VEGETATIVE REPRODUCTION AND THE INTEGRATED MANAGEMENT OF CANADA THISTLE

by

Joanna Katarzyna Sciegienka

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APPROVAL

of a thesis submitted by

Joanna Katarzyna Sciegienka

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Dr. Fabian D. Menalled

Approved for the Department of Land Resources and Environmental Sciences

Dr. Bruce Maxwell

Approved for the Division of Graduate Education

Dr. Carl A. Fox

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Joanna Katarzyna Sciegienka

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ABSTRACT

Canada thistle (*Cirsium arvense* (L.) Scop.) is an aggressive, introduced creeping perennial weed that flourishes in a wide variety of environments. Its deep, creeping root system and colony forming tendencies make it one of the most difficult weeds to control. A strategy that incorporates a better understanding of the biology of Canada thistle into the development of an integrated management plan that includes herbicides and biological control could help reduce the dispersal and impact of this species. The objectives of this work were to: 1) determine how Canada thistle emergence and growth are affected by changes in root size, biomass, burial depth, and soil moisture and 2) compare single and joint impacts of herbicides and biological control agents on Canada thistle growth.

Objective 1 was carried out in a greenhouse and in field conditions. To monitor Canada thistle emergence and growth responses, a completely randomized factorial design was used in the greenhouse, and a randomized complete block design was used in the field. Models were developed to predict emergence and growth patterns based on available water, burial depth, and various root metrics. For the exception of available water, the same predictors were used in field conditions to predict Canada thistle emergence and growth. The variables that were manipulated were able to significantly predict the responses measured, and we concluded that available water, root burial depth, and root weight, length, diameter, and volume are indicators of emergence likelihood, emergence time, shoot and root growth, and shoot number.

Objective 2 was carried out in greenhouses and field settings. Three herbicides were evaluated with and without insects to determine reduction in Canada thistle root and shoot biomass in the greenhouse. One herbicide was also used at a low rate and evaluated singly and in combination with a stem-boring weevil (*Hadroplontus litura* (F.)) and a pathogen (*Pseudomonas syringae* pv. *tagetis*) to determine effect on Canada thistle response in the greenhouse and field. We failed to reject our null hypothesis of additivity between control agents, and concluded that integrating individual control methods yields greater Canada thistle control than any singular method.

CHAPTER 1

PROJECT BACKGROUND AND OBJECTIVES

Introduction

As long as humans have cultivated land to grow food, weeds have been a problem to farmers. With human population increasing, the demand for agriculture and its products is expanding dramatically. The weed problem has followed accordingly with numerous chemical, biological, cultural, and mechanical control methods used to minimize weed impacts and maximize yield. Unfortunately, weeds still pose a problem to many farmers, with a total annual cost of $26.4 billion to the U.S. agricultural economy for crops and over $1 billion for forage losses (Pimentel et al. 2000).

Canada thistle (*Cirsium arvense* (L.) Scop.), also known as Californian thistle, creeping thistle, green thistle, perennial thistle, and small flower thistle, is an aggressive, introduced creeping perennial weed that infests crops, pastures, rangelands, roadsides, and non-crop areas throughout the United States (Donald 1990, Moyer 1991, Morishita 1999, Mamolos and Kalburtji 2001, Grekul and Bork 2004, Lym and Duncan 2005, Mesbah & Miller 2005, Travnicek et al. 2005). Canada thistle is well-known for its deep and creeping root system and colony-forming tendencies (Weeds of the West 2004) and in Montana, it can be found in every county (Skinner 2005).

Although much research has been conducted on individual approaches to manage Canada thistle, this species remains one of the most problematic weeds in the United States and around the world (Schröder et al. 1993, Pimentel et al. 2000, Skinner et al.

2000). A strategy that incorporates a better understanding of the biology of Canada thistle into the development of integrated management practices could help reduce the dispersal and impact of this species. The key principle in long-term Canada thistle control is to stress the plant by forcing it to use stored root nutrients. This goal can be achieved through a management plan that integrates different control practices including biological control agents, mechanical practices, and herbicides (Liebman et al. 2001). The purpose of this research is to 1) gain insight into Canada thistle's vegetative reproductive biology by manipulating roots, moisture, and burial depth and 2) develop an integrated management strategy based on chemical and biological tactics. By using an integrated approach, the hope is that more efficient and effective control can be put into practice.

Literature Review

History and Origins

Canada thistle was first introduced to North America from Europe in the 1600s as a contaminant of grain seed (Moore 1975, Mack and Erneberg 2002). It was thought to be introduced to Canada and the U.S. separately, and did not spread from Canada to New England (Dewey 1901, Hansen 1918). Because of its negative impacts, Vermont was the first US State to enact noxious weed legislation against Canada thistle in 1795; New York followed in 1831 (Detmers 1927, Moore 1975). In 1868, a law was passed in Iowa that fined land owners for letting Canada thistle blossom or mature after receiving a written warning of Canada thistle on their property (Hayden 1934). In 1975, the Federal Noxious Weed Act established a program to control the spread of noxious weeds. Amended in the 1990 Farm Bill, it required each federal land-managing agency to coordinate and adequately fund weed control programs, work cooperatively with states, and establish integrated management systems for undesirable plants.

Distribution

Canada thistle occurs throughout Europe (as far north as 68° N), North Africa (as far south as 30° N), Asia, Japan, South Africa, New Zealand, and Australia (Moore 1975). It has been found at latitudes greater than 37° S in the southern hemisphere, exclusive of Antarctica (Amor and Harris 1974). It is distributed in all provinces and territories throughout Canada except Prince Edward Island (Rice 2008) as far north as 58-59° N, most commonly in agricultural fields. It has spread throughout the United States and can be found as far south as 37° N. It is considered to be naturalized in the northern Great Plains and grows very well where it gets moderate temperature and rainfall (Moore 1975). Canada thistle infests millions of US hectares (Morishita 1999) in 43 states (Rice 2008). Duncan and Jechetta (2005) estimate Canada thistle coverage in 17 western states at just under three million hectares, with an average annual rate of spread of 10-12%. It has been found in every county in Montana (Skinner 2005), infesting over approximately 600,000 hectares (Montana Department of Natural Resources and Conservation 2004).

Habitat

Canada thistle can be found in nearly every type of plant community in its range, including wet and wet-mesic grasslands, irrigation ditches, sedge meadows, prairie marshes, roadsides, croplands, pastures, sand dunes, sandy fields, stream banks, lakeshores, cleared swamps, muskegs, rangeland, riparian areas, and especially in disturbed areas (Nuzzo 1997, Moore 1975). It is not, however, typically found in forests, as it is a shade intolerant species. It grows in many types of soil including deep, well-aerated mesic soils, clay, clay loam, silt loam, sandy loam, sandy clay, sand dunes, gravel, limestone, and chalk, but does not grow as well in waterlogged, poorly aerated soils or peat (Bakker 1960, Moore 1975).

Biology

Morphology: Canada thistle is a member of the Asteraceae family. Its juvenile growth form is a rosette, which bolts into a mature plant up to five feet tall. Its oblong or lance-shaped leaves are 10 to 20 cm long, lack petioles, and are divided into spiny-tipped irregular lobes. Occasionally, plants with spineless and smooth leaves can be found. Four varieties of Canada thistle are recognized and are differentiated by their leaves. Var. *vestitum* has leaves which are gray-tomentose below. Var. *integrifolium* has thin, flat leaves which are glabrous below and entirely or shallowly pinnatifid. Var. *arvense*'s thin, flat leaves are glabrous below and shallowly to deeply pinnatifid. Var. *horridum*, the most common variety in North America, has thick, wavy leaves that are glabrous below and have many marginal spines (Moore 1975).

