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UNDERSTANDING THE BIOLOGY OF CLOVER ROOT CURCULIO AND

IMPROVING THEIR MANAGEMENT USING BIOFUMIGATION

IN ALFALFA

by

Steven J. Price

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

2017

ABSTRACT

Understanding the Biology of Clover Root Curculio and Improving

Their Management Using Biofumigation in Alfalfa

by

Steven J. Price, Master of Science

Utah State University, 2017

Major Professor: Dr. Ricardo A. Ramirez

Department: Biology

Clover root curculio (CRC) is an emergent regional pest of alfalfa whose larvae

damages the root system. Unfortunately, there are limited management options available

for CRC suppression. Much of the biological knowledge of CRC comes from research

conducted in the eastern U.S., making management strategy development problematic in

the West where local information on larval activity and overwintering life stages is

lacking. One option for soil-dwelling pest control is the soil incorporation of

biofumigants, including brassicaceous plants, which release toxic volatile compounds

that have suppressive effects on insect pests. The role of biofumigation in alfalfa pest

suppression or the compatibility in the alfalfa production system has received little

attention. The goals of this research were to determine 1) phenology, population sizes,

and root damage severity of CRC occurring in the Intermountain region and 2) the direct

and indirect suppressive effects of biofumigant cover crop incorporations on CRC and its agronomic compatibility in rotation with alfalfa. First, I observed that larval activity occurred from mid-spring to mid-summer and local larval densities were generally lower than eastern densities. Adults began emerging from the soil in mid-summer having two subsequent population peaks. In the fall, adults had peak oviposition that continued through early winter. Low adult activity in the spring and equal egg counts from fall through spring indicated that CRC most likely overwinter in the egg stage. CRC damage to taproots was cumulative, increasing as stands age, with most damage occurring in the first few years of stand life. While the incorporation of biofumigant crops appeared to be compatible with alfalfa and did not affect yield, in field trials, no effects of biofumigation were seen in adult oviposition, populations, or feeding damage. In one greenhouse trial, biofumigants significantly suppressed adult feeding rates more than non-biofumigant oat treatment but the effect was not consistent. Biofumigant incorporation timing, for field trials in particular, may have contributed to the lack of CRC suppression. Overall, my research provides a better understanding of CRC phenology and activity in northern Utah and will assist in improving the timing of management approaches in alfalfa.

(129 pages)

PUBLIC ABSTRACT

Understanding the Biology of Clover Root Curculio and Improving

Their Management Using Biofumigation in Alfalfa

Steven J. Price

Clover root curculio (CRC) is a pest of alfalfa where larvae feed belowground damaging alfalfa roots. Regional knowledge of CRC activity and biology is limited making the development of pest management strategies difficult. One potential management technique for soil-dwelling pests is the use of biofumigant containing cover crops. Biofumigation can affect the survival and behavior of pest insects. However, biofumigant crops have not been evaluated against CRC or as a rotational crop compatible with alfalfa. The goals of this research were to determine 1) phenology, population sizes, and root damage severity of CRC occurring in the Intermountain region and 2) the direct and indirect suppressive effects of biofumigant cover crop incorporations on CRC and its agronomic compatibility in rotation with alfalfa. First, I observed that larval activity occurred from mid-spring to mid-summer and local larval densities were generally lower than those reported in the eastern U.S. Adult CRC began emerging from the soil in mid-summer having two population peaks. After the second fall peak of adults was when most eggs were deposited which continued through early winter. CRC damage to taproots was cumulative, increasing as stands age, with most damage occurring in the first few years of stand life and mostly occurring in the top 20 cm of roots. While the incorporation of biofumigant crops appeared to be compatible with

alfalfa and did not negatively affect yield, in field trials, biofumigation did not suppress CRC, disrupt egg laying, or decrease feeding damage. In one greenhouse trial, biofumigants significantly suppressed adult feeding rates more than non-biofumigant plants but the effect was not consistent. Biofumigant incorporation timing, for field trials in particular, may have contributed to the lack of CRC suppression. Overall, my research provides a better understanding of CRC phenology and activity in northern Utah and will assist in improving the timing of management approaches in alfalfa.

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Steven J. Price

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CHAPTER I

LITERATURE REVIEW

Clover Root Curculio

Clover Root Curculio (CRC), *Sitona hispidulus* (Fab.) (Cuculionidae: Coleoptera), also referred to as *S. hispidula* in the past, is one of eleven species of *Sitona* in North America (Bright 1994) and is a pest of alfalfa and clovers (*Medicago sativa* L. and *Trifolium* spp.). Native to temperate Europe and Asia, it was first reported in North America in 1875 from Long Branch, New Jersey (Hamilton 1894; Wildermuth 1910). CRC was found throughout the Mid-Atlantic states by the early 1880s, the Midwest in the early 1900s (Marshall and Wilbur 1934), Pullman, Washington by 1909 (Wildermuth 1910), and Salt Lake City, Utah by 1910. CRC had been detected widely throughout the eastern U.S. and parts of the West by 1915 (Webster 1915). It is ubiquitous trans-continentally from the Atlantic to Pacific coasts and from central Alaska and British Columbia to eastern Mexico, although it is less common at these extreme latitudes (Bright 1994, Bright and Bouchard 2008).

During early investigations, adult CRC in clover swards were occasionally considered an important pest when populations were high. Subterranean larval damage was usually misattributed to other pests or simply overlooked (Wildermuth, 1910). It was not until 1914 when the enigmatic root damage noticed in alfalfa stands, which was previously referred to as "pitting of the tap-root", that had "puzzled agronomists" was linked to CRC did it begin to receive much attention (Stewart et al. 1908, Webster 1915a,

Webster 1915b). The historical shift from forage cropping systems involving short-term clover swards to increasingly large acreage alfalfa stands, where they persist for multiple years, may have increased the severity and range of CRC (Wildermuth 1910). Later, other severe alfalfa pests such as alfalfa weevil (*Hypera postica* Gyllenhal) and potato leafhopper (*Empoasca fabae* (Harris)) drew the major research efforts of forage entomologists. CRC was primarily regarded as a pest of red clover where insecticide use was not as prevalent at the time as it was in alfalfa (Leath and Hower 1993). It has been hypothesized that the introduction of a successful biocontrol agent complex to the eastern U.S. to control alfalfa weevil in the mid to late 1970's, reduced broad-spectrum pesticide use and subsequent non-target control of CRC which coincided with premature alfalfa stand degradation (Hower et al. 1995). Leath and Hower (1993) also hypothesized that the increase of alfalfa fusarium wilt that occurred in the 1970's was due to increased CRC damage. Gotlieb et al. (1987) believed that the reduction of alfalfa stand life from six to three years that occurred in the mid-1970's in southern Vermont was a result of cold hardiness reduction caused by CRC and fusarium root rot.

Description

Adult

Adults range from 3–5 mm in length and 1.26-2 mm in width (Wildermuth 1910) being almost 2.5 times longer than wide (Bright 1994). Compared with many North American *Sitona*, the eyes are weakly convex (Bright 1994). The black cuticle is covered with a dense vestiture of dark grey, brown, and tan flat round scales making a stripe and

checkerboard pattern on the elytra. Long, white, semi-recumbent, hair-like setae occurring on the elytra are diagnostic of CRC and do not occur in other North American *Sitona* (Bright 1994). Females are typically larger than males which can be distinguished by the distal abdominal segment (pygidium) overlapping the hypopleurites being dorsally exposed beyond the apex of the elytra with the last sternite being straight (Leibee et al. 1980a). In contrast, the ventral edge of the last sternite is rounded in females (Bright 1994). Wing length and associated thoracic musculature is polymorphic in some European populations (Jackson 1928, 1933) but adults are fully winged and capable of flight in North America (Jackson 1928, Prescott and Newton 1963).

Egg

The eggs are ellipsoid, approximately 0.36 mm × 0.29 mm and initially yellowish-white when laid, later becoming shiny black within a few days if fertilized (Bigger 1920, Jackson 1922, Jackson 1928). They have a slightly granular, shiny cast stemming from the micro-sculpturing of the chorion (Wildermuth 1910, Marvaldi 1999). On rare occasion, misshapen fusiform eggs are laid towards the end of the ovipositional period (Markkula and Roivainen 1961). As in most *Sitona*, eggs are laid singly and at random (Emden, 1952) being laid loosely without cementation on the soil surface, concentrated around the crown of the host plant (Elvin and Yeargan 1985).

Larva

Larvae have five instars (Leibee et al. 1980, Tan and Hower 1991). First instars are 0.68 mm × 0.18 mm (length × width), off-white, semi-translucent, with a 0.16 mm ×

0.19 mm light brown head capsule. Last instars measure up to 5 mm × 1.3 mm,

yellowish-white, with a brownish or ochre head (Wildermuth 1910). The shape of the

head capsule and mandibular anatomy of CRC larvae have been used as diagnostic

characteristics to separate it from larval *Sitona cylindricollis* Fåhraeus (Herron 1953,

Manglitz et al. 1963). Whether these characters could be reliably used to differentiate it

from other U.S. *Sitona* spp. is unknown. Larvae have three thoracic segments, ten

abdominal segments with a small terminal segment, transverse wrinkles, long dorsal

setae, and are legless (Jackson 1920).

Pupa

The pupa is exarate and cream colored. The head is concealed beneath the

prothorax in dorsal view and has hooked, capitate bristles. The first eight abdominal

segments each bare a row of bristled "pap like" protuberances along the posterior portion

of the tergite extending laterally. The ninth abdominal segment possesses a pair of

posterolaterad tooth-like projections armed with auxiliary spicules (Jackson 1920). Pupae

can be sexed by close examination of the seventh sternite. In females, it is greatly

rounded and projects below the eighth sternite in lateral view. In males, the seventh

sternite is slightly rounded meeting the eighth on a comparable plane with an even suture.

In ventral view, the seventh sternite is posteriorly more rounded in females and more

truncated in males (Jackson 1920). Days before eclosion, the eyes and ends of

appendages begin darkening to brown (Bigger 1930, Jackson 1920).

Life History

In North America, CRC is univoltine (Webster 1915) and typically overwinters as both eggs and adults (Bigger 1930, Phillips and Ditman 1962). Eggs may develop enough in fall to hatch late in the season in Kentucky (Leibee et al. 1980a). In Pennsylvania, 52.3% of eggs hatched during an unseasonably warm fall (Quinn and Hower 1985). While overwintering larvae have been reported in some areas, it is unknown to what extent larvae survive and contribute to spring populations (Folsom 1909, Rautapää and Markkula 1966, Morrison et al. 1974). Overwintering larvae would represent an earlier brooded cohort later resuming activity with spring hatched larvae (Quinn and Hower 1985). In North America, a complete second generation has not been observed.

Hatched larvae move quickly belowground where they feed on the root system until pupation. Specializing on legumes, first instar larvae burrow into a root nodule and begin feeding, remaining concealed, by emptying the contents and either leaving behind the hollowed out epidermis or consuming it entirely (Bigger 1930, Marshall and Wilbur 1934, Manglitz et al. 1963). The presumed obligatory nature of nodule feeding by some *Sitona* spp. larvae, has been long debated (Danthanarayana 1967, Byers and Kendall 1982, Aeschlimann 1986, Quinn and Hower 1986b, Wolfson 1987, Gerard 2001, Hackell and Gerard 2004). Regardless, first instar larvae are associated with nodules (Quinn and Hower 1986b). The inner contents of the nodules are high in amino acids (Danthanarayana 1967) and nodule feeding appears to be beneficial for growth (Tan and Hower 1991). Studies suggest that larvae prefer feeding on effectively inoculated, metabolically active nodules and that CRC may use olfactory cues to locate these feeding

sites (Wolfson 1987, Hackell and Gerard 2004). Second instar larvae continue to feed on nodules or small fibrous roots (Tan and Hower 1991). Moderately sized larvae can be found feeding on small rootlets and can completely sever them (Bigger 1930, Marshall and Wilbur 1934). Late instars are found feeding within long groove-like lesions on main taproots and within the crown (Wildermuth 1910, Marshall and Wilbur 1934, Lau and Filmer 1959, Manglitz et al. 1963). Most feeding on Ladino white clover occurs in the uppermost 5.08 cm to 7.6 cm of roots with feeding extending down to 12.7 cm to 15.26 cm with damage occurring deeper into the cortex, sometimes reaching the vascular system, in the uppermost sections (Kilpatrick and Dunn 1958, Powell and Campbell 1983a). Feeding on alfalfa roots can occur up to 71.12 cm deep in the soil although feeding is concentrated in the top 25 cm of the root system and crown. As alfalfa stands age, the soil depth of root damage changes marginally but the severity of the accumulative damage occurring at shallow soil depth increases within just a few years (Dickason et al. 1968, Pesho 1975). Fifth instar larvae stop feeding and create a pupal cell near the soil surface where they remain for a week to multiple weeks before emerging as adults in the summer (Bigger 1930, Marshall and Wilbur 1934).

New generation CRC adults often overlap with the previously overwintering generation in the summer but can be distinguished from older adults by their flexible exoskeleton, higher number of dorsal scales, less worn appearance, and semi-sclerotized, undeveloped reproductive systems (Markkula and Roivainen 1961, Powell and Campbell 1984). Adults are primarily found on the ground during this time either because they are taking advantage of the cool and humid microclimatic conditions under the plant canopy

or because of the die-off of overwintered adults and emergence of the new generation. It is thought that the new generation is active on foliage during midsummer to early fall and feed before aestivation. The aestivation period, or oversummering period, is a time of diapause when adults feed minimally and remain inactive concealing themselves in crevasses in the soil, under rocks, or buried down into the plant crowns (Phillips and Ditman 1962). During extreme summer temperatures, other *Sitona* have been noted to be active during cooler night temperatures (Calkins and Manglitz 1968); a similar trend of nocturnal activity in CRC was briefly noted by Kerr and Stuckey (1956). The emergent adults have been observed to migrate out of the field by crawling into adjoining pastures, field edges, or sheltered wood-edges of fields where populations aggregate and aestivate (Underhill et al. 1955, Pausch et al. 1979, Roberts et al. 1982). Aestivation for this species may be obligatory rather than facultative since reproductively active adults in Finland that have already overwintered still undergo a period of inactivity the following summer resuming activity in the fall (Markkula and Roivainen 1961, Rautapää and Markkula 1966). The activity of adults is primarily initiated by cooler temperature cues and slightly influenced by the seasonal reduction in photoperiod (Leibee et al. 1980a). Returning adults slowly immigrate back into the field by crawling over the course of a few months and begin feeding and maturing (Pausch et al. 1980). Males may become abundant in the field sooner than females (Phillips and Ditman 1962) and may be less likely to leave the field for aestivation (Powell and Campbell 1984). After energy reserves are restored, light flights occur on warm fall days preceding reproduction and are directed out of the fields and may be important in large scale dispersal over heterogeneous landscapes to colonize new fields while fields adjoining one another may

receive founders from the general crawling population (Prescott and Newton 1963, Leibee et al. 1981, Culik and Weaver 1994). CRC spring flights after overwintering, while mentioned by Herron (1953), were not confirmed by Prescott and Newton (1963) and may not occur due to the degeneration of flight muscles over the winter (Jackson 1933).

After fall migration, post-aestivatory adults feed, mate, and oviposit diurnally on foliage, which continues throughout the adult lifespan during times of activity into spring (Jackson 1926, Phillips and Ditman 1962, Rautapää and Markkula 1966, Powell and Campbell 1984). Since eggs are laid in fall and early winter and again after successful overwintering in spring, the relative contribution of fall laid versus spring laid eggs to spring larval populations deserves additional research effort to fully understand CRC populations and timing of life stages, which directly affects management. For example, in central Illinois (Bigger 1930), Kentucky (Ng et al. 1977), and Finland (Rautapää and Markkula 1966), oviposition in fall may not be as important as spring oviposition when the majority (about 75%) of eggs are laid. Fall oviposition is also known to occur in Kansas (Marshall and Wilbur), New Jersey (Lau and Filmer 1959), New York (Kalb et al. 1994), Oregon (Dickason et al. 1958), Utah (Davis et al. 1976), Virginia (Underhill et al 1955) and North Carolina (Powell and Campbell 1984), the extent of which is unknown, and is suspected in Ohio (Herron 1953) and Maryland (Phillips and Ditman 1962). In Pennsylvania, fall oviposition can contribute between 50 to 100% of the egg load found in spring depending on the overwintering mortality of adults (Quinn and Hower 1985). Likewise, the majority of eggs in Delaware come from the fall

oviposital period (Dysart 1990). Adult overwintering mortality can vary widely between years and have a large effect on overall populations (Roberts et al. 1979, Quinn and Hower 1985). In New York, areas with reduced snow cover were thought to have higher overwintering adult mortality, and thus reduced spring oviposition, limiting populations and subsequent damage (Kalb et al. 1994). Eggs retain over 91% viability over the winter in Pennsylvania, hatching in the spring (Quinn and Hower 1985) but general viability of *Sitona* eggs can be impacted by environmental conditions such as drought (Johnson et al. 2010).

In preparation for overwintering, adults feed less and increase their cold hardiness which improves winter survivability and ability to feed and oviposit at lower temperatures (Markkula and Roivainen 1961, Rautapää and Markkula 1972). The physiological processes by which this happens are not understood for CRC (Phillips and Ditman 1962). Adults overwinter in quiescence and are able to resume activity if temperatures rise sufficiently during warm weather (Rautapää and Markkula 1972).

Warming temperatures in early spring stimulate overwintered adult CRC to start feeding and to oviposit. Bigger (1930) noticed adults being most active in late March when temperatures were around 10-21 °C. The increase in crawling activity in spring is mostly contained within a field but a few individuals may disperse to neighboring fields at this time (Leibee et al. 1981, Culik and Weaver 1994).

Hosts and Soil Preferences

Members of the Sitonini are oligophagous on Fabaceae, primarily on

Papilionoidae, with the vast majority of *Sitona* feeding on the "inverted repeat-lacking

clade" of legumes which includes Trifolieae, Cicereae, Hedysareae, and Galegeae (Castro

et al. 2007). CRC may have a wider range of host tolerance than some other *Sitona*

(Murray and Clements 1994). In general, *Trifolium* spp. are preferred hosts over

Medicago and other legumes, although preference can be variable between the host

species being tested and may be influenced by growth stage (Thompson and Willis 1971,

Barratt and Byers 1992). Females fed *Trifolium alexandrinum* L. may oviposit more eggs

than those fed alfalfa, although both are suitable hosts (Melamed-Madjar 1966). The most

historically important clovers in North America, the red (*Trifolium pretense* L.), white

(*Trifolium repens* L.), and alsike clover (*Trifolium hybridum* L.) are all suitable hosts and

are fed on readily by adults and larvae in choice tests (Thompson and Willis 1971, Barratt

and Byers 1992). *Trifolium dubium* Sibth. is also heavily fed on by adults when restricted

to it but it is less preferred than white clover (Murray and Clements 1994). In early

spring, CRC can make up half of the *Sitona* spp. larval population in sweet clover

(*Melilotus* spp.) where they may overwinter as adults immigrating to other clover fields

in spring (Herron 1953). Late instar larvae that feed within the sweet clover root system

are more associated with root lesion damage versus feeding primarily on nodules as *S.*

cylindricollis does (Manglitz et al. 1963). CRC is a minor pest of soybean particularly

when adjacent to alfalfa or clover (Kogan and Kuhlman 1982). *Lespedeza striata*

(Thunb.) (Phillips and Ditman 1962) and black medic (*Medicago lupulina* L.) (Murray

and Clements 1994) have been noted to be hosts for adult CRC. Bigflower vetch (*Vicia grandiflora* var. *kitaibeliana* W. Koch) may also be a suitable host (Byers and Kendall 1982). Trefoils (*Lotus* spp.) and crownvetch (*Coronilla varia* L.) are not preferred hosts and are fed on very little by both adults and larvae and support little larval growth or survival (Thompson and Willis 1967, Thompson and Willis 1971, Byers and Kendall 1982, Barratt and Byers 1992). Pulse crops also do not seem to be suitable hosts for CRC (Melamed-Madjar 1966). Some early researchers speculated that grasses are hosts for *Sitona* larvae, including CRC, but the larvae have not been demonstrated to feed on pasture or small grain grasses and can distinguish them from host legume roots from a considerable distance (Hatch and Murray 1994, Murray and Clements 1998, Johnson et al. 2004).

