INTEGRATED PEST MANAGEMENT OF CANADA THISTLE (*Cirsium arvense* L.)

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Integrated Pest Management of Canada Thistle (*Cirsium arvense* L.)

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MASTER OF SCIENCE

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ABSTRACT

Canada thistle is a clone forming perennial weed that spreads aggressively and is difficult to control. One approach to managing invasive weeds is integrating numerous tactics instead of relying on a single tactic. Therefore, the objectives of this research were: 1) assess impacts of *Hadroplontus litura*, common sunflower competition, and soil nutrients on Canada thistle, and 2) investigate head capsule morphometrics and model *H. litura* developmental timing. Common sunflower competition, low soil nutrients, and *H. litura* herbivory negatively impacted aspects of Canada thistle growth and reproduction, but effects varied. Additionally, *H. litura* effects on thistle morphology were mild whereas the effects of soil nutrition and competition were persistent throughout the experiment. Histogram analysis and verification via Dyar's rule produced adequate larvae categorization by instar number. Logistic thermal time models developed to predict mean developmental time were most accurate for first instar larvae and least accurate for egg stage.

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"For though we may be the Earth's gardeners, we are also its weeds. And we won't get anywhere until we come to terms with this crucial ambiguity about our role – that we are at once the problem and the only possible solution to the problem."

-Michael Pollan

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LITERATURE REVIEW

Canada Thistle Invasive Status and Distribution. Weedy plants have played a large role in human society since agriculture began approximately ten thousand years ago (Radosevich et al. 1997). A weed has been defined by the Weed Science Society of America to be "any plant that is objectionable or interferes with the activities or welfare of man" (Anonymous 2002). Furthermore, an invasive species is defined as "an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (USDA 1999). Estimated production losses and control costs incurred by the presence of invasive plant species in United States range from 6 to 27 million dollars in range and cropland systems, respectively (Pimentel et al. 2005).

One notable problematic invasive plant in the United States is Canada thistle (*Cirsium arvense* L.). Canada thistle is a serious invasive weed in many croplands, rangelands, and recreational areas around the world. In the United States, it is most prevalent and troublesome in the northeastern, mid-Atlantic, Great Lakes, and Northern Great Plains states (McClay 2002). Canada thistle is listed as a noxious weed under state weed control legislation in 33 states, including North Dakota, South Dakota, and Minnesota (USDA 2010). Currently Canada thistle is found in all 53 counties in North Dakota (EDDMapS 2012) and infests over 400,000 ha of non-cropland in North Dakota, making it the most serious noxious weed problem in the state (NDDA 2007).

Canada Thistle Impacts. This invasive weed thrives in disturbed or moist environments (McClay 2002). Canada thistle infestations can negatively impact crop, range, and public lands. In agricultural fields this weed is an economic concern because it competes with crop plants for resources, thereby lowering yields (McClay 2002). Rangelands are also negatively impacted by

Canada thistle infestations. The prickly nature of mature leaves deters livestock from grazing, thus making infested pastures unsuitable for livestock production (McClay 2002). Canada thistle is not only a troublesome weed in crops and rangelands, but also in Conservation Reserve Program (CRP) land in many states. In Minnesota, Canada thistle occurs in over 75% of CRP land and may serve as an important source for possible seed dispersal into nearby agricultural fields (Jewett et al. 1996).

Canada thistle is a particularly difficult challenge for organic and sustainable producers who wish to limit chemical inputs (Lukashyk et al. 2008) and for land managers who need to control Canada thistle in environmentally sensitive areas where herbicide use may cause undesirable contamination or be prohibited. Infestations of Canada thistle in organic systems are becoming an increasing problem (Lukashyk et al. 2008). With regard to organic cropland, the Economic Research Service reported that in 2008 approximately 1.9 million ha in the United States were in organic production, with 87,642 of those ha in North Dakota (ERS 2008).

Canada Thistle Biology. Canada thistle is a clone forming perennial herb with a deep root system that can spread extensively (Donald 1994; McClay 2002) and give rise to adventitious shoots from root buds throughout the growing season (Tiley 2010); the success of this weed is often attributed to these characteristics. For example, in a field experiment, a root fragment 10 cm long from an 18 wk old plant produced approximately 930 shoots (Nadeau and Vanden Born 1989). The extensive root system allows for vigorous vegetative growth, and Canada thistle patches can spread at a rate of 1 to 2 m per yr (Amor and Harris 1974). Additionally, plants can regenerate from fragments only 6 mm in length (Forsberg 1962).

Research has found that increased nitrogen levels can break Canada thistle root bud dormancy and consequently stimulate shoot production and the spread of a Canada thistle patch

(Hamdoun 1970; McIntyre and Hunter 1975; Nadeau and Vanden Born 1990). In plots fertilized with 100 kg ha^{-1} of nitrogen three times over a 2 yr period, total root length was 67% greater than in unfertilized plots (Nadeau and Vanden Born 1990). Root dry weight was also greater in fertilized treatments. An increase in root length and dry weight led to an increase in the total number of root buds, which increased shoot density in fertilized treatments compared to unfertilized treatments. McIntyre and Hunter (1975) also reported that an increase in nitrogen led to increased root bud production and subsequently three times the number of shoots compared to untreated control plants.

Root storage carbohydrate levels also play a large role in the invasiveness and vigor of Canada thistle. Canada thistle roots undergo seasonal fluctuations of carbohydrate reserves with total nonstructural carbohydrates lowest in the spring due to recent use of reserves for active growth and development and highest in fall when the plant is preparing to overwinter (Otzen and Koridon 1970). Adequate amounts of stored carbohydrates in the root system over the winter months are vital to ensure the next season's growth and shoot production (Nkurunziza and Streibig 2011). Therefore, knowledge of carbohydrate reserves and their seasonality in the root system of Canada thistle may aid in understanding when the weed is most vulnerable to control methods (Tworkoski 1992).

Although Canada thistle reproduces primarily via vegetative shoots, it also produces approximately 5,000 to 40,000 seeds per plant in 1 yr (Derscheid and Schultz 1960). Seeds can remain viable in the soil for up to 20 years (Piper and Andres 1995; Tiley 2010), although approximately 60 to 90% of the seeds germinate within 1 yr (Hutchison 1992). Colonization of new areas is primarily by wind dispersed seed (Tiley 2010), but seeds also float and are often dispersed via water (i.e., irrigation and flooding) (Derscheid and Schultz 1960).

Canada thistle flowers bloom from June to September and flowers are imperfectly dioecious, with florets on all flowering shoots of a single plant being either male or female. For flowering to occur, Canada thistle requires a 14-16 h d length and male and female flower heads must be within 50-90 m for insect pollination to be successful (Tiley 2010). Stems are slender, grooved, often highly branched, and covered in fine hairs (Winston et al. 2008). Stems die back in winter and in early spring new shoots emerge from the stem base and root buds (Donald 1994; McClay 2002).

Canada Thistle Management. Numerous control tactics for suppression and management of Canada thistle have been investigated, including chemical, cultural, mechanical, and biological tactics. Many results have been published about using herbicides to control Canada thistle; these have been reviewed by Donald (1990) and include: 2,4-D, atrazine, bentazon, bromoxynil, chlorsulfuron, clopyralid, dicamba, glyphosate, imazapyr, MCPA, metsulfuron, picloram, sulfometuron, and tebuthiuron. In 2005 aminopyralid a fairly new herbicide for Canada thistle control was registered for use in rangelands and noncrop areas (EPA 2005). Research has demonstrated that Canada thistle control 1 yr after treatment with aminopyralid was ≥90% and comparable to picloram and picloram + 2,4-D applied in the spring or fall (Enloe et al. 2007). Additionally aminopyralid was given a reduced risk classification by the United States Environmental Protection Agency (EPA 2005) and can be used up to the water's edge (Enloe et al. 2007). Although aminopyralid is considered to be a reduced risk herbicide, this herbicide does have negative impacts on non-target forbs (Almquist and Lym 2010). Cultural control practices have also been investigated, which focus primarily on the use of competitive crop cultivars (Blackshaw et al. 2008). Mechanical control practices that have been explored include frequent

mowing (Lukashyk et al. 2008), hoeing (Graglia et al. 2006), and tillage (Pekrun and Wilhelm 2004).

Canada Thistle Biological Control. Classical biological control is an ecologically sound pest management tool that has been explored to control Canada thistle. The crux of classical biological control involves the identification of coevolved natural enemies in the invasive plant's home range and subsequent release of these natural enemies into the introduced range of the troublesome weed (Cripps et al. 2011). Several natural enemies have been identified to help suppress Canada thistle (McClay 2002, Reed et al. 2006). Beginning in 1959, surveys were conducted throughout Europe and Asia to identify possible biological control agents (Piper and Andres 1995). Overall, 78 species were identified, although many were later eliminated because they attack non-target plants, including economically important species of the Cardueae tribe such as safflower (*Carthamnus tinctorius* L.) and globe artichoke (*Cynara scolymus* L.) (McClay 2002). When investigating foreign biological control agents for release, host specificity experiments should be conducted to insure that the agent of interest does not attack and damage any desirable plants growing in the region (i.e., non-target species), including economically important crops and threatened or endangered native plants (Zwolfer and Harris 1971).

Canada thistle biological control agents in the United States include insects released deliberately: *Hadroplontus* (formerly *Ceutorhynchus*) *litura* Fabricius (Coleoptera: Curculionidae), *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae), and *Urophora cardui* L. (Diptera: Tephritidae) and unintentionally: *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae), *Cleonus piger* Scop. (Coleoptera: Curculionidae), and *Larinus planus* Fabricius (Coleoptera: Curculionidae) (Cripps et al. 2011). Pathogens have also been identified as biological control agents of Canada thistle and include: *Puccinia punctiformis* (Strauss) Röhling

(Cripps et al. 2011) and *Pseudomonas syringae* pv. *tagetis* (Gronwald et al. 2002). Although many species have established in North America, the majority of research results suggest that classical biological control of Canada thistle has largely been unsuccessful (Cripps et al. 2011).

***Hadroplontus Litura* Origin and History.** However, *H. litura*, a phytophagous stem-mining weevil (McClay 2002) native to Europe (i.e., France, Switzerland, Austria, Germany, Britain, and southern Scandinavia; Zwolfer and Harris 1966) is generally considered the most effective insect biocontrol agent for Canada thistle in North America. With regard to host specificity, testing has shown that feeding, oviposition, and larval development of *H. litura* is limited to exotic and native species in the *Cirsium*, *Carduus*, and *Silybum* genera, which does not include any major economically important crop species grown in the United States. The *Silybum* genus does include one minor economic crop, milk thistle (*Silybum marianum* L.), which is grown commercially and used for medicinal purposes (Jacobs et al. 2002). However, testing performed by Zwolfer and Harris (1966) demonstrated that *H. litura* feeds inconsistently on milk thistle and release of *H. litura* would not cause significant damage to the plant. Additionally, the *Cirsium, Carduus, and Silybum* complex does include native North American thistle species. Laboratory testing (Zwolfer and Harris 1966) has demonstrated that *H. litura* can successfully feed, oviposit, and develop on native North American thistles, including two species found in North Dakota, *Ci. flodmanii* (Rydb.) Arthur (Flodman thistle) and *Ci. undulatum* (Nutt.) Spreng. (wavyleaf thistle), but this has not been observed in the field (McClay 2002). Since *H. litura* does not attack any economically important plant species in the United States, and because the weevil did not appear to attack native North American thistles in field settings, *H. litura* was approved for release as a biological control agent for Canada thistle in 1971 under the Federal Plant Pest Act of 1957 (Julien and Griffiths 1999). This act is largely concerned with the effects biological control

agents have on agronomically important crops, but beginning in the 1970's more focus was given to non-target native plants (Pemberton 2003). This has led to new standards and more rigorous testing on exotic insects for weed biological control that were not in place when *H. litura* was initially released.

In Canada, *H. litura* was first released in 1965 and became established in 1967 after a total of four releases (Rees 1990). In the United States, *H. litura* releases totaling 2,461 adults were conducted in eight states between 1971 and 1975, including Montana and South Dakota. In North Dakota, *H. litura* was released multiple times beginning in the 1970s (Julien and Griffiths 1999). In 2004, the North Dakota Department of Agriculture (NDDA) conducted field releases of *H. litura* in 34 counties at 95 sites totaling 131,460 adults (NASDA 2004).

***Hadroplontus Litura* Biology.** Adult *H. litura* overwinter in the soil and emerge early in the spring in synchrony with Canada thistle emergence. Mating occurs for 4 to 6 wk, and females must feed on the host plant prior to oviposition (Zwolfer and Harris 1966). Eggs are laid on the leaves singly or in groups of up to five eggs in round feeding cavities (1.0-2.5 mm in diameter) females create using their mouthparts. Cavities are located on the leaf mid-vein or in the surrounding palisade parenchyma. In the laboratory, larvae hatch after 5 to 9 d and mine the mid-vein of the leaf, eventually tunneling into the stem. A single stem is often mined by several larvae (heavy infestation 12 larvae, average 3 to 6) and becomes blackened due to larval feeding and frass (Zwolfer and Harris 1966). *Hadroplontus litura* larvae develop via three immature instar stages; the exact range of head capsule widths associated with each instar has not been previously reported. In a laboratory experiment the first ecdysis occurred 3 to 5 d after hatching follow by a second ecdysis (d after hatching not reported), which occurred in the basal region of the stem (Zwolfer and Harris 1966). The third instar larvae exit the base of the stem mid-

summer, construct soil cocoons (4-6 mm), and pupate in the soil. New adults emerge in the fall, feed on Canada thistle foliage, and overwinter in the soil.

Generally, *H. litura* larvae inflict more damage to Canada thistle plants than adult weevils. However, although larval mining stresses the plant, mining only occurs in the stem pith and does not damage the vascular tissue; thus, the plant is not damaged substantially and is able to continue growth during and after attack (Peschken and Wilkinson 1981). Even though *H. litura* herbivory does not kill the shoot, larval feeding may lead to reduced overwinter survival (Rees 1990) and also a reduction in early season root sugar (Peschken and Derby 1992) and starch content (Hein and Robert 2004). Larval feeding may also indirectly contribute to Canada thistle control by increasing the plant's susceptibility to pathogens or adverse environmental conditions (Rees 1990).

***Hadroplontus Litura* and Integrated Pest Management.** Overall, research results concerning *H. litura* efficacy are mixed, and suggest that *H. litura* alone is not a highly effective biological control agent (Peschken and Derby 1992; Reed at al. 2006). However, many researchers have suggested that combining additional control tactics along with *H. litura* might increase Canada thistle suppression (Bacher and Schwab 2000; Ferrero-Serrano et al. 2008; Friedli and Bacher 2001). In general, individual control methods do not provide effective, long-term results (Evans 1984; Travnicek et al. 2005), and are often prohibitively expensive (Sciegienka et al. 2011; Tichich and Doll 2006). Additionally, overreliance on one management tactic may cause a shift in the response of weed communities, leading to the evolution of resistance, which compromises the effectiveness of the tactic (Buhler et al. 2000). In hopes of alleviating this potential problem, integrated pest management (IPM) seeks to diversify the selection pressure applied on weed communities, thus possibly preserving the efficacy of current management practices. Overall,

IPM seeks to integrate multiple control tactics to provide effective pest management solutions that are environmentally, sociologically, and economically sound (Liebman and Gallandt 1997; Thill et al. 1991).

Integrating highly competitive native vegetation along with biological control agents has been shown to effectively suppress Canada thistle and may additively or synergistically enhance the efficacy of *H. litura*. Planting cover crops that are functionally and phenologically similar to the invasive weed of interest provide competitive pressures early in the growing season and are usually most effective in restoration efforts (Ferrero-Serrano et al. 2008; Perry et al. 2009). Perry et al. (2009) reported that competition from common ragweed (*Ambrosia artemisiifolia* L.) and common sunflower (*Helianthus annuus* L.) reduced Canada thistle above-ground biomass in greenhouse experiments. These early successional species compete with weeds for sunlight and nutrients (specifically nitrogen) and can promote the establishment of desired native plant species, especially in highly disturbed areas.

Ferrero-Serrano et al. (2008) investigated the combined impact of *H. litura* and a native cool season grass (*Hesperostipa comata* Trin. & Rupr., needle and thread grass) on Canada thistle above- and belowground biomass. They found that combining these two control tactics greatly reduced thistle root biomass, and hypothesized that *H. litura* had a positive indirect effect on needle and thread grass by decreasing the competitive ability of Canada thistle. Friedli and Bacher (2001) reported that a shoot-base boring weevil (*Apion onopordi* Kirby) and competition from a mixture of three grass species (perennial ryegrass, *Lolium perenne* L.; Italian ryegrass, *Lolium multiflorum* Lam.; and orchardgrass, *Dactylis glomerata* L.) had a negative synergistic effect on both the above- and belowground biomass of Canada thistle. Furthermore, Bacher and Schwab (2000) found that combining high levels of plant competition along with leaf herbivory

from a defoliating shield beetle (*Cassida rubiginosa* Müller) resulted in 50% mortality of Canada thistle above- and belowground plant parts during the duration of the experiment (June to August).

One barrier to the successful implementation of IPM is a lack of knowledge about the complex biological and ecological interactions among weedy plants, control measures, and the system as a whole (Buhler et al. 2000; Buhler 2002; Holt 2004). The challenges of integrating biological control with other non-chemical weed management tactics (e.g., mechanical, physical, and cultural) was reviewed by Hatcher and Melander (2003), who stressed that the timing of applying additional control methods is of critical importance to protect the biological control agent while simultaneously damaging the desired weed. For example, studies have suggested that biological control agents can survive prescribed burning events if safe sites are present during the fire such as in the soil or roots (Fellows and Newton 1999; Hatcher and Melander 2003). Knowledge of the biological control agent's phenology would allow researchers to pinpoint a protected stage of the life cycle in which the insect is potentially sheltered from the prescribed fire (i.e., pupa in the soil, root-mining larvae) (Briese 1996). Additionally, studies evaluating the combined effects of prescribed burning and insect biological control in grassland systems have suggested that re-colonization post-fire is often quick and that post-fire insect populations are greater than pre-fire levels (Fellows and Newton 1999; Swengel 2001). Timing of mechanical control methods can also affect biological control agent survival (Hatcher and Melander 2003). Many insects, including *H. litura* have a pupal stage that resides in the soil. A previous study that evaluated the impact of tillage on biological control performance showed that deep tillage during the time of pupation caused destruction of *Phytomyza orobanchia* Kalt. (Diptera: Agromyzida)

pupae (Klein and Kroschel 2002). Adult emergence from the soil was also reduced because pupae were buried too deeply for successful adult emergence.

The combined impact of mowing/cutting and biological control on perennial weed management has also been investigated. The goal of cutting perennial weeds is to deplete root reserves that are used for regrowth of above-ground structures (Hatcher and Melander 2003). Although cutting alone has not been shown to be effective for Canada thistle control (Donald 1990) and is rather labor intensive, efficacy of cutting events could potentially be enhanced with the addition of biological control agents (Tipping 1991). A study that evaluated the combined impacts of cutting and herbivory by a seed-feeding weevil, *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae), on musk thistle (*Carduus theormeri* Weinm.) control reported that when cutting events were conducted at plant senescence, which corresponded to the time in which *R. conicus* was no longer present in the seed head of the musk thistle plant, no mortality was incurred by the weevil (Tipping 1991). Conversely, when mowing occurred during the bud to flower stage of musk thistle development, 75% mortality of *R. conicus* was observed and subsequently musk thistle plants produced 98% more seeds per plant than plants that were cut at the senescence stage and attacked by *R. conicus*.

Integrating insect biological control with herbicides for weed management has also been evaluated and reviewed by Messersmith and Adkins (1995). Studies reviewed noted variable outcomes, but yielded similar conclusions that herbicide application timing in relation to insect developmental stage was pivotal. Overall, herbicide applications during early egg and larval stages of insect development were often detrimental to insect survival. For example, applications of 2,4-D that occurred within 48 h of oviposition by the thistle seed head weevil *R. conicus* caused greater mortality rates than when applied 3 wk after oviposition (Trumble and Kok 1979).

Additionally, applications of 2,4-D or picloram for leafy spurge control did not adversely impact *Hyles euphorbiae* L. (Lepidoptera: Sphingidae) larvae if they were in the fourth or fifth instar stage (Rees and Fay 1989). Overall, knowledge of the timing of the life stages of *H. litura* would allow land managers to identify invulnerable life stages and apply additional management tactics, such as prescribed burning, mowing, and/or herbicides that would potentially not harm *H. litura* and also provide continual diverse management pressure on Canada thistle. Understanding the phenology of *H. litura* immature stages could enhance IPM of Canada thistle, when the focus is integrating weevil impacts with other control measures.

Insect Development. In general, insect growth is cyclical, with a period of active growth followed by a molt and then a period of relative inactivity (Wigglesworth 1972). This cycle can repeat multiple times and is unique to each insect species. The sclerotized integument or cuticle that covers the insect's body plays a large role in dictating insect growth and development. The cuticle is unable to grow with the insect, thus for the insect to grow it must molt, a process in which a new, larger cuticle is laid down in the place of the previous one, allowing the insect to expand and grow. The amount an insect can grow at each molt is often predictable and theories including Dyar's rule and Przibram's rule have been formed surrounding the phenomenon of insect growth. Most notable of these theories is Dyar's rule, which states that head capsule growth is geometric and increases at each molt by a ratio that is consistent and unique to each species (Dyar 1890). According to this rule, when the logarithm of head capsule width is plotted against instar number, a linear relationship is formed. This rule is not restricted to head capsule measurements and can also be applied to other parts of the insect body (Wigglesworth 1972).

The most important environmental factor that influences insect development rates is temperature (Wigglesworth1972). In general, insects can develop within a range of temperatures;

development is accelerated at high temperatures and is slowed at low temperatures. Below a given temperature, unique to each insect species, development ceases. This pattern in development, when plotted on a graph, gives a characteristic S shape curve (Figure 1.1).

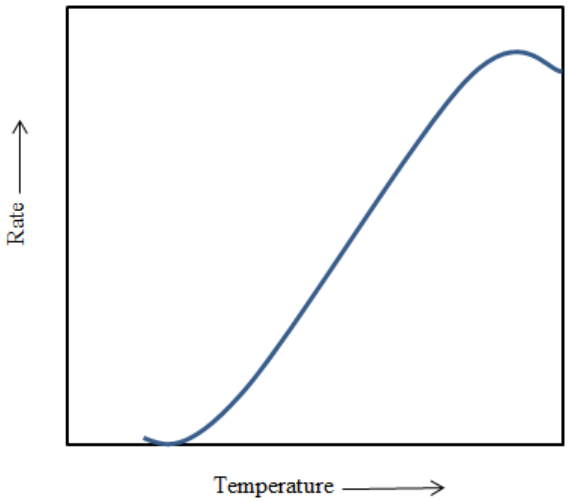

Figure 1.1. The nonlinear relationship between temperature and insect rate of development. Adapted from Wigglesworth (1972).