Hodgson (1964) found considerable and consistent differences in leaf and flower structure, seed weight and dormancy, spring shoot emergence, time of bolting, average height, and formation time of buds and flowers in Canada thistle ecotypes obtained from 10 locations in Montana, Idaho, Washington, and Wyoming. Further research by Hodgson (1970) determined that Canada thistle varieties respond differently to 2,4-D and amitrole applications as well as to cultivation. In western Canada, the variety *horridum* was 40% more susceptible to hexazinone than the variety *integrifolium* (Zand 2002).

Canada thistle can also change morphology in response to environmental conditions. When growing in an area with higher wind and evaporation, Canada thistle leaves produce higher amounts of lipids than those growing in areas with less wind and evaporation. Since lipids help retard herbicide absorption through the leaf tissue, Canada thistle plants in higher wind and evaporation areas have been found to be more tolerant to herbicides, including 2,4-D (Hodgson 1973).

Reproduction: In mid- to late spring, Canada thistle develops from seeds or vegetative buds from its root system. Seedlings grow slowly to form a rosette with irregularly-lobed spine-tipped leaves. For successful growth, the small plants require a 14-16 h photoperiod, with 16 h being preferable (Hunter and Smith 1972, Miller and Lym 1998). As they require full sun for normal development, they are susceptible to competition, particularly due to shading (Bakker 1960). Four to five weeks after emergence, the seedlings initiate growth of lateral roots (Forsberg 1962). Seven to eight weeks after germination these roots are developed enough to reproduce through buds (Bakker 1960). If the rosette is formed early in the season, the plant gradually produces an upright, elongated stalk. Plants that are formed late in the fall do not produce upright stems that year, but remain rosettes until the following summer (Miller and Lym 1998).

Sexual Reproduction: Canada thistle is the only perennial thistle. Mature Canada thistle plants are dioecious with plants usually flowering between June and August and pollinated by insects, another trait that distinguishes it from other thistles. One plant produces an average of approximately 1500 seeds, but large ones can produce over 5000 seeds (Hay 1937). Fruits are approximately 0.3 cm long, flattened, brown, and have tufts of hairs at the top, which enable the seeds to be blown up to 1 km in the wind (Bakker 1960). Although 90% of the seeds will germinate within one year, seeds can remain viable in the seedbank for up to 20 yr (Madsen 1962, cited in Chao et al. 2005). Burnside et al. (1996) found that after 17 yr, Canada thistle seeds buried 20 cm deep in untilled Sharpsburg silty clay loam in Lincoln, NE had a germination rate of 9%. Kumar and Irvine (1971) determined that the germination rate of C. thistle seeds can reach up to 40% in light and 25% in darkness, depending on temperature, while Amor & Harris (1974) found a 25-86% germination rate.

Flowers can grow up to 2 cm in diameter with the majority of them being purple, lavender, or pink, though white flowers and pale blue flowers have also been identified (Hayden 1934, Hodgson 1964). While seeds are the primary source for new invasions (Hayden 1934, Nuzzo 1997, Sole et al. 2004), survival of seedlings and new seeds is secondary to rosette survival in terms of population growth (Lalonde and Roitberg 1994, Heimann and Cussans 1996, Laubhan and Shaffer 2006).

Vegetative Reproduction: Although over half of Canada thistle’s root system tends to grow in the top 30 cm of soil, roots can reach depths of up to 5-7 m (Haderlie et al. 1987). A wide variety of root annual growth rates and rates of spread have been documented. Bakker (1960) determined that Canada thistle roots can spread over a distance of up to 5 m in 2 yr. In Australian pastures, established patches of Canada thistle spread vegetatively at an annual rate of 1.5 m (Amor and Harris 1975). Vegetative spread of Canada thistle roots is highly variable because it is affected by the age of the stand, seasonal conditions, soil type, plant vigor, grazing pressure, and management treatments such as mowing (Amor and Harris 1975). While the rate of root growth is variable, Canada thistle's extensive creeping lateral root system is a trait that distinguishes it from the other thistles. Although the aboveground shoot tissue dies in winter, its roots are capable of surviving and sending up new shoots the following spring (Moore 1975, Rees 1990). As old vertical shoots exhaust their nutrient supplies and die, lateral roots give rise to fresh shoots. The terminology for the underground portion of the Canada thistle plant has varied throughout the literature (Schreiber 1967, Hamdoun 1970, Moore 1975). The terms roots, rootstock, and rhizomes have all been used. McAllister and Haderlie (1981) extensively detail the definition of a root and conclude that Canada thistle has roots, not rhizomes. Donald (1994) furthers this argument by asserting that the adventitious root buds that Canada thistle forms can only form on roots, not rhizomes. However, a visible root bud is not necessary for new shoot formation, as the presence of sub-epidermal shoot-bud initials is enough to begin new shoot growth (Hamdoun 1970).

Cultivation that breaks up the root network encourages many horizontal portions of root as small as 0.3 to 0.6 cm long to produce shoots. Cultivation also stimulates the vertically descending roots to grow deep-running horizontal roots 0.3-0.9 m below the surface and send up new shoots (Hayden 1934). Canada thistle roots are extremely hardy. In one experiment, shoots from roots growing in moist vermiculite were removed at weekly intervals until the root was exhausted. Longer roots lasted much longer than shorter ones, but a root 0.6 cm in length continued to survive and produce shoots for 164 d. Even when dried to 5% of initial water content, some roots still remained viable (Forsberg 1962).

Impacts

Canada thistle is of great concern because it can grow among many types of economically productive crops as well as crowd out and replace natives in rangeland and natural areas. In a review of noxious weed lists in the US and Canada, Canada thistle was the most frequently listed problematic noxious weed (Skinner et al. 2000). It can cause extensive crop yield losses through competition for light, nutrients, and moisture and can create harvest problems. For example, alfalfa (*Medicago sativa*) seed yield can be reduced by over 48% through competition (Moyer 1991) and by an additional 10% after harvesting due to the need for weed seed removal during cleaning (Parker and Krall 1984). In a study done in the northern Great Plains, the relative yield of semi dwarf hard red spring wheat (*Triticum aestivum* L.) decreased linearly as Canada thistle shoot density increased (Donald 1992). Canada thistle has also been shown to have a strong competitive effect on winter wheat, mainly through competition for nitrogen (Mamolos and Kalburtji 2001).

Canada thistle can reduce pasture productivity by crowding out native species, and its spiny leaves deter close grazing, causing poor pasture utilization. Tall, dense flowering stalks can discourage stock movement and make stock handling difficult (Popay and Field 1996). In western Canada, yield losses in perennial pastures due to Canada thistle infestation peak at 2 $kgha^{-1}$ per kg of standing thistle biomass and 4.3 $kgha^{-1}$ for each additional thistle stem per m^2 (Grekul and Bork 2004). In New Zealand, a cost-benefit analysis of Canada thistle management in pastures determined that the corresponding increase in pasture productivity and utilization made both cutting and spraying treatments valuable as Canada thistle management tools (Hartley and James 1979).