CRC larval survival can be affected by soil texture and moisture levels because of their weak burrowing ability as first instars which may also affect their access to root nodules (Tan and Hower 1991). Cracks that occur during dehydration shrinkage of slightly moist, high clay soils improve movement and survival of first instar larvae after hatching, while loamy sand soils limit movement and survival especially when dry or saturated. First instar movement is also high in course sand due to larger pore spaces but also declines with increasing moisture content (Pacchioli and Hower 2004). In a silt loam soil, saturated soil moisture levels reduced larval establishment to 0.9%, while moderately dry to moderately moist (19-27%) conditions had an average 7% larval establishment (Godfrey and Yeargan 1985). Excessive soil moisture reducing larval populations has been observed in the field during a wet spring with twice the average

amount of precipitation (Godfrey and Yeargan 1987). Although the association is

inconsistent (Quinn and Hower 1986b), first and second instar larvae are more positively

associated with field soil moisture content than larger larvae (Quinn and Hower 1986a).

Heterogeneous damage to forages at the landscape level may be influenced by changes in

soil properties (Pacchioli and Hower 2004) particularly if those properties affect nodule

availability (Quinn and Hower 1986b). Whether soil moisture directly affects larval

survival or is mediated through other variables (e.g. changes in entomopathogen

communities or nodule accessibility) is not known (Quinn and Hower 1986b).

Damage

Direct Damage

Adult feeding forms semicircular notches on leaf edges or symmetrical or paired

holes centered on the midrib when feeding on unexpanded leaflets (Folsom 1909, Bigger

1930). The field damage from adult feeding is typically negligible; however, foliar

feeding on seedlings that reduces stand establishment (i.e, seedling densities) can be

detrimental (Jewett 1934). Larvae can reduce stand establishment by severing seedling

roots. Godfrey et al. (1986) found within the first month of seeding a 32-48.8% reduction

in seedling densities from CRC which reduced establishing alfalfa plot yields by 19% one

month afterwards.

Root system damage to established stands may reach economic significance,

although the difficulty in assessing damage is much higher after stand establishment. The

potential for unseen nodule and fine root damage by first instars and small larvae is high

due to the spring synchrony between peak nodule and fine root production and activity of small larvae (Quinn and Hower 1986b, Pietola and Smucker 1995). *Sitona* spp. have been found to damage 25% of sweet clover root nodules (Manglitz et al. 1963) and can reduce alfalfa average dry nodule biomass by 61% (Dintenfass and Brown 1988a). Nodule removal can temporarily interrupt nitrogen fixation putting plants under nitrogen stress once nitrogen accumulations in taproots or stolons are depleted; by reducing photosynthetic efficiency before compensatory nodulation responses can occur (Quinn and Hall 1992, Murray et al. 2002).

Larval feeding on the lateral and fibrous roots, although difficult to assess, can result in heavy damage where larvae completely sever or girdle roots interrupting water or nutrient movement or killing root apices (Jewett 1934, Tan and Hower 1991). Whether roots receive scarring or severing type of damage is related to the proportional size of the larvae to the root (Tan and Hower 1991). Aboveground exposure of the root system due to freezing and thawing action working inadequately anchored roots out of the soil is referred to as winter heaving and can occur in poorly drained, finely textured, heavy soils leading to plant mortality due to freezing, desiccation, or harvest injury (Russell et al. 1978). While the role of CRC taproot feeding in promoting alfalfa winter heaving has been questioned (Perfect 1987), severing of lateral roots by larvae is thought to increase winter heaving issues (Underhill et al. 1955).

The characteristic taproot damage from larger larvae occurs rapidly with individual fourth and fifth instars removing 5.68 mg to 1.90 mg of alfalfa taproot mass per day, respectively (Dintenfass and Brown 1986). Feeding injury is accumulative,

increases as stands age, and is typically not noticed until the second year of damage (Dickason et al. 1968, Pesho 1975, Cranshaw 1985, Godfrey and Yeargan 1987). In an accession trial where alfalfa germplasms were screened for CRC resistance, approximately 45% of alfalfa taproots had an average of over 21.3% (0.05 to 51.84% range) surface area damaged after two years which the author considered as significant loss (Pesho 1975). Similarly, alfalfa has been seen to accumulate 17% of taproot surface area damage by CRC within two years (Quinn and Hower 1986a) which increases to 87.34% by the third year of damage (Hower et al. 1995). Clovers may be less tolerant to CRC and accumulate damage more rapidly than alfalfa because of the shallower rooting system that cannot root beyond larval feeding depth (Dickason et al. 1968). For example, red clover feeding lesions from the first to second year of damage can increase more than tenfold (Lau and Filmer 1959).

The observed impacts of larval CRC root system damage on forage yields have been inconsistent. Studies have seen no significant yield impacts (Dickason et al 1969), rare increases (Godfrey and Yeargan 1987), and direct yield reductions from larvae stunting growth, delaying regrowth, and reducing crown densities. In an experimental cage study, second year alfalfa plots with a history of damage had between 10-18.6% (avg 15.43%) yield reductions (Jewett 1934). Hower et al. (1995) saw a 31.5% average (23.2-38.9% range) reduction in alfalfa stem height across four harvests after three years of damage. Moreover, total yield was reduced by 11.25% resulting in an overall 2,633 kg/ha (2,349 lb/ac) annual yield reduction. Stunting from CRC damage reduced average alfalfa yields in two trial years by 8.4% with residual losses occurring in additional

cuttings three months after larvae had pupated, although CRC presence increased height and yields for one harvest (Godfrey and Yeargan 1987). In multiple harvest production systems, the delayed regrowth after alfalfa harvest, or "green-up", in response to *Sitona* feeding can appear superficially similar to drought stress and is thought to be due to reduced photosynthetic capacity and nutrient reserves (Goldson et al. 1985, Goldson et al. 1987, Goldson et al. 1988). Dormancy induction maybe related to a reduction in total nonstructural carbohydrates (TNC) which are stored within the taproots and later remobilized in the spring or during postharvest regrowth. Taproot larval damage was negatively correlated to TNC reserves and recovered one month after larvae pupated (Dintenfass and Brown 1988b). CRC feeding significantly reduced average alfalfa crown densities 17.9-31.8%, 36.05-36.58%, and 4.25% in the first, second, and third year of damage, respectively (Godfrey and Yeargan 1989) and 15.85-17.11% in one to two years of damage (Dintenfass and Brown 1988b). Immediate yield reductions due to decreases in crown densities have been inconsistently associated with CRC; however, early reductions in crown densities are persistent through the life of the stand therefore reducing the long-term economic viability of fields (Dintenfass and Brown 1988b). Godfrey and Yeargan (1989) predicted an 11.4-15.25% reduction in stand life attributable to CRC. CRC may also be a pest during seed production by reducing seed yields (Leach et al. 1961).

Indirect Injury

Physical damage from larval feeding can indirectly damage plants by predisposing them to a suite of diseases caused by complexes of pathogens such as crown

rots, root rots, and wilts (Graham and Newton 1959, 1960, Graham et al. 1960, Newton et al. 1960, Kilpatrick and Dunn 1961, Leach et al. 1963, Thompson and Willis 1967, Dickason et al. 1968, Hill et al. 1969, Hill et al. 1971). While CRC feeding damage is not necessarily imperative for root pathogen infection (Dunn et al. 1964), evidence indicates that the larval mechanical injury creates an infection site. Here, pathogens like *Fusarium oxysporum medicaginis* or *Corynebacterium insidiosum* can systemically colonize the vascular system causing wilt symptoms or, in the case of deep feeding lesions leading to inner cortex colonization by *Fusarium oxysporum* or *Fusarium solani*, cortical rots (Leath and Hower 1981, Leath and Hower 1993, Kalb et al. 1994). It is possible that larvae may not only open wounds for secondary infections but may also vector pathogens, as many fungi pathogenic to host plants have been isolated from CRC larval head capsules (Kilpatrick 1961, Leath and Hower 1993). Combined larval CRC damage and phytopathogens can work synergistically in reducing yields, plant densities, or rapidly decreasing stand life (Leach et al. 1963, James et al. 1980, Godfrey and Yeargan 1989). For example, CRC and root rot fungi in alfalfa have a synergistic effect reducing second cutting yields by 20.8% where each pest alone only reduced yields by about 8% (Godfrey and Yeargan 1987). CRC injury and *Fusarium* may cause stand decline by reducing the cold hardiness of plants leading to increased winterkill (Gotlieb et al. 1987). Secondary invaders, such as saprophytes, are also associated with CRC feeding lesions and once decay within the crown has begun, colonization by arthropod and other microorganism successional communities begins which furthers the decay process and attracts other pests like the clover root borer (*Hylastinus obscurus* Marsham) (Leath and Byers 1973, Wheeler 1973, Leath and Hower 1993, Kalb et al. 1994).

Although the pattern is not always clear, CRC larval damage can decrease forage competitiveness against weeds and can increase weed invasion into stands (James et al. 1980, Godfrey and Yeargan 1985, 1987, Hower et al. 1995). Weeds do not seem to have an effect on CRC populations nor do CRC directly affect weed growth (Godfrey and Yeargan 1985, Barney and Pass 1987). However, CRC larval feeding can increase the rate of nitrogen transfer from clover to grasses benefitting non-host plant growth (Murray and Hatch 1994). From a forage production context, this may be undesirable although it may indicate that CRC populations may be an important component in nutrient cycling in pastures (Murray and Hatch 1994).

<div align="center">Clover Root Curculio Management</div>

Monitoring

A combination of approaches have been described to monitor CRC because of the cryptic nature of the larvae and eggs in the soil and the mobile adults that live on the soil surface and in the plant canopy. Egg population sizes can be monitored by taking soil samples next to plant crowns and wet sieving using a gentle spray of water through a standard sieve set. After washing, remaining soil can be separated from eggs and organic particulates through floatation and filtration using a high solute solution of MgCl or NaCl (Aeschlimann 1975, Ng et al. 1977, Quinn and Hower 1985c). Such methods have high recovery accuracy and do not effect egg viability but are time intensive requiring large numbers of samples for accurate density estimation due to the aggregated distribution of eggs (Quinn and Hower 1985a). Monitoring for larvae and pupae can be done by using a similar process (Lau and Filmer 1959, Leibee et al. 1980b, Quinn and Hower 1986a).

Recovery of first instar larvae can be difficult since they are concealed within root

nodules (Leibee et al. 1980b). Later instar *Sitona* larvae and pupae can be monitored by

breaking up soil core samples by hand and using a Berlese-Tullgren funnel to recover

active larvae as they move down the funnel into a collection container (Aeschlimann

1979). Unfortunately, the time constraints and logistic challenges that arise with these

monitoring methods impede their usefulness to many growers.

Multiple adult sampling methods developed allow adults to be collected in a

variety of situations. Pitfall traps are useful to monitor adults during times of the year

when they are ground active, such as during fall crawling migration, and are useful for

monitoring movement direction when placed in a series or when directional barriers are

used (Pausch et al. 1979, Leibee et al. 1981, Culik and Weaver 1994). Fall adult flight

activity can be monitored with sticky traps on posts or with motorized rotational aerial

nets (Prescott and Newton 1963). Adult emergence after pupation or aestivation can be

monitored using emergence trap cages (Roberts et al. 1979, Leibee et al. 1981, Roberts et

al. 1982). For research purposes, a suction sampling device (collection vacuum or

motorized aspirator) can be used to collect samples from the soil surface or foliage which

can be actively sorted on a heated metal pan to encourage movement or used with a

Berlese-Tullgren funnel to passively extract adults (James et al. 1980, Roberts et al. 1982,

Goldson 1983, Goldson and French 1983). Most of these methods require high

contributions of time or specialized equipment and are unlikely to be adopted by growers.

Most growers are familiar with sweep nets, being the gold standard sampling method in

forage pest monitoring, and can be used to sample adults when they are active in the

canopy but have limited usefulness when adults are primarily located at the soil surface such as during summer diapause or right after emergence (Thompson and Willis 1967).

One future possibility for advancing monitoring techniques would be using pheromone baited traps. Pea leaf weevil (*Sitona lineatus* L.) males produce an aggregation pheromone during spring pulse crop colonization that attracts both sexes of conspecifics (Blight and Wadhams 1987, Nielsen and Jensen 1993, Quinn et al. 1999). Male CRC may reenter fields in fall sooner than females but the presence of a homologous aggregation pheromone that could be used in IPM monitoring or control is unknown (Phillips and Ditman 1962).

Host Plant Resistance

Research into resistant lines of alfalfa and clovers to CRC and other chewing insects in general have been limited and often inconclusive. 'Chesapeake' red clover may have higher field persistence than 'Kenland' red clover (Phillips and Ditman 1962). Byers and Kendall (1982) did not observe reductions in larval growth or survival in four commercial clover cultivars or twelve alfalfa cultivars versus a check. This included 'Lahontan' which was previously shown as the only commercial cultivar of six tested to show resistance to larval feeding by Pedersen et al. (1975). Pesho (1975) saw variable taproot larval feeding damage in an alfalfa field trial with 32 of 59 entries tested having mean percent damage from introduced CRC below the overall 21.9% average that might be considered tolerant. In another trial, a few imported accessions and half sibling crosses were seen to have reduced root injury in both the field and lab, which could be a source of germplasm (Byers et al. 1996). Within 96 Ladino clover genotypes tested in the

greenhouse and field, four entries had consistently reduced larval survival and size and two tolerant entries had superior growth despite supporting high larval numbers (Powell et al. 1983). In 75% of test years, adult feeding in choice-tests did not show a significant correlation to larval feeding damage, which may indicate different mechanisms of resistance occurring between life stages (Byers et al. 1996). For Ladino clover roots, increased cellulosic and hemicellulosic fiber density may be one mechanism that increases resistance to larval feeding (Powell and Campbell 1983). Transgenic resistant traits have been evaluated little for CRC; alfalfa nodules colonized by recombinant *Rhizobium meliloti* Dang. expressing insecticidal crystal proteins from the addition of *cry*III endotoxin genes isolated from *Bacillus thuringiensis tenebrionis* Berliner had a 26% reduction in larval CRC damage (Bezdicek et al. 1994).

Chemical Control

Chemical control of CRC has long been fraught with difficulties due to year-round population presence and cryptic larval habits. In the past, CRC management primarily relied on heavy applications of chlorinated hydrocarbons (e.g. DDT, cyclodienes, and hexachlorocyclohexanes), carbamates, and organophosphates with long lasting residual activity producing highly variable results. Before seeding alfalfa, fall incorporation of organochlorine cyclodienes, such as aldrin, dieldrin, heptachlor, or chlordane, presumably decreased CRC root damage by killing adults before oviposition leading to larval damage suppression the following season (Underhill et al. 1955). Applications of cyclodienes only provided effective control for one year, until dieldrin, which has longer residual activity, was used which had the potential to reduce root injury

for three years; effective long term control in forage and seed production often required additional applications (Turner 1957, Dickason et al. 1958, Leach et al. 1961). Effectiveness of CRC control by spring applications of chlorinated hydrocarbons ranged from negligible to highly effective depending on timing of application, life stages present, and field age (Underhill et al. 1955, Kerr and Stuckey 1956, Hansen and Dorsey 1957, Turner 1957, Forsythe and Gyrisco 1962, Dunn et al. 1964, Waters 1964). Carbamates, such as carbofuran that have systemic activity, produced inconsistent results for similar reasons and were best used in late summer against pre-ovipositional adults to reduce larval populations the following year especially when coupled with spring treatments of diazinon, a soil active organophosphate (Neal and Ratcliffe 1975, Godfrey and Yeargan 1987). When annual treatments of carbamates are applied for long-term insect control, the effectiveness of the applications is diminished by the enhanced soil bacterial metabolism which rapidly degrades the pesticide (James et al. 1980, Pedigo and Rice 2009). Even when these antiquated broad-spectrum chemical controls were successfully deployed in reducing larval populations and root damage, seed or forage yields, plant populations, and stand longevity were often not improved (Phillips and Ditman 1963, Dunn et al. 1964, Dickason et al. 1968, Neal and Ratcliffe 1975, James at al. 1980, Dintenfass and Brown 1988a). Similarly, foliar applications of other carbamate and organophosphate pesticides with presumed systemic root translocation were ineffective in controlling *Sitona* in alfalfa (Barratt 1985).

After the revocation of carbofuran tolerances in 2009 (EPA 2016) current control options have been limited to short residual insecticides targeting the adults. However, the

prophylactic deduction of adults appears to be ineffective so far to suppress subsequent

larval numbers and is not currently recommended (Wenninger and Shewmaker 2014,

Reitz 2016). For example, even at 10-day spray intervals covering a five-month period in

fall and spring, methyl parathion provided poor control and was not cost effective (Kalb

et al. 1994). Such extensive, field-wide treatments in spring to control adult CRC would

likely have unintended consequences such as reducing the important biocontrol agents of

alfalfa weevil, a primary alfalfa pest, also present at this time. Future techniques

involving band spraying autumn post-aestivation adults during field reentry may be a

more feasible approach (Pausch et al. 1980). As for larval management with insecticides,

there are currently no soil active insecticides registered for management.

Cultural Control

The cultural controls suggested for CRC in the past included burning over forage

stubble in winter or disking and harrowing after first harvest to reduce adult numbers;

such tactics would be a challenge to use in modern production systems (Wildermuth

1910, Webster 1915b). In consecutive alfalfa rotations, larvae may survive spring

plowing by feeding on root debris left in the field, or in no-till operations from plants left

in the soil resulting in heavy damage to new vulnerable plants (Godfrey et al. 1986,

Barney and Pass 1987). One of the few control options available for contemporary

producers is rotation to a non-leguminous, non-host crop to temporarily disrupt CRC

populations before rotating back to alfalfa or clovers. Spring planting forages may be

preferable over summer or fall planting since well-established plants appear to tolerate

more damage from fall migrating adults (Leibee et al. 1981). Byers et al. (1996) did not

see significant differences in larval root damage between alfalfa planted in the spring or summer. However, the roots of spring planted alfalfa are larger, and given that larval feeding on thicker roots may limit feeding superficially to the cortex (Powell and Campbell 1983). Planting in spring may also reduce the effects of larval feeding and is the current recommendation (Wenninger and Shewmaker 2014). In established stands, proper fertilization may help in mediating damage since robust healthy plants may better withstand root damage and recover from stress quicker (Wilson and Barber 1954). Nitrogen applications have been shown to reduce larval establishment and CRC populations by inhibiting plant nodulation which is beneficial for larval growth (Wolfson 1987) but is unlikely to be an economically viable long-term solution for CRC control (Wenninger and Shewmaker 2014, McNeill et al. 2016).

