, we wanted to address the high variability of the distribution of resident BVW populations in the field. In addition, given that *S. kraussei* and *M. anisopliae* each have an optimum temperature range for infectivity, incubating the soil samples at 20ºC theoretically allowed for the quickest possible infection of insects by the pathogens (Ricci et al. 2004, Dimbi et al. 2004).

The day following treatment application, three soil samples approximately 10 to 15 cm in depth were collected from each plot using a 10-cm diameter golf hole cutter (Kemper Golf, Akron, OH, USA). Samples were brought to the laboratory where each of the three samples (approximately 780 cm^3 in volume) from each plot were combined and thoroughly mixed. Any resident BVW larvae found were counted and removed. A 120-g subsample of soil and cranberry roots was taken from each combined sample and placed into a 5-oz. plastic cup. Each cup was inoculated with 10 fourth or fifth instar BVW. Larvae were obtained from a colony maintained at the USDA Horticultural Crops Research Lab in Corvallis, OR (Fisher and Bruck 2004). Soil samples were incubated at 20ºC and percent BVW mortality determined at 14 days. Symptomology of mortality was noted for each treatment in order to observe the cause of mortality in the control treatment as well as the proportion of mortality in each treatment that was caused by the treatment itself.

This process was repeated (in Long Beach and Langlois, respectively) before the cranberry harvest on 6 and 21 October 2009, after the harvest on 9 and 29 November 2009, after the winter dormancy period on 20 and 25 April 2010, and after the growing season, before the next harvest on 13 and 20 September 2010.

3.2.3 Statistical Analysis

BVW mortality over time, as an indicator of treatment efficacy, was compared between treatments for each site using repeated measures ANOVA (STATISTCA 7.1 2010). To assess persistence, BVW mortality at one year after application was compared using single factor ANOVA. Count data of resident BVW from the Langlois site were analyzed using repeated measures ANOVA. Count data from Long Beach were not used, since no larvae were found in the samples collected. Because the number of dead larvae was lower in some treatments than in the control, treatment mortality was directly compared to mortality in control plots.

3.3 Results

3.3.1 Long Beach Field Site

Soil treated with *M. anisopliae* resulted in greater BVW mortality than the control and other treatments during three of the five sampling periods (Fig. 3.1). In September 2009, November 2009 and April 2010, mean percent BVW mortality for