In natural areas, Canada thistle can displace desirable grasses and forbs by forming a dense monoculture. In a riparian area, Krueger-Mangold et al. (2002) found that when Canada thistle was controlled by glyphosate, species richness as well as forb density and forb biomass increased. However, reestablishment of native forbs and grasses could be difficult because of the weed's detrimental effect on the seedbank. Travnicek et al. (2005) found that in an area being controlled for Canada thistle, the seedbank was dominated (>80%) with Canada thistle and Kentucky bluegrass (*Poa pratensis* L.) seed. Since the primary concern in these natural areas is the re-establishment of desirable native species, the main problem is not just controlling Canada thistle, but supplementing the seedbank so the infestation does not recur.

Besides competition and exclusion, it has been suggested that Canada thistle litter can alter plant communities through allelopathy. Redroot pigweed (*Amaranthus retroflexus* L.), green foxtail (*Setaria viridis* L.), and barley (*Hordeum vulgare* L.) growth was reduced when planted into field soil collected from an area with no Canada thistle infestation, but mixed with Canada thistle litter (Stachon and Zimdahl 1980). Canada thistle root and foliage extracts did not effect germination, but did reduce radicle growth of redroot pigweed, green foxtail, cucumber, and barley. Canada thistle roots and foliage incorporated in soil exhibited the same trend. Similarly, greenhouse studies showed that Canada thistle root and shoots had negative effects on growth of sugar beet (*Beta vulgaris* L.), wheat, alfalfa, and even other Canada thistle plants (Wilson 1981).

Management

Canada thistle management is a major and ongoing task with a wide variety of treatment options. Developing an effective, efficient, economical, and environmentally benign strategy depends on the particular set of biotic and abiotic factors a manager faces. Because Canada thistle responds differently to management under different conditions (Hodgson 1964, Friesen 1968, Hodgson 1970, 1973, Donald 1990, Zand 2002), it is beneficial and often necessary to jointly implement several control techniques and continuously monitor their effects. No matter what management approach is used to control Canada thistle, as a perennial species it requires depleting nutrient reserves from its extensive and long-lived root system. This tends to work best in the late summer and early fall (Derscheid 1962, Friesen 1968). Several comprehensive reviews of Canada thistle control methods exist (Friesen 1968, Trumble and Kok 1982, Donald 1990), with integrated management strategies preferred over singular tactics.

Chemical Control: Many studies have evaluated the efficacy of herbicides on Canada thistle with a range of success and failures (Carlson and Donald 1988, Darwent et al. 1994, Hunter 1995, Hunter 1996, Miller and Lym 1998, Tworkoski et al. 1998). For example, when 2,4-D was applied at the bud stage of Canada thistle, control was 22% greater on average than when applied at the flower stage. However, these differences became insignificant after two years (Hodgson 1970). Unfortunately, herbicides commonly used to manage Canada thistle can be ineffective and have damaging impacts on ecosystems and the environment. Concerns associated with herbicides include decreasing water quality (Leistra and Boesten 1989, Ritter 1990), herbicide drift, and human health impacts (Liebman et al. 2001, Ward et al. 2006). Also, the need for more research into limiting herbicide movement into water and the atmosphere has been acknowledged (McWhorter and Barrentine 1988) and modeled (Franklin et al. 1994).

Herbicide resistance is a concern that has worsened since the first resistant biotype was discovered in 1968 (Holt and Lebaron 1990, Warwick 1991). Due to a potential lack of effective herbicides, new management strategies must be developed to deal with resistant weeds and to prevent new ones from developing. Canada thistle plants resistant to synthetic auxin herbicides have been found in Hungary and Sweden (Heap 2008). Strategies suggested to combat resistance include mixing and/or rotating herbicides with differing modes of action and cropping practices, using more cultivation, and controlling seed set of resistant types (Holt and Lebaron 1990).

Despite the negative effects of herbicides, they can provide efficient and cost-effective control of weeds if correctly used. Glenn and Heimer (1994) found that Canada thistle can be effectively controlled in no-tillage corn (*Zea mays* L.) with a mix of 2.2 $kgha^{-1}$ glyphosate plus 560 gha^{-1} 2,4-D applied at planting. Clopyralid applied post-planting with or without 2,4-D also was effective. Donald (1990) provided a comprehensive review of the herbicides and rates used for Canada thistle control. The list is extensive and includes 2,4-D, acifluorfen, amitrole, atrazine, bentazon, bromoxynil, chlorsulfuron, clopyralid, dacamine, dicamba, dichlobenil, glyphosate, hexazinone, imazapyr, MCPA/MCPB, metsulfuron, picloram, sulfometuron, and tebuthiuron. Current strategies for control emphasize reduced use of herbicides in combination with other cultural, mechanical, or biological control practices to ensure that control is attained without too many harmful side effects (Zoschke 1994, Doyle and Stypa 2004).

Mechanical and Cultural Control: Mechanical and cultural control practices commonly used to manage Canada thistle include mowing, fire, tilling/plowing, and competition with crops or other species. In natural communities, fire has been successful in decreasing Canada thistle populations. However, while it is clear that these tactics can reduce the overall fitness and density of Canada thistle stands, none of them can fully control the weed on their own. Early spring burns can decrease weed density, allow grasses to compete, and prevent new Canada thistle invasions (Reever Morghan et al. 2000). Shreiber (1967) observed that mowing repetitively for four years reduced Canada thistle density in alfalfa, and Kluth et al. (2003) reduced overall Canada thistle fitness by cutting thistle stems once a year for two years. However, unsatisfactory results were reported for mowing paired with herbicides by Beck and Sebastian (2000), and to my knowledge no reviewed study clearly stated that Canada thistle was successfully controlled by only mechanical means.

Plowing or tilling is not an effective control practice for Canada thistle, as it breaks up the root system, encouraging new plants to grow from each root fragement (Hayden 1934). Hamdoun (1972) states that "control of *Cirsium arvense* by fragmentation alone is not likely to be successful unless repeated cultivations are carried out, or some other factor is limiting". Edwards et al. (2000) found that a single cultivation increased Canada thistle cover from less than 3% to greater than 20% within 3 months. This effect was due to not only cutting and spreading root fragments, but also eliminating competing vegetation. Even when paired with herbicides, cultivation has not shown promising results (Zimdahl and Foster 1993). In addition, traditional tillage can have many negative impacts on the environment. Increased erosion and runoff, decreased water quality, and unfavorable changes to soil properties are all caused by traditional tillage (Gebhardt et al. 1985). These effects, along with the potential to make Canada thistle infestations worse, make tillage an unsatisfactory control agent.

Competition, most often used in conjunction with other control methods, has been cited as a potential approach to manage Canada thistle (Shreiber 1967, Wilson and Kachman 1999, Mamolos and Kalburtji 2001). Seeded perennial grasses in a pasture were able to provide 53-62% Canada thistle control (Wilson and Kachman 1999). Tall fescue (*Festuca arundinaceae*) and crown vetch (*Coronilla varia*) can reduce Canada thistle biomass by 55% to 85%, the latter occurring at higher levels of moisture (Ang et al. 1994). These studies demonstrate that competition with other plant species can be an important tool in managing such a competitive weed. However, care must be taken in choosing appropriate control tactics. In a grazing/competition study where rabbits (*Oryctolagus cuniculus* L.) were allowed to graze plots with Canada thistle present amongst the native grasses, they preferred the native grasses to the unpalatable thistle, releasing the thistle from competition and enabling it to achieve higher abundance (Edwards et al. 2000).