This pattern is due to the fact that insects are poikilothermic, meaning their internal temperature is not stable and varies with environmental temperature, thus the environment has a strong influence on development, mainly due to the temperature dependence of rates of biochemical enzymatic reactions essential to growth (Higley and Haskell 2001; Wigglesworth1972).

Insect Phenology Models. A great deal of research has been devoted to modeling insect phenology as influenced by temperature (Candy 1991; Damos and Savopoulou-Soultani 2012; Gu and Novak 2005; Manel and Debouzie 1997; Nowierski et al. 1983; Pruess 1983; Worner 1992). Most insect phenology models are based on the principle that poikilothermic insects have a specific thermal constant which corresponds to the required heat units that must accumulate for the insect to complete a specific stage of development (Damos and Savopoulou-Soultani 2012). Heat units are defined as the accumulation of daily mean temperatures above a certain threshold temperature, which is unique to each species (Wang 1960). Heat units have been used in plant

science and entomology to model and predict phenology for over 280 yr and are often interchangeably termed “degree-days,” “growing degree-days,” and “growth units.”

Insect phenology research has been conducted using two different approaches: in controlled laboratory experiments under a constant temperature regime (Briere et al. 1999; Morgan et al. 2001) or in field experiments with natural variable fluctuating temperatures (Manel and Debouzie 1997; Nowierski et al. 1983). In controlled laboratory experiments, cohorts of individuals are reared at various constant temperatures and developmental progress is observed (Wagner et al. 1984). However, research has suggested that models created from data generated by this type of experiment may not be applicable to field conditions (Manel and Debouzie 1997; Wagner et al. 1984; Worner 1992). This is because, in natural environments with fluctuating temperature conditions, a greater degree of insect development can occur at lower and also higher temperatures than can occur at constant low or high temperatures in laboratory experiments (Hagstrum and Milliken 1991).

Kaufmann first described the difference between rates of development predicted by nonlinear models derived from data collected under constant and variable temperature trials (Ludwig and Cable 1933). This difference was subsequently termed the Kaufmann effect (Ludwig and Cable 1933) or rate summing effect (Ratte 1985). The Kaufmann or rate summing effect holds that fluctuations in the lower temperature portion of the development curve, outlined above (Figure 1.1), will cause development to proceed more quickly than what would be predicted from a mean constant temperature in the lower part of the development curve, and conversely fluctuations in the upper temperature range of the development curve will slow development time more than the time predicted from a mean constant temperature in the upper part of the development curve (Higley and Haskell 2001). This is due to the curvilinear nature of

insect development as influenced by temperature. To derive models that are relevant to field conditions, experiments must be conducted either in the laboratory under fluctuating temperatures or directly from field populations (Manel and Debouzie 1997).

How temperature might impact the developmental timing and efficacy of *H. litura* as a biological control agent is unclear. Previous research performed by Zwolfer and Harris (1966) identified the timing of certain life stages (egg hatching, timing of first ecdysis, pupation duration and emergence timing) by observing infested Canada thistle plants under constant laboratory conditions. These results may not be applicable to field situations; therefore developing a mathematical model to predict the developmental timing and duration of the life stages of *H. litura* may aid in the integration of *H. litura* with other control tactics.

CHAPTER 1. INTEGRATING WEEVIL HERBIVORY, A NATIVE COVER CROP, AND SOIL NUTRIENTS FOR CANADA THISTLE (*Cirsium arvense* L.) CONTROL

ABSTRACT

Our objective was to determine the impact of integrating a biological control agent (*Hadroplontus litura* Fabricius, a stem-mining weevil) and a native annual cover crop (*Helianthus annuus* L., common sunflower) on measurements associated with Canada thistle size, vegetative and sexual propagation under two soil nitrogen regimes. Previous research has found that *H. litura* acting alone is a mildly effective control agent; however, integrating multiple control tactics may provide enhanced control of Canada thistle. During the summers of 2010 and 2011, outdoor microcosms (19 L containers of field soil) were established with full factorial combinations of weevil and cover crop presence/absence and high vs. low soil nutrient levels. Canada thistle morphological characteristics were measured weekly and final above and below ground biomass was harvested at the end of the growing season. In 2010, cover crop presence was associated with reduced Canada thistle height. Weevil presence was associated with reduced Canada thistle height, however this effect was weak. Weevil presence, cover crop presence, and low soil nutrients reduced inflorescence production. Cover crop presence and low soil nutrients reduced side shoot production. Cover crop presence reduced both final root and shoot biomass. Increased soil nutrients increased final shoot, but not root, biomass. In 2011, weevil presence was associated with reduced Canada thistle height and this effect was persistent throughout the growing season. Cover crop presence reduced overall Canada thistle height regardless of soil nutrient level. Cover crop presence and low soil nutrients reduced overall inflorescence and shoot production. Cover crop presence was associated with reduced final shoot biomass in high soil nutrient treatments. Increased soil nutrients were associated with greater final shoot biomass compared to the low soil nutrient treatment, regardless of cover crop presence or absence. Cover crop presence and low soil nutrients reduced final root biomass. Inconsistencies between 2010

and 2011 could be due to environmental differences, such as overall greater precipitation in 2010 and colder early season temperatures in 2011. In general, weevil effects on Canada thistle morphology were weak whereas the effects of soil nutrition and cover crop were more persistent throughout the duration of the experiment. Research results also suggest that effects of weevils and cover were additive rather than synergistic, but that integrating plant competition with biological control could provide enhanced Canada thistle control.

INTRODUCTION

Canada thistle (*Cirsium arvense* L.) is a serious invasive weed in many croplands, rangelands, and recreational areas around the world. This invasive weed thrives in disturbed or moist environments and can lower the quality of grazing lands, out-compete native plants, and negatively impact crop yield (McClay 2002; McLennan et al. 1991; O'Sullivan et al. 1982, 1985). Canada thistle is a clone forming perennial with a deep root system that can spread extensively (Donald 1994; McClay 2002) and give rise to adventitious shoots from root buds throughout the growing season (Tiley 2010); the success of this weed is often attributed to these characteristics.

Despite continued efforts, effective Canada thistle management continues to be a challenge, and this weed remains a problematic invasive plant throughout the world (Cripps et al. 2011; Tiley 2010). Part of the problem may be that, in general, individual control methods do not provide effective, long-term results (Evans 1984; Travnicek et al. 2005), and are often prohibitively expensive (Sciegienka et al. 2011; Tichich and Doll 2006). Overall, research suggests that an integrated pest management (IPM) program may be a more effective way to sustainably manage Canada thistle infestations. IPM seeks to integrate multiple control tactics to provide effective pest management solutions that are environmentally, sociologically, and economically sound (Liebman and Gallandt 1997; Thill et al. 1991).

One common weed management tactic is biological control, which is environmentally friendly and ideally self-sustaining. *Hadroplontus* (formerly *Ceutorhynchus*) *litura* Fabricius (Coleoptera: Curculionidae), a phytophagous stem-mining weevil (McClay 2002) is generally considered the most effective insect biocontrol agent for Canada thistle in North America. Adult *H. litura* overwinter in the soil and emerge in early spring in synchrony with Canada thistle

emergence (Zwolfer and Harris 1966). Mating occurs for several weeks and one to five eggs are laid on the leaves in round feeding cavities. Larvae hatch and mine the mid-vein of the leaf, eventually tunneling into the stem. A single stem is often mined by several larvae and becomes blackened due to larval feeding and frass. *Hadroplontus litura* larvae undergo three immature instar stages. Third instar larvae exit the base of the stem mid-summer, then tunnel underground to construct soil cocoons for pupation. New adults emerge in the late summer or early fall and feed on Canada thistle foliage prior to overwintering in the soil.

Generally, *H. litura* larvae inflict more damage to thistle plants than adult weevils. However, although larval mining stresses the plant, the plant is not damaged substantially and is able to continue growth during and after attack (Peschken and Wilkinson 1981). Even though *H. litura* herbivory does not kill the shoot, larval feeding may lead to reduced overwinter survival (Rees 1990), reduction in early season root sugar (Peschken and Derby 1992) and starch content (Hein and Robert 2004), and increased susceptibility to pathogens or adverse environmental conditions (Rees 1990). Overall, research results concerning *H. litura* efficacy are mixed, and suggest that *H. litura* alone is not a highly effective biological control agent (Peschken and Derby 1992; Reed at al. 2006). However, many researchers have suggested that combining additional control tactics along with *H. litura* might increase Canada thistle suppression (Bacher and Schwab 2000; Ferrero-Serrano et al. 2008; Friedli and Bacher 2001).

Integrating highly competitive native vegetation along with biological control agents has been shown to effectively suppress Canada thistle and may additively or synergistically enhance the efficacy of *H. litura*. Perry et al. (2009) reported that competition from common ragweed (*Ambrosia artemisiifolia* L.) and common sunflower (*Helianthus annuus* L.) reduced Canada thistle above-ground biomass in greenhouse experiments. These early successional species

compete with weeds for sunlight and nutrients (specifically nitrogen) and can promote the establishment of desired native plant species, especially in highly disturbed areas.

Ferrero-Serrano et al. (2008) investigated the combined impact of *H. litura* and a native cool season grass (*Hesperostipa comata* Trin. & Rupr., needle and thread grass) on Canada thistle above- and belowground biomass. They found that combining these two control tactics greatly reduced thistle root biomass, and hypothesized that *H. litura* had a positive indirect effect on needle and thread grass by decreasing the competitive ability of Canada thistle.

Based on these previous results, we decided to investigate the potential of combining *H. litura* weevil attack with common sunflower competition in different soil nutrient environments. Common sunflower is a highly competitive annual that is native to North America and found throughout the United States, Canada, and Mexico (Burke et al. 2002). Like Canada thistle, common sunflower is fast growing and often thrives in disturbed areas (Burke et al. 2002; Perry et al. 2009), which are qualities that potentially make it a strong competitor against Canada thistle. In addition, root exudates and foliar tissue extracts of common sunflower have allelopathic activity (Leather 1983; Perry et al. 2009); meaning that these compounds can negatively affect growth of neighboring plants (Rice 1974). Therefore, the objective of our experiment was to investigate the combined impact of *H. litura* and common sunflower competition on Canada thistle height, inflorescence number, side shoot number, final shoot and root biomass under different soil nutrient (N-P-K) regimes.

MATERIALS AND METHODS

Experimental Design. Effects of *H. litura* attack, plant-plant competition, and soil nutrient content on Canada thistle growth and reproductive output were determined using outdoor microcosm experiments. During 2010 and 2011, experiments were conducted using a randomized complete block design with four replications and three factorially combined treatments: 1) plant competition presence versus absence, 2) *H. litura* attack versus no attack, and 3) high versus low soil nutrient (N-P-K) content. Initial Canada thistle and common sunflower plant height were used as blocking factors.

The experiments were conducted from June 2 to September 8, 2010 and June 4 to August 8, 2011. Microcosms were established outdoors on the campus of North Dakota State University (Fargo, ND) to take advantage of natural light and temperature fluctuations. In 2010, microcosms were protected from adverse weather conditions (strong wind/hail) by placing a plastic tarp over the plants when forecasts predicted severe weather (approximately 5 times). In 2011, microcosms were protected from adverse weather conditions by a permanent structure constructed of a wooden frame encased by black polypropylene netting (1.3 cm hole opening) which did not obstruct natural light.

Microcosms consisted of 18 kg of lightly compacted finely sieved (3 mm hole opening) Ulen fine sandy loam (Sandy, mixed, frigid Aeric Calciaquolls) soil (hereafter base soil) containing two different nutrient levels (soil nutrient treatments are explained in detail below), placed in 19 L white plastic buckets (30 cm top diameter by 37 cm high by 26 cm bottom diameter) and spaced 60 cm apart from adjacent microcosms.

Collection of Plant Materials. In 2010, Canada thistle plants were propagated from vegetative root cuttings excavated from a small natural infestation on a south facing drainage ditch slope on

the campus of North Dakota State University (Fargo, ND) to obtain homogeneous plant material from a single ecotype. Vegetative root cuttings were approximately 8 to10 cm in length and treated with 0.1% indole-3-butyric-acid powder[1] to promote rooting prior to planting. Root cuttings were grown in 8 cm wide by 9 cm deep plastic pots containing the base soil. Plants were allowed to grow in the greenhouse (24-26 C, 16:8 L:D h photoperiod) until transplanted into microcosms as small rosettes. In 2011, Canada thistle plants were either propagated as previously described or transplanted from small rosettes obtained from the same natural infestation used in 2010. Two Canada thistle propagation methods were used in 2011 due to low shoot production of vegetative root cuttings. Rosettes were maintained in 8 cm wide by 9 cm deep plastic pots containing base soil and grown in the greenhouse as previously described. The microcosm experiments were blocked by plant propagation method; one replicate received Canada thistle rosettes grown by root cuttings and the remaining replicates received Canada thistle rosettes grown from transplanted small rosettes. In both years, each microcosm received only one Canada thistle rosette.

Common sunflower plants were used as the plant competitor in the microcosm experiments. Common sunflower plants were grown from seed collected in the fall of 2009 and 2010 from wild plants found on the campus of North Dakota State University (Fargo, ND). Approximately 5 seeds were planted 3 mm deep into 4.5 cm wide by 4 cm deep plastic seed pots containing Sunshine Mix #1[2]. Approximately 5 d after planting, sunflowers were transplanted into 8 cm wide by 9 cm deep plastic pots containing the base soil at the vegetative emergence (VE) seedling growth stage (Schneiter et al. 1998). Plants were grown in the greenhouse (24-26

[1] Bonide Products Inc., Oriskany, NY 13424
[2] SunGo Horticulture Canada Ltd., Bellevue, WA 98008

C, 16:8 L:D h photoperiod) until the vegetative V4 to V6 growth stage (Schneiter et al. 1998), when they were transplanted into microcosms receiving the plant competition treatment. In both years, one Canada thistle and one common sunflower plant were transplanted 10 cm away from the center point of the microcosm on opposite sides (Canada thistle left, common sunflower right) in microcosms receiving the plant competition treatment. For microcosms not receiving the plant competition treatment, one Canada thistle plant was transplanted into the center of each microcosm.

***Hadroplontus Litura* Treatment**. Canada thistle and sunflower plants were transplanted into microcosms on the same day and allowed to establish for 1 d, after which they were subjected to *H. litura* attack. *Hadroplontus litura* adults were purchased from a commercial source[3] that originally field collected the insects in May near Bozeman, MT. *Hadroplontus litura* adults were shipped in cardboard containers of approximately 100 weevils each with fresh Canada thistle foliage. They were received on May 10, 2010 and May 23, 2011 and subsequently stored in the refrigerator (4 C). Insects were maintained on Canada thistle foliage until used in experiments, which was within 23 d and 12 d in 2010 and 2011, respectively. *Hadroplontus litura* attack treatments were applied by adding 10 adult insects to each microcosm receiving the weevil treatment. Gender of *H. litura* adults was not assessed due to difficulty in determining their sex without dissection and potential of harm due to excessive handling. Assuming a 50:50 chance of one weevil being either female or male, the probability that all 10 insects would be the same sex is approximately 0.001 (Ferrero-Serrano et al. 2008). All microcosms were individually caged during attack. Cages were constructed of 75 cm wide by 150 cm long nylon mesh sleeves draped

[3] Copeland Biological Inc., Bozeman, MT 59715

over two overlapping 1.5 m metal wires embedded into the soil of each microcosm. Mesh sleeves were securely fastened to the outside rim of the plastic bucket with an elastic cord to ensure no insects escaped. *Hadroplontus litura* adults were allowed to feed and oviposit on Canada thistle plants and were removed 7 to 9 d after release; adults were difficult to find and thus the removal period lasted for 3 d, after which cages were also removed.

Soil Nutrient Treatment. Prior to amendment, the nutrient levels of the original field collected base soil used in microcosm experiments were 60 kg ha^{-1} of nitrogen, 15 kg ha^{-1} of phosphorus, and 132 kg ha^{-1} of potassium. The low soil nutrient treatment consisted of this non-amended base soil, which had N-P-K quantities to simulate average pasture or range soil nutrient levels. The low soil nutrient treatment is in accordance with soil samples from 6 *H. litura* release locations [pastures dominated by Canada thistle and smooth brome (*Bromus inermis* Leyss.)] that were analyzed for N-P-K content and ranged from 2-38 kg ha^{-1} nitrogen, 11-241 kg ha^{-1} of phosphorus, and 568-2046 kg ha^{-1} of potassium. The high soil nutrient treatment consisted of the non-amended base soil with N-P-K additions to achieve a soil with 142 kg ha^{-1} of nitrogen, 55 kg ha^{-1} of phosphorus, and 179 kg ha^{-1} of potassium, which is suitable for corn production [yield potential of 31 metric tons (mt) ha^{-1} and 18 mt ha^{-1} for corn silage and sweet corn, respectively; Franzen 2010]. N-P-K additions were made in the form of ammonium nitrate (NH_4NO_3), potassium chloride (KCl), and triple superphosphate ($Ca(H_2PO_4)_2 \cdot H_2O$), respectively. Additional fertilizer was not provided to any of the soil nutrient treatment levels during the experiment, but microcosms were watered as needed to field capacity.

Data Collection. Plant height, number of inflorescences (flowers and buds combined), and number of side shoots were non-destructively measured each week during the experiment for Canada thistle plants. A ruler was used to measure plant height from the soil level to the top of

the shoot apical meristem. Adult *H. litura* damage was quantified by visually assessing plants after insect attack to ensure damage was similar among treatments.

At the end of the experiment plants were destructively sampled and final shoot and root biomass was quantified. To obtain final biomass, plants were first moved into the greenhouse (24-26 C, 16:8 L:D h photoperiod) and allowed to desiccate until plants visibly wilted and soil moisture levels approached permanent wilting point, approximately 10 to 14 d. The wilting process decreased the water potential of the plant material and of the soil to aid in root dyeing (Murakami et al. 2006). After desiccation in the greenhouse, Canada thistle stems were cut 5 cm above soil surface, split open using a scalpel, and *H. litura* larval damage assessed using the following scale: undamaged (stem interior white/green), light (stem lightly damaged/mined), moderate (stem moderately damaged/mined), and severe (stem severely damaged/mined interior blackened). Shoots of all plants were harvested, placed in paper bags, dried in a 70 C oven for 72 to 120 h, and weighed.

After severing the shoots, we used the Murakami et al. (2006) procedure for root dyeing to aid in separating Canada thistle and common sunflower roots. Systemic floral stem dye[4] was injected into the xylem tissue of both Canada thistle and sunflower plants to differentially stain the root systems of the two species. To inject the dye the severed stem of each plant was fitted with a piece of amber latex rubber tubing of various diameters (32, 22, 12, or 8 mm). The tubes were secured to the stems with plastic cable zip ties to achieve the tightest seal possible. Each tube was then connected to a pipette (30 ml volume), which was filled with red dye for Canada thistle plants and blue dye for sunflower plants. Each pipette was then connected to aquarium plastic airline tubing, the airline tubing was attached to a 2-way air t-joint plastic inline aquarium

[4] Design Master, Bolder, CO 80302

connector, all connectors were attached to each other via airline tubing and the final plants' tubing was attached to an air compressor. The system was pressurized at 0.03 MPa for 120 to 192 h. Pipettes were checked daily and filled with dye as needed. Following the staining process, pots were individually removed and the soil-root mass was placed onto an aluminum mesh screen (3 mm hole opening). Soil was then washed from the roots using a garden hose attached to a spray nozzle. Great care was taken when washing roots to insure root systems stayed intact and all debris was removed. The few root pieces that detached from the main root mass were collected and later sorted when possible via color. Roots were separated in the laboratory according to species based on color and structural differences. Occasionally entire root systems were not completely dyed, which could be due to insufficient pressure or rupture of tubing junctures at high pressures (above 0.03 MPa). When this occurred, caution was used to keep root systems intact during the washing and separating process. Root biomass was dried in a 70 C oven for 72 to 120 h, and weighed.

Statistical Analysis. Prior to conducting ANOVA, Levene's test was performed to assess the homogeneity of variance and normality was assessed visually via residual plots. Data met the assumptions of parametric statistics therefore transformations were not needed to comply with the assumptions of ANOVA. Initially, ANOVA using *H. litura* larvae damage rating as a covariate was conducted, but the covariate was insignificant and thus was dropped from the analysis. Thistle morphological response variables (i.e., plant height, number of side shoots, number of inflorescences) that were measured weekly were each tested separately with repeated measures factorial ANOVA using a first-order autoregressive AR(1) covariance structure using Proc Glimmix in SAS Version 9.2 (2008). *Hadroplontus litura*, plant competition, and soil nutrient level treatments were analyzed as fixed effects and replicate and time as random effects.

Significant interaction and main effects ($P \leq 0.05$) were further analyzed for differences among treatment means using Tukey's HSD post-hoc tests. When second-order interactions involving time were present, the slicediff option within the lsmeans statement in SAS was used to explore the differences in the levels of the fixed effects inside the levels of time. This allowed for pairwise comparisons of the two levels of the fixed effect within each time period. When third-order interactions involving time were present, separate ANOVAs for each time period were conducted using Proc Mixed in SAS Version 9.2 (2008) to obtain P-values for the main effects and interactions at each time period.

Final shoot and root biomass data were analyzed separately with a factorial ANOVA using Proc Mixed in SAS Version 9.2 (2008). *Hadroplontus litura*, plant competition, and soil nutrient level treatments were analyzed as fixed effects and replicate as a random effect. Significant interaction and main effects ($P \leq 0.05$) were further analyzed for differences among treatment means using Tukey's HSD post-hoc tests.

RESULTS AND DISCUSSION

Due to numerous year-by-treatment interactions (data not shown); 2010 and 2011 data were analyzed separately for all Canada thistle characteristics measured.

Canada Thistle Morphology.

Canada thistle Height. In 2010, from 5 weeks after treatment (WAT) to the completion of the experiment, *H. litura* presence and common sunflower competition decreased Canada thistle height when compared to the control (*H. litura* absence x plant competition absence) (Figure 1.2; Table 1.1-1.2). With additional pressure provided by *H. litura* Canada thistle plants grown with common sunflower competition were shorter than Canada thistle plants only grown with common sunflower competition 9 WAT (Figure 1.2). The soil nutrient treatment had no effect on Canada thistle height in 2010.