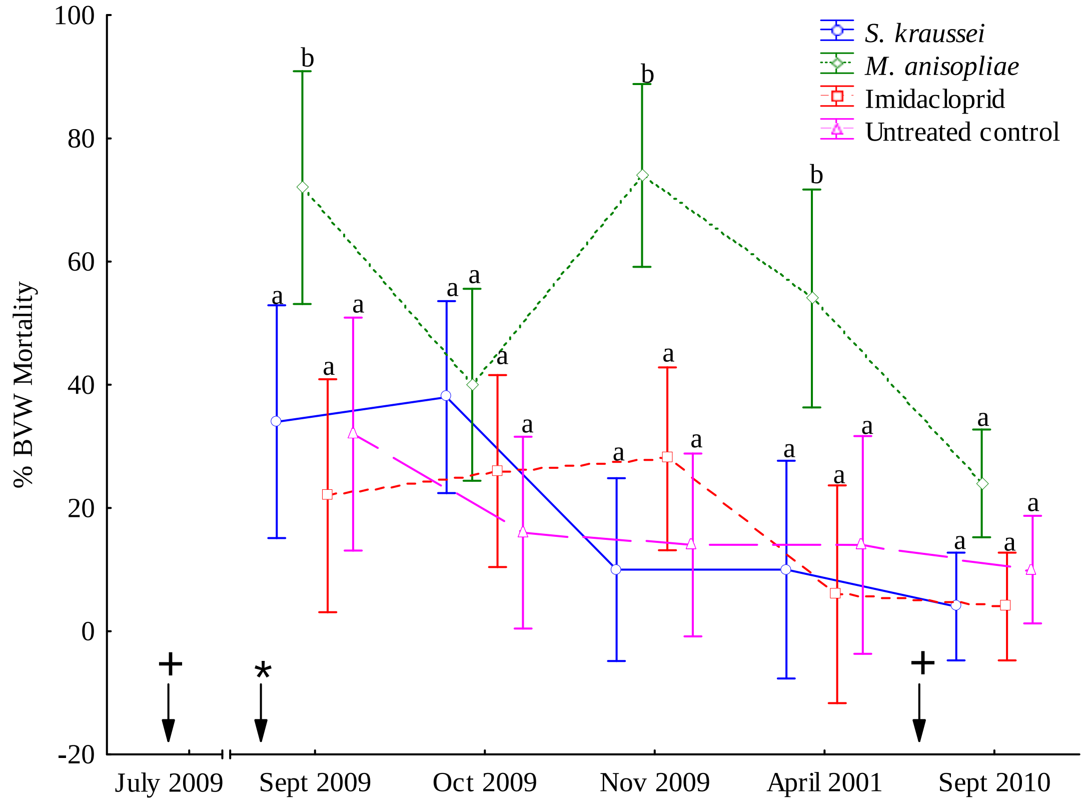

Fig. 3.1. Mean percent BVW mortality in Long Beach soil from September 2009 to September 2010. Means accompanied by the same letter are not significantly different ($P<0.05$, Tukey's HSD, Repeated Measures ANOVA). Arrows with a cross indicate fungicide applications; the arrow with the asterisk indicates when treatments were applied. Bars denote 95% confidence intervals.

Table 3.1. Mean percent BVW mortality after one year in Long Beach soil.

Treatment	1 year post-application	Pooled annual average
S. kraussei	4.00ab	19.20a
M. anisopliae	24.00bc	52.80b
Imidacloprid	4.00ab	17.20a
Untreated control	10.00abc	17.20a

Means followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD).

M. anisopliae treated plots was greater by 40, 60 and 40 percentage points, respectively, compared to the control treatment (F = 2.81; df = 12, 64; P = 0.017). No other treatment means differed significantly from the control. A single factor analysis of BVW mortality one year after application indicated that *M. anisopliae* resulted in significantly greater mean BVW mortality compared to imidacloprid and *S. kraussei*, but did not differ significantly from the control, indicating that none of the treatments were effective by this time (Table 3.1; F = 5.24; df= 3, 16; P = 0.014). An analysis of the average BVW mortality over the entire sampling period indicated that mean percent BVW mortality over a one year period was 2.75 times greater for *M. anisopliae* than for *S. kraussei* and three times greater than imidacloprid and the control (Table 3.1; F = 39.03; df = 3, 16; P < 0.00001). However, no significant differences in natural field populations of BVW between treatments were found.

3.3.2 Langlois Field Site

Mean BVW mortality in *M. anisopliae* treated plots did not differ significantly from any other treatment at any given sampling period (Fig. 3.2). However, a single factor analysis of BVW mortality one year after application did show significant differences (Table 3.2). *Metarhizium anisopliae* resulted in greater mean BVW mortality compared to imidacloprid and the control; however, no differences were found when compared to *S. kraussei* (F = 4.00; df = 3, 28, P = 0.022). Significant differences were detected in the pooled average over the entire sampling period (Table 3.2). In this analysis, *M. anisopliae* increased mean BVW mortality by 12 and 12.25