<u>Biological Control:</u> Many biological agents have been surveyed and tested to control Canada thistle based on host specificity, availability, and degree of damage (Peschken & Beecher 1973, Ang et al. 1995, Green and Bailey 2000, Gronwald et al.

2002, Louda & O'Brien 2002, De Bruijn & Bork 2006). Even though few biological control agents have shown enough success to merit use without supplemental control (Julien and Griffiths 1998, Reed et al. 2006), the combined use of various biocontrols has been proposed to enhance control via positive interactions (Peschken and Derby 1992, Kluth et al. 2001). For example, the stem-boring weevil *Apion onopordi* functioned as a vector for pathogen transmission during its oviposition. In turn, the pathogen, a rust fungus called *Puccinia punctiformis* (Str.) Rohl., made infected plants more attractive to the weevils, which were heavier, had higher fecundity, and laid larger eggs than those utilizing healthy shoots (Friedli & Bacher 2001). Peschken and Beecher (1973) showed a similar trend between this rust and *Hadroplontus litura* (F.) (Colonnelli 2004), another stem-boring weevil.

Studied Biological Control Agents: *H. litura*'s predominate host is Canada thistle, and it is thought to have originated in Europe (Zwölfer and Harris 1966). Releases of this weevil were made in Canada beginning in 1965 (Peschken and Beecher 1973) and throughout the 1970s (Peschken and Wilkinson 1981). Releases in the U.S. were made between 1971 and 1975 in CA, CO, ID, MD, MT, NJ, SD, and WA (Rees 1990). *H. litura* is ideal for biological control because it thrives on thistle growing in cultivated land and attacks it in early spring, before it has thoroughly established (Zwölfer and Harris 1966). Although this weevil belongs to a genus with a broad range of insects that attack agricultural crops as well as many other plant species, *H. litura* is in a group separate from these potentially harmful insects. It has only been found on three genera: *Cirsium, Silybum,* and *Carduus,* and in feeding choice tests, it would not accept any other plants. It is very likely that this insect's feeding pattern has been stable since around the Pleistocene glaciation and is unlikely to change in a new environment (Zwölfer and Harris 1966). This high degree of host specificity makes *H. litura* an ideal biological control candidate.

Zwölfer and Harris (1966) provide a comprehensive review of the weevil's life cycle. In April and May, adults lay groups of eggs (1-5 eggs in each group) in 1-2.5 mm cavities on the underside of leaves at least 5 cm long. The larvae hatch in 5-9 d and promptly mine down the leaf mid-veins into the root crown, and sometimes into the upper root, feeding on callus tissue. If there are only one or two larvae in the stem, the plant's formation of callus tissue can overwhelm and kill them (Zwölfer and Harris 1966). However, a larger number of larvae (usually 3-6, but sometimes up to 12 or more) can create more tunnels, which then coalesce to create a woody gall 3-4 cm long. Mature third-instar larvae pupate in cocoons of soil particles for 2-3 wk, before emerging as adults in late summer or early fall. The adults feed on both sides of the leaves, making punctures of 2-4 mm^2, but leaving the opposite epidermis undamaged. They overwinter in soil litter and emerge in early spring at the time of Canada thistle rosette emergence (Zwölfer and Harris 1966).

In Canada, *H. litura* had one of the best establishment rates out of 12 biocontrol agents released (Harris 1979). In Montana's Gallatin valley, Rees (1990) found that the insects infested over 80% of Canada thistle stems in the study area over a period of 10 yr and spread up to 9 km from the release site in that time. He also found that underground parts of Canada thistle plants generally do not survive when attacked by the weevil, and attack proved to decrease shoot recruitment the following year by reducing overwintering survival (Rees 1990). Reed et al. (2006) found that although infestation rates of *H. litura* were high (up to 90% in some areas), the weevils did not statistically reduce thistle flowering, stem length, or over-winter survival. By feeding on the plants, however, the weevils lowered root carbohydrate reserves, weakening them. Combining weevil feeding with other stressors could increase the potential for successful control (Hein and Wilson 2004). Additionally, the exit holes left in the root crown by weevil larvae can make the weed more susceptible to pathogens and allow arthropods and nematodes access which they otherwise would not have (Agrios 1980, Rees 1990, Johnson et al. 1996).

Pseudomonas syringae pv. *tagetis* (PST) is a bacterial pathogen that causes leaf spot and apical chlorosis in some members of the Asteraceae family, including Canada thistle (Styer and Durbin 1982a, Styer and Durbin 1982b, Rhodehamel and Durbin 1985, Johnson et al. 1996, Gronwald et al. 2002, Gronwald et al. 2004). Other Asteraceae species affected include sunflower (*Helianthus annuus* L.) (Gulya et al. 1981, Gulya et al 1982, Styer and Durbin 1982a, Seifers and Stegmeier 1983, Shane and Baumer 1984, Johnson et al. 1996), woollyleaf bursage (*Ambrosia grayi* (A. Nels.)) (Sheikh et al. 2001), common ragweed (*Ambrosia artemisiifolia* L.) (Styer and Durbin 1982b, Shane and Baumer 1984, Rhodehamel and Durbin 1985, Johnson and Wyse 1992, Gronwald et al. 2004), common dandelion (*Taraxacum officinale* Weber in Wiggers) (Rhodehamel and Durbin 1985, Gronwald et al. 2004), common cocklebur (*Xanthium strumarium* L.) (Johnson and Wyse 1992, Abbas et al. 1995), horseweed (*Conyza canadensis* Less.), prickly lettuce (*Lactuca serriola* L.) (Johnson and Wyse 1992), Jerusalem artichoke

(*Helianthus tuberosus* L.) (Shane and Baumer 1984) and marigold (*Calendula officinalis* L.) (Styer et al. 1980, Shane and Baumer 1984, Rhodehamel and Durbin 1985, Gronwald et al. 2004). Several species from other families have also been documented and include houndstongue (*Cynoglossum officinale* L., family Boraginaceae) (Zidack et al. 2000), green foxtail (*Setaria viridis* L., family Poaceae), ladysthumb (*Polygonum maculosa* L., family Polygonaceae), smartweed (*Polygonum hydropiper L.*, family Polygonaceae) and velvetleaf (*Abutilon theophrasti* Medik., family Malvaceae) (Johnson and Wyse 1992).

PST produces a tagetitoxin that directly inhibits chloroplast RNA synthesis through interaction with RNA polymerase III, a class of eukaryotic nuclear RNA polymerase that transcribes small, stable RNAs (Steinberg et al. 1990, Mathews and Durbin 1994). RNA polymerase III can be further broken down into two groups. The polymerase enzymes sensitive to tagetitoxin belong to the group containing multimeric enzymes found in archaebacteria, eubacteria, chloroplasts, and the eukaryotic nucleus (Mathews and Durbin 1994). Since inhibition of chloroplast RNA synthesis has the biggest effect on developing chloroplasts, chlorosis is observed in tissues where chloroplast maturation is incomplete at the time of toxin application (Mathews and Durbin 1990, Steinberg et al. 1990, Johnson et al. 1996). Thus PST does not affect plant development (Steinberg et al. 1990).