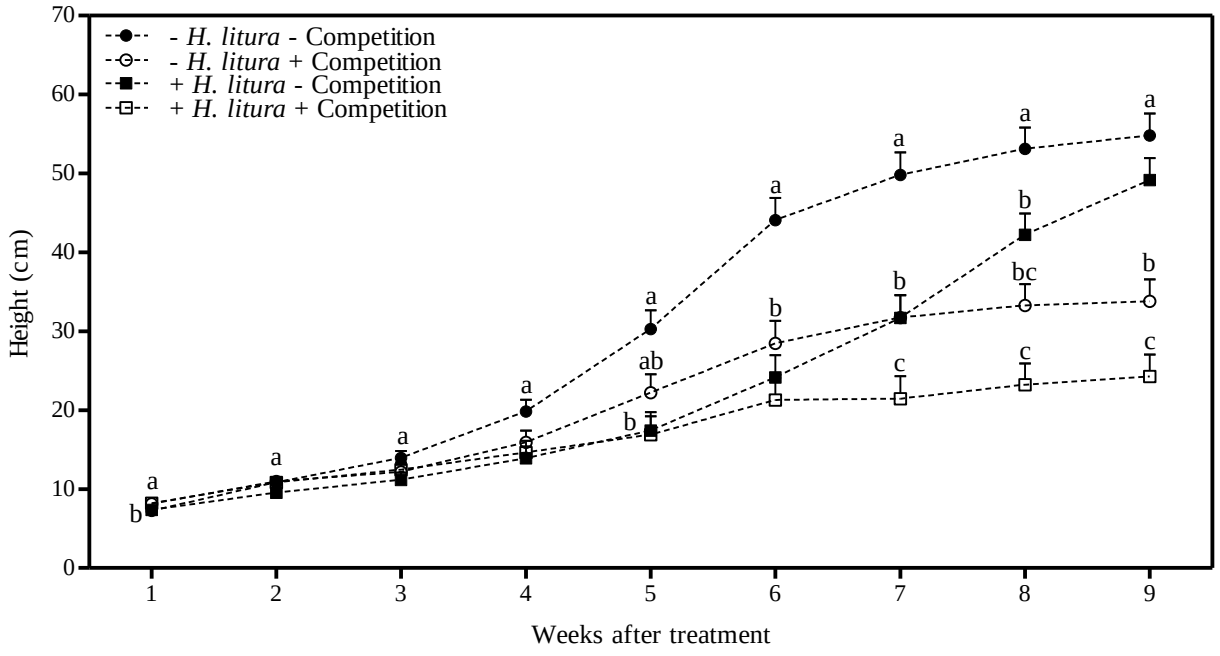

Figure 1.2. Impact of *Hadroplontus litura* attack and common sunflower plant competition on Canada thistle height in 2010. Symbols indicate mean values plus standard error of the mean. Symbols labeled with different letters within each date differ ($P \leq 0.05$).Unlabeled points have the same letter designation as the point directly above.

Table 1.1. ANOVA results for Canada thistle height, inflorescence number, and side shoot number as affected by *Hadroplontus litura*, plant competition, and soil nutrients in 2010 and 2011.

	Canada thistle morphological characteristics					
Treatment	Height	Inflorescence number	Side shoot number	Height	Inflorescence number	Side shoot number
	2010			2011		
H[a]						
df[b]	1, 36	1, 30	1, 41	1, 36	1, 33	1, 36
F	8.22	4.28	0.87	4.75	0.66	1.74
P	0.007	0.047	NS[e]	0.036	NS	NS
PC[c]						
df	1, 36	1, 30	1, 41	1, 36	1, 33	1, 36
F	11.16	7.19	13.15	0.37	3.49	17.97
P	0.002	0.012	0.001	NS	NS	0.001
SN[d]						
df	1, 36	1, 30	1, 41	1, 36	1, 33	1, 36
F	3.11	4.74	6.02	50.31	15.67	14.96
P	NS	0.038	0.019	<0.001	0.001	0.001
Time (T)						
df	8, 269	8, 224	8, 235	8, 257	8, 255	8, 216
F	74.22	16.33	113	120.03	23.05	25.81
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
H x PC						
df	1, 35	1, 30	1, 41	1, 36	1, 33	1, 36
F	0.64	0.87	0.17	0.24	0.09	0.03
P	NS	NS	NS	NS	NS	NS
H x SN						
df	1, 35	1, 30	1, 41	1, 36	1, 33	1, 36
F	0.10	1.05	1.87	0.08	0.04	0.70
P	NS	NS	NS	NS	NS	NS
PC x SN						
df	1, 35	1, 30	1, 41	1, 36	1, 33	1, 36
F	0.67	0.86	0.79	4.18	1.4	2.23
P	NS	NS	NS	0.048	NS	NS
H x T						
df	8, 269	8, 224	8, 235	8, 257	8, 255	8, 216
F	7.07	3.05	0.98	3.13	0.63	1.44
P	<0.001	0.003	NS	0.002	NS	NS

Table 1.1. Continued.

	Canada thistle morphological characteristics					
Treatment	Height	Inflorescence number	Side shoot number	Height	Inflorescence number	Side shoot number
	2010			2011		
PC x T						
df	8, 269	8, 224	8, 235	8, 257	8, 255	8, 216
F	11.09	6.16	14.74	2.82	4.04	8.39
P	<0.001	<0.001	<0.001	0.005	0.001	<0.001
SN x T						
df	8, 269	8, 224	8, 235	8, 257	8, 255	8, 216
F	1.15	3.31	6.85	19.05	10.00	4.80
P	NS	0.001	<0.001	<0.001	<0.001	<0.001
H x PC x SN						
df	1, 42	1, 30	1, 41	1, 36	1, 33	1, 36
F	0.52	0.50	0.08	0.63	0.12	1.21
P	NS	NS	NS	NS	NS	NS
H x PC x T						
df	8, 271	8, 224	8, 235	8, 257	8, 255	8, 216
F	2.96	1.27	0.19	0.16	0.48	1.53
P	0.003	NS	NS	NS	NS	NS
H x SN x T						
df	8, 270	8, 224	8, 235	8, 257	8, 255	8, 216
F	0.85	1.85	2.12	0.44	0.46	0.46
P	NS	NS	NS	NS	NS	NS
PC x SN x T						
df	8, 270	8, 224	8, 235	8, 257	8, 255	8, 216
F	0.75	1.87	1.17	1.25	1.80	1.89
P	NS	NS	NS	NS	NS	NS
H x PC x SN x T						
df	8, 282	8, 224	8, 235	8, 257	8, 255	8, 216
F	0.64	1.44	0.34	0.89	0.60	1.39
P	NS	NS	NS	NS	NS	NS

[a] H = *H. litura* = presence, absence
[b] Degrees of freedom present are: numerator, denominator
[c] PC = Plant competition by common sunflower (*Helianthus annuus*) = presence, absence
[d] SN = soil nutrients = low, high
[e] NS indicates P > 0.05

Table 1.2. P-values from separate time period ANOVAs for Canada thistle height as affected by *Hadroplontus litura* and plant competition in 2010 from 1 to 9 WAT.

	Weeks after treatment								
Treatment	1	2	3	4	5	6	7	8	9
H[a]	NS	NS	NS	NS	0.030	0.005	0.003	0.009	NS
PC[b]	0.034	NS	NS	NS	NS	0.047	0.003	<0.001	<0.001
H × PC	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] *H. litura* = presence, absence
[b]Plant competition by common sunflower (*Helianthus annuus*) = presence, absence

In 2011, *H. litura*, soil nutrients, and plant competition treatment main effects reduced Canada thistle height (Table 1.1; Figure 1.3). *Hadroplontus litura* attack was associated with a slight reduction in Canada thistle height during the latter half of the experiment (Figure 1.3a). Canada thistle plants grown in low soil nutrient environments were considerably shorter than Canada thistle plants grown in high soil nutrient environments (Figure 1.3b). Unlike 2010, in 2011 Canada thistle plants grown along with common sunflower competition were only slightly shorter than Canada thistle plants grown alone and this negative impact only occurred for the final 2 wk of the experiment (Figure 1.3c). Overall the soil nutrient treatment had the greatest negative impact on Canada thistle height than *H. litura* herbivory and common sunflower plant competition.

Although results from ANOVA indicated a second-order interaction between soil nutrient and the common sunflower plant competition main effects (Figure 1.4; Table 1.1), the P-value for this interaction (P=0.048) was marginally significant and, as illustrated by figure 1.4, no interaction is evident. Canada thistle plants grown in high soil nutrient environments were taller than Canada thistle plants grown in low soil nutrient environments, regardless of plant competition presence or absence (Figure 1.4).

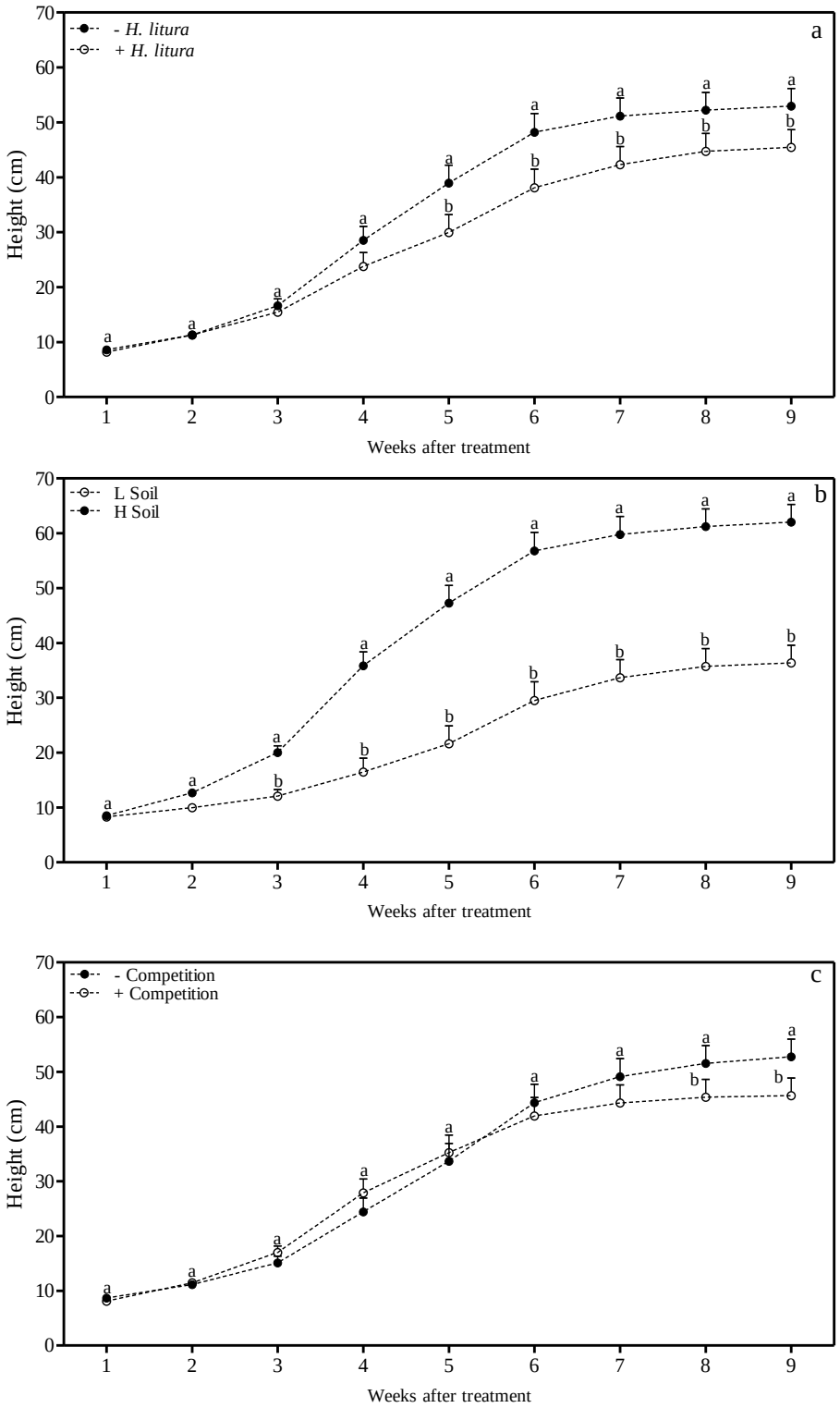

Figure 1.3. Impact of *Hadroplontus litura*, soil nutrients, and common sunflower plant competition on Canada thistle height in 2011. Symbols indicate mean values plus standard error of the mean. Symbols labeled with different letters within each date differ ($P \leq 0.05$). Unlabeled points have the same letter designation as the point directly above.

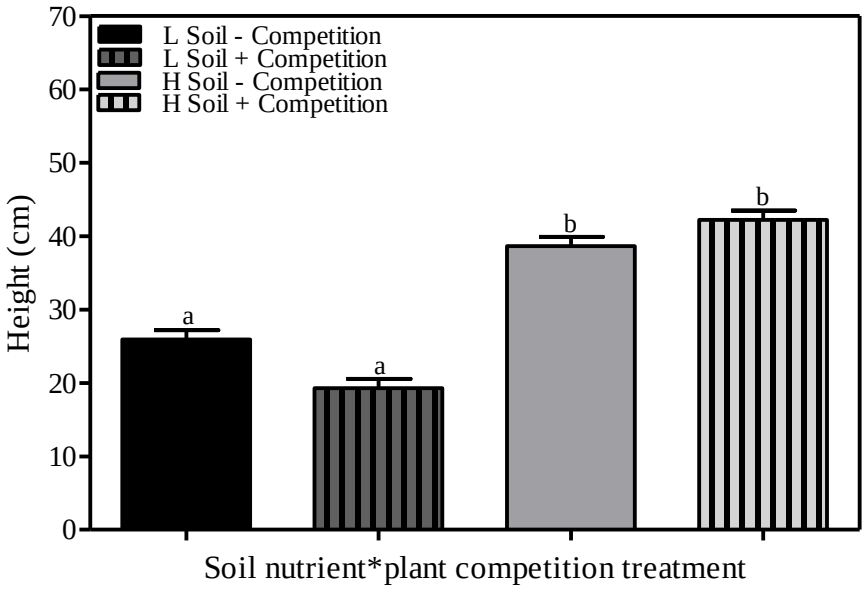

Figure 1.4. Impact of soil nutrients and common sunflower plant competition on Canada thistle height in 2011. Bars indicate mean values plus standard error of the mean. Pairwise comparisons labeled with different letters differ ($P \leq 0.05$).

This result is supported by McIntyre and Hunter (1975) who reported that Canada thistle plants grown in soil treated with 21.0 ppm nitrogen were 37.4 cm tall and plants grown in soil treated with 5.25 ppm nitrogen were only 21.5 cm tall.

The reduction in Canada thistle plant height associated with competition found in both 2010 and 2011 is not supported by other studies that measured Canada thistle height as affected by plant competition and may be attributed to functional differences in the plant competitors used. Bacher and Schwab (2000) used an herbaceous seed mix and Cripps et al. (2010) chose a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) as the plant competitors. Both studies reported no difference in height between Canada thistle plants grown alone and along with plant competitors (Bacher and Schwab 2000; Cripps et al. 2010). Common sunflower, the plant competitor in our experiment, has a fast growth rate which gives the plant the ability to quickly shade plant neighbors, thus reducing the neighboring plant's

growth (Perry et al. 2009). Previous research has reported that Canada thistle is a shade intolerant species (Evans 1984); this effect may help to explain our results in which common sunflower negatively impacted Canada thistle height. Although competition had a negative impact on Canada thistle height in this study, competition can lead to an over-compensatory response by the target plant via competition for light (Jaremo et al. 1996). Plants grown under shade may express strong apical dominance and result in rapid growth of the main shoot which can lead to a taller plant (Aarssen 1995).

The reported reduction in Canada thistle height in 2011 after *H. litura* herbivory agrees with previous research in which Bacher and Schwab (2000) reported a sustained negative impact on Canada thistle height when attacked by the biological control agent *C. rubiginosa*, a leaf defoliating beetle, on an artificially infested Canada thistle plot in Switzerland. The sustained impact of *H. litura* on Canada thistle height that occurred in 2011 is contradictory to the transient 2010 *H. litura* impact and results reported by Cripps et al. (2010), which suggested a transient impact of a biological control agent *C. rubiginosa* on Canada thistle height in an experiment performed on a natural infestation of Canada thistle in a Switzerland pasture system. Herbivory may also stimulate plant overcompensation, but in a very different manner than overcompensation occurring in relation to competition for light (Agrawal 2000). Plants subjected to main stem attack by herbivores may experience release of apical dominance and thus an increase in lateral shoot production and overall increased growth. The difference in previously reported research results may be attributed to experimental differences, as Bacher and Schwab (2000) used artificially infested Canada thistle plots and Cripps et al. (2010) used a naturally infested field population of Canada thistle.

Canada thistle Final Shoot Biomass. In 2010, Canada thistle shoot biomass was affected by soil nutrient and plant competition main effects (Table 1.3; Figure 1.5). Canada thistle plants grown in low soil nutrients or along with plant competition produced less above-ground biomass than Canada thistle plants grown in high soil nutrients or without plant competition (Figure 1.5a,b).

Table 1.3. ANOVA results for Canada thistle shoot and root biomass as affected by *Hadroplontus litura*, plant competition, and soil nutrients in 2010 and 2011.

	Canada thistle dry biomass			
Treatment	Shoot	Root	Shoot	Root
	2010		2011	
H[a]				
df[b]	1, 21	1, 21	1, 21	1, 21
F	1.88	2.06	0.01	0.54
P	NS[e]	NS	NS	NS
PC[c]				
df	1, 21	1, 21	1, 21	1, 21
F	119.77	10.62	36.74	26.58
P	<0.001	0.004	<0.001	<0.001
SN[d]				
df	1, 21	1, 21	1, 21	1, 21
F	11.56	0.28	61.88	39.76
P	0.003	NS	<0.001	<0.001
H x PC				
df	1, 21	1, 21	1, 21	1, 21
F	0.54	1.67	0.32	0.77
P	NS	NS	NS	NS
H x SN				
df	1, 21	1, 21	1, 21	1, 21
F	0.57	0.11	0.12	0.09
P	NS	NS	NS	NS
PC x SN				
df	1, 21	1, 21	1, 21	1, 21
F	3.87	0.08	8.08	3.32
P	NS	NS	0.001	NS
H x PC x SN				
df	1, 21	1, 21	1, 21	1, 21
F	0.39	0.10	0.06	3.46
P	NS	NS	NS	NS

[a] H = *H. litura* = presence, absence
[b] Degrees of freedom present are: numerator, denominator
[c] PC = Plant competition by common sunflower (*Helianthus annuus*) = presence, absence
[d] SN = soil nutrients = low, high
[e] NS indicates P > 0.05

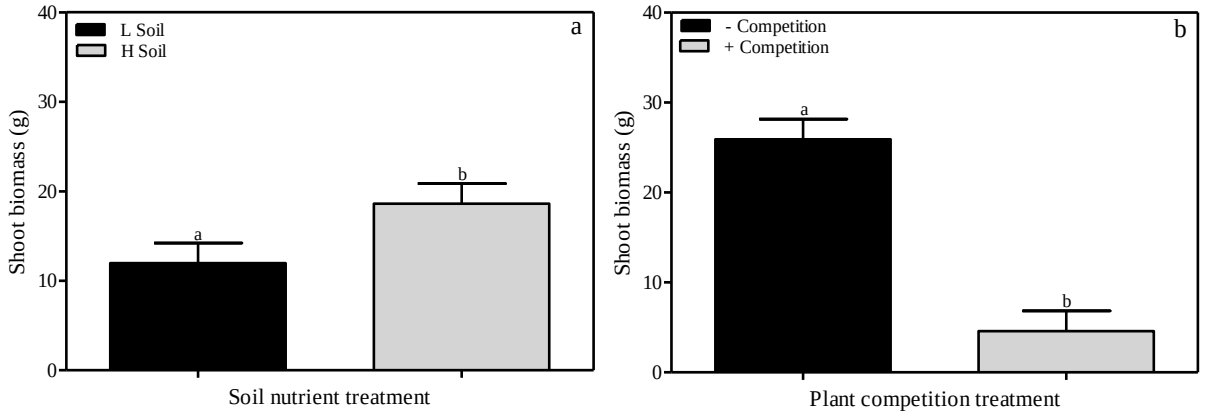

Figure 1.5. Impact of soil nutrients and common sunflower plant competition on Canada thistle final shoot biomass in 2010. Bars indicate mean values plus standard error of the mean. Pairwise comparisons labeled with different letters differ ($P \leq 0.05$).

In 2011, Canada thistle plants grown in high soil nutrient environments had the greatest shoot biomass when compared to all other treatment combinations (Figure 1.6). Canada thistle plants grown in high soil nutrients and additionally with common sunflower had less shoot biomass when compared to Canada thistle plants grown only in high soil nutrients. The low soil nutrient x common sunflower competition presence treatment reduced Canada thistle shoot biomass most when compared to all other treatment combinations (Figure 1.6).

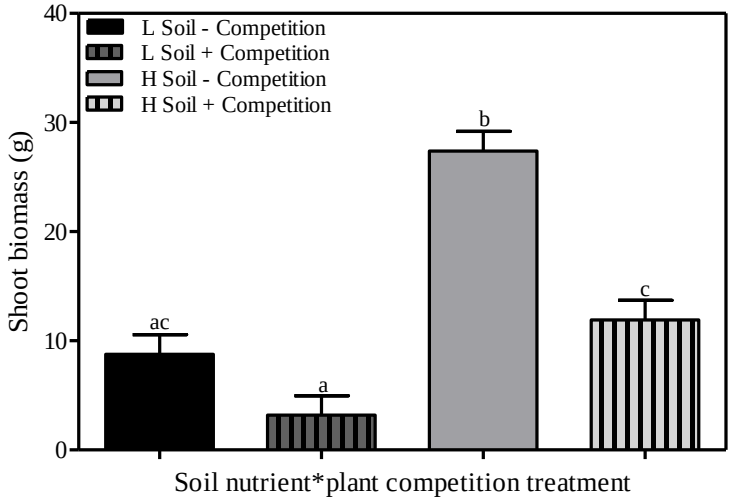

Figure 1.6. Impact of soil nutrients and common sunflower plant on Canada thistle final shoot biomass in 2011. Bars indicate mean values plus standard error of the mean. Pairwise comparisons labeled with different letters differ ($P \leq 0.05$).

The reduction in Canada thistle shoot biomass when plants were grown in low soil nutrient environments found in this study was also reported by McIntyre and Hunter (1975). Canada thistle plants grown in soil treated with 210 ppm nitrogen produced a mean of 5.6 g total shoot dry biomass while Canada thistle plants grown in soil treated with 5.3 ppm produced only a mean of 1.4 g total shoot dry biomass, which represents a 75% reduction.

A similar reduction in shoot biomass when Canada thistle plants were grown with common sunflower competition was reported by Perry et al. (2009) in a controlled greenhouse experiment and also by Friedli and Bacher (2001), who grew Canada thistle in competition with three grass species (perennial ryegrass, Italian ryegrass, and orchardgrass). In contrast, when Canada thistle was grown with plant competition by alkali sacaton and needle and thread grass (alone or in combination with these two grasses), Canada thistle total shoot biomass was not affected by competition from either species (Ferrero-Serrano et al. 2008). Again, this difference may be attributed to the physiological and morphological differences in the plant competitors used in the experiments.