Biofumigation

Current phase-out of soil active, broad-spectrum insecticides and synthetic soil fumigants (i.e. carbofuran and methyl bromide) by the Environmental Protection Agency has left growers of a wide variety of crops without chemical control options for soil-dwelling pests. Biofumigation has received increased interest in organic systems and has been used as a component of integrated pest management programs in some other cropping systems (McGuire 2003). The practice of using cover crops or plant biomass containing volatiles that deter or are toxic to pests, and subsequently incorporating them into the soil as green manures or soil amendments for agronomic benefit has been coined "biofumigation" (Brown and Morra 1997, Rosa et al. 1997, Kirkegaard and Sarwar 1998, Sarwar and Kirkegaard 1998, Sarwar et al. 1998, Fahey et al. 2001, Matthiessen and

Kirkegaard 2006). Many plants contain metabolic compounds that counteract herbivory

including plants in the order Capparales, specifically the Brassicaceae (Cruciferae) (Kjaer

1976, Rosa et al. 1997). Primarily, the biocidal properties of these crops come from a

suite of volatile compounds released into the soil during the hydrolytic degradation of

glucosinolates, a group of about 120 described compounds belonging to 10 different

chemical classes (Fahey et al. 2001). The suite of these chemicals contained within plant

tissues can differ in composition and concentration by plant species, tissue type,

developmental stage, genetics, and physical/biotic environment of the cover crop (Kjaer

1976, Sang et al. 1984, Mojtahedi et al. 1993, Fahey et al. 2001, Buskov et al. 2002,

Morra and Kirkegaard 2002, Matthiessen and Kirkegaard 2006, Velasco et al. 2008).

Biofumigation is one approach to managing soil dwelling insects. Isothiocyanates, a

common product of glucosinolate hydrolysis, are reported to have insecticidal properties.

Eggs of the black vine weevil (*Otiorhynchus sulcatus* Fab.) exhibit mortality positively

correlated with exposure to isothiocyanates with increased molecular weights and greater

lipophilicity (non-polarity) (Borek et al. 1998). A 1.93% and 8.69% soil incorporation

rate of active ground 'Dwarf Essex' rapeseed meal kills 50% and 90% of black vine

weevil larvae, respectively, from released isothiocyanates (Borek et al. 1997). After

pressing oil from the seeds, high surface incorporation rates of meal in potted plants can

reduce larval survival up to 70%; however, such high incorporation rates were not

considered economically viable and were moderately phytotoxic to the strawberry host

plants (Elberson et al. 1997). First instar whitefringed weevil (*Naupactus leucoloma*

(Boheman)) larvae exposed to the vapors of high glucosinolates containing Indian

mustard (*Brassica juncea* (L.) Czern.) seed meal and plant tissues had higher mortality

than larvae exposed to canola seed meal or aerial portions of fodder rape (*Brassica napus*

(L.) which have been bred for low glucosinolate levels (Matthiessen and Shackleton

2000). Beyond direct mortality, biofumigants can have indirect suppressive effects on

pests that could be used as part of an integrated pest management program. For example,

for insects with limited larval mobility, such as CRC, ovipositional site selection by

females can have large fitness consequences and females are expected to show high site

selectivity (Johnson et al. 2006). Biofumigation using Ethiopian mustard (*Brassica*

carinata A. Braun) seed meal can reduce oviposition in Colorado potato beetles

(*Leptinotarsa decemlineata* Say) by 50% (Henderson et al. 2009). It is currently unknown

if any direct or indirect effects of biofumigation, such as direst mortality or changes in

reproductive behavior, are suppressive against CRC and could be used successfully as

part of a forage production system.

Biocontrol

Multiple species of entomopathogenic fungi are known to infect larvae and adult

CRC populations and are especially common in laboratory settings (Kilpatrick 1961,

Crow et al. 1968, Wildermuth 1910, Aeschlimann 1980). However, the role filled by

these fungi in regulating CRC populations is not well understood. *Beauveria bassiana*

(Balsamo) Vuillemin and *Beauveria globulifera* (Spegazzini) Picard field infection rates

of adults can reach high levels and is thought to be important in CRC population

regulation (Turner 1957, Kilpatrick 1961, Crow et al. 1968). Others have argued that *B.*

bassiana may most likely be acting as a secondary pathogen or saprophyte and may be a

low-level mortality factor in adult populations (Quinn and Hower 1985a).

Entomopathogenic nematodes (EPN), such as *Diplogaster* sp., have also been recovered from larval CRC (Marshall and Wilbur 1934). EPNs have been used in the long-term successful control of other belowground weevil pests of alfalfa, where there are also no current chemical control options available, which has led others to investigate their potential use in CRC control (Shields et al. 2009). In the laboratory, *Heterohabditis bacteriophora* Poinar, *Steinernema feltiae* Filipjev, and *Steinernema bibionis* Steiner infect and reproduce in early and late instars, pupa, and even adult CRC. Later instars exhibit quick mortality from EPNs with *S. feltiae* and *H. bacteriophora* being particularly effective (Jaworska and Wiech 1988, Wiech and Jaworska 1990). The Oswego strain of *H. bacteriophora* may be especially useful when targeted towards second to fifth instars and pupae; late instars in particular support high nematode infectivity and reproduction (Loya and Hower 2003). When applied in the field, this strain reaches stable populations quickly, persists for multiple years, and can reduce adult emergence and larval root damage (Loya and Hower 2002).

Little research has been done on generalist predator effects on CRC populations. With few management techniques available, conservation biological control may become a crucial component in IPM programs designed for CRC control. Ground beetles (Coleoptera: Carabidae) are abundant during the CRC ovipositional period with multiple species able to feed on CRC eggs. *Pterosticus lucoblandus* Say, *Agonum* (*Olisares*) *cupripenne* Say and, especially, *Amara* (*Amara*) *aenea* DeGeer have been reported to be important egg predators in Pennsylvania where egg predation rates may reach 28% (Quinn and Hower 1987). Likewise, *Cyclotrachelus* (*Evarthrus*) *sodalist* (LeConte),

Pterostichus (*Abacidus*) *permundus* (Say), *Harpalus* (*Pseudoophonus*) *pennsylvanicus*
DeGeer (Carabidae) and *Gryllus pennsylvanicus* Burmeister (Orthoptera: Gryllidae) may
be significant field edge predators in autumn preying on CRC adults during aestivation
and subsequent field migration (Barney et al. 1979, Barney and Armbrust 1980). Birds
may also be significant predators of adult CRC. Nine species of birds have been found to
prey on adult CRC with chimney swifts (*Chaetura pelagica* L.) and song sparrows
(*Melospiza melodia* Wilson) heavily consuming them with 15 adult CRC found in one
individual (Wildermuth 1910). CRC adults may be an important food item for European
starlings (*Sturnus vulgaris* L.) later in the year making up 9.3% of the diet in August
alone (Lindsey 1939).

The mymarid CRC egg parasitoid *Anaphes diana* (Girault) (=*Patasson lameerei*
Debaucheis) (Schauff 1984), was first introduced from France in 1977 into the United
States in Newark, Delaware and subsequently released around Delaware, Illinois,
Kentucky, and Idaho to control CRC and other *Sitona* (Dysart 1990). Within a few days
of emerging, the short-lived females oviposit single eggs into CRC host eggs, preferring
black eggs in which the chorion has not completely hardened (Leibee et al. 1979,
Yeargan and Shuck 1981). Parasitoids likely find their host by olfactory cues from
volatiles released from damaged plants or adult CRC frass (Bloem and Yeargan 1982b).
The introductions ultimately failed for unknown reasons but, since the parasitoid is
tolerant of a wide range of temperatures, thermal extremes were not considered to be the
main culprit for reduced establishment and success (Bloem and Yeargan 1982a, Dysart
1990).

Three braconid parasitoid wasps, *Pygostolus falcatus* (Nees), *Perilitus rutilus* (Nees)*, Microctonus aethipoides* Loan and one tachinid fly, *Campogaster exigua* (Meigen), that parasitize both *Sitona* spp. and *Hypera* spp. weevil adults were introduced from Europe into North America for investigation in Manitoba, Canada (Loan 1961) and North Dakota, United States (Munro and Post 1948, Berry and Parker 1950). While releases targeted sweet clover weevil, *S. cylindricollis*, they were known to naturally parasitize CRC and other *Sitona* in their native ranges and in controlled environments (Berry and Parker 1950, Loan and Holdaway 1961a, Loan and Holdaway 1961b). A population with unknown origin of *P. falcatus* from Prince Edward Island, Canada, exhibited modest levels of CRC parasitism but is unlikely to be a dependable biocontrol agent due to poor host synchronization (Loan and Thompson 1972, Milbrath and Weiss 1998). Overall, these introductions failed to establish in North American *Sitona* spp. (Loan 1961, Loan 1965).

Surveys to recover introduced or native parasitoids of adult *Sitona* in Missouri (Crow et al. 1968), Northern California, and Oregon (Phillips et al. 2000) have also resulted in limited success. CRC has proven to be an unsuitable host for the native parasitoid *Microctonus sitonae* Mason which regularly infects adult *Sitona scissifrons* Say (Loan 1960, Loan 1963). One native tachinid, *Hyalomyodes triangulifer* Loew (=*triangularis*), has been found parasitizing CRC but is unlikely to be useful in management being a generalist beetle parasitoid (Loan 1963).

Conclusion

Much remains to be learned about the biology of clover root curculio. The majority of the previous research on CRC biology and phenology was carried out in the eastern U.S. quite some time ago. Our understanding of the lifecycle timing and pest status of CRC in the western U.S. up to this point has been insufficient. In addition, our knowledge of CRC damage and management, which certainly has never been very complete, also comes from the eastern U.S. based on research that may not still have the relevance it once did. Since the time of past active investigation into CRC as a forage pest, forage production systems have adapted along with modern advances in technology changing dramatically. Cost effective production of high yielding, top quality forages is greater than it has ever been because of these changes. Unfortunately, our knowledge of CRC has not advanced at the same pace leaving producers with limited management options capable of fitting into modern production systems when needed. Because management decisions must be timed to target pests when control is most effective, the scarcity of phenological and biological information for CRC in the West has been an impediment to basic research regarding this pest in the Intermountain West specifically. The future development of management tools will require an updated understanding of regional CRC phenology. As previously described, the options available to producers for CRC control are limited. Finding replacements for antiquated pesticide chemistries requires researchers to look to novel pest control methodologies. Only through the experimental application of alternative approaches, such as biofumigation, paired with up-to-date phenological information, will regional management options for CRC control

be able to be developed that are both complimentary and effective in contemporary forage production.

Research Objectives

CRC has become a regional pest of increased interest with limited management options available to producers. Pest management strategies that are effective and compatible with production must be designed around the biology of the pest in question. The lack of understanding of CRC biology and phenology in the western U.S. has been an obstacle to researchers as they have begun developing modern management approaches. One management tactic used for soil-dwelling pests in other cropping systems is biofumigation although it has yet to be investigated in the management of CRC and has received little attention in alfalfa production. An effective integrated pest management program must not only reduce pests as warranted but also be compatible with the agronomic system as a whole in order to provide sustainable benefit to growers. The overall goal of this research was to increase the regional understanding of CRC biology and evaluate the use of biofumigation as a management tactic. Specifically, field surveys, field, and greenhouse experiments were conducted to determine:

1. The regional timing of CRC life stages including the damaging larval stage and overwintering stages and to quantify the current extent of CRC damage occurring in our area (Chapter II, formatted for Journal of Economic Entomology).

2. The direct and indirect suppressive effects of biofumigation on CRC and better understand the compatibility of using biofumigant cover crops in rotation with alfalfa (Chapter III, formatted for Journal of Economic Entomology).

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CHAPTER II

CLOVER ROOT CURCULIO PHENOLOGY AND DAMAGE

Abstract

Clover root curculio (CRC), *Sitona hispidulus* (Fab.), a root-feeding pest of

clovers (*Trifolium* spp.) and alfalfa (*Medicago sativa* L.), has the ability to reduce forage

yield and stand life, and increase crop exposure to plant pathogens. Historically, CRC

remained a minor pest because of the effectiveness of soil active pesticides and has

mostly been researched in the eastern U.S. However, coincident with the federal ban on

carbofuran, CRC has become a notable pest, yet the understanding of CRC biology and

phenology in the western U.S. has previously been too inadequate to begin strategizing

management approaches. The objectives of this study were 1) to determine the timing of

CRC life stages including the damaging larval stage and overwintering stages during the

production season, and 2) quantify the current extent of CRC damage occurring in our

area. We conducted a field survey in northern Utah during the 2015-2016 field seasons

by sampling nine different fields. We used insect suction samples to collect adult CRC

aboveground, and soil core samples to collect larval and pupal stages belowground. In

addition, we recorded the root damage of CRC larvae. We found that in contrast to some

areas in the eastern U.S. the overwintering stages in our area were primarily in the egg

stage since the majority of eggs were laid in fall and adult survival through winter was

low. Newly established fields accumulated damage faster than in older fields which can

reduce the life span of stands. This suggests that successful management of CRC early in

the life of the stand may provide a long-term benefit. Given our new understanding of CRC phenology in northern Utah, we can begin to better time and develop management toward susceptible life stages.

Introduction

Clover root curculio (CRC), *Sitona hispidulus* (Fab.), is an economically damaging weevil pest of clovers (*Trifolium* spp.) and alfalfa (*Medicago sativa* L.) that can reduce yields, decrease stand life, and increase phytopathogen infection such as fusarium rots and wilts from feeding on plant roots (Dickason et al. 1968, Godfrey and Yeargan 1987, Hower et al. 1995). The majority of what is known about CRC biology, damage, management, and phenology is based on research from the eastern U.S. during an era when heavy applications of soil active, highly toxic, broad-spectrum insecticides with high environmental persistence were common measures for alfalfa pest management (Underhill et al. 1955, Dickason et al. 1958, Phillips and Ditman 1962, Neal and Ratcliffe 1975, Godfrey and Yeargan 1987, Dintenfass and Brown 1988b). The regulatory phase-out of these pesticide chemistries over the past few decades, including the 2009 carbofuran revocation (EPA 2016), has left producers with limited management options and only a partial regional knowledge of CRC biology in western North America. The development of management techniques that are compatible with contemporary western forage production requires a better understanding of CRC biology, phenology, and damage in the region.

The damaging life-stage of CRC comes from larval feeding below ground. Root damage often goes unnoticed by growers and aboveground symptoms may be misattributed to other causes, including plant pathogens or nutrient deficiencies (Tietz 2012). Although CRC has been in northern Utah since at least 1910, the enigmatic damage to roots was not connected to the larval stage initially (Webster 1915). Since then, little CRC research has been done in Utah alfalfa or in the West in general (Dickason et al. 1958, Waters 1964, Davis et al. 1976). Based on observation from the eastern U.S., our current understanding is that CRC overwinters as eggs and adults, CRC larvae may prefer silty-clay loams over loamy sand soils, and adults are active and have peaks in the spring and fall undergoing a summer aestivation (Leibee et al. 1981, Hower et al. 1994, Pacchioli and Hower 2004, Wenninger and Shewmaker 2014). Current CRC pest status and prevalence in the western U.S. is largely unknown.

In order to develop appropriate management strategies targeting CRC, an understanding of CRC phenology during the season is imperative to determine when susceptible life stages are occurring to better time management strategies. Over the course of two field seasons (2015-2016), production alfalfa hay fields in Cache County, Utah were sampled to 1) determine the timing of CRC life stages including the damaging larval stage and overwintering stages during the year, and 2) quantify the current extent of CRC damage occurring in our area.

Materials & Methods

Phenology

Alfalfa production fields distributed across Cache Valley, Utah were surveyed for two field seasons (2015-2016). In 2015 and 2016, four and seven fields, respectively, were visited from April to December (specifically May 31 to September 23 in 2015 and April 2 to December 3 in 2016; Table 2-1). Field age ranged from two to eight-year old production fields. The average field size was 17.53 hectares. Fields varied in irrigation method, soil type, pest management, and harvest schedule (Table 2-1). Only one field applied insecticide (chlorpyrifos and dimethoate, both organophosphates) targeting pea aphid (*Acrythospihon pisum* Harris). CRC adults were collected using suction sampling and eggs, larvae, and pupae were collected from soil samples. The sampling area at each site was a 115×243 meter (ca. 2.8 hectares) area plot divided into four, 57×121 meter quadrats with a 6 meter buffer along the edge to minimize sampling edge effect.

CRC adults were sampled using an insect suction sampling device made from a leaf blower/vacuum (Echo ES-250) outfitted with a fine mesh organdy collection bag (Rincon-Vitova Insectories #DVAC401) secured around the 12 cm diameter opening of the suction tube. Sampling captured individuals in the plant canopy during times of activity as well as on the soil surface when CRC was not active during summer aestivation. One suction subsample was taken in each quadrat totaling four subsamples per field each collection period. A suction subsample consisted of placing the vacuum at full throttle over an alfalfa crown and contacting the soil surface for a one-second interval

in one motion. This was repeated every one meter in a linear 30 meter transect in succession 30 times to produce one suction subsample. Samples were stored on ice in a cooler, transported back to the lab, and refrigerated until processed. Adults were sampled from June 30 to September 24, 2015 (total 5 sample dates) and from April 9 to December 3, 2016 (total 10 sample dates) and were counted.

Larvae were sampled by taking soil cores 11 cm in diameter and ca. 28 cm long centered around alfalfa crowns. In 2015, four larval soil cores were collected each sampling period from each quadrat totaling 16 cores per field. In 2016, soil core samples were reduced to two samples per quadrat totaling eight cores per field each visit. Soil cores contained plants with intact taproots as well as surrounding soil. They were refrigerated to slow down larval development and activity until they could be processed. Larval soil cores were processed using modified methods of Lau and Filmer (1959) and Leibee et al. (1980b). Cores were soaked in water for ten minutes in an 11.4-liter plastic tub and broken apart with gentle agitation and a water spray. As the tub filled with water, the supernatant containing small soil particulates, organic matter, soil mesofauna, and floating CRC larvae was decanted off the top through a U.S. standard sieve set (#5, #10, #35, #60). The process was repeated until cores were completely disaggregated and contents could be thoroughly agitated and suspended to recover all larvae. Alfalfa roots were removed, cleaned, and stored frozen for future damage assessment (see "Damage assessment" below). CRC larvae and pupae were counted and head widths of CRC larvae were measured using an ocular micrometer to determine larval size throughout the season as in Leibee et al. (1980b). In order to have a size standard for first instars, five eggs were

taken from each egg soil-core and placed in a 3.5 cm petri dish lined with Whatman®

filter paper moistened with distilled water. To prevent desiccation or newly emerged

larval escape, Parafilm M® was used to cap dishes before the lids were added. Dishes

were kept in a Percival I-30BLL incubator (21 °C, 100% RH, 24 hour dark period) and

checked every 2-3 days to count hatched eggs, and remove eclosed larvae and chorion

remains, to avoid potential cannibalism. In 2015, larval cores were collected from June 1

to July 30, 2015 (totaling 5 sample dates), and from May 15 to August 2, 2016 (totaling 6

sample dates). Additionally, in order to check for any larval stages that might become

present in the fall, eight soil cores were collected per field on October 26 and December

3, 2016 from the two fields which had the highest CRC populations during the season

(Caine Dairy 6 and Richmond 6; Table 2-1).

Eggs were sampled similarly to the larval cores; four and two egg soil-cores were

collected in 2015 and 2016 per field quadrat, respectively. To sample for eggs which are

oviposited on the soil surface and aggregated around alfalfa crowns (Ng et al. 1977), soil

samples were taken using a 7.62×7.62 cm collecting template positioned so that at least

two sides were in contact with alfalfa crowns. The soil within the square sampling

template was removed at a depth of 2.5 cm, bagged, and transported in an ice chest.