Johnson and Wyse (1991) isolated PST from infected Canada thistle plants and determined it to be the causal organism of apical chlorosis in its host. Results of infection included reduced seed production, thistle height, and horizontal spread. These symptoms demonstrated the potential of PST as a bioherbicide. Although water is crucial for dispersal of the bacteria in an aerosol form, an appropriate surfactant is critical for host infection (Johnson et al. 1996). The nonionic organosilicone surfactant Silwet L-77 (polyaklyleneoxide modified heptamethyltrisiloxane; GE Silicones, Friendly, WV) facilitates bacterial infections by penetrating stomata even in the absence of wounds or free moisture on the leaf surface (Zidack et al.1992, Zidack 1996, Bailey et al. 2000, Gronwald et al. 2002). High levels of infection in the field were obtained even in hot, dry, and sunny conditions, when stomata are generally closed. In a field study, infection of Canada thistle by PST facilitated with Silwet L-77 reduced populations of Canada thistle by 57% compared to controls. Canada thistle that was damaged but not killed exhibited chlorosis and inhibited seed production (Johnson et al. 1996). Organic producers are not able to utilize Silwet L-77 because it is a synthetic compound. Tichich et al. (2006) found that rain may facilitate PST entry into Canada thistle leaves and suggest that an application of PST just before a significant rain event could increase the probability of infection. Although more research needs to be done, there is a possibility that PST could still be applied in organic agroecosystems.

In general, Canada thistle control with PST has been sporadic because even though PST exhibits high degrees of infection, it does not always lead to high levels of control. In growth chambers, PST reduced chlorophyll content of newly emerging leaves by up to 90% and reduced shoot dry weight by 52%, but did not kill any plants. In the field, 50-71% of Canada thistle plants sprayed exhibited chlorosis, but at the time of flower bud formation, few plants exhibited this symptom. Plant height was sometimes reduced by up to 30%, and number of flower buds was sometimes reduced by up to 80%, but other times neither was significantly affected (Gronwald et al. 2002). In another experiment by Gronwald et al. (2004), PST reduced Canada thistle shoot growth by 31%. Tichich and Doll (2006) found that four consecutive weekly applications caused more infection than one or two, but it was still not sufficient to effectively control Canada thistle.

Although PST alone may not be able to control Canada thistle, it has the potential to be more successful when paired with other tactics. Pairing with glyphosate can increase disease levels in the plants and significantly reduce fresh and dry weight (Bailey et al. 2000). Since wounds to the plant can provide vectors for pathogen entry, it is possible that insects, cutting, mowing, or grazing could boost PST's effectiveness. For example, Kluth et al. (2003) observed that cutting Canada thistle and infecting it with the rust *Puccinia punctiformis* led to reduced proportions of fertile flowerheads and more impact than either tactic alone.

Integrated Weed Management: Integrated pest management (IPM) began with the advent of overreliance on pesticides in the late 1950s and early 1960s (Thill et al. 1991) when managers were forced to develop new techniques to compensate for the failure of many insecticides. Unfortunately, the idea of IPM was mainly applied to insect pests, as weed control has been dominated by herbicides and tillage (Hodgson 1958, Zimdahl and Foster 1993, Beck and Sebastian 2000). However, the environmental contamination, human health impacts, herbicide resistance, adaptation, and soil erosion that arise from these tactics have prompted alternative methods of managing weeds (Buhler et al. 2000) that are very similar in theory and scope to IPM. Integrated weed management (IWM) is “the integration of effective, environmentally safe, and sociologically acceptable control tactics that reduce weed interference below the economic injury level” (Thill et al. 1991).

Instead of targeting a single population in a single year, integrated weed management focuses on developing a holistic approach to weed management that can be sustained through multiple years and considers how the cropping system fits in to a larger ecosystem. This systems approach not only protects crop yields, but also plays an important role in soil protection, water and soil quality, and crop diversity. Current agricultural practices favor large-scale production, homogenization, short-term profits, and minimization of financial risk. IWM emphasizes long-term returns and a more complex system of management to reduce problems that are prevalent in the status quo (Buhler et al. 2000). An integrated approach to management can provide a more sustainable, economic, and successful method of weed control (Liebman et al. 2001, Masters and Sheley 2001), especially for perennials that are difficult to manage using singular methods.

Integration of management strategies involves applying very different control methods at different times of the plant’s life cycle in order to provide continuous stress. According to Donald (1990), individual control measures applied at only one part in the thistle’s life cycle are never completely effective. Because stress is continuously weakening the plant, there is potential to reduce harmful inputs like herbicides. Consequently, most research within the last few decades has focused on combining singular methods to find a suitable integrated approach. One of the many benefits of IPM is that the variety of tactics to choose from allows managers to develop a site-specific approach to weed control (Masters and Sheley 2001). Such an approach can focus not only on the interactions between treatments, but also on the interactions between weed and arthropods as well as other organisms in the system as a whole (Norris and Kogan 2000).

The principles of IWM, whether intentionally or unwittingly, have been practiced for decades on Canada thistle. Hodgson (1958) used combinations of cropping, cultivation, and chemical spraying over consecutive years to control Canada thistle. 2,4-D was found to be more effective when combined with a competitive crop. Cover crops such as alfalfa or a mix of grass and white clover (*Trifolium repens* L.) combined with 2,4-D and mowing had a 99% success rate. Cultivation every 21 days and spraying 2,4-D in spring wheat in alternating years resulted in almost complete elimination of Canada thistle in four years. Graglia (2006) also saw reduction in Canada thistle biomass with suppressive crops of red clover (*Trifolium pratense* L.) and a mix of grass and white clover combined with mowing or hoeing in an organic cropping system. Herbicide rates can remain effective even at lower rates when combined with other control methods like cultivation (Zhang et al. 2000). Combining biological control with herbicides is also an effective way of reducing Canada thistle biomass. When *Hadroplontus litura* weevils were combined with 2,4-D, glyphosate, or clopyralid, Canada thistle root biomass was reduced between 72-92% (Collier et al. 2007).

Project Justification and Objectives

Canada thistle can flourish in a wide variety of environments (Schröder et al. 1993, Pimentel et al. 2000, Skinner et al. 2000). Much research has been conducted on the physiology, biology, invasion ecology, and single-tactic management approaches of Canada thistle, but the literature is still lacking in information on its reproductive biology and integrated management. For example, little information exists on the impact of burial depth, root size, and soil moisture on Canada thistle vegetative reproduction. Integrated management strategies do exist, but they have not been successful enough to merit large-scale implementation. To fill these knowledge gaps, our main objectives are:

Objective 1: Determine the effects of root size, root biomass, soil moisture, and root burial depth on Canada thistle emergence and growth in greenhouse and field conditions.

Objective 2: Compare the single and joint impacts of herbicides and biological control agents on the growth of Canada thistle in greenhouse and field conditions.

Any further understanding gained from research of this noxious weed can serve to further management goals. A strategy that incorporates a better understanding of the biology of Canada thistle into the development of integrated management practices could help reduce the dispersal and impact of this species. Our work focuses on root and shoot responses to abiotic factors as well as single and combined control tactics. It is our hope that with this knowledge, we will be able to develop an informed, efficient, effective, integrated, and environmentally sustainable approach to Canada thistle management.