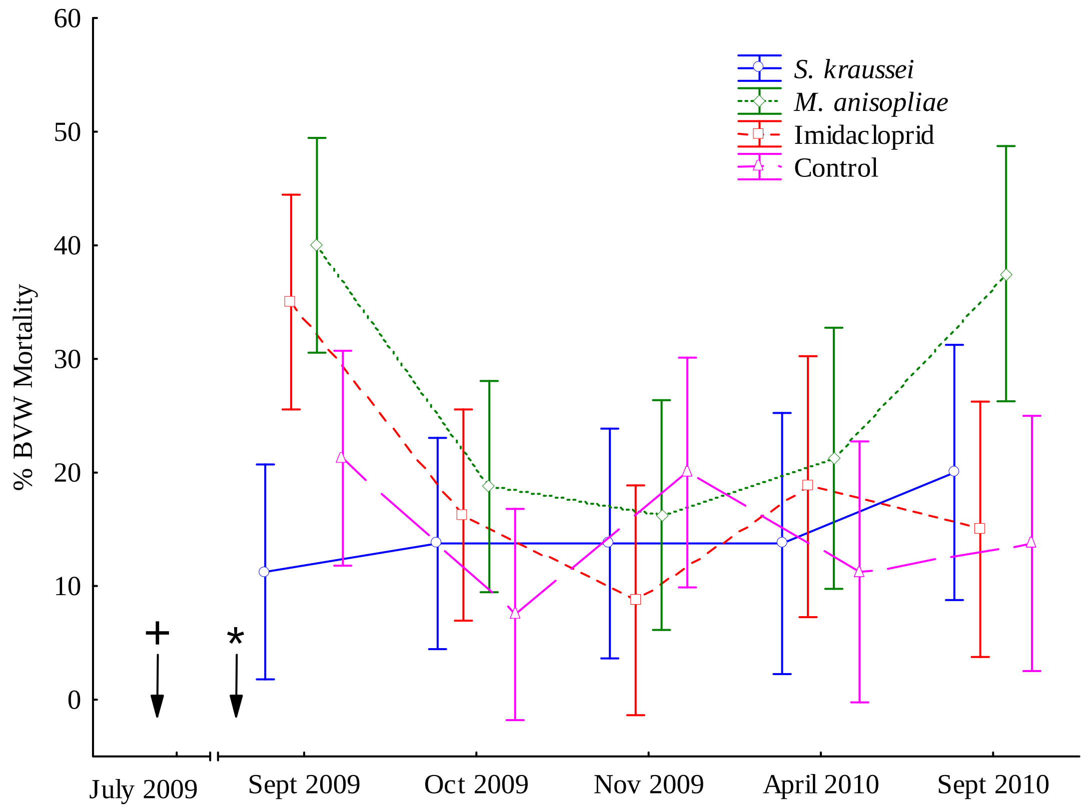

Fig. 3.2. Mean percent BVW mortality in Langlois soil from September 2009 to September 2010. Differences between treatments are not significant ($P<0.05$, Tukey's HSD, Repeated Measures ANOVA). The arrow with a cross indicates fungicide application; the arrow with the asterisk indicates when treatments were applied. Bars denote 95% confidence intervals.

Table 3.2. Mean percent BVW mortality after one year in Langlois soil.

Treatment	1 year post-application	Pooled annual average
S. kraussei	20.00abc	14.50a
M. anisopliae	37.50ac	26.75b
Imidacloprid	15.00ab	18.75ab
Untreated control	13.75ab	14.75a

Means followed by the same letter are not significantly different ($P<0.05$, Tukey's HSD) percentage points compared to the control and *S. kraussei* treatments, respectively (F = 6.00; df = 3, 28; P = 0.003, ANOVA). There was no significant difference between *M. anisopliae* and imidacloprid treatment means.

3.3.3. Symptomology of Mortality

The mortality caused by each treatment had a distinct appearance (Fig. 3.3) and in each treatment, some portion of the mortality appeared to be unrelated to the treatment itself. Mortality in the control was relatively inconsistent and sometimes higher than that in the treatments.

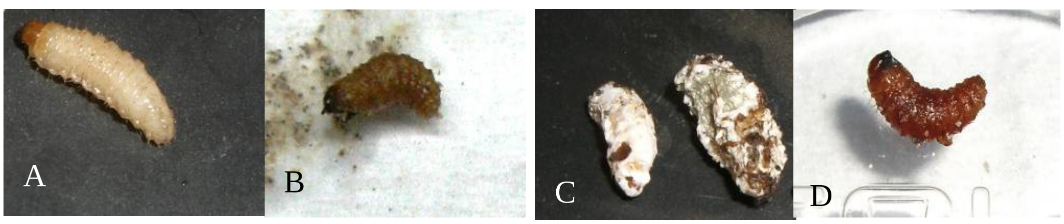

Fig. 3.3. Treatments cause distinct mortality. (A) A healthy BVW larva and larvae infected with (B) *S. kraussei*, (C) *M. anisopliae*, and (D) poisoned by imidacloprid.