Moreover, niche complementary and plasticity in resource use are additional factors that could attribute to the differences in research results. The niche complementary hypothesis suggests species coexistence is primarily due to resource use differences that result in different plants occupying distinct niches and utilizing resources in a complementary fashion (Kahmen et al. 2006; Pacala and Tilman 1994). Although niche complementary is often noted as a factor explaining plant community diversity, resource use plasticity in resource use could also be a factor (Ashton et al. 2010). Plasticity in resource use means that, when different plant species are grown together in competition for a particular limiting resource, one individual may switch to an alternative less-used form of that resource and continue growth (Hector et al. 1999). For

example, microcosm experiments demonstrated that when prairie bluebells (*Mertensia lanceolata* (Pursh) DC.) were grown with either Bellardi bog sedge (*Kobresia myosuroides* (Vill.) Fiori), Ross' avens (*Geum rossii* (R. Br.) Ser.), or curly sedge (*Carex rupestris* All.), each of the latter species preferred the ammonium form of nitrogen, whereas all species preferred nitrate when grown in monoculture. (Ashton et al. 2010). This change in the dominate form of nitrogen utilized allowed each competing species to maintain growth when grown with prairie bluebells. Differences in outcomes of competition between Canada thistle and various competitors may possibly have been due to either niche complementarity and/or plasticity in resource use outlined above.

In neither of our experimental runs did we detect a negative impact of *H. litura* on final Canada thistle shoot biomass (Table 1.3). Our findings are similar to those of Collier et al. (2007) and Ferrero-Serrano et al. (2008), who investigated the combined impacts of *H. litura* and three different herbicide treatments and *H. litura* and native grass competition, respectively, for Canada thistle control; both reported no difference in Canada thistle final shoot biomass of plants attacked by *H. litura* and unattacked plants. Friedli and Bacher (2001) also reported that the shoot base boring weevil *A. onopordi* had no impact on Canada thistle total shoot biomass. In contrast to this trend, Sciegienka et al. (2011) reported that *H. litura* herbivory had a negative impact on Canada thistle shoot biomass in greenhouse experiments. The reported differences in impacts of *H. litura* and Canada thistle biological control agents on Canada thistle total shoot biomass could be attributed to inherent genetic differences of the insects used, the severity of the attack, and also the impacts of differential environmental conditions on biological control agent performance (Menalled et al. 2004).

Canada Thistle Sexual Reproduction.

Canada thistle Inflorescence Number. In 2010, all three treatments were associated with a reduction in Canada thistle inflorescence production (Table 1.1; Figure 1.7). Both *Hadroplontus litura* attack and low soil nutrients were associated with approximately a 50% reduction in Canada thistle inflorescence production (Figure 1.7a,b). Common sunflower plant competition had the strongest negative impact on inflorescence production compared to the *H. litura* and soil nutrient treatments (Figure 1.7c).

In 2011, Canada thistle plants grown in the low soil nutrient treatment or with common sunflower competition produced fewer inflorescences than Canada thistle plants grown in high soil nutrient environments or without common sunflower competition (Table 1.1; Figure 1.8). Overall, the low soil nutrient treatment was associated with the greatest reduction in Canada thistle inflorescence number (Figure 1.8a).

The negative impact of *H. litura* attack on inflorescence production in the 2010 experimental run is not supported by previous research. In a similar study, herbivory by *C. rubiginosa* had a negligible effect on Canada thistle reproductive output (Cripps et al. 2010). Furthermore, herbivory by another biological control agent, *A. onopordi*, a stem boring weevil, had no impact on Canada thistle flower production (Friedli and Bacher 2001). These previously reported results are similar to our 2011 results, in which we found no effect of *H. litura* attack on Canada thistle inflorescence production. Plant competition negatively impacted Canada thistle flower production during both 2010 and 2011 experimental runs and also in a previous study in which a mixture of three grass species (perennial ryegrass, Italian ryegrass, and orchardgrass) reduced Canada thistle flower production by 81% compared to Canada thistle plants grown without grass competition (Friedli and Bacher 2001).

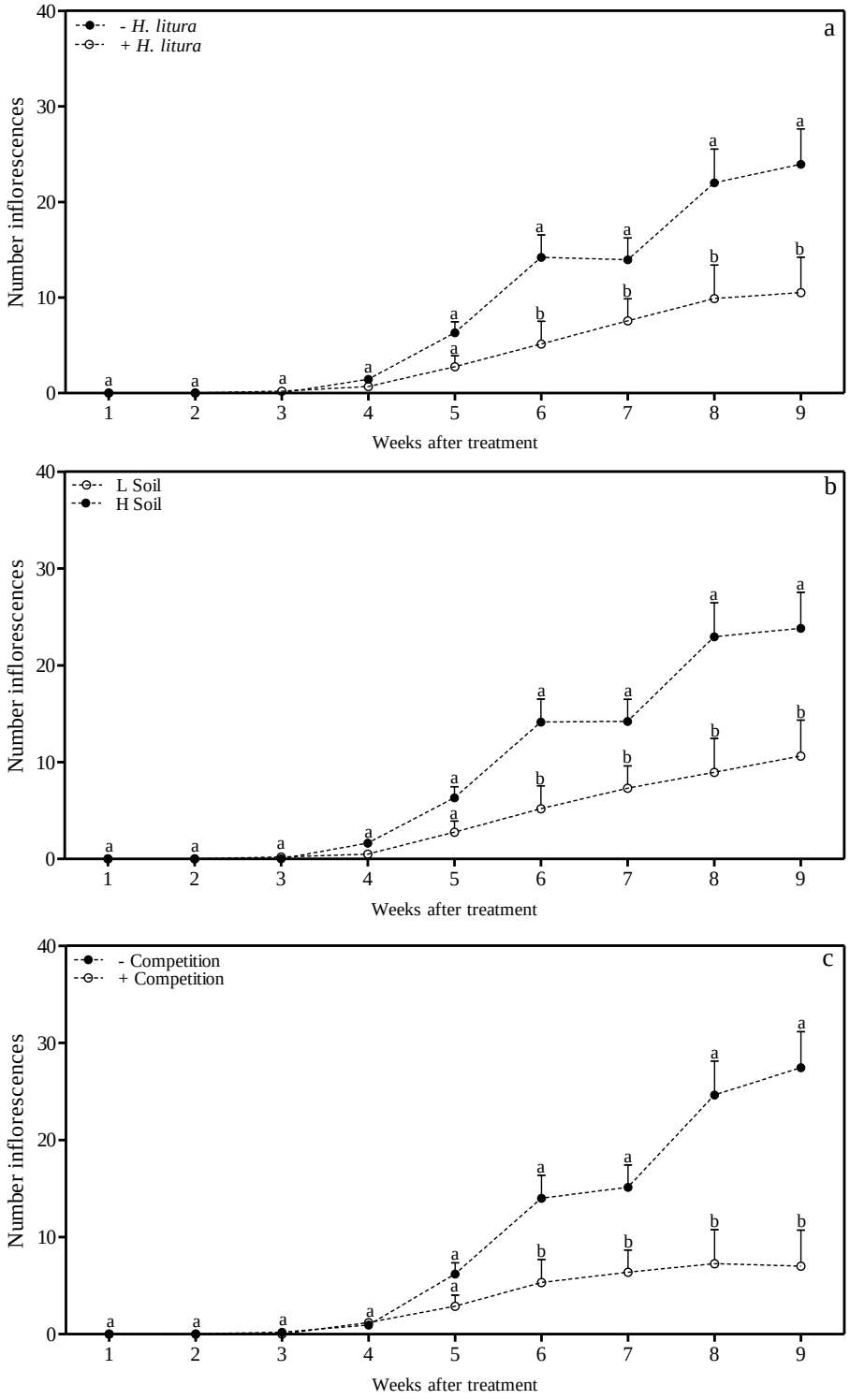

Figure 1.7. Impact of *Hadroplontus litura*, soil nutrients, and common sunflower plant competition on Canada thistle inflorescences in 2010. Symbols indicate mean values plus standard error of the mean. Symbols labeled with different letters within each date differ ($P \leq 0.05$). Unlabeled points have the same letter designation as the point directly above.

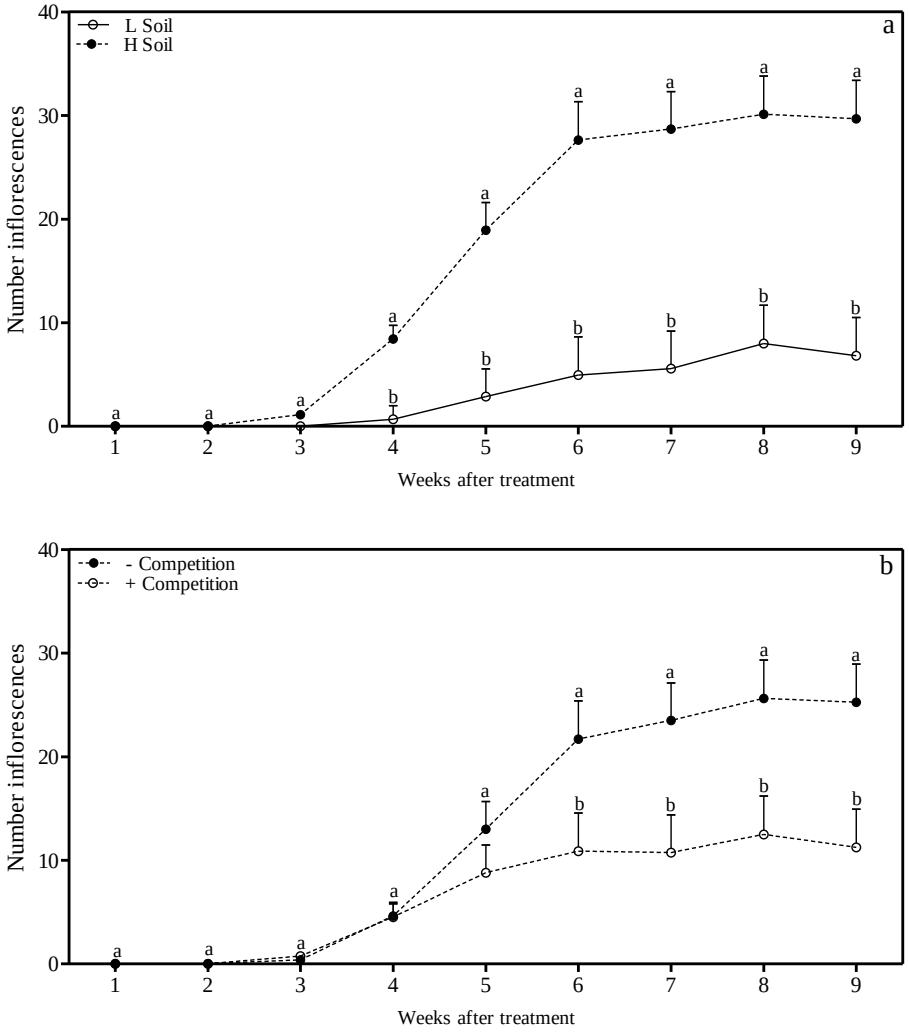

Figure 1.8. Impact of soil nutrients and common sunflower plant competition on Canada thistle inflorescences in 2011. Symbols indicate mean values plus standard error of the mean. Symbols labeled with different letters within each date differ ($P \leq 0.05$). Unlabeled points have the same letter designation as the point directly above.

Previous studies (Loehle 1987), along with our study, have demonstrated that increased soil nutrients are associated with production of more reproductive structures by perennial plants. A model constructed by Loehle (1987), which evaluated energy partitioning in clonal plants, predicted that increased soil nutrients would be associated with a decrease in the cost of producing sexual reproductive structures, which ultimately leads to increased seed production. Additional theories outlined by Gadgil and Solbrig (1972) and Newell and Tramer (1978)

suggested that under conditions of low stress plants will allocate more resources to seed production than in stressful environments. These theories support our 2010 and 2011 results, in which Canada thistle plants grown in stressful low soil nutrient environments produced fewer inflorescences than Canada thistle plants grown in less-stressful high soil nutrient environments.

Canada Thistle Vegetative Reproduction.

Canada thistle Side Shoot Number. In 2010, soil nutrients and plant competition main effects contributed to a decrease in Canada thistle side shoot production for the last 2 wk of the experiment (Table 1.1). Canada thistle plants grown in low soil nutrient environments or with common sunflower competition on the final sampling date produced 36 and 51% fewer side shoots than plants grown in high soil nutrient environments or without common sunflower competition, respectively (data not shown).

Similar to 2010, in 2011 the low soil nutrient and common sunflower plant competition treatments reduced Canada thistle side shoot production (Table 1.1; Figure 1.9). At the end of the experiment Canada thistle plants grown with common sunflower competition produced the fewest number of side shoots (Figure 1.9b) followed by Canada thistle plants grown in low soil nutrient environments (Figure 1.9a).

The negative impact of low soil nutrients on Canada thistle side shoot production in both 2010 and 2011 is similar to findings by McIntyre and Hunter (1975), who reported a 68% decrease in side shoot production by Canada thistle plants grown in soil treated with 21 ppm nitrogen compared to Canada thistle plants grown in soil treated with 210 ppm nitrogen.

During both years of our study, common sunflower competition reduced side shoot production, but this result is not supported by research performed by Ferrero-Serrano et al. (2008), who reported plant competition by alkali sacaton (*Sporobolus airoides* (Torr.) Torr.) and

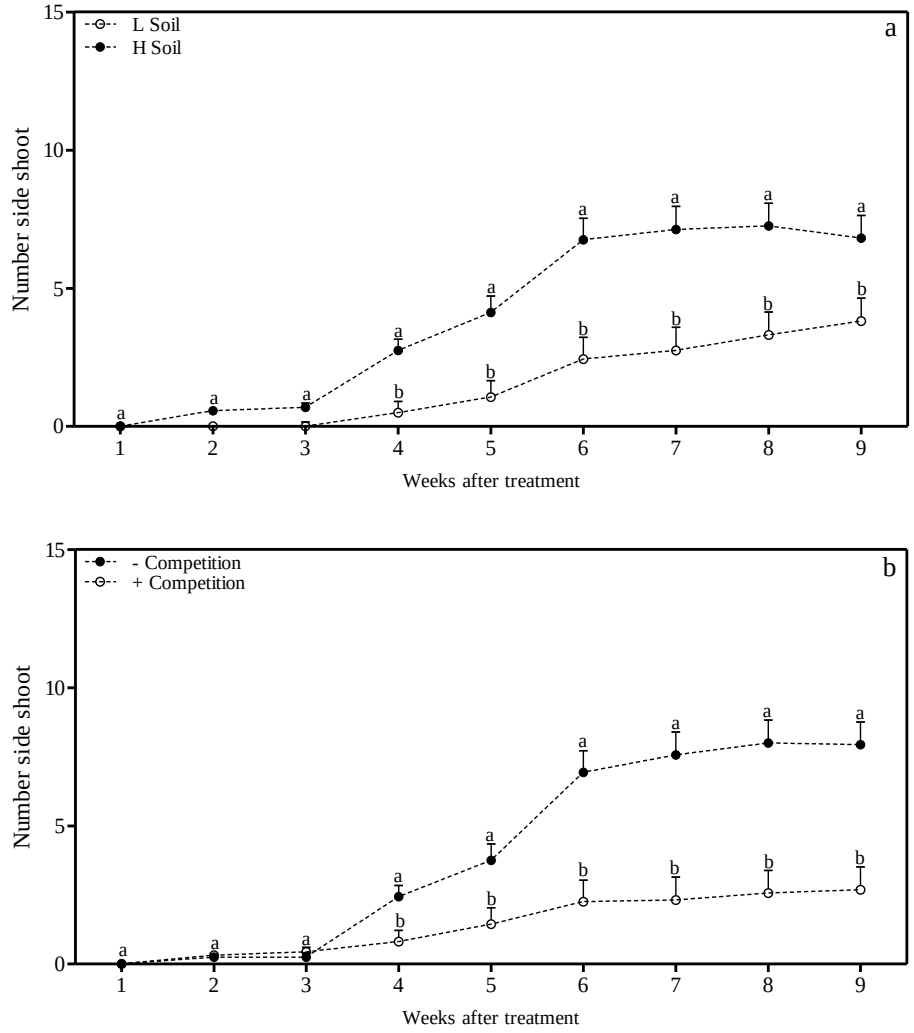

Figure 1.9. Impact of soil nutrients and common sunflower plant competition on Canada thistle side shoot number in 2011. Symbols indicate mean values plus standard error of the mean. Symbols labeled with different letters within each date differ ($P \leq 0.05$). Unlabeled points have the same letter designation as the point directly above.

needle and thread grass (*Hesperostipa comata* (Trin. & Rupr.) Barkworth) grown together and alone with Canada thistle in microcosm experiments had no impact on the number of side shoots produced by Canada thistle throughout the experiment.

We failed to detect any impact of *H. litura* on Canada thistle side shoot production in either 2010 or 2011 experimental runs (Table 1.1). In a microcosm study performed under controlled greenhouse settings Sciegienka et al. (2011) reported a negative impact of *H. litura*

attack on Canada thistle side shoot production. Canada thistle plants attacked by *H. litura* produced on average 29% fewer side shoots than Canada thistle plants not attacked. This difference may be attributed to greenhouse vs. outdoor field conditions in which the two experiments were conducted and also the age of the *H. litura* adults used in the two experiments. In our experiment *H. litura* adults were slightly older and maintained in the refrigerator longer than Sciegienka et al. (2011) and this may have led to the reduced impact of *H. litura* in our study. Additionally, the severity of *H. litura* damage may be different between our study and Sciegienka et al. (2011). In our study not every Canada thistle plant that received the *H. litura* treatment sustained larval mining stem damage. Sciegienka et al. (2011) did not report the extent of *H. litura* larval mining; if the damage was more extensive than in our study this might be another source of variation between the two studies that could play a large role in the discrepancy between results.

*Canada thistle Final Root Biomass***.** In 2010, common sunflower plant competition was the only treatment to have a negative impact on Canada thistle root biomass (Table 1.3; Figure 1.10). Common sunflower plant competition had a strong negative impact on mean root biomass and was associated with a reduction of 89% (Figure 1.10).

Similar to 2010, in 2011 common sunflower competition reduced Canada thistle mean root biomass although the impact was not as strong as in 2010 (Table 1.3; Figure 1.11b). Additionally in 2011, Canada thistle plants grown in low soil nutrient environments produced less root biomass than Canada thistle plants grown in high soil nutrient environments (Table 1.3; Figure 1.11a).

In regards to the impact of soil nutrient levels, similar results were found when Canada thistle infestations were treated with 100 kg ha^{-1} nitrogen fertilizer; root biomass excavated from

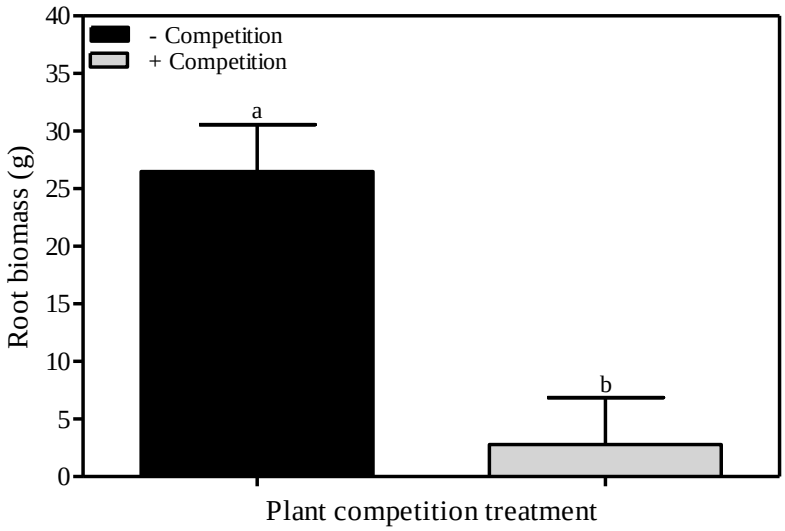

Figure 1.10. Impact of common sunflower plant competition on Canada thistle final root biomass in 2010. Bars indicate mean values plus standard error of the mean. Pairwise comparisons labeled with different letters differ ($P \leq 0.05$).

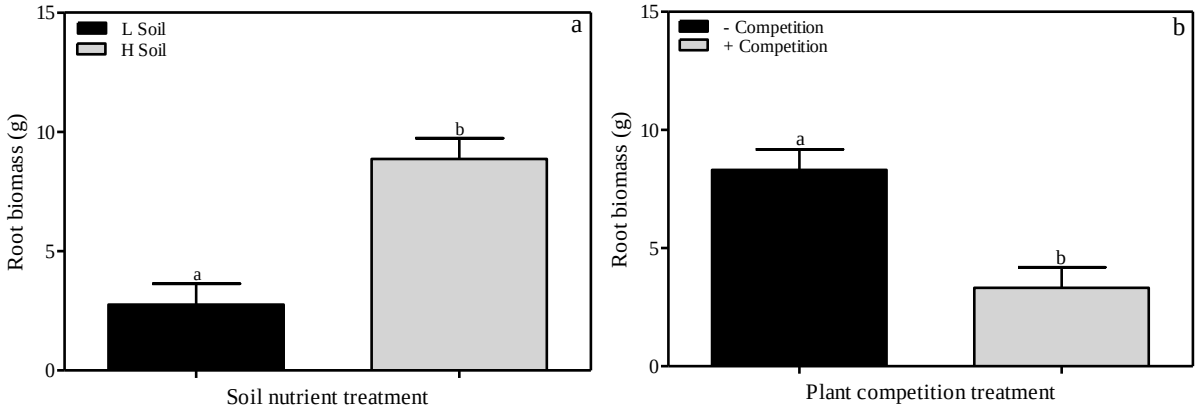

Figure 1.11. Impact of soil nutrients and common sunflower plant competition on Canada thistle final root biomass in 2011. Bars indicate mean values plus standard error of the mean. Pairwise comparisons labeled with different letters differ ($P \leq 0.05$).

a 1 and 2 yr old stand from the top 20 cm of soil was approximately three times greater than the root mass from unfertilized plots (Nadeau and Vanden Born 1990).

Similar experiments have also reported a reduction in Canada thistle root biomass via plant competition. Ferrero-Serrano et al. (2008) reported a decrease in Canada thistle root biomass when plants were grown in competition with alkali sacaton and needle and thread grass,

separately or in combination. Friedli and Bacher (2001) reported that when Canada thistle was grown in competition with three grass species (perennial ryegrass, Italian ryegrass, and orchardgrass) root biomass was less than when Canada thistle plants were grown alone.

In neither of our two experimental runs did we detect any impact of *H. litura* on Canada thistle root biomass (Table 1.3). These results are somewhat supported by Collier et al. (2007), who reported that *H. litura* herbivory failed to reduce Canada thistle root biomass in two of the three experimental runs. In the run that did detect an impact of *H. litura* on Canada thistle root biomass, the authors reported a 54% decrease in root biomass of attacked Canada thistle plants. Ferrero-Serrano et al. (2008) and Sciegienka et al. (2011) both reported a negative impact of *H. litura* on Canada thistle root biomass. These two studies found that *H. litura* attack decreased Canada thistle root biomass by 81 and 18%, respectively. The variety in research results is perplexing when trying to determine the impact of *H. litura* when used in absence of other control methods. The main difference from previous studies that reported a negative impact of *H. litura* herbivory on Canada thistle root biomass production to our study is the settings in which these studies were performed.