Samples were frozen for storage in 2015; however, in order to assess egg viability in

2016, samples were refrigerated to stop egg development and retain viability (Quinn and

Hower 1985). The methods of Ng et al. (1977) and Aeschlimann (1975) were used with

slight modification to process egg samples because of their high recovery rates and egg

viability retention. Eggs were separated from soil as described for larvae. It was

determined that a #60 sieve was sufficient in collecting ca. 97% of whole eggs and was the finest sieve used. Eggs were then counted using a stereomicroscope (Leica S6D).

Damage Assessment

To assess larval root damage, roots were washed from larval soil cores as they were being processed and frozen for later evaluation. Roots were initially scored for crown and taproot larval CRC feeding damage on a 0-5 number scale: 0- damage absent, 1- damage present but minimal, 2- light damage, 3- moderate damage, 4- moderate-high damage, 5- extensive/severe damage. Pictures were taken of roots for comparison. To quantify lateral root pruning damage in 2016, a semi-cylindrical counting template made from a 15 ml centrifuge tube by longitudinally bisecting it and drilling 10 mm diameter holes at 25, 50, and 75 mm from the top of the tube. Once the template was placed at the junction of the taproot and crown, all lateral roots and rootlets arising from within each of the three 78.54 mm^2 area holes, being associated with the different soil depths, were counted. In order to better quantify the percentage of taproot damage and its occurrence at differing soil depths, a modified method similar to Pesho (1975) was used. Crowns, lateral roots, and rootlets were removed from the taproot. A longitudinal incision was made deep enough into the root to cut through the vascular tissue so that the epidermal tissue, outer cortex and cambial layer could be peeled away from the inner cortex. When multiple codominant roots were present, each was sliced and peeled intact. The excised outer root layers were flattened and overlain with a transparency film sheet with a printed 5 mm grid. The root outline was traced onto the film using permanent marker and all identifiable larval feeding lesions were transcribed on the film. The sheet was then

scanned (Canon CanoScan LiDE 60). Scans were quantified using ImageJ (ImageJ 1.49f;

http://rsbweb.nih.gov/ij/) where each root was divided into 25 mm sections and the area

of taproot scarring and the total taproot area was recorded for each section so that percent

damage could be calculated per section as well as for the entire root.

Analysis

Correlative data analysis of CRC life stages consisted of a Pearson correlation test

using the PROC CORR procedure (SAS Studio 3.5). Correlations were also made to

determine if counts of root damage lesions were correlated to percent taproot damage.

The procedure was also used to analyze the relationship between the damage variables

measured after larvae had pupated (i.e. end of season visual damage estimates and

computer imaging estimates) and larval densities in 2015. Additional correlations were

completed for 2016 data to compare the pre and post larval period change in damage

metrics between roots collected while larval densities were low (May 15, 2016) and after

pupation had occurred (August 1, 2016).

Pearson correlations were used to determine whether field age had an overall

relationship to taproot damage or peak larval numbers. The effect of field age on the

amount of accumulated taproot damage was assessed further by comparing the average

amount of the 2015 and 2016 post larval period taproot damage in fields in their first ($N =$

3), second ($N = 2$), third ($N = 2$), and fourth or more ($N = 3$) years of damage by using the

PROC GLM procedure and Tukey's HSD posthoc means comparison. Planned contrasts

were used to further assess differences in taproot damage and larval numbers occurring in

stands of different age classes. The PROC REG procedure was used for a single regression to compare field age and larval populations. For fields that were sampled both years (Creech and Larson; Table 2-1), both sets of annual data were included in the analysis for the appropriate age category during the sampling year.

Results

Phenology

In 2015, CRC adults were captured in samples from June 30 to September 24 in high abundances (>10 adults/sample) suggesting that CRC adult activity also occurs both before and after this period of time (Fig. 2-1a). As a result, sampling in 2016 was expanded to April 9 to December 3, 2016 to capture complete activity. Spring populations were low both years until July when adult populations began to increase from 0.74 adults per sample to >10 adults per sample from early August through November (Fig. 2-1b). In 2015 and 2016, there were two distinct peaks of adult abundance occurring in mid-July and late-August in 2015 and early August and early October in 2016.

In 2015, from the first collection period on June 1 to the second collection period on June 18, larval numbers dropped from 6.91 to 5.38 larvae per sample indicating that peak larval numbers were occurring or had already occurred before early June (Fig. 2-1a). Larval sampling was started earlier in 2016 and on April 9 larval numbers were very low at 1.08 larvae per sample. By May 30, 2016 larval numbers reached their peak (6.42 larvae per sample) and began to decline reaching very low numbers by August 1, 2016 (Fig. 2-1b). In order to better understand growth over the larval period, head widths were

measured as an estimate of larval size. In 2015, larval size changed little from June 1 to July 29 indicating that when samples were collected larvae were primarily in their final fifth larval stage (Fig. 2-2a). In 2016, larvae recovered in early April were generally small in size (Fig. 2-2b). Although there were a few larger sized larvae present, 58.82% of larvae recovered ($N = 54$) had head widths matching the first instars hatched in the incubator that were used as a size standard. Average larval head width continued to increase until plateauing June 13 indicating that most larvae where fifth instars at this time. Pupal densities peaked between June 28 and July 1 both years (Fig. 2-1).

In 2015, egg densities were fairly low (< two eggs per sample) from June 16 through August 24. The highest egg densities observed in 2015 were from September 23 samples which contained an average of 2.91 eggs per sample (Fig. 2-1a). Again, sampling in 2016 was expanded to better understand oviposition throughout the year. When egg cores were collected April 9, 2016, egg densities (6.75 per sample) were higher than when sampling was stopped in late September 2015 (2.91 per sample). By May 15, egg numbers were low (0.9 eggs per sample) and continued to decline to 0.15 eggs per sample on August 2. In fall, egg numbers began to increase from 4.18 eggs per sample on September 30 to 13.09 eggs per sample on December 3 when sampling was stopped (Fig. 2-1b).

To better understand the population dynamics seen among average CRC life stages within fields, correlative analyses using 2016 data were completed. The peak densities of eggs recovered in December were not significantly correlated to either peak of adult numbers occurring in August ($r = -0.239$; $P = 0.606$) or October ($r = 0.519$; $P =$

0.232). No significant correlation was observed between peak June larval densities and the peak of new generation adults occurring in late July to early August ($r = 0.248$; $P = 0.591$). Peak larvae numbers were also not correlated significantly to the October peak of adults ($r = 0.386$; $P = 0.393$).

Damage Assessment

There was a significant, positive correlation ($N = 741$; $r = 0.468$; $P < 0.001$) between the number of larval feeding lesions and the percentage area of taproot damage calculated for individual roots. Counting lesions could be an efficient way to quantify taproot damage as it can be achieved at a much faster rate. June 2015 and 2016 peak larval densities were not significantly correlated to visual taproot damage ratings ($N = 11$; $r = 0.033$; $P = 0.923$), crown damage ratings ($r = 0.107$; $P = 0.753$), area of taproot damage ($r = -0.261$; $P = 0.438$), or percent taproot damage ($r = -0.191$; $P = 0.574$) from roots collected in July 29, 2015 and August 1, 2016. The roots from these collection periods were chosen for analysis to represent the end of season damage levels because larvae had pupated at this time. The correlation between overall end of season amount of taproot damage and percent of taproot damage was not significantly correlated ($r = 0.501$; $P = 0.116$).

Because taproot damage is accumulative (Dickason et al. 1968) and old damage is difficult to distinguish from new damage, the change between damage metrics within fields for roots collected before (May 15) and after (August 1) the 2016 larval period were compared with peak larval densities. Significant positive correlations existed

between larval densities and average difference between pre to post larval period visual crown damage rating ($N = 7$; $r = 0.867$; $P = 0.012$), taproot damage rating ($r = 0.928$; $P = 0.002$), average amount of taproot damage ($r = 0.913$; $P = 0.051$), and average percent taproot damage ($r = 0.782$; $P = 0.038$) meaning that higher larval densities increased the severity of root damage more than lower densities. These results may explain why larval populations were not correlated with end of season damage measurements overall which could have been attributed to the masking effect of old and new damage being indistinct. The correlation between field age and reduction in rootlets over the season was positive ($r = 0.740$; $P = 0.057$). A significant negative correlation was found between average peak larval populations and the pre to post-larval period difference in the total number of rootlets counted per root ($r = -0.907$; $P = 0.005$). This pattern was driven by only two of the fields, which were in their second year of production and had the highest numbers of larvae ($F = 4.58$; df = 1; $P = 0.076$), having a lower number of rootlets after the larval period than before it accounting for a 31.92% reduction in rootlet density.

Stand age was significantly and positively correlated to area of taproot damage collected at the end of the 2015 and 2016 larval period ($N = 11$; $r = 0.657$; $P = 0.028$) but not percentage of taproot damage ($r = 0.359$; $P = 0.279$). The average amounts of accumulated taproot damage for fields in their first, second, third, or fourth or more year of damage, which corresponds to the second, third, fourth, or at least fifth year of production, were statistically unequal ($F = 6.87$; df = 2, 8; $P = 0.017$). Posthoc means comparisons and planned contrasts of the average total amount of damage indicated that the mean damage from first damage year fields was significantly less than fields in their

second to fourth or more year of damage (F = 16.32; df = 1; P = 0.005; Fig. 6). Stands in their second year of damage also had statistically less damage than fields with three or more years of damage (F = 4.31; df = 1; P = 0.077). Stands in their third year of damage did not have significantly different amounts of damage than fields at least in their fourth year of damage (F = 0.11; df = 1; P = 0.747). However, the average percentages of damage between field age classes were not statistically different overall (F = 2.03; df = 2, 8; P = 0.198). The correlation found between 2015 and 2016 field ages and peak larval numbers was not significant (r = -0.405; P = 0.217). A regression (R^2 = 0.082, F = 0.80; df = 1, 9; P = .0395) did not exhibit a significant relationship between field age and peak larval densities.

Discussion

Most of what is known about the general biology, timing of life stages, and levels of damage associated with CRC in North America comes from research in the eastern U.S. (Phillips and Ditman 1962, Roberts et al. 1979, Leibee et al. 1981, Powell and Campbell 1984, Godfrey and Yeargan 1985, Quinn and Hower 1985). Our current understanding, broadly speaking, is that CRC eggs hatch in the spring, larvae pupate midsummer, and the new generation of adults go on to overwinter resuming activity and depositing eggs in the spring thus completing one generation per year. In a general sense, this is similar to our regional observations with the exception of a few important differences. Dissimilarities found in ovipositional timing, occurring mostly in fall in our area, and timing of peak adult densities, which are locally quite low in spring, demonstrate key regional differences in life stages present during the sensitive time of

overwintering, which has been considered to play a large role in CRC population dynamics (Kalb et al. 1994). Larvae are reported to occur as early as April in New Jersey (Lau and Filmer 1959). A few larvae, which were primarily first instars, were present in three fields checked in early April 2016 along with a soil egg load that continued to decline afterward suggesting that the larval period was just beginning at this time (Fig. 2-1b, Fig 2-2b.). The slightly larger, more abundant larvae occurring in mid-May coupled with low whole egg densities suggests that peak egg hatching occurred between April to early May 2016 beginning the larval period which continued until mid-July peaking in June. Overall, the average peak larval densities found in our study (27.71 per m^2 in 2015; 67.66 per m^2 in 2016) were generally reported from eastern North America. Some of the highest average reported densities (converted for comparison) have been reported from by Godfrey and Yeargan (1987) in Kentucky (1232.44 per m^2) and by Thompson and Willis (1967) from the Maritime Provinces, Canada (548.34 and 731.81 per m^2). Our densities were higher than reported by Lau and Filmer (1959) in New Jersey (5.02 per m^2).

CRC has been suggested to be multivoltine in warmer areas although this has not been supported (Webster 1915, Bigger 1930, Powell and Campbell 1984). To confirm CRCs univoltine lifecycle in Cache Valley, two fields were checked for fall and winter second generation larvae since oviposition occurred before this time. A small number of newly hatched ca. first instars were found in late fall to early winter (Fig. 2-1b, 2-2b). Similar small numbers of *Sitona* hatching in the fall, presumed to be CRC, were recorded in the Maritime Provinces of Canada (Thompson and Willis 1967) but was not seen in

North Carolina where the growing season is longer making the a second generation more

likely (Powell and Campbell 1984). While overwintering larvae have been recognized, it

is unknown what level of survivorship a partial second generation of larvae would have

or if they would contribute substantially to spring larval populations. In 2016, during

early April sampling, very few large larvae were recovered (4 of 35 recovered) during

this time with the other larvae being within the first couple stadia of growth (Fig. 2-2b).

The large larvae were tentatively identified as CRC based on mandibular anatomy

(Manglitz et al. 1963) and two were able to be reared to adulthood to confirm their

identity as CRC. It did not appear that overwintering larvae, if they continue to grow over

winter, significantly contributed to 2016 spring populations.

In both 2015 and 2016, peak densities of pupae were observed around the very

end of June and the first of July. While this occurs a month or two later than in North

Carolina (Powell and Campbell 1984), the general occurrence of pupation in mid to late

June and early July is similar to the time established for many other areas of the United

States (Bigger 1930, Marshall and Wilbur 1934, Lau and Filmer 1959, Thompson and

Willis 1967, Leibee et al. 1981). Thus it appears that the larval period spans April to late

June and early July in Cache Valley with peak larval densities occurring at the end of

May and early June when taproots are damaged from fourth and fifth instar feeding (Fig.

2-1, 2-2).

Adult capture rates peaked twice in fall with the first peak occurring two weeks

earlier in 2015 than in 2016 and the second peak occurring a month earlier in 2015 than

in 2016. A distinct seasonal reduction in recovered adults was observed both years

occurring a month earlier in 2015 than 2016. A similar seasonal reduction of adults

recovered from the soil surface was seen in eastern Canada (Thompson and Willis 1967).

It may be that this reduction was due to reproductively immature adults emigrating from

fields to bordering habitat or seeking shelter in secluded areas at the soil surface,

precluding sampling collection, for summer aestivation at this time (Phillips and Ditman

1962, Roberts et al. 1979, Culik and Weaver 1994). The overall lack of oviposition at this

time for 2015 and 2016 may further support this hypothesis as oviposition occurs after

summer aestivation once the reproductive system develops (Rautapää and Markkula

1966, Powell and Campbell 1984). Since post-aestivatory activity is initiated by seasonal

temperature reduction instead of photoperiod (Leibee et al. 1980a), annual timing

differences in activity and oviposition are expected. Further monitoring and collection

during this crucial transition in life history would provide valuable insight into the

reproductive timing of CRC, which may have implications for population control (Pausch

et al. 1980).

Although more collection information in spring would be useful in our area, for

2015 and 2016, adult survival overwinter appeared to be low. In some areas, such as New

Jersey (Lau and Filmer 1959) and Maryland (Phillips and Ditman 1962), adult

populations in spring may be high with mortality rates increasing heavily in mid to late

May (Bigger 1930, Jewett 1934). CRC oviposition in many eastern locales is known to

occur in spring and, depending on the overwintering capacity of adults, may contribute

the majority of individuals to spring larval populations (Bigger 1930, Phillips and Ditman

1962, Ng et al. 1977). In other areas, adult populations may be highly reduced over

winter and contribute little to new generation CRC spring populations (Thompson and

Willis 1967, Kalb et al. 1994). In these situations, oviposition in fall may contribute the

majority of the spring egg loads (Roberts et al. 1979, Quinn and Hower 1985, Dysart

1990, Kalb et al. 1994). Fall oviposition has been noted to occur in Utah, although the

degree to which had remained unknown (Davis et al. 1976). Egg densities in 2016 were

highest in early December during the last sample period. It appears that during our

research heavily ovipositing adults in fall influenced Cache Valley populations the most

since relatively few adults were present in spring (Fig. 2-3). If this was the case, then low

counts of CRC adults during spring scouting would not be indicative of the true scale of

CRC populations present in the field which produce root damaging larvae some time

later. Since only two fields could be visited both years, additional expansion of sampling

to more fields through the fall to spring transition would enable more thorough

quantitative analyses than was possible in this research. Additional collection information

is also needed to see if the overwintering dynamics we have hypothesized are the typical

annual patterns given that the climactic conditions faced by organisms during inter-

seasonal transitions in our area are extreme and highly variable both during the season

and between years. More information on the correlation between stages is also needed

from a population monitoring and predictive standpoint. Using 2016 data, we did not see

significant correlations between either peak of adults within fields or peak egg densities

found in December which would presumably overwinter and hatch in the spring. It is not

known how those eggs loads were correlated to larval densities the following year, but it

would already seem that using adult populations, which although are easily sampled and

can be collected by growers with little training, to predict damaging larval densities would not be met with success.

The significant correlation between the number of CRC larval feeding lesions and percentage of taproot area damaged by larvae was similar to the results of Pesho (1975) who found that the number of feeding lesions had a positive linear relationship with percentage of surface area damaged close to a 1 to 1 ratio. Since calculating the amount of taproot damage from scanned images as described here was time consuming, taking approximately two to three hours total per soil core to process and generate data, researchers with limited resources needing to more efficiently quantify damage may consider counting lesions instead. Manually measuring the length and width of scars and approximating area by multiplication has also been used to quantify damage and could be less time intensive but was not tested here (Hower et al. 1995).

The differences in correlations between overall end of season damage metrics and peak larval densities, being non-significant, when compared with the change in damage measurements occurring during the larval period, was interesting but was perhaps an unsurprising result. Where alfalfa stands have been under larval feeding pressure accumulating damage for multiple years (Dickason et al. 1968), the addition of damage from earlier generations of CRC to estimates of root damage present at the time was confounding. Because of this effect, comparing a single generation of larval densities to end of season damage in a field that has had multiple years of additive damage was misleading. A better understanding of damage accumulation rate is necessary in linking larval populations to root damage and, with the ultimate goal of understanding the

economic effects of CRC, responses in yield. When larval populations and damage were investigated further by comparing the increase in damage metrics from before and after the larval period, significant positive correlations were seen.

Another noteworthy observation was the pattern in rootlet reduction during the larval period. The fields that were seen to have reductions in rootlet density were in their first damage year and hosted the highest larval densities. In older fields, rootlet density was low overall having only 53.81% of the density two year old fields had. Whether this was due to accumulative larval pruning damage over time was unknown but loss of fine, subsurface roots could especially have an impact on water absorption (Houston 1955). Drought conditions further aggravate yield reductions by CRC which may mean that producers in drought prone areas may need to consider the pest status of CRC in their region (Godfrey and Yeargan 1985). Alternatively, if lateral rootlet densities decline in stands over time as part of an overall shift in root morphology, the same pattern may be seen where young stands with high lateral root counts could potentially support higher larval CRC populations due to increased food resources available to small larvae reducing intraspecific competition and density dependent mortality (Goldson and French 1983, Quinn and Hower 1986a).

A significant positive relationship between stand age and area of damage was observed. Based on the fields sampled, fields accumulated damage rather quickly. Stands at the end of their first year of damage already had 61.35% of the damage level seen the following year. Damage only would increase from fields in the second year of damage 25.82% compared to the damage averaged across all fields in their third or more year of

damage. There was a minimal 4.97% increase in damage between third year of damage fields to fourth of more year of damage fields. The 53.05% increase observed between first and third year of damage stands is lower than the 87.34% increase reported by Hower et al. (1995) but still may be considered a significant result. Initial reductions in plant densities caused by pest feeding in second year fields can still be tracked through additional seasons (Godfrey and Yeargan 1989). While growth compensation by plants in fields with reduced crown densities can recover from potential reductions in yield (Dintenfass and Brown 1988b), it is currently unknown how larval populations damaging second year stands in our region are contributing to premature stand declines. Future research into the effects of controlling CRC yield reductions during this stage of stand development and how control responses are carried over the life of the stand could provide insight on not only damage, but how CRC control methods applied during this time to delay the accumulation of damage could be used economically. If timed correctly, it could mean that even expensive control options like entomopathogenic nematodes which kill CRC larvae (Jaworska and Wiech 1988, Loya and Hower 2002) could be optimized for economic control.