3.4 Discussion

The results of this study support previous findings that *M. anisopliae* has potential as an effective biological control agent of BVW when compared to other options. In addition, the results indicate the potential of *M. anisopliae* to provide sustained control of BVW in Pacific Northwest cranberries for up to one year after application. Treatment of *M. anisopliae* resulted in higher overall mortality than *S. kraussei* at both field sites. Mortality trends at Long Beach are consistent with the findings of Booth and Shanks (1998). A seasonal trend in infectivity similar to that seen at Langlois was reported with *M. anisopliae* in container-grown ornamentals (Bruck and Donahue 2007). The seasonality may have been due to increased spore release from infected cadavers. Mortality due to *S. kraussei* treatments decreased over time at Long Beach, while it increased at Langlois. This is most likely due to high numbers of resident BVW at Langlois, a condition not shared with Long Beach, as demonstrated by counts of BVW in the soil samples. While *S. kraussei* may be able to contribute to short-term control of BVW, the apparent inability of the nematodes to persist in the cranberry field favors *M. anisopliae* as a more suitable long-term biological control agent (Smits 1996, Willmott et al. 2002).

Unfavorable application conditions were suspected to result in the inconsistent performance of *M. anisopliae* at Langlois. At Long Beach, where performance of the fungus was best overall, environmental conditions during application were more favorable to both *M. anisopliae* and *S. kraussei*.

Soil moisture throughout the course of the experiment is a likely explanation for the inconsistent performance of *M. anisopliae* at our field sites. At the well-drained Long Beach location, efficacy of *M. anisopliae* was reduced during the driest and warmest period of the year. At Langlois, where drainage was poor, BVW mortality caused by *M. anisopliae* trended upward during the dry pre-harvest period in September (Agrimet 2011). Our findings are consistent with those of Booth et al. (2000), who suspected saturated soils as a factor reducing efficacy of *M. anisopliae* in cranberry fields, and appear to support what Peng et al. (2010) demonstrated as an optimum soil moisture content range.

Metarhizium anisopliae is able to persist in the environment for a period of over one to four years (Booth and Shanks 1998, Booth et al. 2000, Rath et al. 1995a) and is better able to persist in undisturbed soils with extensive root networks (St. Leger 2008); our results from the cranberry soil environment support these findings.

Fungicide applications may have an impact on the performance of *M. anisopliae* (Bruck 2009, Shah et al. 2009, Prabhu et al. 2007, Gupta et al. 2002, Luz et al. 2007, Mandava et al. 1988). Virulence of *M. anisopliae* at Long Beach decreased from April to September 2010, following chlorothalonil and ferbam applications, and was significantly reduced compared to September 2009. In contrast, at Langlois, where no fungicides were applied during the study, virulence of *M. anisopliae* in September 2010 was equal to that in September 2009, despite having reduced virulence on all other sampling dates.

In recent years, researchers have demonstrated a synergy between *M. anisopliae* and EPNs for optimum control of BVW and other pests in container-grown plants (Ansari et al. 2004, 2008, 2010; Anbesse et al. 2008). Ansari et al. (2008, 2010) achieved 100 percent control of third instar BVW in overwintering ornamental and strawberry plants with a combination of *S. kraussei* and *M. anisopliae*.

M. anisopliae appears to be more effective than imidacloprid. A good strategy may be to maintain resident populations of *M. anisopliae* in the field to provide sustained, low level control of BVW, leaving expensive EPNs to be used in pest outbreak situations. Future studies should focus on field application of combinations of *M. anisopliae* and *S. kraussei* in order to assess their efficacy on field populations of BVW under ambient environmental conditions. Application timing and the effects of soil moisture and fungicides should be carefully studied.