Our study was performed outdoors and subject to variable light, temperature, and moisture conditions, whereas the previous studies were conducted in highly controlled greenhouse environments. The natural weather fluctuations in our study may have played a large role in the results and future research would benefit from additional studies conducted in outdoor field settings to add to our findings. Additionally, studies that reported a negative impact of *H. litura* herbivory on Canada thistle root biomass production were conducted in much smaller microcosms than ours. In our study, microcosms were 19 L plastic containers whereas the studies conducted by Ferrero-Serrano et al. (2008) and Sciegienka et al. (2011) were conducted in 7.6 L

plastic containers. Experiments have demonstrated that plant growth can be negatively affected by pot size and that often when plants are grown in pots that are too small they may become root bound and a general reduction in growth occurs (Townend and Dickinson 1995). The smaller microcosm size in the previous studies may have constrained plant growth and therefor *H. litura* herbivory was more detrimental due to potentially stressed root bound Canada thistle plants. In our study microcosms were much larger and through visual inspection of the Canada thistle root system at the end of the experiment the roots did not appear to be constrained by pot size. This difference in microcosm size may have played a large role in the observed impacts of *H. litura* herbivory on Canada thistle biomass.

CONCLUSION

In general, common sunflower plant competition, low soil nutrient levels, and *H. litura* herbivory negatively impacted some aspect of Canada thistle growth and reproductive output, but specific effects varied between experimental runs. Differences in the results may be attributed to the environmental differences between 2010 and 2011 and also methodological differences. Environmental sources of variation include slight differences in precipitation and temperature. These environmental differences could be attributed to the main difference observed between the 2010 and 2011 experimental runs. The main difference observed was that, during 2011, high soil nutrients were associated with greater Canada thistle height and root biomass than low soil nutrients, but had no effect in 2010. During 2010, Canada thistle plants were exposed to 52 mm more rain than during 2011 (NDAWN 2012). As noted above, N-P-K additions were only made once at the beginning of the experiment, thus the additional rain during 2010 may have leached the soil nutrients out of the microcosms more than in 2011.

Temperature may also have played a role in the observed differences between years in the effect of the soil nutrient treatment. The common sunflower plants in 2010 were on average taller and produced more above and below-ground biomass (data not shown) than the 2011 common sunflower plants. In 2010, common sunflower plants experienced 345 more growing degree days (GDD) than the sunflower plants in 2011 (NDAWN 2012a). This increase in GDD may have led to larger plants and potential increased nutrient uptake from the soil, which possibly may have lessened the impact of the soil nutrient treatment in 2010. Additionally, this could explain the differences in magnitude of the common sunflower plant competition treatment impact among study years.

The main methodological difference between years was the Canada thistle propagation method used. In 2011 Canada thistle plants were propagated not only by vegetative root cuttings as in 2010, but were also propagated via field excavated intact small rosettes. Two Canada thistle propagation methods were used in 2011 due to low shoot production of vegetative root cuttings. To ensure propagation method did not play a large role in our experiment in 2011, propagation method was included as the blocking factor in the experimental design. Therefore, this methodological difference should have had minimal effects on the results, but is noteworthy.

Although this experiment was conducted outside to simulate field conditions there may be some discrepancies in what our study found and results that may be obtained from field experiments. First, during 2010 and 2011, not all Canada thistle plants attacked by *H. litura* sustained stem damage, suggesting that although we observed mating pairs on every Canada thistle plant and visual adult feeding damage on Canada thistle plants that received the *H. litura* treatment either females did not successfully oviposit or eggs failed to hatch and transition into first instar larvae. As a result the *H. litura* larval pressure in this study is lighter than that often noted in the field (personal observation) and may have contributed to the non-significant impacts of *H. litura* on many of the Canada thistle characteristics observed. Second, we conducted our study with Canada thistle plants grown from small vegetative root cuttings or from excavated intact rosettes, which is unlike the extensive root system of established Canada thistle plants in the field. The observed negative impacts of common sunflower plant competition and soil nutrients on Canada thistle root biomass found in this study potentially might not have the same strong negative impacts on established Canada thistle stands with highly extensive root systems in field settings.

Regardless of the variation in the impact of *H. litura*, soil nutrients, and plant competition treatments across 2010 and 2011 experimental runs, our research consistently failed to detect any synergistic impacts of control methods. Lack of significant statistical interactions amongst main effects would suggest additive effects, whereas significant interactions amongst main effects would suggest synergistic impact of control methods (Collier et al. 2007; Rees and Brown 1992). A lack of synergistic impacts is similar to findings by previous studies that combined various herbicides (Collier et al. 2007; Sciegienka et al. 2011), pathogens (Sciegienka et al. 2011), and plant competition (Ferrero-Serrano et al. 2008) with *H. litura* attack. Instead of synergistic responses amongst control methods these studies noted a rather additive nature of control methods when combined.

Although we were not able to demonstrate any synergisms amongst control methods, numerous conclusions can be drawn from this research. First, our research demonstrated the ability of *H. litura*, soil nutrients, and plant competition treatments to influence inflorescence production by Canada thistle. Colonizing of new areas by Canada thistle is primarily via wind dispersed seed (Tiley 2010) and control methods that could impact seed production may aid in reducing the ability of Canada thistle to invade new areas. Canada thistle seed also remains viable in the soil for long periods of time (Piper and Andres 1995; Tiley 2010), therefore decreasing seed inputs into the soil seed bank would be advantageous for future restoration efforts. Restoration successes and failures are often influenced by the ratio of desirable species seed to invasive species seed present in the soil seed bank (Travnicek et al. 2005).

Second, this research demonstrated the ability of control measures to negatively impact adventitious side shoot production. Canada thistle patches spread and creep into new territories via adventitious shoot production throughout the growing season, making the patches larger and

more difficult to manage (Tiley 2010). In our study, low soil nutrients and plant competition reduced side shoot production in both experimental runs. Planting common sunflower and experimentally manipulating soil nitrogen levels (Morghan and Seastedt 1999; Paschke et al. 2000; Vasquez et al. 2008) could potentially slow down the rate of expansion of Canada thistle patches.

Third, this research demonstrates the utility of using common sunflower as a plant competitor against Canada thistle. Competition by common sunflower negatively impacted multiple morphological characteristics of Canada thistle, most notably a reduction in root biomass in 2010 and 2011. Similar previous studies investigating the impacts of plant competition on Canada thistle were unable to quantify root biomass impacts (Edwards et al. 2000; Perry et al. 2009) and difficulties in controlling Canada thistle are often attributed to the deep, extensive, and regenerative root system that allows the plant to be extremely invasive (Lukashyk et al. 2008; Tiley 2010). This result is additionally promising because often chemical control measures fail to translocate sufficiently (Armel et al. 2005; Petersen and Swisher 1985) to the root system of Canada thistle to cause detrimental damage to the entire network of clonal plants. Control measures that could target and negatively impact this prolific root system would be beneficial and this research has identified a possible native plant competitor with this capacity. Future studies should be performed to further explore common sunflower as an effective competitor against Canada thistle in different environments and also combined with other control tactics.

Finally, although *H. litura* only had a weak effect on some Canada thistle morphological characteristics, our results could potentially be used to identify times when Canada thistle plants that are exposed to *H. litura* are the most vulnerable to additional control treatments. For

example in 2010, *H. litura* negatively impacted thistle growth 5 to 8 WAT (Figure 1.2), but this impact was not sustained at the last sampling date 9 WAT; another control measure could be applied to additionally stress the plant during the more vulnerable stage from 5 to 8 WAT. Future research on this system could focus on applying the treatments at different times and assess impacts on Canada thistle growth and reproductive output.

CHAPTER 2. MODELING DEVELOPMENT OF IMMATURE CANADA THISTLE STEM-MINING WEEVILS (*Hadroplontus litura*) USING A THERMAL TIME MODEL

ABSTRACT

Head capsule morphometrics and the effect of ambient air temperature on phenology of immature Canada thistle stem-mining weevil (*Hadroplontus litura* Fabricius), a biological control agent of Canada thistle (*Cirsium arvense* L.), were determined from field data collected in eastern North Dakota. Insect and temperature data were collected from sites with established *H. litura* populations that were sampled weekly from May through July during 2010 and 2011. At each sampling date, 6 Canada thistle plants were excavated and scanned for eggs and larvae under a stereomicroscope. Head capsule widths of larvae were measured at the widest point and plotted on a frequency histogram to establish the range of head capsule widths associated with a specific instar. We found the head capsule width ranges associated with first, second, and third instar *H. litura* larvae were 165-324 µm, 346-490 µm, and 506-736 µm. Logistic regression models were constructed to estimate the proportions of *H. litura* eggs, first, and second instar larvae in the population as a function of thermal time. Model estimates of the median development duration in degree-days (DD) for egg, first, and second instar stages ranged from 181 ± 35 DD to 226 ± 58 DD, 159 ± 22 DD to 185 ± 22 DD, and 113 ± 39 DD to 167 ± 40 DD. Overall, model estimates based on the validation statistics were the most accurate for the first instar stage and least accurate for egg and second instar stages.

INTRODUCTION

Canada thistle is a clone forming perennial herb with a deep root system that can spread extensively (Donald 1994; McClay 2002) and has spread throughout the temperate regions of the world (Tiley 2010). In the United States, this weed is most prevalent and troublesome in the northeastern, mid-Atlantic, Great Lakes, and Northern Great Plains states (McClay 2002). Canada thistle is listed as a noxious weed under state weed control legislation in 33 states (USDA 2010). This invasive weed thrives in disturbed or moist environments and can lower the quality of grazing lands, out-compete native plants, and negatively impact crop yield (McClay 2002; McLennan et al. 1991; O'Sullivan et al. 1982, 1985).

Classical biological control is an ecologically sound pest management tool that has been explored to control Canada thistle. A previously identified biological control agent is *Hadroplontus* (formerly *Ceutorhynchus*) *litura* Fabricius (Coleoptera: Curculionidae), a phytophagous stem-mining weevil (McClay 2002; Zwolfer and Harris 1966) native to Europe (Zwolfer and Harris 1966). Adult *H. litura* overwinter in the soil and emerge in the spring in synchrony with Canada thistle emergence. Mating occurs and eggs are laid on the leaves singly or in groups in round feeding cavities (Zwolfer and Harris 1966). In the laboratory, larvae hatch after 5 to 9 d and mine the mid-vein of the leaf, eventually tunneling into the stem.

A single stem is often mined by several larvae and becomes blackened (Zwolfer and Harris 1966). *Hadroplontus litura* larvae develop via three instar stages, the exact range of head capsule widths associated with each instar has not been previously reported. In a laboratory experiment the first ecdysis occurred 3 to 5 d after hatching follow by a second ecdysis (d after hatching not reported), which occurs in the basal region of the stem (Zwolfer and Harris 1966). The third instar larvae exit the base of the stem mid-summer, construct soil cocoons, and pupate

in the soil. New adults emerge in the fall, feed on Canada thistle foliage, and overwinter in the soil.

Despite the extensive effort at controlling Canada thistle with biological organisms, the majority of research suggests that classical biological control of Canada thistle has largely been unsuccessful (Cripps et al. 2011) and this plant still remains a problematic weed (Cripps et al. 2011; Tiley 2010). Integrated pest management (IPM) seeks to integrate multiple control tactics to provide effective pest management that is environmentally, sociologically, and economically sound (Liebman and Gallandt 1997; Thill et al. 1991). One barrier to the successful implementation of IPM is a lack of knowledge about the complex biological and ecological interactions among weedy plants, control measures, and the system as a whole (Buhler et al. 2000; Buhler 2002; Holt 2004). The need to precisely time application of other control tactics can be an additional impediment to successful integration of additional management practices with biological control. Management practice application timing needs to be carefully assessed and optimized to minimize damage to the biological control organism and maximize damage to the weed (Hatcher and Melander 2003). Therefore knowledge of insect biology, especially when an insect control agent is in a vulnerable life stage, would be advantageous in an IPM program.

The most important environmental factor that influences insect development rates is temperature (Wigglesworth1972). Considerable research has been devoted to modeling insect phenology as influenced by temperature (Candy 1991; Damos and Savopoulou-Soultani 2012; Gu and Novak 2005; Manel and Debouzie 1997; Nowierski et al. 1983; Pruess 1983; Worner 1992). Most insect phenology models are based on the principle that insects have a specific thermal constant which corresponds to the required heat units that must accumulate for the insect to complete a specific stage of development (Damos and Savopoulou-Soultani 2012). Insect

phenology research has been conducted using two different approaches: in controlled laboratory experiments under constant temperatures (Briere et al. 1999; Morgan et al. 2001) or in field experiments with natural variable fluctuating temperatures (Manel and Debouzie 1997; Nowierski et al. 1983).

Models created from data generated by controlled laboratory experiments may not be applicable to field conditions (Manel and Debouzie 1997; Wagner et al. 1984; Worner 1992). This is because, in natural environments with fluctuating temperature conditions, a greater degree of insect development can occur at lower and also higher temperatures than can occur at constant low or high temperatures in laboratory experiments (Hagstrum and Milliken 1991).To derive models that are relevant to field conditions, experiments must be conducted either in the laboratory under fluctuating temperatures or directly from field populations (Manel and Debouzie 1997).

How temperature might impact the developmental timing and efficacy of *H. litura* as a biological control agent is unclear. Previous research by Zwolfer and Harris (1966) identified the timing of certain life stages by observing infested Canada thistle plants under constant laboratory conditions. These results may not be applicable to field situations; therefore developing a mathematical model to predict the developmental timing and duration of the life stages of *H. litura* may aid in the integration of *H. litura* with other control tactics.

The first objective of this study was to establish the range of head capsule widths associated with the three larval instars of *H. litura* using head capsule morphometrics (Dyar 1890). The second objective was to construct a phenological thermal time model to predict immature development time of *H. litura* (egg, first instar, and second instar) under variable field temperatures.

MATERIALS AND METHODS

Study Site. The effect of ambient air temperature on phenology (or developmental timing) of three immature *H. litura* life stages was determined in eastern North Dakota. Insect and temperature data to support this objective were collected from sites with established *H. litura* populations that were sampled weekly from May through July during 2010 and 2011. Sites were located in Pembina, Nelson, Traill, and Richland counties and were located along a north to south gradient with variable soil texture and characteristics (Table 2.1). The vegetative cover at all locations consisted predominantly of smooth brome (*Bromus inermis* Leyss.) and Canada thistle.

***Hadroplontus Litura* Collection.** At each sampling date, 6 Canada thistle plants were excavated at a soil depth of 8 cm, placed in plastic bags, and transported to the laboratory in coolers. Plants were chosen based on the visual presence of adult *H. litura* feeding damage (Prischmann et al. in press). Canada thistle plants were stored in controlled temperature chambers at 3 C and processed within 1 wk of collection. Egg and larvae counts were assessed for each plant. First, plants were placed under a stereomicroscope[5] equipped with a microscope illuminator[6] and both sides of all leaves were scanned carefully for egg masses. If egg masses were present, eggs were carefully loosened from the leaf epidermis with a metal tweezer and placed in a small plastic vial labeled with the site name, plant number, and date. Each vial contained 70% ethanol to preserve eggs. After leaves were scanned for eggs, stems were processed for larvae by carefully splitting the stem open with a scalpel and counting all larvae. All larvae found from the same plant were placed in a plastic vial labeled with the site name, plant number, and date. Vials were filled with 70% ethanol to preserve larvae for later head capsule assessment. Descriptions and photographs

[5] Stemi 2000-C, Carl Zeiss Microscopy, Thornwood, NY
[6] Model A20500, Schott AG, Mainz, Germany

of the appearance of *H. litura* immatures and adults are provided by Winston et al. (2008) and were referenced to insure only *H. litura* individuals were used for analysis.

Table 2.1. Site and soil information for study sites utilized in *Hadroplontus litura* phenology study. Sites are arranged on a north to south gradient.

County	GPS coordinates	Sand	Silt	Clay	Soil texture	Soil series
	——N, W——	——————% ——————				
Pembina	48° 42’ 5.9’’, 97° 25’ 31.7’’	20	30	50	Clay	Grano
Nelson I	48° 5’ 9.7’’, 98° 22’ 53.6’’	55	5	40	Sandy clay	Vallers; Parnell
Nelson II	48° 4’ 44.5’’, 98° 19’ 20.0’’	43	14	43	Sandy clay	Vallers; Parnell
Traill I	47° 19’ 27.8’’, 97° 25’ 14.8’’	70	6	24	Sandy clay loam	Towner
Traill II	47° 19’ 6.1’’, 97° 24’ 37.4’’	75	5	20	Sandy loam	Arveson
Richland	46° 21’ 46.1’’, 96° 44’ 10.6’’	16	16	68	Clay	Wahpeton

Head Capsule Data Analysis. *Hadroplontus litura* larvae were extracted as discussed above from sampled Canada thistle stems and their developmental stage (i.e., larval instar) was determined using head capsule morphometrics (Dyar 1890). Larval instar determination via head capsule width is one of the most frequently utilized methods to categorize larvae into stages (Panzavolta 2007). First, larvae were placed on a glass microscope slide dorsal side up. Next, a glass coverslip was placed over the larvae to insure the preserved larvae did not tilt and remained flat against the microscope slide. Finally, head capsule widths were measured at the widest point (Godin et al. 2002; Panzavolta 2007) using a Wild M5 stereo microscope fitted with Precision Digital Positioners[7] connected to Microcode Digital Dials[8] calibrated to an accuracy of 0.001. The distribution of head capsule widths were plotted on a frequency diagram and visually

[7] Model 3486-1, Boeckler Instruments, Tucson, AZ
[8] IKL Inc., Newport Beach, CA

inspected for instar determination (Godin et al. 2002; Panzavolta 2007). Additionally, Dyar's rule was used to verify the histogram data (Dyar 1890).

Temperature Data Collection. Air temperature data used for this analysis was collected by North Dakota Agriculture Weather Network (NDAWN; http://ndawn.ndsu.nodak.edu) weather stations, which measure and record air temperatures hourly. The following NDAWN weather stations were used for Pembina, Nelson I/II, Traill I/II, and Richland sampling sites: St. Thomas, Crary, Mayville, and Wyndmere, respectively (Table 2.2). These weather stations were the closest available to the established *H. litura* locations sampled and in no instance was the weather station more than 30 km from the sampling site.

Table 2.2. Study site and weather station location information used in *Hadroplontus litura* phenology study. Sites are arranged on a north to south gradient.

Study site	GPS coordinates	Weather station	GPS coordinates	Distance from site
	N, W		N, W	km
Pembina	48° 42' 5.9'', 97° 25' 31.7''	St. Thomas	48° 36' 3.5", 97° 29' 34.8"	12
Nelson I	48° 5' 9.7'', 98° 22' 53.6''	Crary	48° 3' 0.1", 98° 36' 21.6"	17
Nelson II	48° 4' 44.5'', 98° 19' 20.0''	Crary	48° 3' 0.1", 98° 36' 21.6"	21
Traill I	47° 19' 27.8'', 97° 25' 14.8''	Mayville	47° 29' 52.5", 97° 16' 12.9"	22
Traill II	47° 19' 6.1'', 97° 24' 37.4''	Mayville	47° 29' 52.5", 97° 16' 12.9"	23
Richland	46° 21' 46.1'', 96° 44' 10.6''	Wyndmere	46° 15' 30.6", 97° 5' 34.8"	30

Statistical Modeling. Logistic regression models, as outlined by Manel and Debouzie (1997), were constructed to estimate the proportions of *H. litura* eggs, first instar larvae, and second instar larvae in the weevil population as a function of thermal time, or degree-days. Counts of field collected *H. litura* eggs and larvae and air temperature data from weather stations (Table 2.2) were used to construct the models.

Logistic regression analysis is typically conducted when the dependent variable is dichotomous or binary (Allison 1999). Logistic regression analysis is an alternative to ordinary least squares linear regression and must be performed when the dependent variable is binary due

to violations of the assumptions of linear regression, namely the assumptions of homoscedasticity and normality of the error term. Logistic regression corrects these violations and offers better statistical properties for analyzing binary data.

The logistic model parameter values were estimated by maximum likelihood (ML) methods. Maximum likelihood methods select parameter values that give the observed result the greatest probability. This is accomplished in two parts. The first part is known as the likelihood function. The likelihood function is chosen by the researcher and simply entails choosing a probability distribution; in regards to this research the distribution is binomial. After a distribution is chosen, a function form or link is selected that will relate the distribution parameter values to the values of the explanatory variables. The second part is known as the maximization step, which in approximates the parameter values iteratively until the greatest parameter estimate is achieved for a particular data set.

The first step in the logistic regression analysis to estimate the proportions of *H. litura* eggs, first instar larvae, and second instar larvae in the population as a function of thermal time is to identify the proportion of larvae, p_{ij}, in stage j at time t_i, which was defined as:

$$p_{ij} = \frac{n_{ij}}{\sum_{k=j}^{a} n_{ij}}, j = 1, 2, 3 \quad [1]$$

Where stage j = 1 is the egg stage, j = 2 is the first instar, j = 3 is the second instar, a = 4 (the total number of stages, including the third instar), and n_{ij} is the number of individuals in stage j counted at time i.

Subsequently, logistic regression models were used to express the proportion of individuals in each life stage as a function of thermal time t_i (Dennis at al. 1986):

$$p_{ij} = \frac{\exp(\alpha_{0j} + \alpha_{1j} t_i)}{1+\exp(\alpha_{0j} + \alpha_{1j} t_i)}, j = 1, \ldots, a - 1 \quad [2]$$

A logit model composed of two parts was then used to estimate the two parameters of the regression equation, α_{0j} and α_{1j}. The first component of the model described changes in n_{ij} as a linear function of t_i.

$$n_{ij} = \alpha_{0j} + \alpha_{1j} t_i \qquad j = 1,\ldots, a - 1 \qquad [3]$$

The second component of the model, the logit link, transforms the probability to an odds ratio to make the model unbounded and conform to the assumptions of linear regression. This component also describes the stochastic nature of the individuals in state S_j at time t_i:

$$\text{logit } p_{ij} = \log \frac{p_{ij}}{1 - p_{ij}} = n_{ij} \qquad [4]$$

The model is assumed to be distributed as a binomial variable $\text{Bin}(N_i, p_{ij})$ (N_i is the total number of individuals collected at time t_i).

Time t_i was measured in degree-days (DD), calculated by summing hourly ambient air temperatures over the relevant development period for each life stage according to the following expressions:

$$t_i = DD_{B_{j,i}} = \sum_{k=u}^{i} \left(\frac{\theta_k - B_j}{24} \right), \theta_k > B_j \qquad [5]$$

$$t_i = DD_{B_{j,i}} = 0, \theta_k \leq B_j \; j = 1,\ldots 3,$$

where B_j is the physiological base for stage j, θ_k is the temperature at time k, and u is the median molt date of the previous life stage. The median molt dates (time zero points) for the first and second instars were calculated from the field collected data. For the egg stage, there was no previous stage, so the zero point was set as the estimated time of weevil mating, 1 wk prior to the first egg collected.