Conclusion

In the past, highly mobile and residually persistent pesticides, which have since been phased-out, were used to combat alfalfa weevil larvae may have offered non-target soil-borne pest suppression (Leath and Hower 1993, Hower et al. 1995). Carbofuran specifically, which has systemic plant action, may have inhibited CRC populations (Neal and Ratcliffe 1975, DiSanzo 1981). Modern insecticide chemistries for alfalfa weevil

control are applied before mid-May in Cache Valley when CRC populations are in the

larval stage below ground presumably being unaffected by contemporary pesticide

applications. Current chemical control options only exist for adult populations and not

larvae (Reitz, 2016). Spraying for adults in an effort to control larval numbers has not

been recommended (Wenninger and Shewmaker, 2014). Alternative approaches will

need to be considered to control CRC such as breeding for resistance (Powell et al. 1983,

Byers et al. 1996). Other novel control measures such as the use of entomopathogenic

nematodes (Loya and Hower 2002, 2003) or using biofumigation to suppress larvae may

be a useful component of a pest management program. Applicative control measures

need to be timed when pests are most susceptible to control to effectively reduce

economic damage as part of an integrated pest management program. The newfound

knowledge of CRC phenology in northern Utah provides an improved framework to

advance the future development of management strategies targeting susceptible life

stages in our region.

Table and Figures

Table 2-1 Field collection site location and characteristic information

Field identification	Location	Coordinates	Year sampled	Field age (years)	Topsoil texture [1]	Additional characteristics	Field size (Hectares)
Creech	Cornish, Utah	41°59'28.57"N, 111°57'25.90"W	2015, 2016	3, 4	Loamy fine sand (7.5% clay, 85.0% sand)	Wheel line irrigation	24.08
Larson	Trenton, Utah	41°52'51.51"N, 111°56'58.13"W	2015, 2016	2, 3	Silt loam (22.5% clay, 9.5% sand)	Center pivot irrigation; voles present	62.93
Nielson	Hyrum, Utah	41°38'14.06"N, 111°49'20.11"W	2015	7	Silt loam (20 % clay, 11.4% sand), rocky	Wheel line irrigation; many weeds (*Latuca serriola*, grasses), and voles present	14.49
Spackman	Trenton, Utah	41°54'7.34"N, 111°55'53.15"W	2015	4	Fine sandy loam (12% clay, 67.6% sand)	Flood irrigation	3.32
Caine Dairy 5	College Ward, Utah	41°38'59.00"N, 111°54'9.98"W	2016	5	Loam (22.5% clay, 33.3% sand)	Flood irrigation	12.90
Caine Dairy 6	College Ward, Utah	41°38'58.45"N, 111°54'12.85"W	2016	2	Loam (22.5% clay, 33.3% sand)	Flood irrigation	3.83
Richmond 5	Richmond, Utah	41°53'16.35"N, 111°49'44.67"W	2016	5	Silty clay loam (37.5% clay, 7.6% sand)	Center pivot irrigation; manure application (2016)	16.57
Richmond 6	Richmond, Utah	41°53'5.47"N, 111°49'46.06"W	2016	2	Silt loam (12.5% clay, 30.9% sand)	Center pivot irrigation; manure application (2016)	14.66
Wellsville	Wellsville, Utah	41°39'43.07"N, 111°54'56.42"W	2016	3	Loam (22.5% clay, 33.3% sand)	Center pivot irrigation; synthetic fertilizer & insecticide for aphid control (2016)	4.96

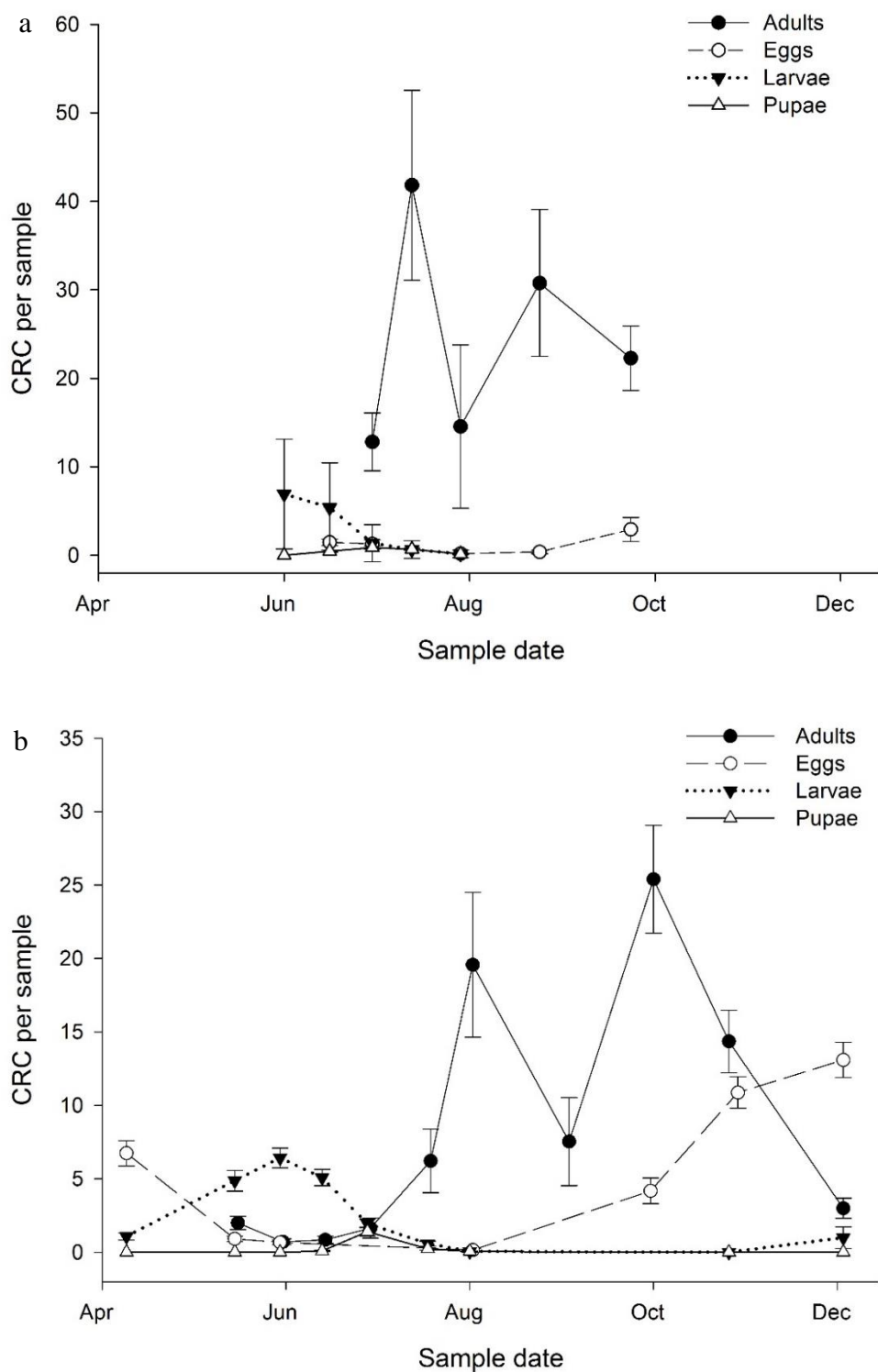1- Soil information obtained from SoilWeb (2017)

Figure 2-1 Seasonal distribution of CRC life stages found in Cache Valley, Utah in (a) 2015 and (b) 2016. Values are means ±1 SE.
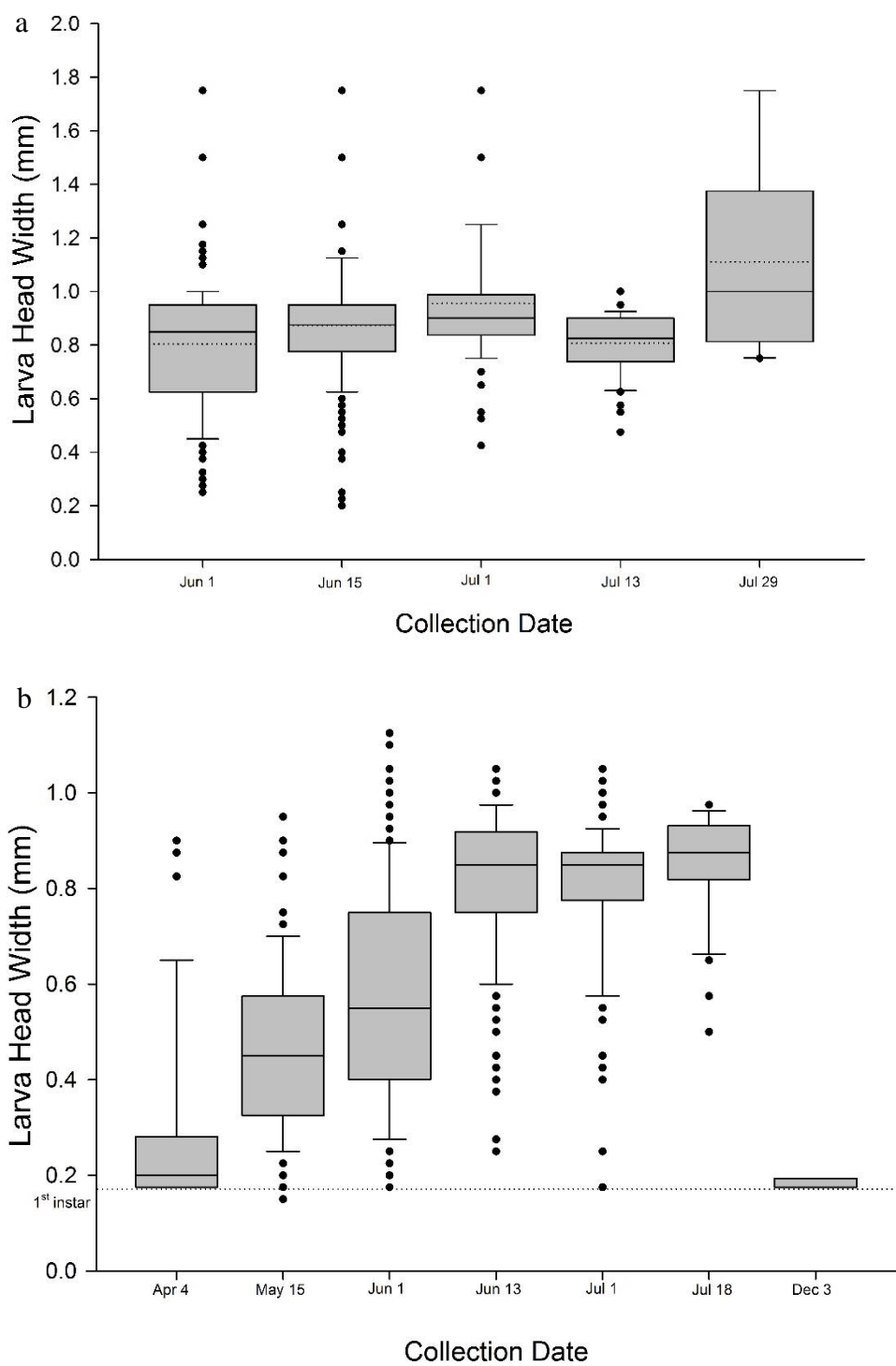
Figure 2-2 Larval size across the field season in (a) 2015 and (b) 2016. Box divisions are means with upper and lower quartiles.
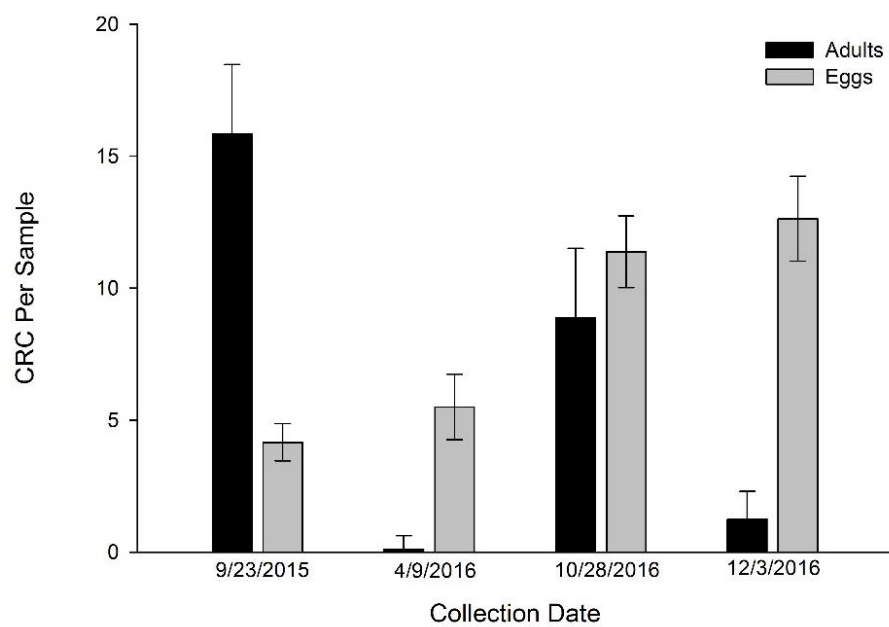
Figure 2-3 Potential overwintering stages of CRC present in the spring and fall. Adult numbers are shown in black bars and eggs are shown in grey bars. Values are means ±1 SE.
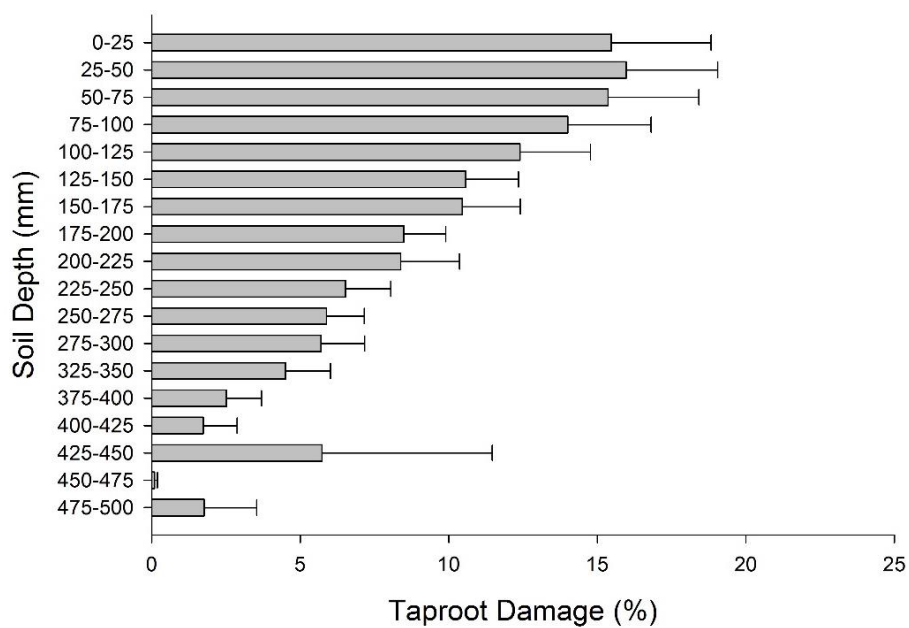
Figure 2-4 Progression of taproot damage through the soil profile across all field sites. Values are means ±1 SE.

Figure 2-5 Accumulation taproot damage occuring in different field age classes ($N = 10$). Tukey's HSD groupings of taproot damage with different letters are significantly different ($P < 0.05$). Values are means ±1 SE.

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CHAPTER III

BIOFUMIGATION EFFECTS ON CLOVER ROOT CURCULIO

Abstract

Clover root curculio (CRC), *Sitona hispidulus* (Fab.), is a root-feeding pest of clovers (*Trifolium* spp.) and alfalfa (*Medicago sativa* L.), which can reduce crop yield, stand life, and increase crop exposure to plant pathogens. Following the federal ban of carbofuran and other synthetic soil fumigants, soil-dwelling pests in alfalfa have received increasing attention due to the difficulty in managing them in established stands and having few management options. Biofumigation is an alternative to synthetic soil fumigants where biofumigant plants are grown, then chopped and incorporated into soil where toxic plant chemicals volatilize suppressing soil pests. Biofumigant cover crops, such as *Brassica* and *Sinapis* spp., provide one possible management option that suppresses soil-borne pests in other cropping systems but has received little attention in alfalfa production. The objectives of this study were 1) to determine the direct and indirect suppressive effects of biofumigation on CRC, and 2) to determine the agronomic benefits of using biofumigant cover crops in rotation with alfalfa. We conducted a repeated field experiment in northern Utah along with supplementary greenhouse experiments in 2015 and 2016. We quantified the effects of biofumigation on adult feeding, area avoidance, oviposition suppression, subsequent larval damage, and stand establishment. We found that the soil incorporation of cover crops can reduce CRC larval damage and that biofumigant soil incorporation can suppress CRC adult feeding behavior. Overall, the response of CRC to biofumigants was variable. We did not see any

direct benefits or disadvantages of biofumigant cover crops on alfalfa production but future integration of mustards to achieve integrated pest management goals as part of an agronomically feasible alfalfa rotation requires additional research and optimization.

Introduction

The recent phase-out of soil active, broad-spectrum insecticides and synthetic soil fumigants (e.g. carbofuran and methyl bromide) by federal agencies has left growers of a wide variety of crops without chemical control options for soil-dwelling pests. Biofumigation is the practice of incorporating plant biomass with fumigant properties, such as cover crops grown as a green manure or applying seed meals, into the soil for pest suppression (Brown and Morra 1997, Rosa et al. 1997, Kirkegaard and Sarwar 1998, Sarwar and Kirkegaard 1998, Sarwar et al. 1998, Fahey et al. 2001, Matthiessen and Kirkegaard 2006). Primarily, the biocidal properties of these biofumigant crops come from a suite of volatile compounds released into the soil during the hydrolytic degradation of glucosinolates which are commonly found in brassicaceous plants (Kjaer 1976, Sang et al. 1984, Mojtahedi et al. 1993, Fahey et al. 2001, Buskov et al. 2002, Morra and Kirkegaard 2002, Turk and Tawaha 2003, Matthiessen and Kirkegaard 2006, Velasco et al. 2008). Isothiocyanates, a common product of glucosinolate hydrolysis, are reported to have insecticidal properties. For example, vapors from Indian mustard (*Brassica juncea* L.) plant tissues and seed meal resulting from glucosinolate degradation negatively affected whitefringed weevil (*Naupactua leucoloma* (Boheman)) larval survival (Matthiessen and Shackleton 2000). Eggs of the black vine weevil (*Otiorhynchus sulcatus* Fab.) exhibited increasing mortality correlated with exposure to isothiocyanates

with increased molecular weights and greater lipophilicity (non-polarity) (Borek et al.

1998). A 1.93% and 8.69% soil incorporation rate of active ground 'Dwarf Essex'

rapeseed meal killed 50% and 90% of black vine weevil larvae, respectively, from

released isothiocyanates (Borek et al. 1997). Furthermore, biofumigation using Ethiopian

mustard (*Brassica carinata* A. Braun) seed meal reduced oviposition in Colorado potato

beetles (*Leptinotarsa decemlineata* Say) by 50% (Henderson et al. 2009). Together, this

suggests that biofumigation can directly affect soil pest survival and indirectly affect their

reproductive behavior and can be a key component of soil pest management.