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CHAPTER 4

GENERAL CONCLUSION

Elizabeth A. Miller

Black vine weevil (BVW) is a sporadic but serious pest in Pacific Northwest cranberry beds. Risk of damage by BVW in cranberries is great; however, the actual potential for plant injury in this crop has not been well quantified. We attempted to expand knowledge in this area by investigating the effects of increasing BVW egg densities and subsequent larval feeding on potted cranberry plants. Our results indicated that with increasing egg density, damage by BVW larvae increased, in the form of girdled underground stems and loss of fine roots. This damage reduced the plant's ability to translocate water from the roots to the foliage, as evidenced by a reduction in water use by plants treated with higher egg densities. This effect was seen most prominently in August, during the warmest period of the growing season. In addition, plants with the most BVW damage had reduced shoot growth. This effect was long lasting, even after the differences in water use were no longer apparent. Similar drought-stress related responses to root herbivory have been seen in natural ecosystems where biological control was implemented to control noxious weeds (Gange and Brown 1989, Hou et al. 1997).

Time-zero reference sampling in our study showed that cranberry leaf-area is closely correlated with shoot length. Other research has shown that photosynthesis in young plants is directly proportional to leaf area (Koyama and Kikuzawa 2009). It then follows that reduced shoot growth reduces the net photosynthetic potential of cranberry plants. In our study, we observed that cranberry plants treated with higher BVW egg densities displayed greater purple leaf coloration and less green leaf area compared to those treated with lower densities. This syndrome is a phenotypic expression of senescence, indicating plant stress. Reduction of shoot growth, green leaf area, and photosynthetic tissue is believed to ultimately impact yield; however, we did not look at yield parameters in our study.

Little is known about the potential of certain varieties to exhibit tolerance or resistance to BVW feeding damage. In our research, we observed ‘Stevens’ plants to have larger root systems that supported greater numbers of BVW larvae, and more feeding damage than ‘McFarlin’ plants. Interestingly, the levels of root damage observed did not compromise subsequent shoot growth or leaf area of ‘Stevens’ compared to ‘McFarlin.’ We interpret this as a level of BVW tolerance by ‘Stevens.’ In both varieties, compensatory growth was observed as a response to low levels of root herbivory. Others have reported this phenomenon as well (Ridenour and Callaway 2003, Weed et al. 2011).

Effective options for managing BVW in cranberries are limited. Chemical controls tend to be inconsistently effective (Kim Patten, unpublished data). Proper timing, negative effects on pollinators, long pre-harvest intervals and contamination of runoff water remain problems (Fisher, personal communication). As such, biological controls are an attractive alternative. Entomopathogenic nematodes (EPNs) are one option available to growers, but commercial availability, high cost and practical challenges relating to application reduce grower adoption. In order to find a more suitable biological control option, we evaluated the entompathogenic fungus *Metarhizium anisopliae* for long-term persistence and efficacy against BVW in Pacific Northwest cranberry beds in comparison to EPN *Steinernema kraussei* and a standard pesticide imidacloprid. Our results indicated that *M. anisopliae* was persistent in the cranberry soil for up to 12 months after application and that plots treated with *M. anisopliae* resulted in BVW mortality ranging from 24 to 37 percent at this time. The annual average mortality over the entire sampling period was between 26 and 52 percent. *M. anisopliae* resulted in greater mortality than *S. kraussei* and imidacloprid. Unfortunately, on average, it did not provide a level of control that would be satisfactory to cranberry growers, and efficacy was inconsistent. Factors suspected to be related to this inconsistency are soil moisture and fungicide applications. The impacts of these factors should be investigated in detail in future studies.

Future research should focus on the effect of BVW root damage on the quality and quantity of fruit yield, as well as preventative and remedial practices for BVW management. In addition, based on our research with ‘Stevens’ variety, we recommend screening current and potentially new cranberry varieties for tolerance to BVW damage, as a possible cultural control method for BVW.

As for biological control, recent studies have examined an apparent synergy between EPNs and *M. anisopliae*, which can provide up to 100 percent mortality of BVW in potted plants when applied in combination (Ansari 2008, 2010). Studies of this nature should be done under field conditions in the cranberry bed to determine efficacy as well as the impact of suboptimal temperatures, moisture levels and applications of other pesticides. A possible strategy for cranberry growers is to maintain populations of *M. anisopliae* in the field for low level control, while reserving EPNs for use in pest outbreak situations.

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