Maximum likelihood estimates for parameters, α_{0j} and α_{1j}, were derived by using Proc Genmod in SAS Version 9.2 (2008). For each developmental stage, the physiological base used

for the parameter estimates was estimated by the model with the smallest scaled deviance value (Manel and Debouzie 1997). Finally, the median development duration of each stage j, DD_{50} was calculated from $p_{ij} = 0.5$, by $-(\alpha_{0j}/\alpha_{1j})$. The DD_{50} corresponds to the point in which 50% of the individuals are in a particular stage j. Confidence intervals were estimated for the DD_{50} for each stage j using Fieller's theorem (Fieller 1940):

$$SE_{\frac{\alpha_{0j}}{\alpha_{1j}}} = \frac{\alpha_{0j}}{\alpha_{1j}} \sqrt{\frac{SEM_{\alpha_{0j}}{}^2}{\alpha_{0j}{}^2} + \frac{SEM_{\alpha_{1j}}{}^2}{\alpha_{1j}{}^2}} \quad [6]$$

Where SE is the standard error and SEM is the standard error of the mean.

A cross validation approach was used to test the ability of the logistic models we developed to predict observed values. Cross validation is a simple and widely used model validation approach whereby single observations or subsets of observations are left out of the model development process and then are used to assess the predictive power of a model. This approach is necessary because assessing model fit to the data used to generate the model is not a valid test of the predictive ability of a model. Predictive ability can only be assessed using validation datasets that are independent and not included in the construction of the model. We used a K-fold cross validation approach whereby data were split into k subsets and these data were fit to the logistic model leaving out each k^{th} subset in an iterative fashion and subsequently using each k^{th} omitted data subset as the validation dataset (Hastie et al. 2001). We considered each site/year to be a k^{th} subset of the data; therefore, for each life stage, the logistic model was fit to 12 separate k-1 subsets of the data. The omitted site/year subset of the data was then used to validate, or test the predictive power, of the model. Root mean square error (RMSE) and the index of agreement (d) values were calculated to assess model fit to the validation dataset (Willmott 1981). The RMSE evaluates the magnitude of the error produced by the model. When

comparing models, the model with the smaller RMSE indicates a better and more unbiased model, meaning the model predictions do not tend to over or underestimate the actual observed values. The index of agreement evaluates the degree to which a model predicted values are error-free. The index of agreement's values lie within $0 \leq d \leq 1$, with values closer to 1 indicating a stronger agreement between the observed and predicted values.

RESULTS AND DISCUSSION

Instar Determination. *Hadroplontus litura* head capsule data were plotted on a frequency histogram (Figure 2.1) to establish the population range of head capsule widths associated with a specific instar (first, second, or third), and Dyar's rule was used to verify the histogram data.

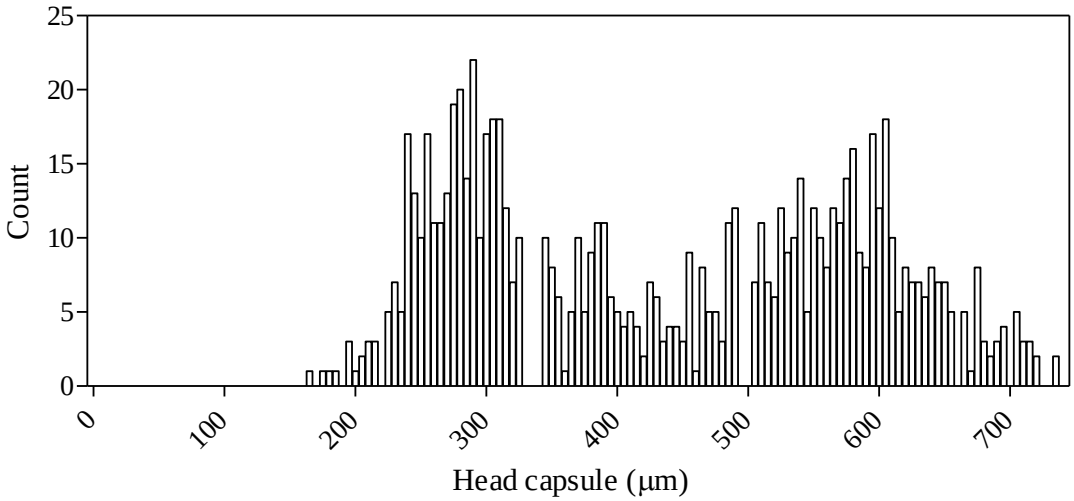

Figure 2.1. Histogram of *Hadroplontus litura* larval head capsules collected from North Dakota in 2010 and 2011.

Dyar's rule states there is a consistency of the growth ratios among instars and when the logarithm of head capsule is plotted against instar number, a linear relationship is formed (Figure 2.2; Dyar 1890). When plotting the natural log of the mean head capsule width against the instar number for the *H. litura* larvae collected in 2010 and 2011, a strong linear relationship was formed with an coefficient of determination value of $r^2 = 0.998$. We found the head capsule width ranges associated with first, second, and third instar *H. litura* larvae were 165-324 μm (mean = 274 μm; SE = 1.9; n = 292), 346-490 μm (mean = 415 μm; SE = 3.5; n = 182), and 506-736 μm (mean = 591 μm; SE = 2.9; n = 339), respectively.

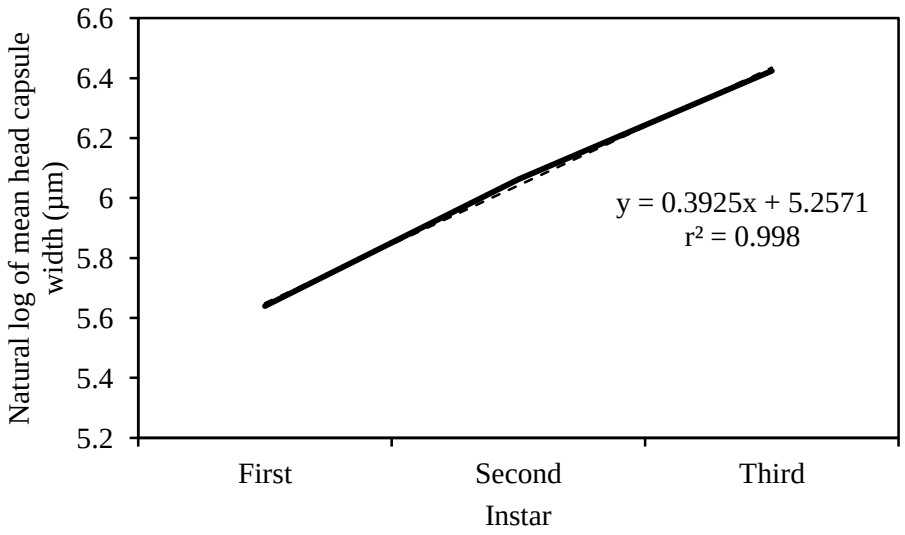

Figure 2.2. Regression relationship between the natural logarithm of the mean larval head capsule widths and the instar number of *Hadroplontus litura*.

Previous research about head capsule widths and instar determination of *H. litura* has not been reported; therefore, our data cannot be directly compared to previously reported information about *H. litura*, but can be compared to results about other *Ceutorhynchus* species including: *C. obstrictus* Marsham (Coleoptera: Curculionidae) (Dosdall and McFarlane 2004), *C. subpubescens* LeConte (Coleoptera: Curculionidae) (Dosdall et al. 2007), and *C. trimaculatus* Fabricius (Coleoptera: Curculionidae) (Kok and McAvoy 1983). *Ceutorhynchus obstrictus*, the cabbage seedpod weevil, infests the siliques of canola (*Brassica napus* L.) plants and develops via three instar stages similar to *H. litura* (Dosdall and McFarlane 2004). Head capsule width ranges for the first, second, and third instar stages were reported as: 205-245 µm (mean = 231 µm), 332-411 µm (mean = 371 µm), and 506-593 µm (mean = 543 µm), respectively. Similarly *Ceutorhynchus subpubescens* develops via three instar stages within the stem tissue of flixweed (*Descurainia Sophia* L.) and ranges of head capsule widths have been reported for first, second, and third instars as: 260-350 μm (mean = 310 μm), 400-540 μm (mean = 470 μm), and 570-740

μm (mean = 640 μm), respectively (Dosdall et al. 2007). *Ceutorhynchus trimaculatus*, which has been identified as closely related to *H. litura* by Zwolfer and Harris (1966), feeds on musk thistle rosettes, and also develops via three instar stages. Mean head capsule widths associated with the first, second, and third instar stages are: 310 µm, 480 µm, and 670 µm (Kok and McAvoy 1983). Results from our study are similar to the head capsule width sizes reported for *C. obstrictus, C. subpubescens,* and the closely related *C. trimaculatus*.

Although larval instar determination via head capsule width is one of the most frequently used methods to categorize larvae into stages, occasionally the application of Dyar's rule and frequency histograms do not reveal clear cut separations between peaks that would delineate successive instars (Panzavolta 2007). In the previously reported *Ceutorhynchus* literature mentioned above and in our data, Dyar's rule confirmed the frequency histogram data and measurements did not overlap. When data form a continuum and Dyar's rule fails to predict the number of instars from frequency histogram head capsule data, additional statistical analysis is needed (Godin et al. 2002; Panzavolta 2007). Logan et al. (1998) developed a program called Hcap to address this problem when frequency histograms show no distinct separations. Hcap was used to identify the separation points, range of head capsule widths, mean, and probability of misclassification of the larval stages of the weevil, *Pissodes castaneus* (Coleoptera: Curculionidae) (Panzavolta 2007). Although further statistical analysis of *P. castaneus* larvae was needed to identify separation between instar stages, no further analysis was needed for our data due to distinctive separations between successive stages in frequency histogram data. This approach was further confirmed by previous research surrounding other *Ceutorhynchus* species in which instar determination was conducted in a similar manner (Dosdall et al. 2007; Dosdall and McFarlane 2004; Kok and McAvoy 1983).

Duration of Life Stages. Model estimates for the DD_{50} (point in which 50% of the individuals are in a particular stage j) for the egg stage ranged from 181 ± 35 DD to 226 ± 58 DD (Table 2.3).

Table 2.3. Estimates of maximum likelihood parameters, median development durations, and 90% confident intervals for *Hadroplontus litura* thermal-time logistic models of egg, instar 1, and instar 2 development for each site/yr that was left out of the model and used as a validation dataset.

Site removed	T_b[a]	α_{0j}	α_{1j}	DD_{50}[b]	CI[c]	α_{0j}	α_{1j}	DD_{50}	CI
	C	2010				2011			
Pembina									
Egg	0	1.2579	-0.0061	206	165-247	1.2541	-0.0063	199	161-234
Instar 1	0	2.9787	-0.0161	185	163-207	3.2081	-0.0187	172	148-196
Instar 2	0	0.9679	-0.0066	147	112-182	1.0573	-0.0077	137	106-168
Nelson I									
Egg	0	1.1597	-0.0064	181	145-217	1.1633	-0.0059	197	157-237
Instar 1	0	3.1920	-0.0188	170	148-192	3.2143	-0.0182	177	153-201
Instar 2	0	0.9107	-0.0063	145	109-182	0.6542	-0.0058	113	74-152
Nelson II									
Egg	0	0.9661	-0.0044	226	168-284	1.0821	-0.0052	208	161-255
Instar 1	0	2.9437	-0.0185	159	137-181	3.5092	-0.0211	166	143-189
Instar 2	0	1.0276	-0.0064	161	123-199	0.9005	-0.0063	143	105-181
Traill I									
Egg	0	1.4990	-0.0068	220	182-258	1.1693	-0.0057	205	163-247
Instar 1	0	3.3650	-0.0198	170	147-193	3.1650	-0.0190	167	144-190
Instar 2	0	1.0117	-0.0065	156	118-194	0.8723	-0.0063	138	101-175
Traill II									
Egg	0	1.0202	-0.0052	196	149-243	1.1081	-0.0056	198	156-240
Instar 1	0	3.3158	-0.0199	167	144-190	3.3454	-0.0193	173	150-196
Instar 2	0	1.1490	-0.0083	138	108-168	1.0609	-0.0068	156	119-193
Richland									
Egg	0	1.5612	-0.0073	214	179-249	1.4139	-0.0068	208	171-245
Instar 1	0	3.2794	-0.0190	173	149-197	3.1879	-0.0180	177	153-201
Instar 2	0	1.1570	-0.0069	167	127-207	0.7866	-0.0063	125	90-160

[a]T_b = physiological base
[b]DD_{50} = median development duration in degree-days
[c]CI = 90% confidence interval of DD_{50}

Many model-predicted DD_{50} estimates were separated by only 1 DD, had similar confidence intervals, and showed low variability amongst each other, with a few exceptions (Table 2.3). One notable exception is the model constructed by omitting the 2010 Nelson II site; this model predicted the longest DD_{50}, 226 DD, and also had the widest confidence interval, 168-284 DD (Table 2.3). Overall, the observed proportion of eggs collected at lower cumulative DD was consistently greater than what was predicted by the model (Figure 2.3-2.4). Furthermore, the observed proportion of eggs collected at greater cumulative DD was consistently lower than what was predicted by the model (Figure 2.3-2.4).

In general, the majority of the observed first instar individuals fell within the 90% confidence interval predicted by all the first instar models (Figure 2.5-2.6). Median development duration of first star individuals ranged from 159 ± 22 DD to 185 ± 22 DD (Table 2.3). Model results suggest that the transition for egg to instar one was well documented by the collection practices.

Overall, observed data were the most variable for the second instar stage and demonstrated no clear visual trend. Median development duration ranged from 113 ± 39 DD to 167 ± 40 DD (Table 2.3). Overall, DD_{50} values predicted by the second instar models were shorter than those predicted by the egg and first instar models, which may have played a role in the scattered nature of the observed vs. predicted plots (Figure 2.7-2.8). Because sites were sampled weekly, the second instar stage may have been not captured sufficiently when compared to the other more slowly developing stages (i.e., instar one). This potential deficiency in our sampling methods made the second instar stage more difficult to capture and the models probably could have been improved by incorporating more frequent sampling periods.

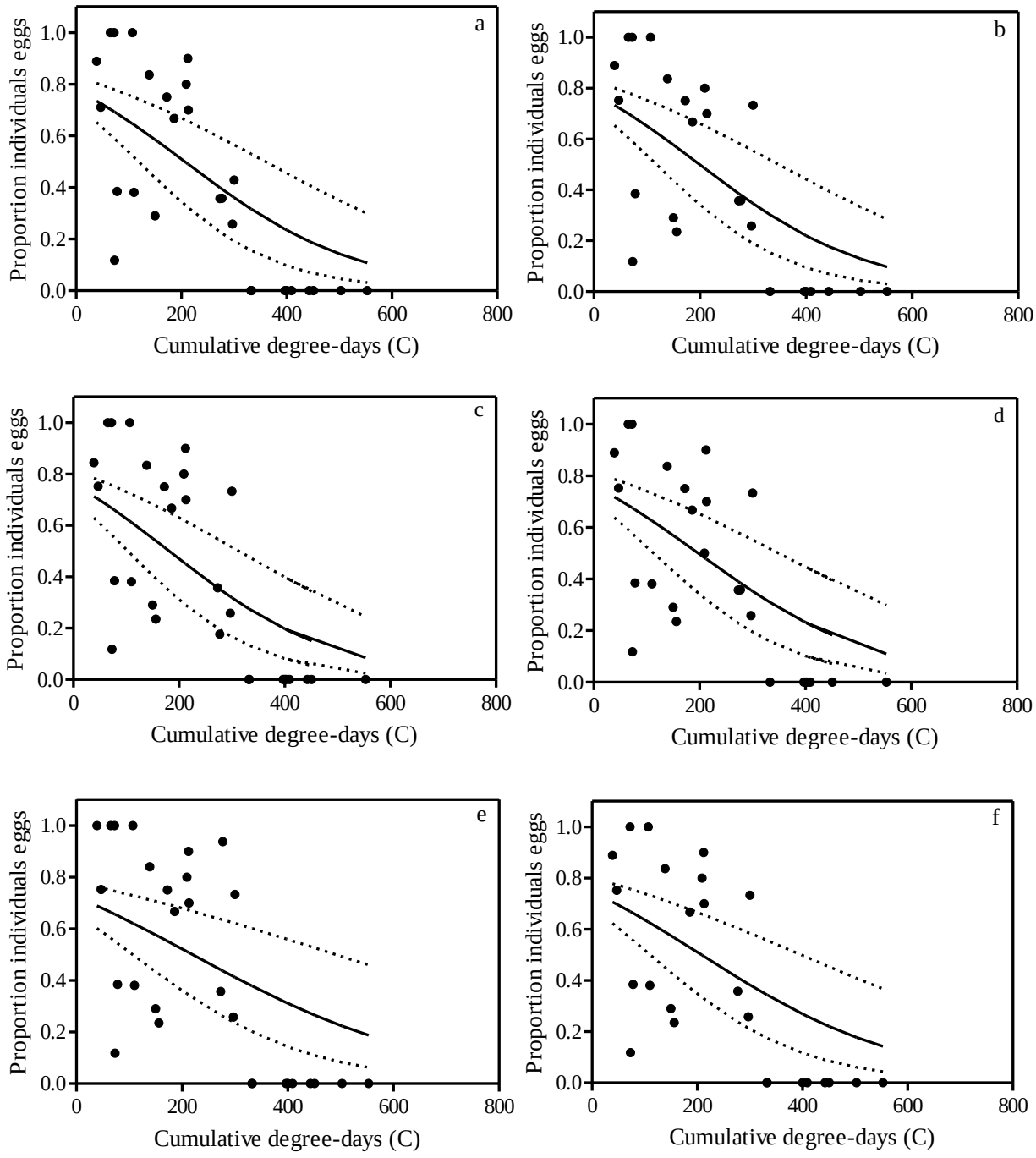

Figure 2.3. Proportion of *Hadroplontus litura* eggs vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Pembina 2010 b) Pembina 2011 c) Nelson I 2010 d) Nelson I 2011 e) Nelson II 2010 f) Nelson II 2011. Degree-days accumulated from 1 wk prior to first egg collected at each location. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

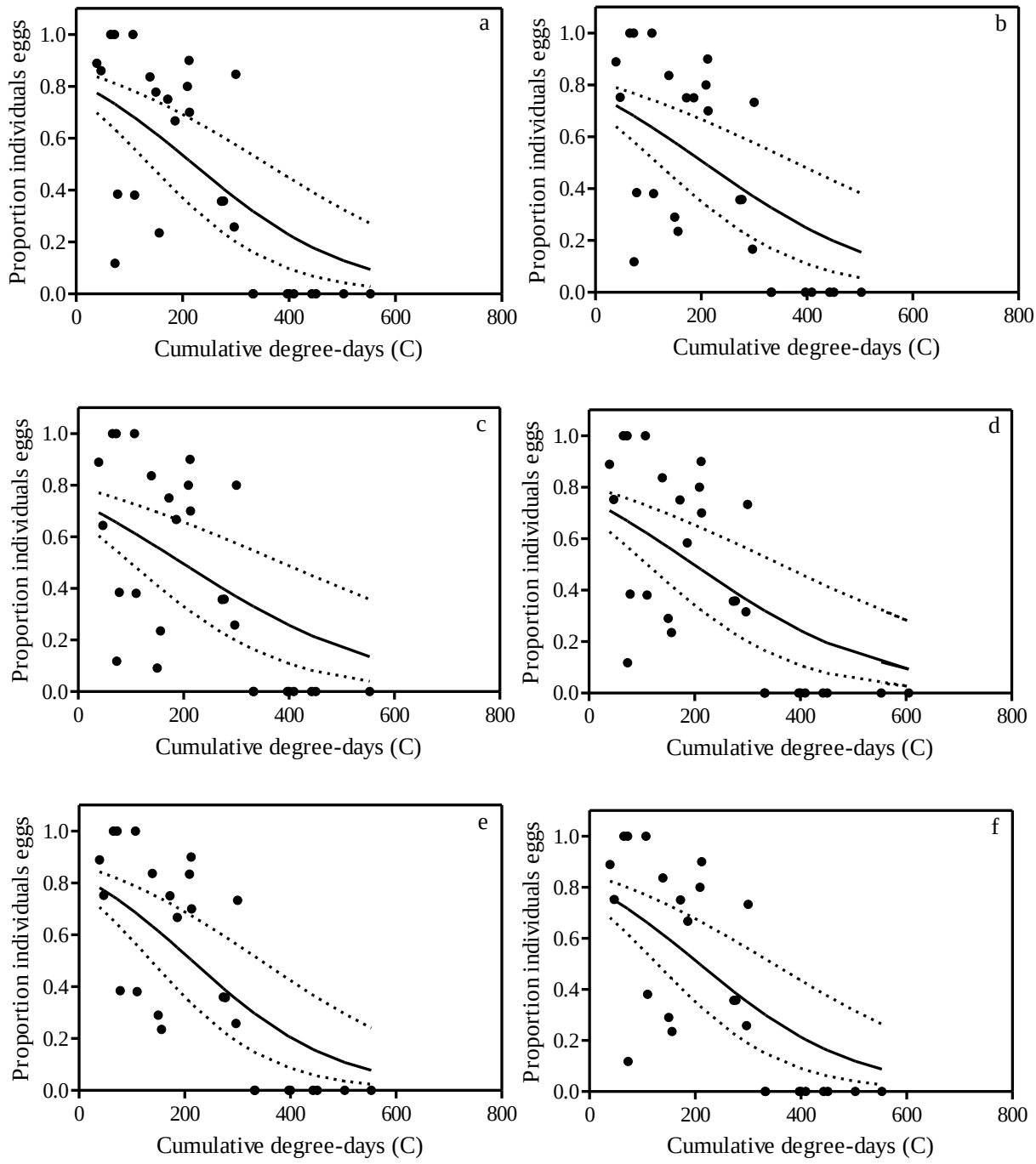

Figure 2.4. Proportion of *Hadroplontus litura* eggs vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Traill I 2010 b) Traill I 2011 c) Traill II 2010 d) Traill II 2011 e) Richland 2010 f) Richland 2011. Degree-days accumulated from 1 wk prior to first egg collected at each location. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