Clover root curculio (CRC), *Sitona hispidulus* (Fab.), damages legume forage

crops throughout the U.S. (Jewett 1934, Marshall and Wilbur 1934, Phillips and Ditman

1962, Dickason et al. 1968, James et al. 1980) and can reduce yields by 8.4% to 18.6%

(Godfrey and Yeargan 1987, Hower et al. 1995), reduce forage quality (Godfrey and

Yeargan 1987, Godfrey et al. 1987, Hower et al. 1995), and accelerate stand decline

(Dintenfass and Brown 1988b, Godfrey and Yeargan 1989). In particular, the larval

stages are the damaging life stage where first instars feed within root nodules and later

instars feed on lateral roots and the taproot (Bigger 1930, Marshall and Wilbur 1934, Tan

and Hower 1991). Currently, there are no soil active insecticides registered for use

against CRC larval stages, leaving growers with non-host crop rotation to a non-legume

crop as one of the only fully accepted management options available (Wenninger and

Shewmaker 2014). The life cycle of CRC is closely associated with the soil during the

egg, larval, and pupal stages making biofumigation ideally suited for management of

CRC.

Biofumigation has broad-spectrum activity and is known to alter the non-target microbial ecology of the rhizosphere (Mazzola et al. 2007, Cohen et al. 2005, Bressan et al. 2009, Omirou et al. 2011). Legumes rely on symbiotic nitrogen fixing rhizobia bacteria inhabiting root nodules that mediate plant nutrition and defenses which can subsequently affect pest herbivore preference and performance (Dean et al. 2009, Katayama et al. 2010, Dean et al. 2014). Biofumigant crops can also have phytotoxic effects that can inhibit weed seed germination as well as subsequent crop germination if seeded into green manure amended soil too soon after incorporation (Campbell 1959, Vera et al. 1987, Haramoto and Gallandt 2004). Therefore, it is important to keep in mind the possible negative interactions between the biofumigant and desired crop when developing a sound rotational system. Alternatively, there is also the possibility of biofumigant containing green manure incorporation enhancing the desired crop not only by suppressing pests but also providing agronomic benefits such as improving soil condition (Głąb and Kulig 2008) or scavenging nutrients making them available for the next crop (Justes et al. 1999).

It is currently unknown what direct effects, such as changes in mortality, or indirect effects, such as changes in feeding or ovipositional behavior, biofumigation has against CRC and whether it could be used successfully as part of a forage production system. The compatibility of biofumigation in the alfalfa (*Medicago sativa* L.) cropping system is also not well known. Although the ability of alfalfa to fix nitrogen may not be affected by cover crop amendment (Waddington 1978, Waddington and Bowren 1978), cover crop incorporation can have negative agronomic effects on legumes especially after

Brassica spp. incorporation (Vera et al. 1987). The objectives of this study were 1) to determine the direct and indirect suppressive effects of biofumigation on CRC, and 2) to determine agronomic benefits of using biofumigant cover crops in rotation with alfalfa.

Materials & Methods

Field Experiment 1: Effect of Biofumigation on Resident CRC Activity and Alfalfa Yield

A field trial comparing two varieties of mustard biofumigant crops ('Andante' yellow mustard and 'Caliente 199' an oriental mustard blend) with low and high levels of glucosinolates, respectively, to two control treatments (fallow and 'Monida' oats) was arranged to evaluate subsequent alfalfa production, CRC activity, and oviposition in each treatment. The study was conducted in 2015 (trial 1) and 2016 (trial 2) at the Greenville Utah Agricultural Experimental Station in Logan, Utah. In 2016, an additional mustard treatment of 'Centennial' brown mustard was added for evaluation.

Experimental units were 4.27 m × 9.14 m plots set in a completely randomized block design. Each treatment was replicated five times in trial 1 ($N = 20$) and trial 2 ($N = 25$). The study area was treated in spring with glyphosate (Roundup WeatherMAX®) to remove the previous alfalfa crop and weeds, then tilled by disk. The final seedbed was prepared with a cultipacker. Plots were seeded ca. 6 mm into the soil on June 6, 2015 (trial 1) and on May 31, 2016 (trial 2) with an experimental plot cone seeder at an 8.9 cm inter-row spacing and a rate of 19.2 kg per ha for mustards and 26.8 kg per ha for oats. Oats ('Monida') were used as a biomass control as they are not known to have biofumigant properties to control for any confounding effects that the general

incorporation of organic biomass may have had in the mustard treatments. Plots were supplied with overhead irrigation *ad libitum*. In both trials, an application of lamda-cyhalothrin was made to all plots to suppress an outbreak of cereal leaf beetle (*Oulema melanopus* L.) in the oat treatment (trial 1) and an outbreak of flea beetle (*Phyllotreta cruficerae* Goeze) in mustard treatments (trial 2). Approximately one month after seeding (July 7, 2015, trial 1; June 29, 2016, trial 2), mustards and oats were chopped using a flail head or deck mower and tilled into the soil within each respective plot by using a rotary tiller. Weeds in the fallow treatment plots were removed before tilling the soil. To calculate biomass being incorporated into plots, two subsample clippings per plot (230 cm^2, trial 1; 0.1 m^2, trial 2) were removed to calculate wet and dry weight using a drying oven at 35 °C over four days. The plots were cultipacked to seal volatiles within the soil and received irrigation to saturate the soil profile to increase glucosinolate hydrolysis (Morra and Kirkegaard 2002). Approximately three weeks after biofumigant incorporation, plots were again cultipacked to prepare the soil for planting, and seeded with 'Ranger' alfalfa (commercially pretreated with rhizobia inoculant) ca. 6.4 mm into the soil at a rate of 11.5 kg per ha. Plots were supplied with overhead irrigation *ad libitum* until the end of the growing season.

Effect of Biofumigation Treatment on Resident CRC

To assess the effects of mustard treatments on resident adult CRC during fall colonization, the recently seeded alfalfa was sampled by using an insect suction sampling device made from a leaf blower/vacuum (Echo ES-250) outfitted with a fine mesh organdy collection bag (Rincon-Vitova Insectories #DVAC401) around the 12 cm

diameter opening of the suction tube attachment. One suction sample was taken from a transect down the middle of each plot by placing the vacuum at full throttle over the alfalfa, contacting the soil surface for a one second interval and, in one motion, raised, moved down the transect, and reapplied to the soil surface over the next alfalfa plant. This was repeated 30 times per plot per sample. Samples were taken back to the lab and the number of adult CRC was counted.

To evaluate resident adult CRC feeding activity in trial 1, two clippings of alfalfa were taken per plot from within a 10 cm diameter ring. All of the alfalfa stems were clipped at the soil surface, bagged, and frozen for later processing where the number of characteristic, semicircular feeding notches made by CRC adults were counted (Bigger 1930, Jewett 1934). The total number of separate leaf notches per stem and the percentage of leaflets with notches per sample were recorded.

To evaluate mustard treatment effects on resident CRC oviposition, two 58.06 cm^2 egg soil core subsamples were taken from the center of each plot 2.54 cm below the soil surface near the crown of an alfalfa plant, bagged, then stored in the refrigerator. A modified method of Ng et al. (1977) and Aeschlimann (1975) was used to process egg samples by disaggregating the soil in a 11.4 liter plastic tub of water then washing it through a U.S. standard sieve set (#35, #60) with a gentle water spray. Eggs were then counted with the aid of a stereomicroscope (Leica S6D).

The following field season on July 8, 2016, which was the first year of larval damage, the taproot damage accumulated over the larval period in trial 1 was quantified

by taking two soil cores 11 cm in diameter and ca. 28 cm long from the middle of the plots. Soil cores were soaked in water, broken apart with gentle agitation and a water spray, and the supernatant containing small soil particulates, organic matter, and soil mesofauna was decanted off the top through a U.S. standard sieve set (#5, #10, #35, and #60). The process was repeated until cores were completely disaggregated so that soil-dwelling CRC life stages (larvae, pupae, and un-emerged adults) could be counted.

Alfalfa roots were cleaned and a longitudinal incision was made deep enough into the root to cut through the vascular tissue so that the epidermal tissue, outer cortex and cambial layers could be peeled away from the inner cortex. The excised outer root layers were flattened and overlain with a transparency film sheet with a printed 5 mm grid. The root outline was traced onto the film using permanent marker and all identifiable larval feeding lesions were transcribed on the film. The sheet was then scanned (Canon CanoScan LiDE 60) and larval damage was quantified using ImageJ (ImageJ 1.49f; http://rsbweb.nih.gov/ij/) by calculating the area and percent damage of the taproot.

Effect of Biofumigation Treatment on Yield

In trial 1, one month after seeding, the number of seedlings in three 58.06 cm^2 areas per plot were counted to evaluate germination. The first week of October for both trials, two sample clippings (232 cm^2, trial 1; 0.1m^2, trial 2) were taken from each plot which were dried after the alfalfa was separated from weeds and weighed. For trial 2, two 0.1 m^2 stem counts per plot were also made to provide an additional metric to evaluate stand establishment. For trial 1, spring (May 12, 2016) stem counts and dry biomass

yields for weeds and alfalfa were taken as previously described to evaluate any effects the cover crops may have had on winterkill or spring green-up.

Field Experiment 2: Effect of Biofumigation Treatment on CRC Adult Feeding and Survival in Cages

To evaluate the effect of biofumigation treatments on CRC, an additional experiment was conducted to quantify changes in adult CRC feeding. The experiment was conducted at the Greenville Research Station using the plots and plot setup described in the 2016 Field Experiment (trial 2). Two trials were conducted within one week of each other (August 16 and 22, 2016).

Experimental units were foam clip cages ($36.5 \times 25.4 \times 9.5$ mm, enclosed with mesh no-thrips screen, Bioquip) attached to an alfalfa plant within each plot. Two plants per plot were chosen at the center of each plot ($N = 50$). One plant received a clip cage fastened by staples on the most apical fully expanded trifoliolate leaf. The other plant received a clip cage on the most basal trifoliolate leaf. Cages were supported by being taped to an adjacent marking flag so that plants would not bow with the cage weight. The field was irrigated before adding adult CRC to cages to avoid overhead irrigation affecting the experiment.

Field collected CRC adults were previously kept in a 9-dram vial at 22.5° C, starved for one week, and provided water with a moistened cotton roll (Patterson Brand™). A single adult was added to each field cage and left to feed for 48 hours. Cages containing the leaflets and CRC adults were collected and brought back to the lab to

determine beetle survivorship and feeding damage. To calculate CRC feeding damage, leaflets were flattened between two sheets of transparency film and scanned. ImageJ was used to calculate the area of leaf consumed by each beetle.

Greenhouse Experiment: Effect of Biofumigation on CRC Feeding Behavior and Oviposition

To better understand biofumigant effects on adult CRC mortality, feeding, and oviposition, a greenhouse study was conducted. Similar to the field experiment, three mustard varieties ('Centennial' brown, 'Andante' yellow, and 'Caliente 199' biofumigation blend) were compared with oats ('Monida') and a fallow control. Each of the four crop amendments and the "fallow" control without any amendment were replicated nine times (N=45) across two trials. Each amendment treatment was sown into potting mix (Sun Gro® #3 Professional Growing Mix) and ca. 10 ml of 15-9-12 granular fertilizer (Osmocote® Plus Premium) in 6.65 liter pots (HC Companies™). Treatment plants were grown for three weeks and then the entire plants (roots and shoots) were washed to remove potting soil debris, and cut into ca. 0.5 cm sections and put into a plastic tub. Based on the cover crop green weight averages seen in the field experiment 1 (trial 2), 4.52 grams of cut plant material was weighed to match field incorporation rate for 7.62 cm square pots (Landmark™), the experimental unit. Soil was collected from Greenville, in an area growing corn to ensure there was not a population of CRC in the soil, and sifted through a #35 mesh sieve to remove rocks and to verify no CRC life stages were present. Plant material was thoroughly mixed with 250 ml of soil and added to each respective treatment pot. Three replicate pots of each treatment were arranged in a completely randomized block design. To avoid any possible cross contamination of

biofumigant effect through draining water, a 5 cm layer of pea gravel was added to each tray containing pots. Four 'Ranger' alfalfa seeds were added to each pot, and thoroughly watered to initiate germination and growth. Each pot was fixed with a 14 cm long × 7 cm diameter transparency film sheet cylinder cage to contain alfalfa plants and CRC. After four weeks of alfalfa growth, one starved adult CRC male-female pair was added to each cage and secured with tulle mesh netting.

CRC were exposed to alfalfa plants for 48 hours then removed. Because of the small leaf area available for feeding, trials were conducted for short time otherwise damage would have been so severe feeding responses would not be able to be compared. After beetles were removed, mortality was assessed, alfalfa seedlings were scanned, and the area of damage was estimated using ImageJ. If a leaflet was entirely consumed and damage could not be estimated, the average area measured for undamaged leaves within that trial for that leaflet position (i.e. unifoliolate, first trifoliolate, second trifoliolate, etc.) was used. The amended soil from each pot was processed using the same methods as previously described to determine the number of eggs deposited.

Analysis

For field experiment 1, measurements taken of the fumigant crop biomass yield; subsequent alfalfa yields; CRC adult and egg populations; and CRC adult and larval feeding damage were analyzed using the PROC GLM procedure (SAS Studio 3.5) followed by Tukey's HSD posthoc means comparisons when appropriate. For Pearson's correlative analyses of CRC densities and damage, the PROC CORR procedure was used.

PROC GLM was used to analyze adult survival, trial effects, and feeding rates from field experiment 2; for feeding rate, only measures from cages with surviving adults were included in the analysis. The estimated area of leaf consumed by adults was fit with a square root transformation to the meet assumption of normality.

PROC GLM was used for the greenhouse experiment to analyze mortality, oviposition, and feeding rate. Because of unequal seedling germination (one to four plants per pot), the total amount of damage occurring on all leaves was used for analysis. When live adults were not recovered from the cage, oviposition and feeding data were not included in the analysis. Ovipositional and mortality data were transformed using a square root transformation.

Results

Field Experiment 1: Effect of Biofumigation on Resident CRC Activity and Yield

Effect of Biofumigation Treatment on Resident CRC

The number of resident adult CRC collected during fall colonization of plots in their seeding year was not affected by biofumigant treatment in either trial 1 ($F = 0.03$; df $= 3, 16$; $P = 0.992$) or trial 2 ($F = 0.61$; df $= 4, 20$; $P = 0.658$). Likewise, average number of eggs recovered from soil cores was not significantly different among treatments in trial 1 ($F = 0.95$; df $= 3, 16$; $P = 0.442$) or 2 ($F = 0.44$; df $= 4, 20$; $P = 0.777$). The average number of CRC captured per plot was 4.64 ($\pm$ 0.55 SE) for adults and 1.36 ($\pm$ 0.17 SE) for eggs which appeared low for local populations during this time (S. Price, personal observation). Additionally, there was no significant difference in the average number of

distinct leaf notches occurring per stem (F = 0.97; df = 3, 16; P = 0.431) or the average number of damaged leaflets present per stem (F = 1.47; df = 3, 16; P = 0.260) among biofumigant treatments in trial 1.

When soil cores were collected to evaluate the suppressive effects of biofumigants on resident larval populations and root damage for trial 1 the year after seeding, pupation was already underway and larval populations had started to decline evidenced by larvae making up 72.58% of recovered CRC life stages. Recovered life stage densities between treatments were not significant for larvae (F = 0.66; df = 3, 16; P = 0.588), pupae (F = 0.17; df = 3, 16; P = 0.915), or adults (F = 0.08; df = 3, 16; P = 0.970) (Fig. 3-1). When CRC life stages that had recently been feeding on taproots (larvae and pupae) were combined into "soil stages" for analysis, no significant differences were found among treatments (F = 0.33; df = 3, 16; P = 0.804). Adults were not included in the soil stage analysis; only 29.63% of them were still teneral leaving the majority of individuals having already emerged and being without a reliable origin within the plot. If soil amendment had affected belowground CRC life stage populations since hatching, the pattern was no longer evident.

The average percent of larval damage occurring on taproots was not significant among all treatments (F = 2.04; df = 3, 16; P = 0.149) (Fig. 3-2). However, planned contrasts showed a significant difference in larval damage between treatments when both mustard varieties were compared with the oat treatment (F = 3.62; df = 1; P = 0.075) with oat treatment plots having 22.51% less damage than mustard plots. Larval and soil stage densities were not significantly different among treatments for this comparison (F = 0.13;

df = 1; P = 0.72; F = 0.00; df = 1; P = 0.988, respectively). When the larval damage across all treatments with crop incorporations were compared with the fallow treatment, differences were not significant (F = 0.00, df = 1; P = 0.98). To better understand the indirect effects of fumigants on larval feeding rates per individual, correlations between soil stage densities and percent taproot damage were made. Surprisingly, there was no significant correlation between soil stage densities and percent taproot damage across all treatments (N = 20; r = 0.308; P = 0.187) even though no confounding factor existed stemming from past annual larval damage. Within treatments, no significant correlation between soil stage densities and taproot damage existed in 'Andante' mustard (N = 5; r = -0.107; P = 0.864), oat (N = 5; r = 0.571; P = 0.315), or fallow (N = 5; r = 0.357; P = 0.555) treatments but there was a significant positive correlation between the soil stages and taproot damage in the 'Caliente 199' treatment (N = 5; r = 0.894; P = 0.041).

Effect of Biofumigation Treatment on Yield

The yields of the different cover crops before incorporation were not significantly different in green biomass (F = 0.76; df = 2, 12; P = 0.491) or dry biomass (F = 3.20; df = 2, 12; P = 0.077) in trial 1 (Fig. 3-1a). In trial 2, yields were significantly different across all cover crops in green biomass (F = 4.92; df = 16, 19; P = 0.013) but were not significantly different in dry biomass (F = 1.75; df = 16, 19; P = 0.198) (Fig. 3-1b). Planned contrasts revealed that oat biomass was significantly less than the average mustard green biomass (F = 7.03; df = 1; P = 0.017) and dry biomass (F = 5.12; df = 1; P = 0.038). As biomass incorporation differences between treatments would have been logistically problematic to control for, no attempt was made to equalize them in the field.

Early counts did not show any significant differences in seedling density ($F = 0.25$; df = 3, 16; $P = 0.863$) implying that biofumigants incorporated did not have a phytotoxic effect on the germination of alfalfa seeds. There was not a significant difference in alfalfa yield ($F = 0.27$; df = 3, 16; $P = 0.846$) or weed yield ($F = 0.45$; df = 3, 16; $P = 0.724$) between biofumigant treatments in trial 1 (Fig. 3-4a) indicating that the cover crop treatment, whether or not it contained biofumigant action from glucosinolates, did not have any positive or negative effect on stand establishment. The following spring, the possible effects of biofumigation on alfalfa winterkill was evaluated by stem counts which were not significantly different between treatments ($F = 0.77$; df = 3, 16; $P = 0.53$). Spring green-up measurements were not significantly different between treatments in dry alfalfa ($F = 1.02$; df = 3, 16; $P = 0.411$) or weed yields ($F = 1.15$; df = 3, 16; $P = 0.359$). Biofumigation did not appear to affect wintering health of the alfalfa. Weeds averaged 2% of dry yield at this time. The expectation of observing mustards affecting alfalfa or weed growth specifically because of biofumigant action over the effect of adding additional organic matter may have been higher in trial 2 since oat green biomass incorporation rate was only 58.90% of the average mustard green biomass rate. However, biofumigant treatments did not significantly affect alfalfa stem counts ($F = 0.20$; df = 4, 20; $P = 0.937$), yields ($F = 0.31$; df = 4, 20; $P = 0.867$), or weed yields ($F = 2.66$; df = 4, 20; $P = 0.063$) in trial 2 indicating no positive or negative agronomic effects of the cover crop on alfalfa stand establishment (Fig. 3-34b).