CHAPTER 2

EFFECTS OF ROOT SIZE, ROOT BIOMASS, WATER REGIME, AND BURIAL DEPTH ON CANADA THISTLE EMERGENCE AND GROWTH

Introduction

Canada thistle (*Cirsium arvense* (L.) Scop.) is a widespread problem in agriculture and disturbed non-crop habitats due to its ability to grow in many different types of environments (Moore 1975, Donald 1990, 1994, Morishita 1999, Grekul and Bork 2004, Mesbah & Miller 2005, Travnicek et al. 2005). Its extensive and vigorous root system makes infestations hard to control and easy to spread, since cultivation merely fragments the root system, transplanting segments across a field. Portions from vertical and horizontal roots can produce new shoots (Hayden 1934).

Canada thistle's susceptibility to mechanical disturbance is governed in part by the size of the root system and the depth at which it is buried (Gustavsson 1997). While fragments only 5 mm in size can produce new shoots, bigger fragments have very high success rates of producing new shoots (Hamdoun 1972). Although deeper burial provides Canada thistle roots with protection from desiccation, it is also associated with lower emergence rates (Gustavsson 1997, Rask & Andreasen 2007).

Moisture is another factor impacting Canada thistle growth, spread, patch dynamics, and control. Tworkowski et al. (1998) found that glyphosate absorption was higher in well-watered Canada thistle plants, but translocation of the herbicide was higher only in shoots. Similarly, Lauridson et al. (1983) determined that glyphosate exhibited lower rates of absorption and translocation in water-stressed Canada thistle plants, while picloram and dicamba were unaffected by moisture stress. At high rates of water stress, Zimdahl et al. (1991) found that increasing the chlorsulfuron application rate did not have any additional negative effect on shoot mass. However, at high moisture levels they observed decreased Canada thistle shoot weight with increasing application rates.

Understanding the joint impact of energy reserves stored in the root and burial depth of roots can help predict when Canada thistle is weakest, and therefore easiest, to control (Gustavsson 1997). However, the relative importance of soil moisture alone or in conjunction with burial depth, root size, and/or root biomass on the management and spread of Canada thistle has received little attention. Our objective in this study was to investigate the relative importance of these variables on Canada thistle emergence, growth, and establishment in greenhouse and field settings. We hypothesized that while an increase in burial depth would have a negative effect on emergence time and growth of Canada thistle, soil moisture, root size, and root biomass would be positively correlated with Canada thistle performance.

Materials and Methods

Greenhouse Study

This study was conducted in a temperature and light controlled greenhouse during late winter and early spring 2008. The experiment followed a completely randomized factorial design with burial depth (2, 10, or 20 cm) and water regime (low, medium, or high) as main factors, and nine replications per treatment. Water regimes represented a 38-yr average (medium), one standard deviation below the average (low), and one standard deviation above the average (high) rainfall amount from April 15 to June 15 in a typical growing season at the Montana State University Arthur Post Research Farm, located 9 km west of Bozeman, MT (Table 2.1). Greenhouse lights supplemented ambient light to provide a 16L:8D photoperiod to facilitate plant emergence and establishment. The bulbs used were 1000 watt GE Multi-Vapor MVR1000/C/U metal halide lamps. Temperature in the greenhouse ranged from a nighttime minimum of 13-15° C to a daytime maximum of 30-40° C.

Experimental units consisted of Canada thistle grown in 43 cm tall pots constructed with 15 cm diameter thin wall PVC plastic irrigation pipes. Each pot was lined with 0.1 mm thick plastic liner to create a bottom for the pot. Approximately 7.6 L of soil were put into each pot. The soil blend consisted of a 1:1:1 ratio of mineral soil, Canadian Sphagnum peat moss, and washed concrete sand. A wetting agent, Aqua-Gro 2000G (Aquatrols Company), was added at a rate of 5.9 kgL^{-1}. To create a homogeneous soil medium and kill any diseases, weeds or insects, the mix was aerated steam pasteurized using the Lindig soil treatment system for 45 min at 80°C. Before planting, the soil was mixed with water to create a medium that would absorb water evenly. After filling pots with soil, the plastic liner was perforated at the bottom to provide drainage.

Canada thistle roots were collected from the Montana State University Arthur Post Research Farm in Sept. 2007. The fresh roots were stored in peat moss at 4°C until ready for use in Jan. 2008. Lateral roots of known length, diameter, and weight were planted in pots to the treatment depths on 10 Jan. 2008. The pots were watered every three days in accordance with water treatments, and emergence was monitored daily and noted for each individual plant. Terminal bud height, two perpendicular plant canopy diameters (the longest and perpendicular to the longest), and number of shoots per pot were measured approximately every week starting two weeks after planting. In addition, two perpendicular stem diameters were measured after the stem was big enough to ensure the calipers would do no damage. Plants were grown for 62 d and at the end of the experiment, shoots were clipped at the soil surface and weighed. Roots were separated from the soil and also weighed. Shoots and roots were dried at 49°C for at least 72 hr and then weighed to the nearest 0.01 g.

Field Study

The second part of this experiment was conducted during the 2007 and 2008 growing seasons at the Montana State University Arthur H. Post Agronomy Farm in Bozeman, MT, 45º 40’29” N, 111º09’14”W, 1423 m elevation. Soil in the area is classified as an Amsterdam-Quagle Silt Loam (Brooker, 2002). Canada thistle roots were dug from the farm in May 2007 and 2008 and planted 24 May in 2007 and 29-30 May 2008. Each year, a different fallow field was used. The experiment followed a randomized complete block design with four 30 x 10 m replicates and a total of 252 roots per replicate each year. Individual root length, diameter, and weight were measured before planting following a grid design. Individual roots were randomly assigned to one of three depth treatments (2, 10, or 20 cm) and planted approximately one meter apart.

In 2007, Canada thistle emergence was monitored daily for six weeks and noted when the first shoot was visible. Measurements of individual plants were taken on a biweekly basis and included terminal bud height, two perpendicular plant canopy diameters, two perpendicular stem diameters, and number of shoots per plant. Canada thistle growing in the field margin began encroaching into the 2007 experiment, so 18% (9 out of 50) of the data points in Block 4 had to be discarded. This encroaching thistle was sprayed with a 1.5% solution of glyphosate (Roundup Original Max®, Monsanto Company) on 1 and 11 June 2007. On 16 June 2007 the fallow field was sprayed for monocot weed control with clethodim (Select®, Arysta LifeScience Corporation) at the labeled rate of 0.125 Lha^{-1}. Finally, on 6 July 2007, all the experimental Canada thistle shoots were covered with plastic cups (6 cm diameter, 6 cm height), and the field was sprayed for dicot weed control with glyphosate at a rate of 2.34 Lha^{-1}. On 21 Sept. 2007 all Canada thistle plants were clipped at the soil surface and weighed fresh to the nearest 0.01g. All samples were then dried at 49°C for at least 72 hr and then weighed again to the nearest 0.01g.

In 2008, a cold and wet spring delayed Canada thistle emergence. After the first shoots emerged on 10 June, emergence was noted every day for two weeks, then every few days after that. On 2 July, the shoots were covered as described previously, and glyphosate was applied at a rate of 2.34 Lha^{-1} to control all other weeds. Plant height, canopy diameter, stem diameter and shoot number per plant were measured once on 16 July before a hail storm struck on 22 July, destroying the Canada thistle shoots. Some plants grew back, but the shoots that had already been measured did not recover. The plants were harvested on 12 and 15 September 2008, dried in an oven at 49°C for at least 72 hr and weighed to the nearest 0.01 g.