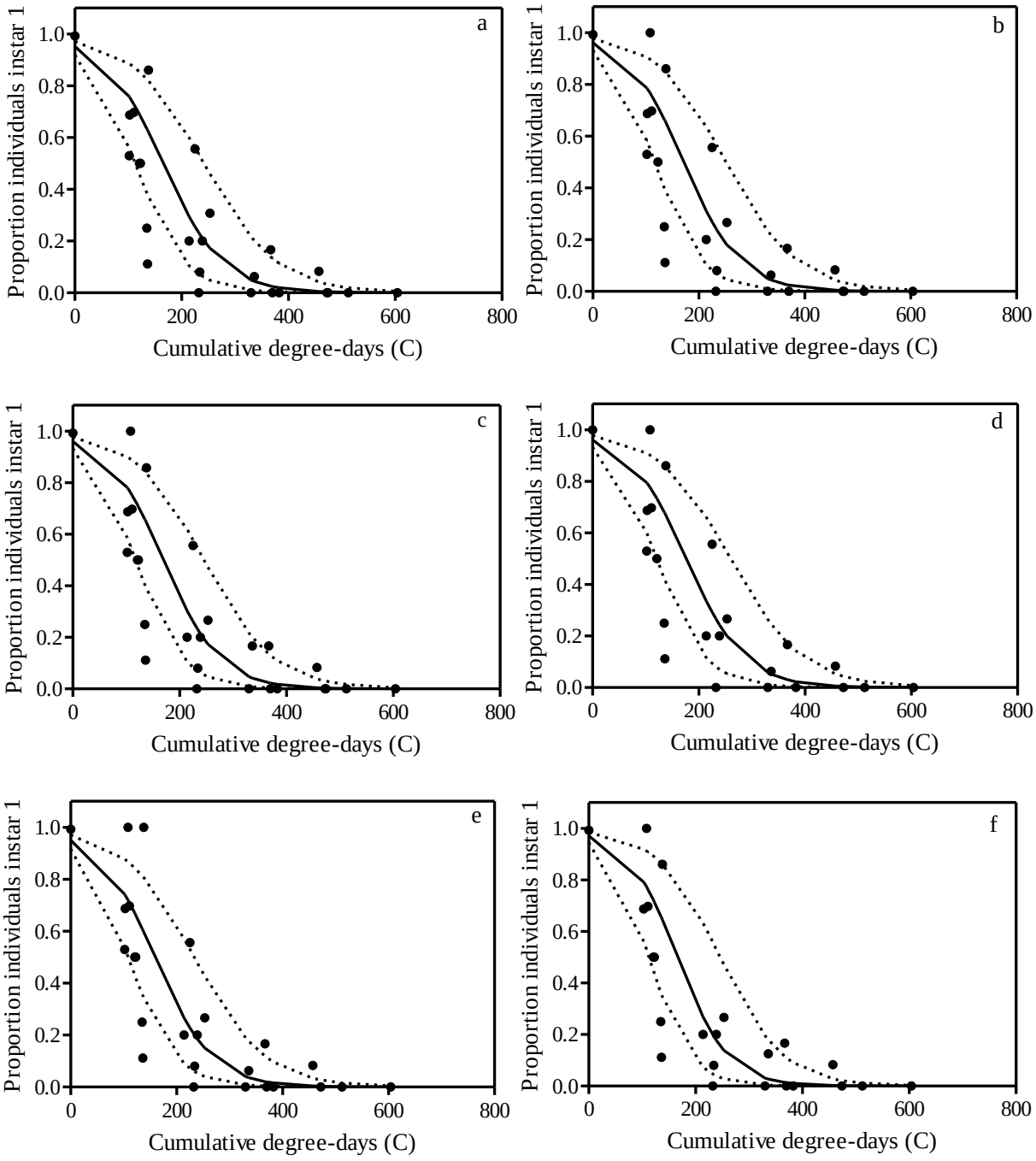

Figure 2.5. Proportion of *Hadroplontus litura* instar 1 vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Pembina 2010 b) Pembina 2011 c) Nelson I 2010 d) Nelson I 2011 e) Nelson II 2010 f) Nelson II 2011. Degree-days accumulated from median molt date of the egg stage. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

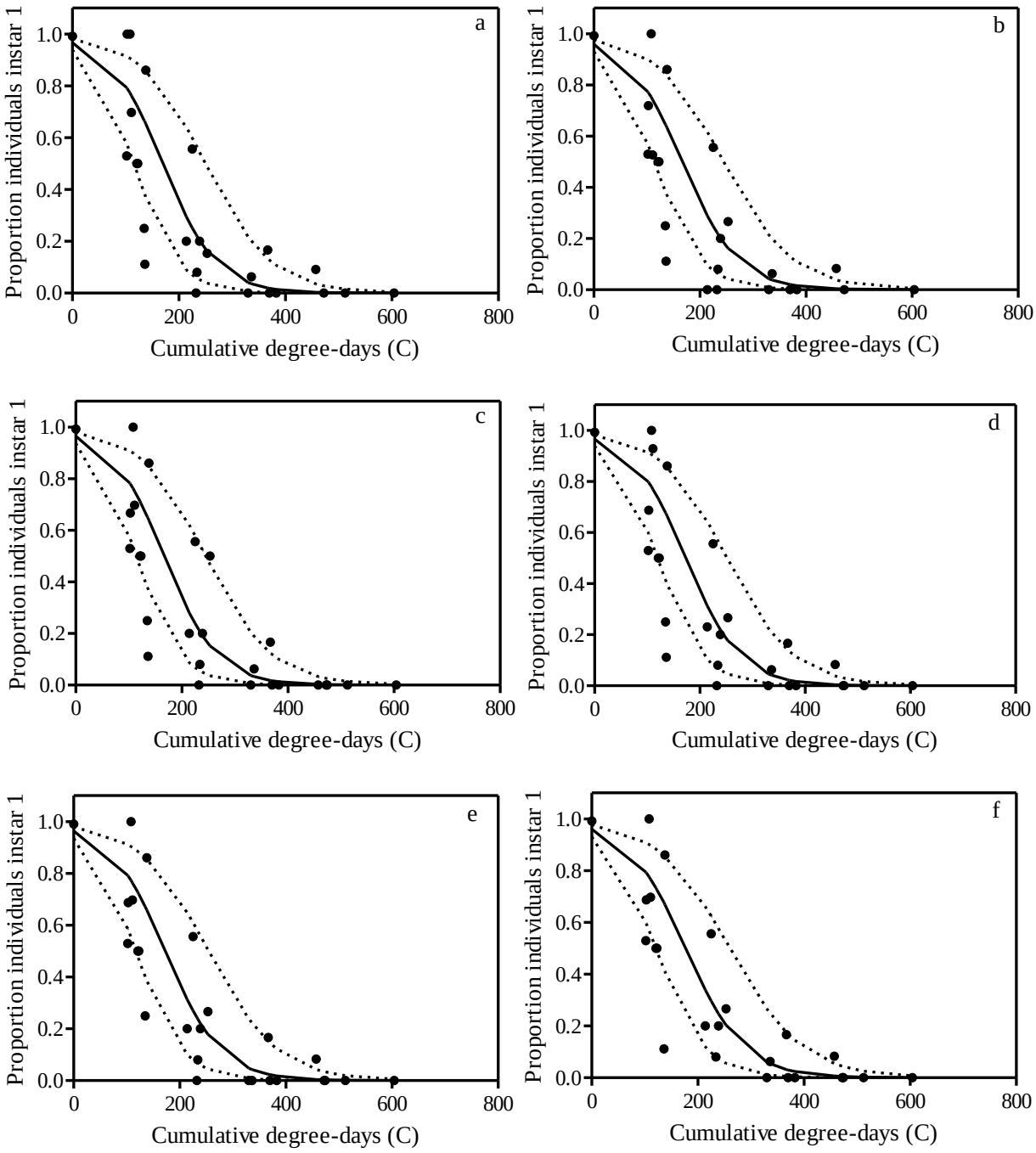

Figure 2.6. Proportion of *Hadroplontus litura* instar 1 vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Traill I 2010 b) Traill I 2011 c) Traill II 2010 d) Traill II 2011 e) Richland 2010 f) Richland 2011. Degree-days accumulated from median molt date of the egg stage. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

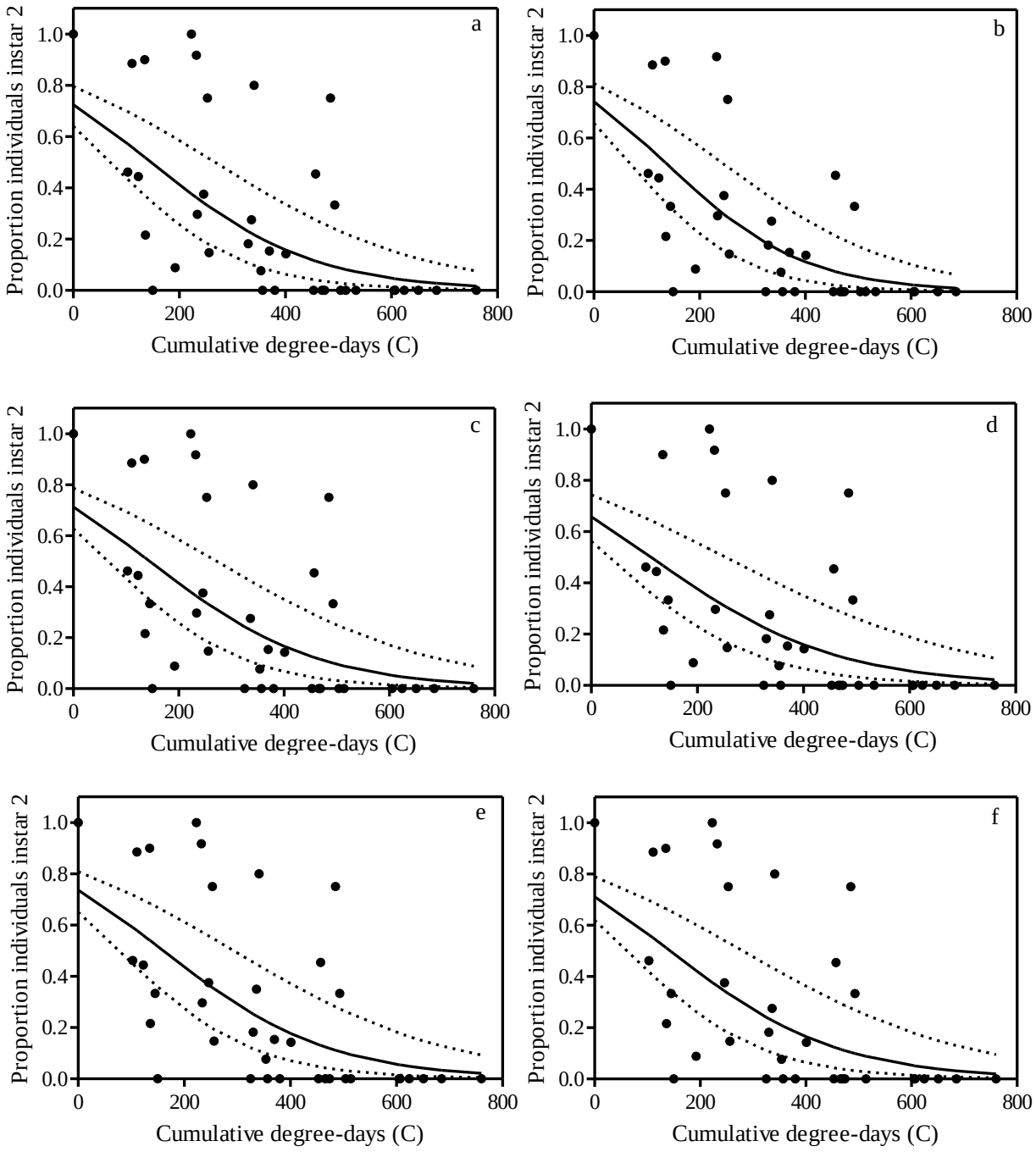

Figure 2.7. Proportion of *Hadroplontus litura* instar 2 vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Pembina 2010 b) Pembina 2011 c) Nelson I 2010 d) Nelson I 2011 e) Nelson II 2010 f) Nelson II 2011. Degree-days accumulated from median molt date of the first instar stage. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

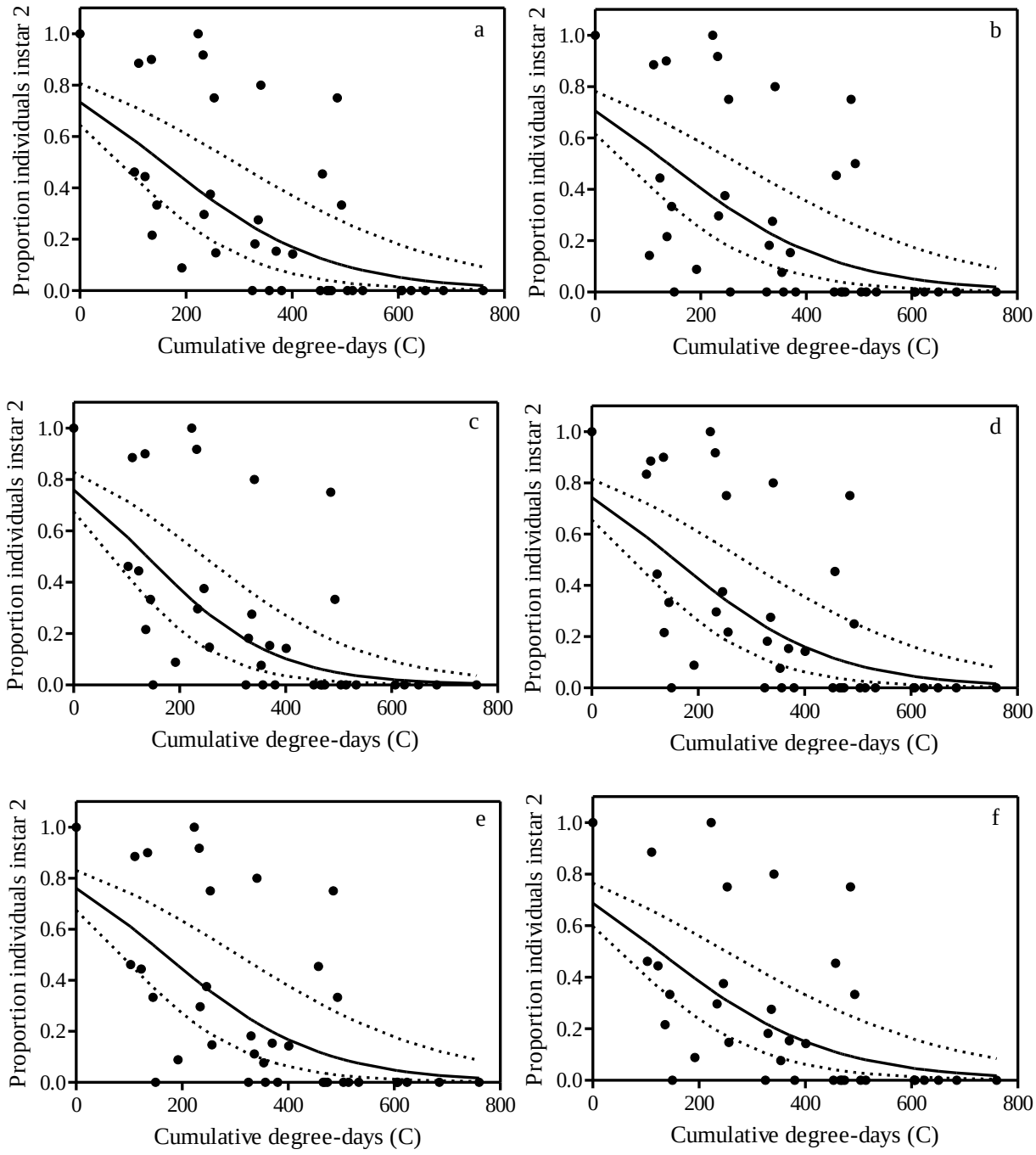

Figure 2.8. Proportion of *Hadroplontus litura* instar 2 vs. cumulative degree-days (base 0 C) for thermal time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr: a) Traill I 2010 b) Traill I 2011 c) Traill II 2010 d) Traill II 2011 e) Richland 2010 f) Richland 2011. Degree-days accumulated from median molt date of the first instar stage. Round symbols represent observed values, the solid lines represent the model prediction curve, and dashed lines represent the model 90% confidence interval band.

When visually evaluating the observed vs. predicted plots for each stage (Figure 2.3-2.8), the first instar stage appears to have the best model fit, due to the majority of the observed values falling within the 90% confidence interval (Figure 2.5-2.6). The confidence intervals for the first instar stage are also the narrowest compared to other stages (Table 2.3). Additionally, when evaluating the model validation statistics, instar one models yielded consistently high d values and low RMSE values compared to the egg and second instar stage models (Table 2.4). The

Table 2.4. Validation statistics for *Hadroplontus litura* thermal-time logistic models constructed from pooled (k-1) site/yrs for each k^{th} removed (validation) site/yr.

Site removed	RMSE[a]	d[b]	RMSE	d
	2010		2011	
Pembina				
Egg	0.4266	0.3151	0.3059	0.6237
Instar 1	0.1830	0.9470	0.1127	0.9774
Instar 2	0.1788	0.7846	0.5097	0.6082
Nelson I				
Egg	0.3729	0.2279	0.3134	0.7487
Instar 1	0.2079	0.9403	0.1797	0.9327
Instar 2	0.0778	0.8287	0.2470	0.8507
Nelson II				
Egg	0.2253	0.7156	0.2360	0.8041
Instar 1	0.1343	0.9757	0.1906	0.9392
Instar 2	0.2414	0.8637	0.1452	0.9333
Traill I				
Egg	0.4286	0.5036	0.1918	0.8883
Instar 1	0.4239	0.7511	0.1007	0.9840
Instar 2	0.3414	0.7623	0.1825	0.9128
Traill II				
Egg	0.1991	0.8656	0.2602	0.7345
Instar 1	0.1206	0.9794	0.1989	0.9371
Instar 2	0.3527	0.6212	0.3304	0.7345
Richland				
Egg	0.3745	0.4656	0.2719	0.0000
Instar 1	0.3252	0.8444	0.2949	0.8528
Instar 2	0.1891	0.6583	0.3705	0.6919

[a]RMSE = root mean square error
[b]d = index of agreement

striking ability of this model to predict development duration of the first instar could be related to the relative duration of this stage. Median development duration times were quickest for the second instar followed by first instar and then the egg stage (Table 2.3). As mentioned above, sampling occurred once weekly and, due to the relatively long duration of the first instar stage, weekly sampling was sufficient to capture this stage. Although the median development duration of the egg stage was also relatively long, the median molt date or zero point for the accumulated DD calculation was unknown because there was no previous stage, so the zero point was set as the estimated time of weevil mating, 1 wk prior to the first egg collected. Estimating the zero point for the eggs could have played a role in the observed variability in egg stage plots (Figure 2.3-2.4). Variability in second instar plots was discussed above and might be a result of the relative quick duration of this stage coupled with the weekly sampling frequency. Second instar individuals were present in Canada thistle stems later in the summer than the egg and first instars and subsequently experienced greater temperatures and accumulated DD faster than the previous stages (Figure 2.9), which ultimately could have sped the duration of this stage, making our sampling frequency insufficient.

Little is known about how temperature impacts the development time of *H. litura* under variable field temperatures. There are two previously published studies in which *H. litura* phenology is briefly described as observed under constant laboratory conditions (Peschken and Beecher 1973; Zwolfer and Harris 1966). In one study wherein temperature was not reported, larvae hatched 5-9 d after oviposition and the first molt occurred 3-5 d after hatching (Zwolfer and Harris 1966). In a study conducted by Peschken and Beecher (1973), the lengths of the egg stage were 4, 5, and 8 d at constant temperatures of 27, 22, and 17 C respectively. Our results cannot be easily compared to those of Zwolfer and Harris (1966) because the temperature

regimes used in their experiments were not reported. The results of our study are not in accordance with those of Peschken and Beecher (1973). Our study evaluated the DD needed for half of the individuals to reach a certain developmental stage, thus theoretically double the DD_{50} would equal the duration of that stage. When this calculation is performed, for example, on the model constructed by omitting the Richland 2010 site, the DD required for the egg stage would be 428 DD. In 2010, at the Richland location 428 DD corresponds to 29 d, which is much longer than the duration reported by Peschken and Beecher (1973) and could be a result of the actual field setting our study was performed in with variable temperatures.

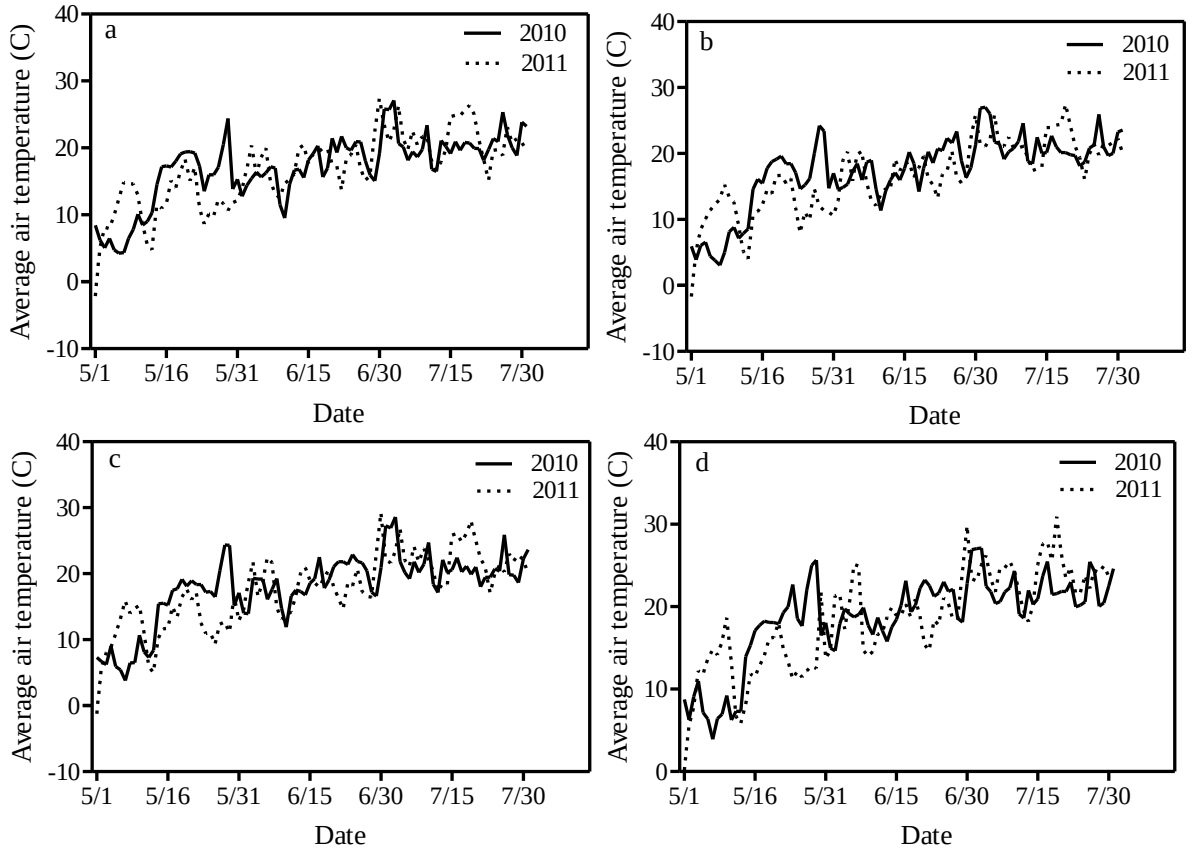

Figure 2.9. Average daily air temperature from May to July 2010 and 2011 as recorded by North Dakota Agricultural Weather Network (NDAWN) weather stations and utilized in the *Hadroplontus litura* phenology study. The following NDAWN weather stations were used for Pembina, Nelson I/II, Traill I/II, and Richland sampling sites: a) St. Thomas, b) Crary, c) Mayville, and d) Wyndmere, respectively.