Field Experiment 2: Effect of Biofumigation Treatment on CRC Adult Feeding and Survival in Field Cages

The overall direct effect of biofumigant treatments on CRC mortality was not significant ($F = 1.35$; df = 4, 95; $P = 0.246$) with only 3% of individuals dying over the course of the two trials ($N = 100$). There was a significant difference in the overall amount of leaf area removed by CRC adult feeding between the two trials ($F = 55.80$; df = 1, 95; $P < 0.0001$) with the damage occurring in trial 2 averaging 28.68% of the damage observed in trial 1 (Fig. 3-5). There also was a significant effect of cage location on feeding rates ($F = 7.57$; df = 1, 95; $P = 0.007$) with beetles caged on bottom leaves removing 49.66% less of the area removed by individuals restricted to leaves at the top of the plants (Fig. 3-6). There also was a significant interaction between trials and cage location ($F = 35.60$; df = 3, 93; $P < 0.0001$; Fig. 3-7). The interactions between treatment and trial ($F = 1.30$; df = 4; $P = 0.275$) and treatment and cage location ($F = 0.36$; df = 4; $P = 0.839$) were not significant. Given this, the significant differences among overall feeding rates modeled with biofumigant treatments, cage location, and time main effects ($F = 6.58$; df = 19, 77; $P < 0.0001$; Fig. 3-8) are driven by differences stemming from time and location. The effect of biofumigation treatments across trials and cage location was not significant ($F = 0.52$, df = 4; $P = 0.722$).

Greenhouse Experiment: Effect of Biofumigation on CRC Mortality, Oviposition, and Feeding Behavior

Adult mortality within cages was 22.58% for trial 1 and 28.57% for trial 2 but the difference was not significant ($F = 0.97$; df = 1, 57; $P = 0.33$). Mortality data for the two trials were analyzed together; treatment did not have a significant direct effect on

mortality ($F = 1.08$; df = 4, 54; $P = 0.376$). Overall ovipositional rates between the two

greenhouse trials were not significantly different ($F = 2.34$; df = 1, 61; $P = 0.131$) so

were pooled for analysis. No significant differences were seen in ovipositional rates in

pots ($N = 63$) receiving different soil amendment treatments ($F = 0.58$; df = 1, 58; $P =$

0.681). Since amount of damage occurring was significantly different between the two

trials ($F = 8.57$; df = 1, 50; $P = 0.005$) with the overall damage in trial 2 being 65.29% of

trial 1 damage, the two trials were analyzed separately. For trial 1, soil amendment did

not have a significant effect on damage ($F = 0.75$; df = 4, 23; $P = 0.566$) (Fig. 3-9a).

Contrasts of the three mustard varieties and non-biofumigant treatment ($F = 0.94$, df = 1;

$P = 0.341$), three mustard varieties and oats ($F = 2.20$, df = 1; $P = 0.152$), and all

amendments and fallow ($F = 0.27$; df = 1; $P = 0.608$) did not show a significant

difference in damage. For trial 2, there was a significant difference in damage between

treatments overall ($F = 5.37$; df = 4, 19; $P = 0.005$) (Fig. 3-9b). Contrasts between

mustards across all three varieties and the average of non-biofumigant treatments ($F =$

13.44; df = 1; $P = 0.002$) and the three mustard varieties and oats ($F = 20.16$, df = 1; $P =$

0.0003) showed significant differences with plants grown in mustard amended soil

receiving lower amounts of damage than controls. Contrasts of the average damage

across the three mustards and fallow ($F = 1.31$; df = 1; $P = 0.265$) and all amendments

and fallow ($F = 0.02$; df = 1; $P = 0.876$) did not show significant differences.

Discussion

Traditional pest management practices that were once widely used in alfalfa

production, such as the use of carbofuran, are no longer an available option in

belowground pest management. Alternative methods of control that are both cost effective and efficient are needed. Biofumigants have the potential to suppress soil-borne insect pests both directly and indirectly by either inducing mortality or affecting normal activity like feeding or oviposition. The effect of biofumigation on CRC and the potential role of biofumigant containing crops as part of an alfalfa production rotation have received little attention. In field experiment 1, we did not see any effect of biofumigant soil amendment on resident adult CRC fall colonization into plots or in their feeding or oviposition activity. Overall, both adult and egg populations were lower than locally expected. Egg samples were collected once in early October, but it is unknown if egg populations may have continued to increase afterward since adults continue to oviposit after this time in our area (S. Price, personal observation). The plot size may have not been large enough to fully observe biofumigant effects on adult behavior. Phillips and Ditman (1962) state that small plots are not suitable for insecticide testing against CRC adults and although our plots were ca. 88% larger than those reported in their study, they may still not have been large enough to account for the high level of adult mobility observed in fall (Culik and Weaver 1994).

The following year after biofumigant incorporation and stand establishment, no effects of soil amendments were observed on larval or soil-dwelling stage densities. We also did not find that larval or soil-dwelling stages densities were correlated to taproot damage measured. When samples were taken in early July, pupation had begun but larval populations were still in the soil and may have continued feeding at this time. Although it has been speculated that large, fifth instars may feed on other root resources moving

away from taproot feeding before pupation (Quinn and Hower 1986a), it has long been assumed that overall larval densities are correlated to taproot damage (Lau and Filmer 1959). It may be that enough small larvae, which feed on smaller lateral roots, were present at this time so that the full severity of taproot damage inflicted by the larval generation was not captured making full assessment of soil amendments difficult. The percentage of taproot damage observed in the plots was 8.08% which falls between the 6.09% damage for fall seeded alfalfa and 14.09% damage for spring seeded alfalfa in their first year of larval damage seen in Kentucky (Dintenfass and Brown 1988b). This level of damage is lower than the 17% (Quinn and Hower 1986b) and 21.3% (Pesho 1975) area of taproot damage noted in fields in their second year of damage when it is most often noticed (Dickason et al. 1968, Cranshaw 1985).

The result of oat plots receiving significantly less larval damage only having 77.49% of the taproot damage observed in plots receiving mustard incorporation was unexpected. A significant difference in larval numbers was not seen, although there may have been a trend for higher larval numbers, making the mechanism of damage suppression obscure. It is unknown if oats have directly biocidal properties against CRC but it does not seem likely. Since the addition of organic biomass, even without biofumigant properties, to the soil can change edaphic microbial communities that are antagonistic to fungi and nematodes (Cohen et al. 2005, Oka 2010), soil amendment with oats may be a possible disruptor of soil-dwelling biocontrol agents with effects similar to biofumigants which suppress entomopathogenic nematodes (Henderson et al. 2009, Ramirez et al. 2009). It is also possible that that oat incorporation benefited alfalfa plant

health making plants more resistant to root damage or improving their ability to compensate for the larval damage occurring. Whatever the mechanism, this result may deserve further attention due to oats, and other small grains, being common in crop rotations where alfalfa and CRC co-occur.

In field experiment one, no differences were seen in alfalfa establishment or alfalfa and weed yields between incorporation treatments indicating biofumigation *per se* did not offer any agronomic benefit nor impairment in either trial. Sequential rotations of alfalfa are not recommended and often result in poor stand establishment due to the autoallelopathic toxicity of medicarpin retained in the soil from the previous stand. The time interval required to alleviate this effect can be affected by numerous factors such as soil type, irrigation quantity, and previous stand density (Mueller et al. 2007). The effects of rotating a short-lived biofumigant crop between alfalfa stands on autotoxicity has not been investigated as is known so far. The average stem counts of 19.9 (trial 1) and 55.16 (trial 2) per 0.1 m^2 are low compared with standard stem density recommendations for profitable alfalfa production (Canevari and Putnam 2007). It is not known to what extent autotoxicity may have reduced stand establishment throughout the field trials; but, if the effects were present, they did not seem to be altered by biofumigation. Alfalfa roots from the previous stand were an issue when establishing the cover crops. The need to drag harrow the field so that the small seeded mustards could be planted shallow was problematic when roots bound in the harrow making seedbed preparation difficult. The amount of time needed to produce a heavy crop of mustards precluded alfalfa establishment until hot and dry field conditions prevailed which also made alfalfa

establishment challenging. In areas where biofumigation using green manures has been used as a component in controlling soil-borne pests such as nematodes, brassicaceous cover crops are often established in fall. Mustard establishment at this time might be a more viable option as autotoxic compounds and roots from the previous alfalfa stand would have a longer time to degrade in the soil. The possibility also exists that a fall seeded mustard green manure rotation could be better integrated into alfalfa production to control soil-borne pests if grown later after a rotational crop, such as winter wheat is harvested in summer, further reducing potential allelopathic effects of the previous alfalfa stand.

In the field experiment 2, feeding rate responses to biofumigation treatments were affected by cage placement (top versus bottom of the plant) and time (occurring within two trial times). It is unknown what variables present influenced the result of feeding on the top leaves being higher than on the bottom leaves and damage during the first trial being higher than during the second trial. Daily environmental patterns between dates or microclimatic variables between cage locations could have influenced feeding behavior. For other *Sitona*, host leaf maturity influences concentrations of both feeding stimulants and deterrents which affects adult diet preference (Akeson et al. 1969). The influence of leaf maturity on CRC feeding preference has not been studied but could also have influenced our results. Future experimental studies restricting CRC adults feeding to leaves of different maturity, location on plants, or on dates with differing weather variables could provide valuable insight into adult CRC dietary preferences, vertical use

of habitat, or behavioral responses to climatic conditions which could further be used in developing control strategies.

In the greenhouse trial, soil incorporation treatments did not affect adult CRC survival or oviposition. Adult feeding responses between trials and treatments were inconsistent. In trial 1, no significant effect of treatments, whether or not they have biofumigant properties, were observed. However, results from trial 2 were interesting in that, when averaged across mustard treatments, feeding was suppressed by 34.80% and 50.62% when compared with non-biofumigant treatments (oats and fallow) and oat incorporation respectively. This supports the hypothesis that biofumigant incorporation is expected to have additional suppressive effects against CRC over a non-biofumigant containing amendment. In comparison, the result of the fallow treatment not being significantly different from mustards overall does not indicate that biofumigants had any effect on feeding. The reason for inconsistent results between trials and between control treatments remains unknown. However, it appears that continued study on biofumigants against CRC may be reasonable especially in quantifying the long-term impacts on adults. Oviposition, mortality, and feeding was measured after only 48 hours of feeding due to small plant size which might not fully measure feeding deterrence after hunger had been satiated or account for the potential effects of biofumigant amendment grown alfalfa consumption by the beetles.

Most studies investigating biofumigation suppressive effects on insect pests have utilized defatted mustard or rapeseed meal often being incorporated into the soil at high rates (Brown et al. 1991, Elberson et al. 1996, Borek et al. 1997, Elberson et al. 1997).

The rates needed may not be economically viable (Elberson et al. 1996, Borek et al. 1997) especially if there are not regional seed meal processing facilities available where oil seed varieties with high biofumigant potential are being pressed. The economic viability of seed meal incorporation becomes even more important in field crops such as alfalfa where incorporations would need to be made across large areas. As a seed meal alternative, plant tissues may be an economically viable source of biofumigant biomass which deserve more research attention. Interestingly, whole plant *Brassica* incorporation for biofumigation can affect potato yields even if soil-dwelling insect pests are not suppressed (Laznik et al. 2014). Because the effects of whole plant incorporation are more variable than the use of defatted seed meals in controlling insect pests, research on whole plant incorporation requires more effort to truly understand insecticidal effects (Furlan et al. 2010). Mustard varieties used (Mojtahedi et al. 1991, Mojtahedi et al. 1993) and timing of cropping (Kirkegaard and Sarwar 1998) can all affect biofumigation potential of mustard green manures leaving many sources of variation to be optimized for pest control (Matthiessen and Kirkegaard 2006).

Conclusion

With no current chemical controls available against the soil-associated pests occurring in multiple cropping systems, biofumigation may be a viable management option in some cropping systems. Biofumigant soil incorporations can negatively affect soil-dwelling pests, including insects, by acting directly on mortality or indirectly by altering pest behaviors such as oviposition. However, biofumigant green manures and other organic soil amendments can also have complex non-target effects that are difficult

to predict, which may either benefit desired crops or negatively affect them due to the phytotoxic properties of biofumigants. Because of non-target effects, incorporation of biomass with potential biofumigation action, such as mustard cover crop green manures, as a component of pest management requires that crop rotations as part of the cropping system be evaluated for compatibility. Alfalfa and other legumes that rely on nitrogen fixing rhizobia bacteria may be particularly sensitive to incompatibilities of biofumigant cover crops. In field trials, we did not see any effect of whole plant soil amendments on resident CRC adult colonization, oviposition, or feeding activity or on resident CRC larval numbers during the first year of alfalfa damage. A statistically significant reduction in CRC larval damage was seen in plots receiving the non-biofumigant containing biomass incorporation of oats. Soil amendment did not have a direct effect on alfalfa establishments or yields in either trial year, however general alfalfa establishment in both trial years was low. In caged adult field trials, the main effect of biofumigation on caged adult feeding rate was confounded by unknown variables being affected by placement of cages and trial. Adult feeding responses to soil amendments in the greenhouse were variable. Significant effects of feeding in one trial indicated that mustard green manure incorporations have the ability to suppress adult CRC feeding. Future research aimed towards mustard biofumigation in controlling soil-borne alfalfa pests may be able to be optimized by changing the varieties used or timing of biofumigant crop rotations.
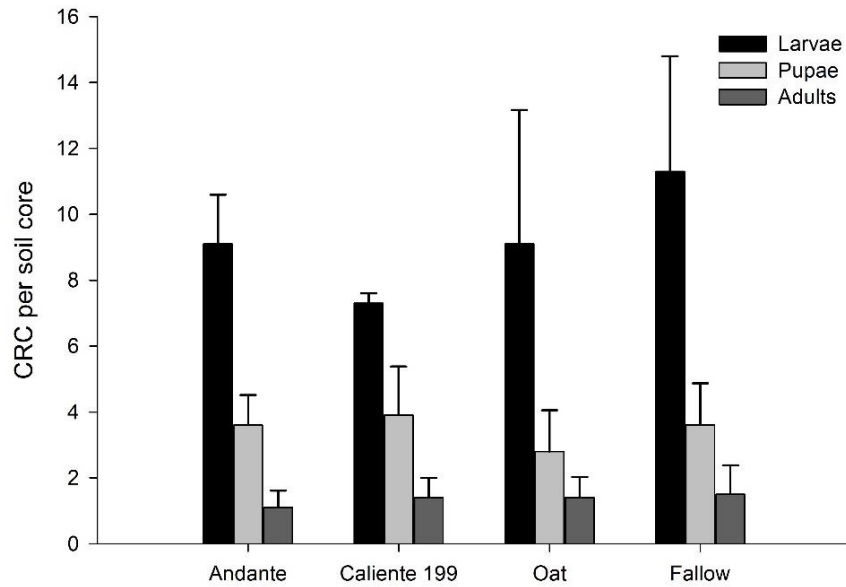
Figures