Data Analysis

R statistical software (version 2.7.2) was used to analyze the data (R Development Core Team 2008). For the greenhouse and field data, a logit regression was used to predict Canada thistle emergence using water regime, initial weight, length and diameter of the root segment, and burial depth of the root segment as independent variables. A backward elimination stepwise regression analysis was performed on a generalized linear mixed model to eliminate nonsignificant variables one at a time until a model containing only significant variables emerged (p=0.05). Year, and rep nested in year, were random effects in the model. The best model was determined based on lowest Akaike's information criterion (AIC) value. To determine each significant variable's effect on the probability of Canada thistle emergence , an odds ratio was calculated from the slope estimates of the model using Equation 2.1 (Kutner et al. 2005),

$$OR = \exp(b) \qquad \text{Eq 2.1}$$

where OR = odds ratio and *b* = the estimated regression coefficient in the fitted logistic response function. To find the OR for an increase of > 1, the OR for that variable is taken to the power of the intended increase. For example, the odds ratio for a 10 mL increase in water will be the odds ratio for water to the power of 10.

Using an analysis of covariance (ANCOVA), linear models for the greenhouse and field were developed to describe emergence time (the time between planting and shoot emergence) and dry shoot and dry root weight at harvest as a function of the independent variables described in the logit regression above. Initial root weight, initial root length, and initial root diameter were the covariates used in the analysis. The response variable emergence time was natural log transformed as necessary to fulfill the assumptions of the analysis, particularly constant variance. A Bonferroni multiple comparisons test was used to determine difference between factor levels. For the greenhouse, emergence time was represented as days after planting (DAP). In the field, emergence time was represented as Celsius growing degree days (GDD), calculated using the formula described in McMaster and Wilhelm (1997):

$$GDD = \left[\frac{(T_{MAX} + T_{MIN})}{2} \right] - T_{BASE} \qquad \text{Eq 2.2}$$

$$\text{where if } \left[\frac{(T_{MAX} + T_{MIN})}{2} \right] < T_{BASE}, \text{ then } \left[\frac{(T_{MAX} + T_{MIN})}{2} \right] = T_{BASE}$$

where GDD is growing degree days, T_{MAX} is the daily maximum air temperature, T_{MIN} is the daily minimum air temperature, and T_{BASE} is the point at which the process of interest (growth) does not progress. Donald (2000) determined that an appropriate T_{BASE} to use for Canada thistle would be 0°C.

As explained above, a backward elimination stepwise regression analysis calculated the model with the lowest AIC. This was done with R statistical software's stepAIC command and also manually to check the results. To potentially eliminate variables and decrease the complexity of the models, we combined root length and root diameter into a new predictor called volume by using the following formula,

$$volume = \Pi r^2 * l \qquad \text{Eq 2.3}$$

where volume refers to the initial root volume, *r* refers to the radius of the root, and *l* refers to the length of the root. For plant canopy diameter and stem diameter, two measurements were taken and averaged: one along the longest axis of the plant and the other perpendicular to it. Initial root volume was used to simplify models, but was used only when it made a significant contribution. The same was true of initial root length and initial root diameter.

To determine how plant height, plant canopy, stem diameter, and number of shoots were affected by growing conditions during the course of the experiment, each response variable was tested with every combination of predictors through an additive step-wise process using a repeated measures analysis. Linear mixed-effects models fit by restricted maximum likelihood (REML) in R statistical software (version 2.7.2) were developed for each combination of predictors. Dependent variables with non-significant p-values (alpha level of 0.05 or higher) were not included in increasingly complex models. AIC values were compared between models to determine the one that best described each particular response variable. In some cases, the response variable in question had to be power transformed prior to the analysis to meet the constant variance and normality assumptions. This transformation was based on the Box Cox analysis in R statistical software (version 2.7.2) (Box and Cox 1964, Teugels and Vanroelen 2004). Effort was made to use common transformations such as natural log, square root, and reciprocal transformations instead of ones that would lead to exact normality but would increase the difficulty in data interpretation (a 0.3 power transformation, for example). The 2007 and 2008 field data sets were combined for the emergence likelihood and emergence time analyses. Because of the hail that destroyed the 2008 experiment, only the 2007 data was analyzed for biomass and plant size over time.

Results

Greenhouse Study

Emergence Likelihood: Water regime and initial root weight were positively related to the probability of Canada thistle emergence (Table 2.2). The odds ratio analysis indicated that for every 10 mL increase in total water applied, the likelihood of a Canada thistle shoot emerging increased by 10.5 and for every 1 g increase in root weight, the odds of emerging increased by 2.5.

Emergence Time, Plant Biomass, and Plant Size: Emergence time, estimated as ln(DAP), was best predicted by the depth of root planting, water regime, and initial root weight (Table 2.3). Increasing burial depth increased emergence time; roots planted at 20 cm took significantly longer to emerge than roots planted at 2 and 10 cm. Increasing water amount decreased emergence time, as roots receiving a high amount of water took significantly less time to emerge than roots receiving a low amount of water (Fig. 2.1). While initial root weight was a continuous variable in the models, it was divided into categories in Figure 2.2 for ease of interpretation of results, with greater initial root weight increasing emergence time slightly (Fig. 2.2).

Increasing water amount and initial root weight positively influenced Canada thistle shoot weight and root weight at harvest (Table 2.3). Plants receiving a high amount of water produced heavier roots and shoots than plants receiving a low or medium amount of water (Figure 2.3). Increasing initial root weight increased final shoot (r = 0.50) and root weight (r=0.41) (Figure 2.3).

Both Canada thistle plant height and stem diameter increased with increasing water amount and initial root weight throughout the growing season. Canopy diameter increased throughout the growing season with increasing water amount and burial depth. Increasing the initial root depth decreased the number of shoots per plant throughout the experiment, while increasing initial root volume increased it (Table 2.4).

Field Study

Emergence Likelihood: Depth of root burial, initial root length, and initial root diameter were significant predictors of Canada thistle emergence under field conditions (Table 2.5). Results from the odds ratio calculation indicated that an increase in burial depth led to a slight decrease in likelihood of emergence, and that an increase in initial root length led to a slight increase in likelihood of emergence. On the other hand, initial root diameter had a relatively large impact on likelihood emergence with an increase of 1 cm in initial root diameter increasing the odds of emerging by 99.5. Year, and rep nested within year, were both significant random effects ($p<0.01$, for both) and they did not significantly interact with the predictors.

Emergence Time and Plant Biomass: The emergence time of Canada thistle was predicted by a highly complex model (Table A.1 in the Appendix). To be useful to managers, a simpler model was developed (Table 2.6) in which all included terms were significant, although the AIC value (599) was higher than that for the complex model

(581). Results of the simplified model indicated that as initial root depth, the initial root depth: initial root volume interaction, and the initial root volume: initial root diameter interaction increased, emergence time increased. Decreasing initial root diameter, initial root weight, and the initial root depth: initial root length interaction also increased emergence time. Although not significant, initial root volume and initial root length were included in the model because they exhibited significant interactions with other terms.

Initial root diameter and an initial root depth: initial root length interaction predicted Canada thistle final shoot biomass (Table 2.7). Increasing initial root diameter increased shoot biomass, while increasing the interaction term decreased it. Initial root depth and initial root length were insignificant but were included in the model because they exhibited significant interactions. Year and rep were significant ($p<0.01$) but did not interact with any predictors.