Although much is unknown about how temperature affects the phenology of *H. litura*, the impacts of temperature on the phenology of closely related *Ceutorhynchus* species has been previously evaluated. *Ceutorhynchus trimaculatus*, identified as closely related to *H. litura* by Zwolfer and Harris (1966), feeds on musk thistle rosettes, and develops via three instar stages. In a laboratory experiment that evaluated the impact of temperature on *C. trimaculatus* immature phenology, the development period was reported for egg, first, second, and third instar stages to be 12.4, 6.0, 5.5, and 31 d when reared on musk thistle plants at a constant temperature of 21 ± 1 C (Kok and McAvoy 1983). Dosdall et al. (2007) evaluated the development duration from pupation of mature third instar *Ceutorhynchus subpubescens* to emergence of new adults. Similar to *H. litura*, mature third instar *C. subpubescens* exit the stem of their host plant, flixweed, and pupate in soil constructed cocoons. The mean development duration of the transition from mature third instar larvae to adult emergence of *C. subpubescens* was reported to be 18 d and ranged from 16.5 to 22 d under laboratory conditions of 21 C. Phenology of *Ceutorhynchus portulacae*, a biological control agent of the weed common purslane, has also been evaluated under constant laboratory conditions of 24 ± 4 C (Visalakshy 2007). Duration of egg, first, second, and third instar stages of *C. portulacae* was found to be 2.3, 1.4, 2.8, 3.7 d.

When comparing the previously reported results for the development duration of other *Ceutorhynchus* species a general trend was noted-the development duration of *H. litura* in our study was much longer than the development duration of the immature stages of the three species mentioned above. For instance, the development duration of egg, first instar, and second instar individuals predicted by the model constructed that omitted the Traill II 2011 site yr is 396, 346, and 312 DD (twice the DD_{50}), respectively (Table 2.3). Converting the accumulated DD to actual d experienced at the Traill II location in 2011 the duration of egg, first, and second instar *H.*

litura stages was 28, 19, and 17 d (data not shown). The longer duration of all stages observed and predicted by the model constructed in this study demonstrates the impact of variable temperature on insect development. As discussed above, insects typically develop within a wide range of temperatures; development is accelerated at high temperatures and is slowed at low temperatures (Figure 1.1; Wigglesworth1972). In this study, *H. litura* immature individuals experienced cooler and fluctuating temperatures (Figure 2.9) compared to the constant laboratory conditions that experiments were conducted in which evaluated the development duration of closely related *Ceutorhynchus* species. Additionally in our study we modeled the development of field populations in which there is variability in development times of individuals within that population, with some developing faster or slower than others. In contrast the previous laboratory studies with C*eutorhynchus* species, development was observed on an individual basis and observations were more controlled. These differences may have played a large role in the longer development durations of *H. litura* egg, first instar, and second instar stages as estimated and observed in this study.

Model Validation. As previously described, model validation was performed in an iterative fashion by fitting the logistic model to pooled 2010 and 2011 minus one site/yr for each iteration. The omitted site/yr was then used to verify the model constructed without that site/yr data and RMSE and d values were calculated and model fit was assessed. This process was performed for each site/yr.

Overall, when a 2010 site/yr was omitted from the construction of the egg stage thermal-time model, the models produced poorer fits than when a 2011 site/yr was omitted (Table 2.4). For example, when the 2010 Traill I site/yr was omitted from the model and then that model was used to predict the omitted data, d = 0.5036 and RMSE = 0.4286. Conversely, when the 2011

Traill I site/yr was omitted from the model and the model produced was used to predict the omitted data, d was much greater and RMSE value was much lower, 0.8883 and 0.1918, respectively (Table 2.4). One noteworthy exception is the model that omitted Richland 2011, where d = 0.0000 (Table 2.4). Richland 2011 consisted of only 2 data points and therefore the model was unable to accurately predict the egg stage data. This tendency in poorer model fits for 2010 site/yrs during the egg stage is potentially a result of not sampling early enough to properly capture egg development in 2010. During 2010, the first time all locations were visited, Canada thistle plants had emerged and eggs were collected at each site (data not shown). Noting this, in 2011 sites were visited earlier and the beginning of the egg stage was more accurately assessed than in 2010. Although visual inspection of the egg stage observed vs. predicted plots (Figure 2.3-2.4) look scattered, the 2011 validation statistics were quite strong, with high d values and relatively low RMSE values (Table 2.4).

Index of agreement and RMSE values were excellent when either a 2010 or 2011 site yr was omitted from first instar model construction (Table 2.4). Index of agreement values ranged from 0.7511 to 0.9840 and RMSE values ranged from 0.1007 to 0.4239 (Tale 2.4). These statistics confirm the strong visual fit displayed by the first instar observed vs. predicted plots (Figure 2.5-2.6).

When visually assessing second instar model fit, the observed data appear to be scattered and do not follow a definite trend (Figure 2.7-2.8). Despite the apparent scattered nature of the data, the validation statistics are reasonably strong, but generally lower than the other stage validation statistics with d values ranging from 0.6082 to 0.9333 and RMSE values ranging from 0.0778 to 0.5097 (Table 2.4).

The k-fold cross validation strategy was chosen for two reasons. The first reason was to assess if any of the site/yrs were anomalous and difficult to predict. One site/yr did yield rather poor validation statistics when compared to the others, Traill I 2010 (Table 2.4). Although the sample size (data not shown) and temperature data (Figure 2.9c) are relatively similar to other locations and years, this site/yr was more difficult than others to predict, which may be attributed to factors beyond the scope of this research that possibly impacted insect development, such as maternal effects (Fox et al. 1999), oxygen uptake (Hagstrum and Milliken 1991) and larval density (discussed below). The second reason was to see if this model could be used to predict *H. litura* immature development at a single location scale or if more data than one location was needed. With some exceptions d and RMSE values were relatively strong for most locations, suggesting that this model can be utilized on location-specific data with relative confidence.

Although the logistic model has many advantages and positive attributes, as outlined above, there are a few drawbacks to this modeling approach. First, our model does not take into account how larval density within the stem might impact development duration. Resources available for development are usually finite because often larvae are unable to move from resource to resource (Fox et al. 1999). This observation would apply to *H. litura* females, who lay their eggs on Canada thistle leaves. Once eggs hatch, the first instar larvae burrow into the stem and all larval development occurs there; thus, larvae are unable to move from plant to plant.

Overall, research results evaluating impacts of density on developmental time of coleopteran are mixed (Fox 1997; Fox et al. 1999; Murata 2012; Weaver and McFarlane 1990). How *H. litura* density might have impacted the median development duration model estimates is unknown and future research evaluating the impacts of *H. litura* immature density could be used

to potentially refine model estimates, although typically phenological models of insect development have not included such adjustments.

The second drawback to this model is the need to know the median molt date of the previous stage to set a zero point for the accumulated DD calculation. The median molt dates (time zero points) for the first and second instars were calculated from the field collected data, but for the egg stage, there was no previous stage, thus the zero point had to be estimated.

The final pitfall of this model is that we were unable to model the phenological development of *H. litura* third instars, which would be beneficial to incorporate into an IPM program. Knowledge of when the third instar larvae had left the stem and began to pupate in the soil would allow for another management tactic to be applied with potentially little harm to the *H. litura* population present at that location. The logistic regression model used in this experiment is binomial in nature and the response variable is the proportion of larvae in a particular stage. Therefore, to model third instar development, numbers of pupae and adults present would need to be quantified to express the number of third instar larvae as a proportion of all individuals present. Collection of such data is extremely challenging because *H. litura* pupates in the soil and also adults are elusive, cryptic, and thus extremely difficult to collect (personal observation; Peschken and Beecher 1973). In an adult collection effort in Belleville, Ontario, a 37.5 cm net was used to sweep Canada thistle plants 7,400 times from July to September and yielded only 38 adult *H. litura* weevils (Peschken and Beecher 1973). At the same location and time, wooden box traps equipped with a funnel were set up and, after 22 d, only 13 adults were found in the trap. Further exploration into pupa and adult collection methods should be conducted in the future to facilitate modeling the phenology of the vital third instar immature stage of *H. litura*.

CONCLUSION

To our knowledge this is the first attempt at classifying the head capsule width ranges associated with the three instars of *H. litura*. Prior to this study, knowledge that *H. litura* undergoes three instars in development was known (Zwolfer and Harris 1966), but exact ranges of head capsule widths associated with each instar was unknown. Knowledge of the head capsule sizes associated with each instar of *H. litura* was essential in the phenology model construction, but also could be potentially used in the field to help estimate population dynamics.

Furthermore, this study was also the first attempt to construct a logistic model that predicted immature development time of *H. litura* (egg, first, and second instar) under variable field temperatures. As noted above, models developed from experiments conducted under constant laboratory temperature conditions may not be applicable to actual field situations (Manel and Debouzie 1997; Wagner et al. 1984; Worner 1992). Moreover, there is variability in development durations of closely related species, which supports the idea that species-specific phenology models are needed and beneficial.

Ultimately, this research will potentially help enhance the efficacy of *H. litura* in a Canada thistle IPM program. The main challenge of integrating biological control with other management tactics is the need to time additional control methods precisely to protect the biological control agent while simultaneously damaging the desired weed (Hatcher and Melander 2003). Employing this model of *H. litura* phenology would allow researchers to make recommendations to land managers about when invulnerable *H. litura* life stages are present in their field and when to apply additional management tactics, such as prescribed burning, mowing, and/or herbicides, that would potentially not harm *H. litura* populatons and would also provide continual diverse management pressure on Canada thistle populations.

SUMMARY

Despite continued efforts, effective Canada thistle management continues to be a challenge, and this weed remains a problematic invasive plant throughout the world (Cripps et al. 2011; Tiley 2010). Part of the problem may be that, in general, individual control methods do not provide effective, long-term results (Evans 1984; Travnicek et al. 2005), and are often prohibitively expensive (Sciegienka et al. 2011; Tichich and Doll 2006). Overall, research suggests that an integrated pest management (IPM) program may be a more effective way to sustainably manage Canada thistle infestations.

This research sought to fill in current knowledge gaps for using the biological control agent *Hadroplontus litura* in an IPM system. The first objective was to investigate the combined impact of *H. litura* and common sunflower competition on Canada thistle height, basal stem diameter, leaf number, reproductive output, side shoot number, final shoot biomass, and root biomass under different soil nutrient (N-P-K) regimes in an outdoor field setting. In general, common sunflower plant competition, low soil nutrient levels, and *H. litura* herbivory negatively impacted some aspect of Canada thistle growth and reproductive output, but specific effects varied between experimental runs.

Although we were not able to demonstrate any synergisms amongst control methods, numerous conclusions can be drawn from this research. First, our study demonstrated the utility of all three control measures investigated to impact leaf production by Canada thistle. Second, our research demonstrated the ability of *H. litura*, soil nutrients, and plant competition treatments to reduce inflorescence production by Canada thistle. Third, this research found all three control measures negatively impacted adventitious side shoot production. Fourth, this research demonstrated the efficacy of using common sunflower as a plant competitor against Canada

thistle. Competition by common sunflower negatively impacted multiple morphological characteristics of Canada thistle, most notably a reduction in root biomass in 2010 and 2011. Finally, although *H. litura* only had a transient impact on some Canada thistle morphological characteristics, our results could potentially be used to identify times when Canada thistle plants that are exposed to *H. litura* are the weakest and most vulnerable to additional control treatments.

The main challenge of integrating biological control with other management tactics is the need to time additional control methods precisely to protect the biological control agent while simultaneously damaging the desired weed (Hatcher and Melander 2003; Messersmith and Adkins 1995). Therefore, the second objective of this study was twofold: 1) establish the range of head capsule widths associated with the three larval instars of *H. litura* using head capsule morphometrics (Dyar 1890) and 2) construct a phenological thermal time model to predict immature development time of *H. litura* (egg, first instar, and second instar) under variable field temperatures.

To our knowledge this is the first attempt at classifying the head capsule width ranges associated with the three instars of *H. litura*. We found the head capsule width ranges associated with first, second, and third instar *H. litura* larvae were 165-324 µm (mean = 274 µm; SE = 1.9; n = 292), 346-490 µm (mean = 415 µm; SE = 3.5; n = 182), and 506-736 µm (mean = 591 µm; SE = 2.9; n = 339), respectively. Knowledge of the head capsule sizes associated with each instar of *H. litura* was essential in the phenology model construction, but also could be potentially used in the field to help estimate population dynamics. Furthermore, this study was also the first attempt to construct a logistic model that predicted immature development time of *H. litura* (egg, first instar, and second instar) under variable field temperatures. Model estimates of the median

development duration in degree-days (DD) for egg, first instar, and second instar stages ranged from 181 ± 35 DD to 226 ± 58 DD, 159 ± 22 DD to 185 ± 22 DD, and 113 ± 39 DD to 167 ± 40 DD. Overall, model estimates based on the validation statistics were the most accurate for the first instar stage and least accurate for egg and second instar stages.

Ultimately, the goal of this research was to investigate ways in which to enhance the efficacy of *H. litura* in a Canada thistle IPM program. This research identified potential for *H. litura*, common sunflower competition, and low soil nutrients to negatively affect the Canada thistle characteristics measured in an additive manner. Additionally utilizing the results of the *H. litura* phenology models would allow researchers and extension personnel to make recommendations to land managers of when invulnerable *H. litura* life stages are present in their field and when to apply additional management tactics, such as prescribed burning, mowing, and/or herbicides that would potentially not harm *H. litura* and also provide continual diverse management pressure on Canada thistle populations. Further development of this approach would benefit from future research investigating the impacts of *H. litura* and common sunflower plant competition on established Canada thistle stands with an extensive root system. Furthermore identification of novel methods to collect the pupae and adult life stages of *H. litura* would allow modeling and predicting the median development duration of the third instar stage of development, which would greatly enhance the utility of the *H. litura* thermal time model.

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APPENDIX

Hadroplontus litura immature density data for the collection sites (Table 2.1) visited from May through July 2010 and 2011. X-axis on figures represents the collection dates.

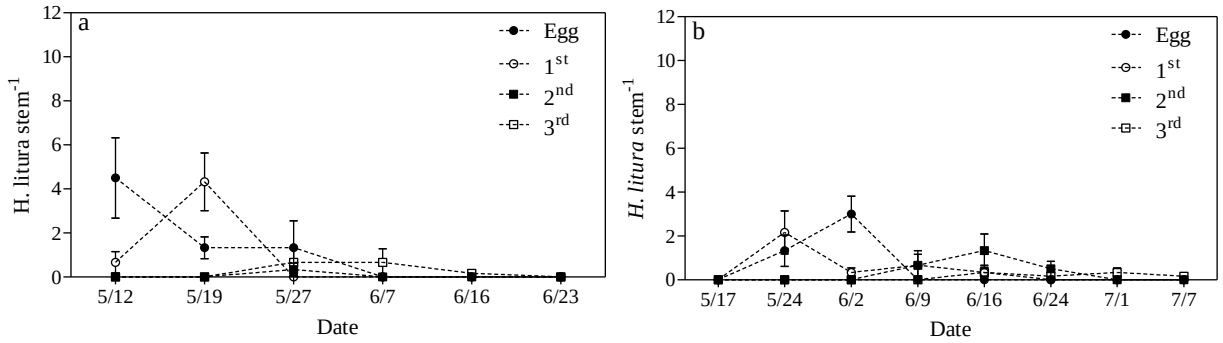

Figure 1. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Pembina County, ND 2010 (a) and 2011 (b). Symbols indicate mean values plus standard error of the mean.

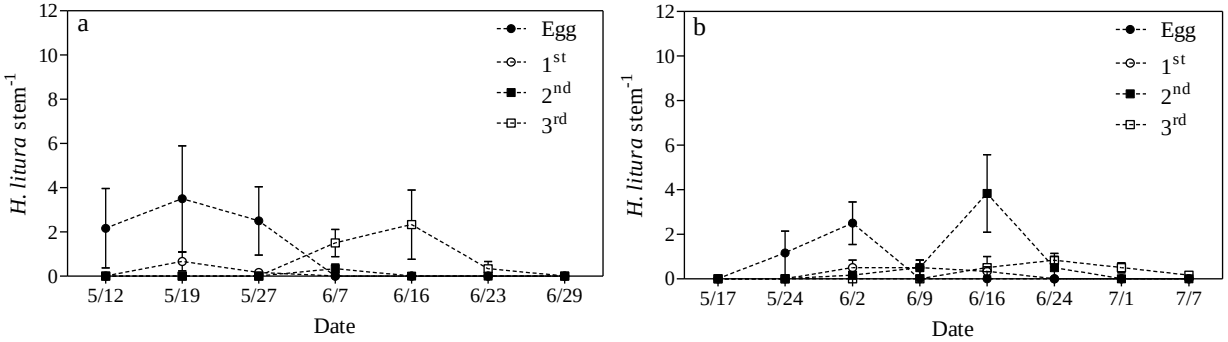

Figure 2. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Nelson County, ND 2010 (a) and 2011 (b) at location I. Symbols indicate mean values plus standard error of the mean.

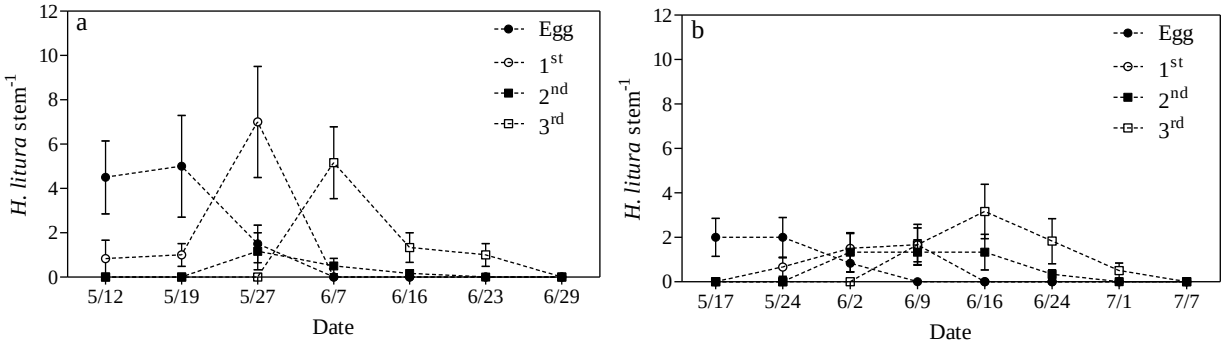

Figure 3. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Nelson County, ND 2010 (a) and 2011 (b) at location II. Symbols indicate mean values plus standard error of the mean.

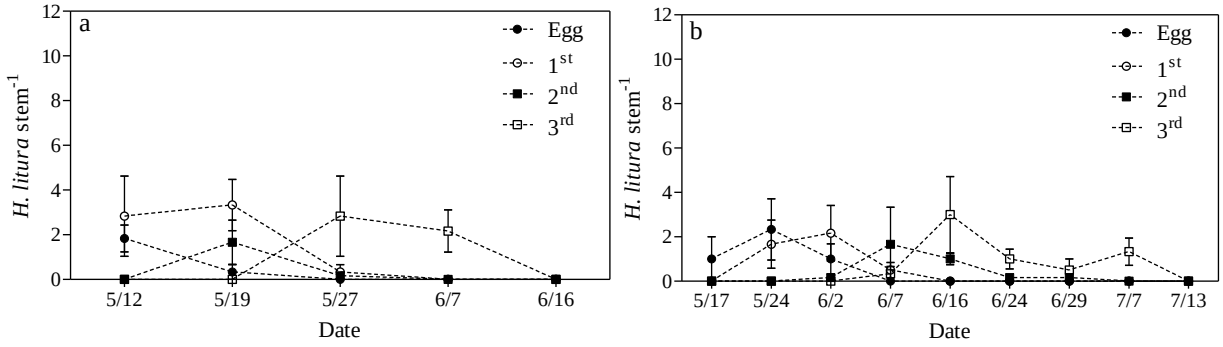

Figure 4. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Traill County, ND 2010 (a) and 2011 (b) at location I. Symbols indicate mean values plus standard error of the mean.

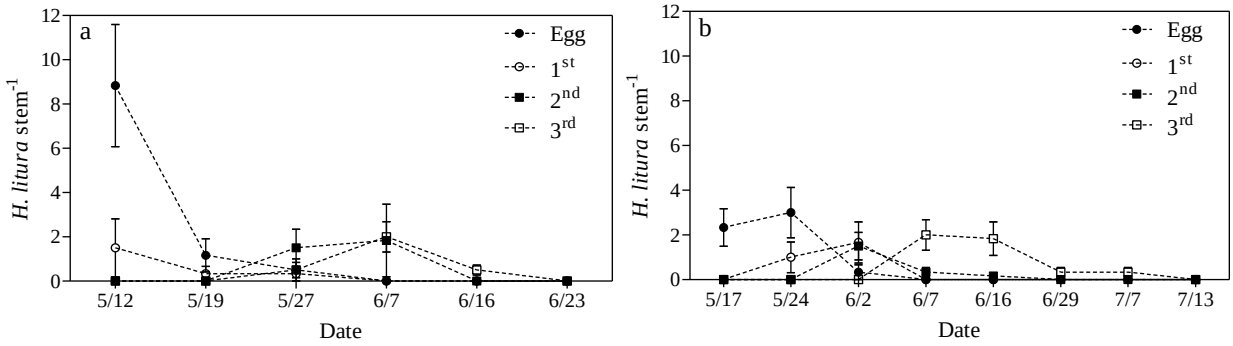

Figure 5. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Traill County, ND 2010 (a) and 2011 (b) at location II. Symbols indicate mean values plus standard error of the mean.

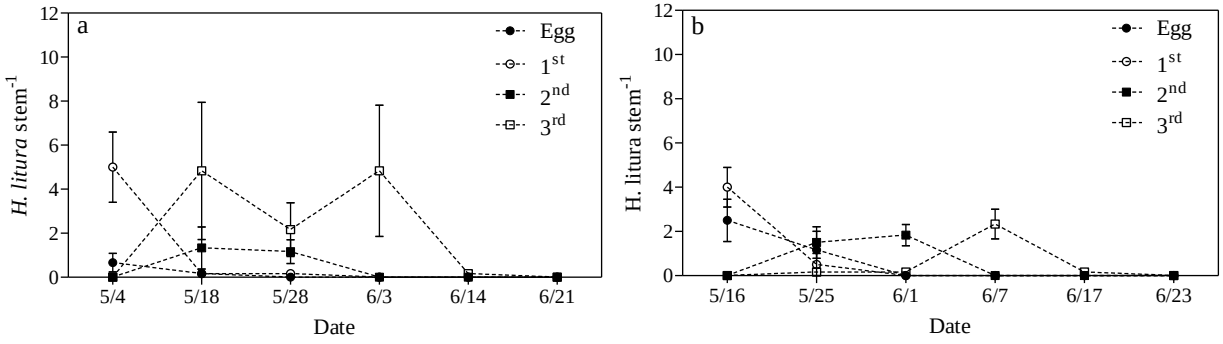

Figure 6. Density of immature *Hadroplontus litura* life stages collected from Canada thistle plants in Richland County, ND 2010 (a) and 2011 (b). Symbols indicate mean values plus standard error of the mean.