Figure 3-1 Effect of different biofumigation treatments on recovered belowground CRC life stage densities in field experiment 1, trial 1 ($N = 20$). Larvae numbers are shown in black bars, pupae are shown in light grey bars, and adults are shown in dark grey bars. Values are means ±1 SE.
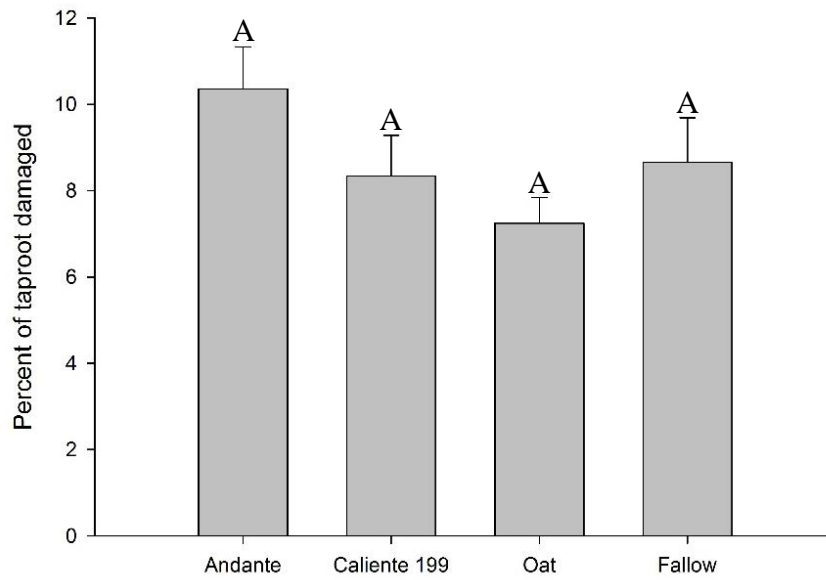
Figure 3-2 Effect of different biofumigation treatments on percent of taproot larval damage in field experiment 1, trial 1 ($N = 5$). Tukey's HSD groupings of taproot damage with different letters are significantly different ($P < 0.05$). Values are means ±1 SE.
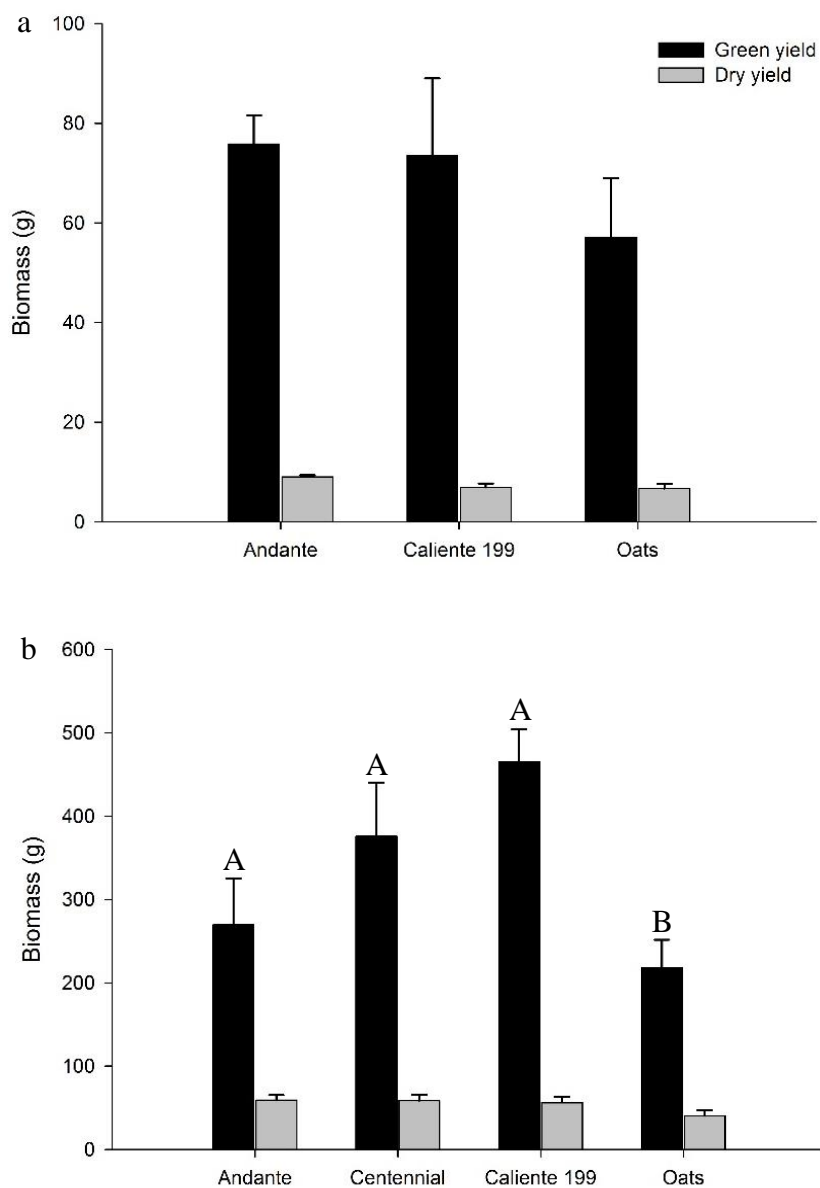
Figure 3-3 Biomass measurements of cover crops before soil incorporation in (a) 2015 ($N = 20$) and (b) 2016 ($N = 25$). Tukey's HSD groupings of green yields with different letters within trial are significantly different ($P < 0.05$). Green weights are shown in black bars and dry weights are shown in grey bars. Values are means ±1 SE.
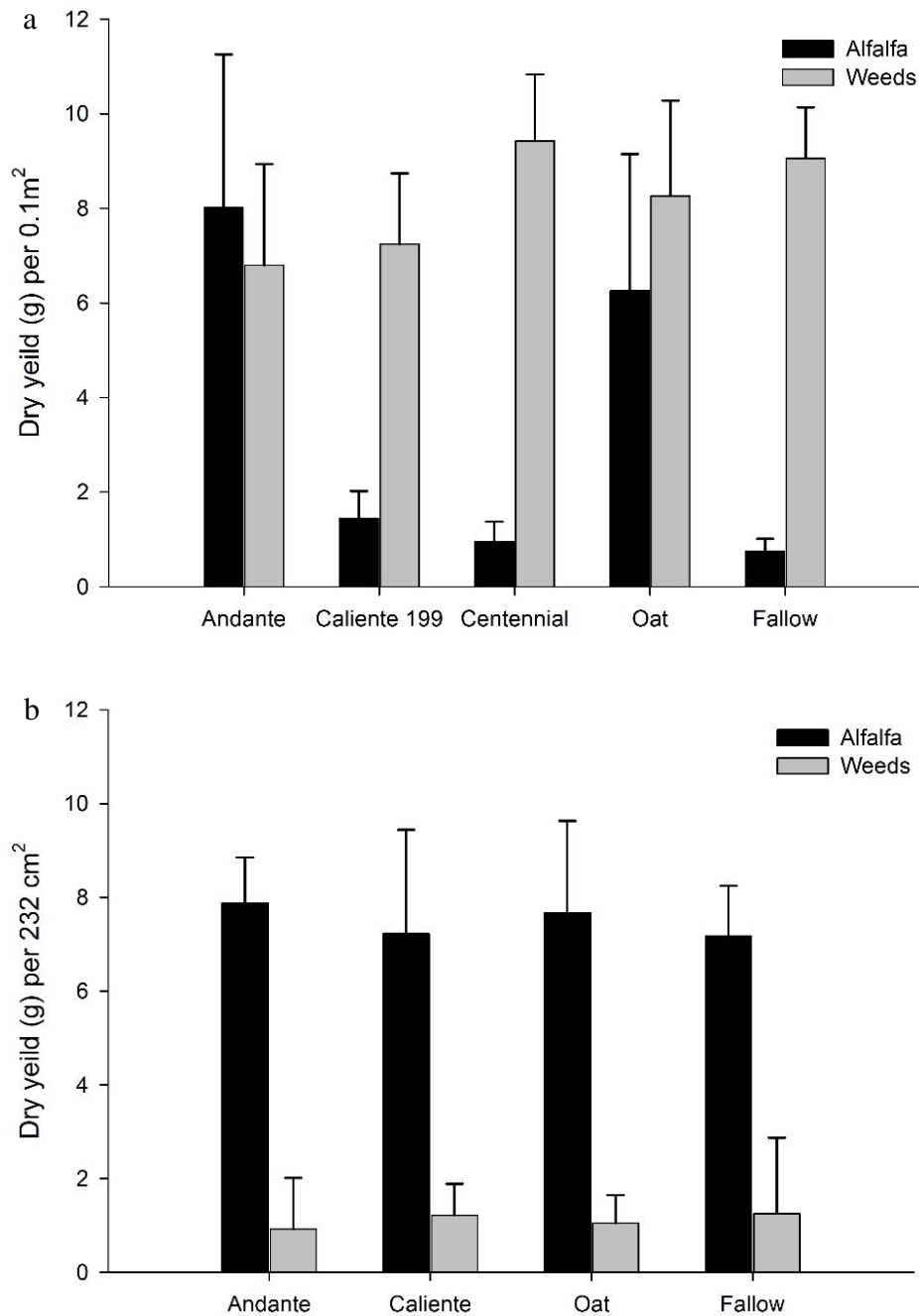
Figure 3-4 Yield response of alfalfa and weeds to biofumigation in (a) 2015 ($N = 20$) and (b) 2016 ($N = 25$). Alfalfa yields shown in black bars and weed yields are shown in grey bars. Values are means ±1 SE.
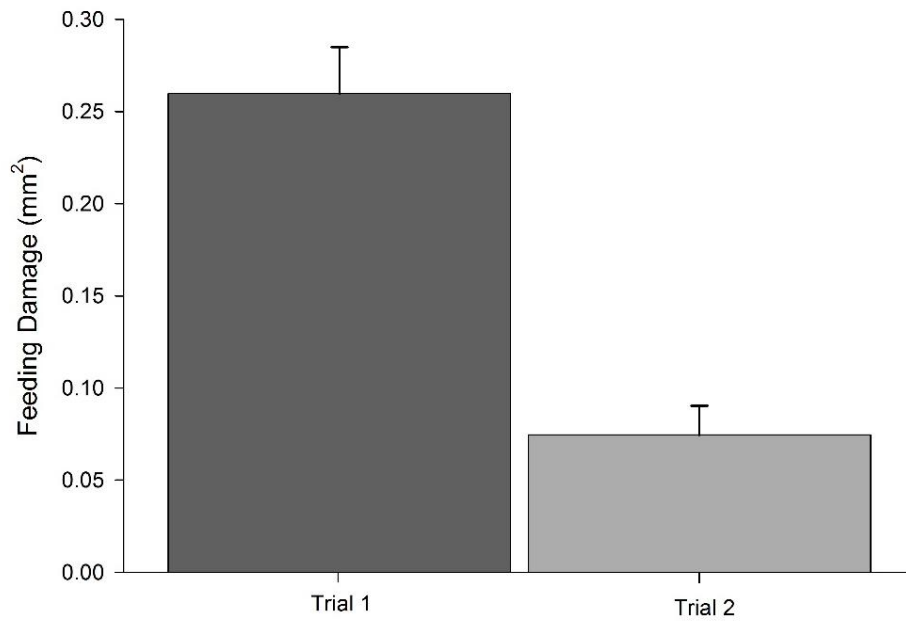
Figure 3-5 Leaf area of adult CRC damage in field experiment 2 between trials. Damage in trail 1 is shown in the dark grey bar and trail 2 in the light grey bar. Values are means ±1 SE.
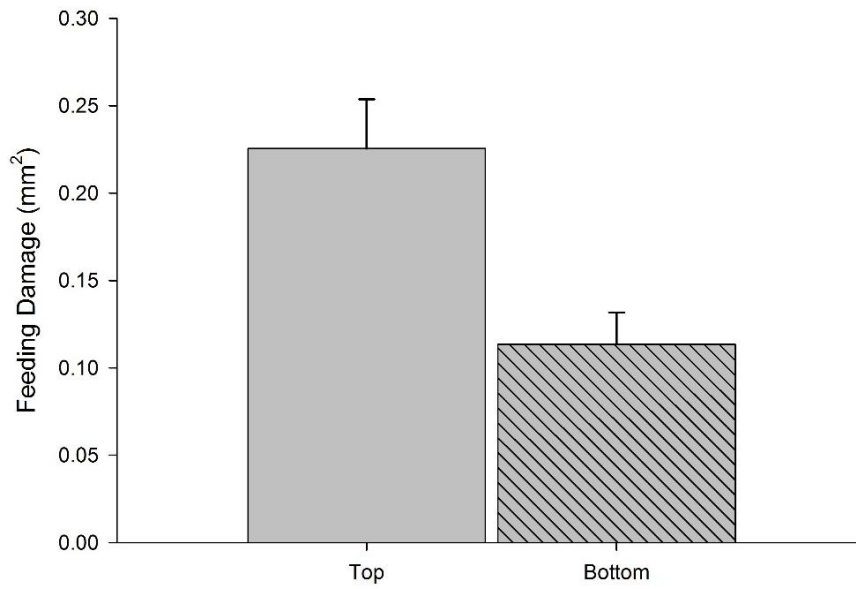
Figure 3-6 Leaf area of adult CRC damage in field experiment 2 between cage location. Damage in cages placed on the top of plants is shown in the solid grey bar and the bottom of plants in the striped grey bar. Values are means ±1 SE.
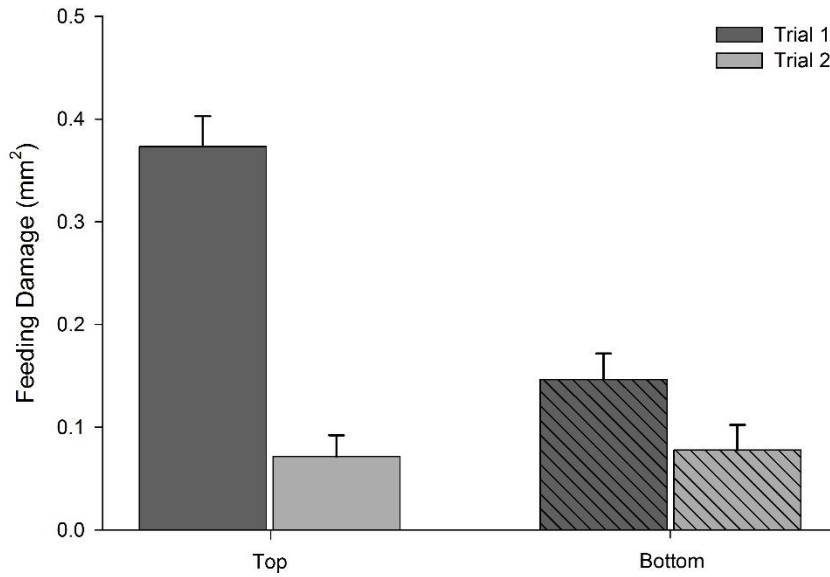
Figure 3-7 Leaf area of adult CRC damage in field experiment 2 between trial and cage location. Damage in cages placed on the top of plants in trial 1 and trial 2 are shown in the solid dark grey and light grey bars, respectively. Damage in cages placed at the bottom of plants in trial 1 and trial 2 are shown in the striped dark grey and light grey bars, respectively. Values are means ±1 SE.
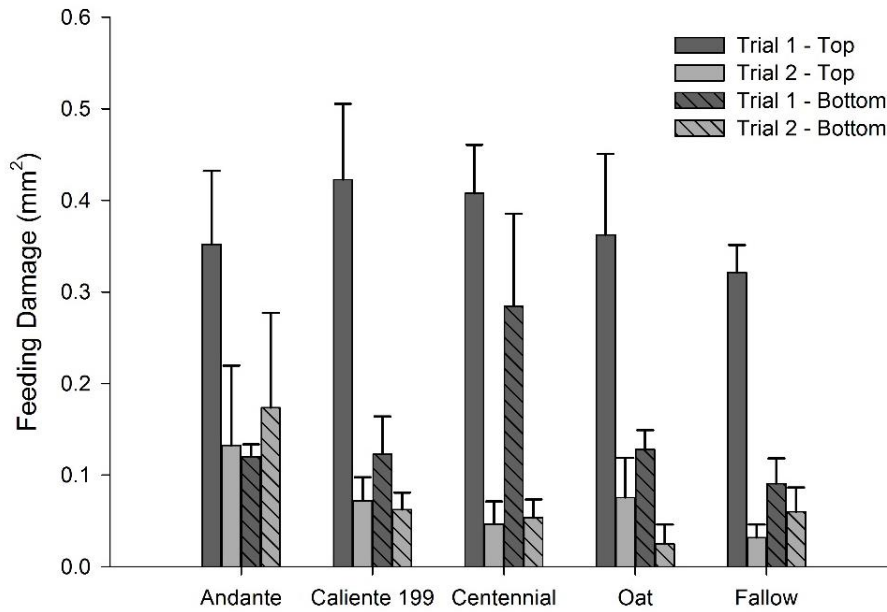
Figure 3-8 Leaf area of adult CRC damage in field experiment 2 between trial, cage location, and biofumigation treatments. Damage in cages placed on the top of plants in trial 1 and trial 2 are shown in the solid dark grey and light grey bars, respectively. Damage in cages placed at the bottom of plants in trial 1 and trial 2 are shown in the striped dark grey and light grey bars, respectively. Values are means ±1 SE.
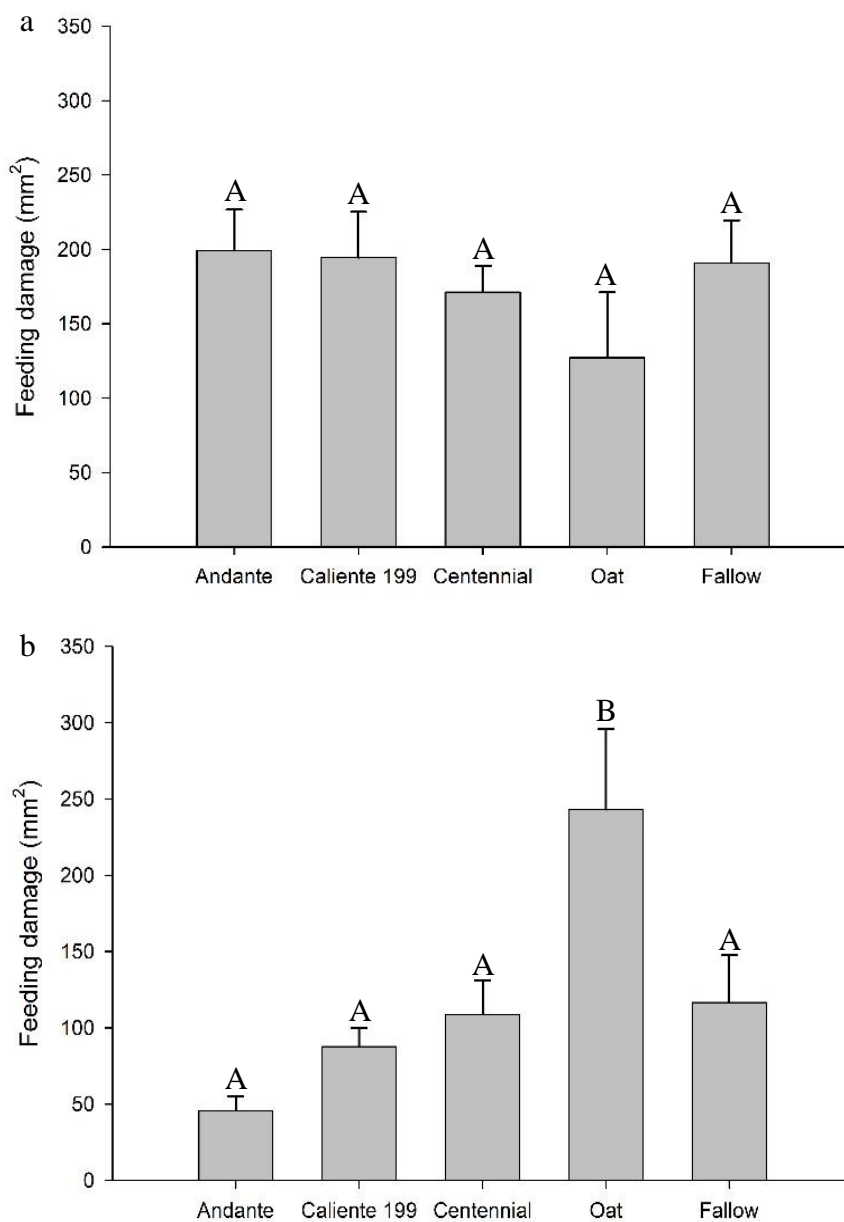
Figure 3-9 Leaf area of adult CRC damage between biofumigation treatments in greenhouse experiment (a) trial 1 and (b) trial 2. Tukey's HSD groupings of feeding damage with different letters within trial are significantly different ($P < 0.05$). Values are means ±1 SE.

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CHAPTER IV

GENERAL SUMMARY AND CONCLUSION

Summary

In order for integrated pest management strategies to be successful, management techniques need to comprehensively integrate both the biological knowledge of the pest and the agronomic knowledge of the crop in question. Clover root curculio (CRC) biology and pest status was poorly understood in the West. Our current knowledge primarily comes from the eastern U.S. and from research conducted many decades ago. The major knowledge gaps in our understanding of CRC in the Intermountain West have been an impediment to the advancement of basic research on this insect and the development of modern control strategies. Without a working knowledge of CRC phenology in our region, even predicting when sampling should occur for belowground life stages of CRC or when damage needs to be assessed within fields has been inaccurate and mostly speculative up to this point.

In my first study, I investigated the timing of CRC life stages, including the damaging larval stage and overwintering stages, during the season and quantified the current extent of CRC damage occurring in our area. I found that eggs start hatching in April to early May and larvae were found in the soil until late June to early July with peak larval densities occurring at the end of May to early June. For Cache Valley, this is about the time of the first alfalfa harvest. I also found that after pupation, adult densities reached two peaks that varied by about one month with the first occurring in mid-July

and early August and the second occurring in late-August and early October based on the year.

In my second study, I evaluated the direct and indirect suppressive effects of biofumigation on CRC and the agronomic benefits of using biofumigant cover crops in rotation with alfalfa. I found that incorporating biofumigants preceding alfalfa planting did not affect fall CRC adult colonization of newly established alfalfa, soil egg loads, or adult feeding. Furthermore, populations of resident larvae damaging roots the following year were also not affected by biofumigant amendments. Incorporation of biofumigants within an alfalfa system appeared to be compatible as alfalfa yield was not affected by amendments. In focused greenhouse trials, biofumigants were seen to have a suppressive effect on adult feeding compared with the non-biofumigant controls. However, this effect was inconsistent across trials. Some of the differences seen in field trials versus the greenhouse suggests that timing of when fumigants are incorporated may be important to investigate.

Conclusion

My research focused on CRC, a pest whose recent increase has gained interest from growers and researchers alike due to a need for more management options. The lack of basic regional knowledge concerning CRC phenology and damage has been a major obstacle to researchers attempting to understand the pest status of CRC in the Intermountain West and develop comprehensive management strategies utilizing modern control methods that are compatible with current alfalfa production systems. The research

presented here represents the first phenology developed for CRC in the western U.S. The differences in overwintering stages and timing of oviposition as compared with the eastern U.S. will need to be taken into account as regional research in CRC control progresses. From my work, we can determine the timing of susceptible life stages and begin to develop programs for monitoring specific life stages and alternative management strategies. One of the first attempts to evaluate an alternative to soil-active insecticides against CRC in alfalfa was the novel application of biofumigant cover crop amendments. Although direct effects on CRC were not seen, the indirect effects of mustards suppressing adult feeding, indicate that, although inconsistently, cover crops can affect CRC. Many variables can affect the biofumigant properties of mustard cover crops and how they can be added into crop rotations; if changes in management to better account for these variables are made to optimize biofumigation impacts, it may become a more attractive option for CRC suppression and as pest management tool for alfalfa in general. In any case, our newfound knowledge of CRC phenology and biology in the region will assist in developing an integrated pest management approach that can improve alfalfa production.