Plant Size: Burial depth was a significant predictor of changes in plant height and canopy diameter over time (Table 2.8). Weight was a significant predictor of changes in plant height over time. While increasing initial root depth decreased plant height throughout the growing season, increasing initial root weight had the opposite effect. Increasing initial root depth decreased plant canopy diameter throughout the growing season. Stem diameter could not be predicted using the measured variables because there was too little variability in the patterns across replicates to do the mixed-effects model analysis. The number of shoots also could not be predicted for the same reason.

Discussion

This study indicated that initial root weight, root size, root burial depth, and water regime affect Canada thistle establishment and growth. In accordance with the proposed hypothesis, Canada thistle emergence was positively impacted by water availability and initial root fragment size in greenhouse conditions and by burial depth, root length, and root diameter in field conditions. In accordance with Rask and Andreasen (2007), our greenhouse experiment indicated that increasing root burial depth delayed Canada thistle emergence. However, this pattern was not as clearly detected in our field experiment, where roots at the 10 cm depth, rather than ones at the 2 cm depth, had the highest emergence. Potential mechanisms for this differential response include the desiccation of roots occurring at shallow depth as well as energy expenses necessary to reach the soil surface from deeper depth (Gustavsson 1997 and Rask & Andreasen 2007). It is possible that roots growing in field conditions were negatively impacted by the hot weather occurring in 2007 and the unseasonable cold weather occurring in spring 2008 (Table 2.9). Also, it is also possible that some deep roots rotted due to the presence of pathogens, including common *Fusarium* species, found associated with the Canada thistle roots (personal observation). Finally, naturally occurring abiotic variables such as wind and water availability that could not be controlled in the field experiment may have resulted in the discrepancies in trends between greenhouse and field experimental conditions.

Under greenhouse conditions and similar to Tworkoski et al. (1998), soil water positively impacted Canada thistle biomass and size. However, water availability did not impact formation of new shoots most likely because the main shoot inhibited its root buds by reducing their xylem water potential, thus reducing their ability to compete for water (Hunter et al. 1985, Tworkoski et al. 1998). Initial root weight's positive impacts on final shoot and root weight as well as plant height and stem diameter were likely due to the increase of resources at the start of growth. In accordance with Gustavsson (1997), initial root diameter and length also followed this trend, with more plant biomass at harvest produced by larger roots. A deeper initial root burial depth slightly decreased number of shoots produced as well as decreased plant height and canopy diameter throughout the growing season. Conversely, it increased plant biomass. Thus plants with roots at deeper depths may have smaller growth forms and fewer stems, but compensate by producing more biomass in the form of leaf tissue.

Since Canada thistle's susceptibility to mechanical disturbance is governed in part by the size of the root fragment (Gustavsson 1997) and its ability to produce new roots and shoots (Hamdoun 1972), it is critical to understand how root size can affect an eventual infestation. In addition, the amount of water these roots receive can also have important implications for their overall health, vigor, and infestation tendencies. Overall, this study indicates that Canada thistle proliferates better in areas with more soil water and that heavier and bigger root pieces will grow bigger shoots. Furthermore, shoot emergence dynamics are affected by some of these variables. Thus managers working on a limited budget could prioritize decisions based on where shoots are more likely to proliferate or where infestations are likely to increase rapidly due to prime growing conditions, such as in low points around the landscape or riparian areas.

A key area of research that merits investigation is how the age of Canada thistle roots and patches affects growth, reproduction, and spatial dynamics. Further research could assess if root age follows the same patterns as root size, root weight, water regime, or depth in determining Canada thistle emergence and growth, as these variables have been shown to determine the growth potential of other species (Dietz and Ullman 1998, Dietz 2002). For example, older infestations of Canada thistle could grow deeper and form bigger networks of roots. Breaking up such a root system could yield different results than segmenting up a very young infestation.

Integrated weed management is an important strategy that could successfully control Canada thistle (Liebman et al. 2001, Masters and Sheley 2001). For it to do so, managers must develop a holistic approach that can be sustained through multiple years. The foundation of such an approach is information about the weed itself (Bhowmik 1997). This study provides information about how Canada thistle roots determine the emergence and growth of individual ramets. It also demonstrates the impact of water availability and depth of burial on shoot emergence and growth. In doing so this study contributes to the biological framework for understanding more about Canada thistle's behavior and what factors to consider in developing an integrated weed management plan.

Table 2.1: Water regime in a greenhouse study aimed at assessing the effect of burial depth, root size, and water availability on Canada thistle emergence and growth.

Water Regime	April		May		June	
	Average Precipitation	Water per pot	Average Precipitation	Water per pot	Average Precipitation	Water per pot
Low	0.08 cm	14 mL	0.12 cm	23 mL	0.13 cm	24 mL
Medium	0.14 cm	26 mL	0.22 cm	43 mL	0.22 cm	41 mL
High	0.20 cm	38 mL	0.33 cm	63 mL	0.32 cm	59 mL

Average precipitation is the average amount of rainfall (in cm) received per day for that particular month at the Arthur Post Research Farm near Bozeman, MT for 1970 through 2008. Water per pot is calculated from average precipitation and reflects the volume of water (in mL) that each pot received per day for that particular month. Medium is the average amount of rainfall, low is one standard deviation below medium, and high is one standard deviation above medium.

Table 2.2: Odds ratio and model coefficients for emergence likelihood of Canada thistle grown in greenhouse conditions.

Predictor	Odds Ratio	Model Coefficient
Water Regime (10 mL)	10.45	0.19
Initial Root Weight (1 g)	2.50	0.92
Intercept	-	-2.27

Table 2.3: Model parameters to predict greenhouse-grown Canada thistle emergence time, dry shoot biomass at harvest, and dry root biomass at harvest.

Predictor	ln(DAP)	Dry Shoot Weight	Dry Root Weight
Initial root depth	0.04	-	-
Water regime	-1.31 e-3	0.02	0.02
Initial root weight	-0.05	0.49	0.28
Intercept	2.27	-1.83	-0.82
p-value	< 0.01	< 0.01	< 0.01
Adjusted R-squared	0.65	0.51	0.45

DAP stands for days after planting and is represented here in its natural log transformed state. Dry shoot and root weight are in grams.

Table 2.4: Model parameters for Canada thistle plant height, average canopy diameter, stem diameter, and number of shoots in greenhouse conditions.

Predictor	Height	Canopy Diameter	Stem Diameter	Shoots
Water regime	0.32	0.15	0.34	-
Initial root weight	0.28	-	0.17	-
Initial root depth	-	0.07	-	-0.20
Initial root volume	-	-	-	0.15
Intercept	0.94	2.60	2.28	1.34

Height, canopy diameter, and stem diameter are in cm.

Table 2.5: Odds ratio and model coefficients for emergence likelihood of Canada thistle growing in fallow field conditions.

Predictor	Odds Ratio	Model Coefficient
Initial root depth (1 cm)	0.97	-0.03
Initial root length (1 cm)	1.05	0.05
Initial root diameter (1 cm)	99.49	4.60
Intercept	-	-3.30

Table 2.6: Model parameters for field-grown Canada thistle emergence time.

Predictor	GDD	p value
Initial root depth	4.65	<0.01
Initial root volume	-57.50	0.34
Initial root length	4.20	0.48
Initial root diameter	-38.59	<0.01
Initial root weight	-5.73	<0.01
Initial root depth: Initial root volume	4.23	<0.01
Initial root depth: Initial root length	-0.43	0.02
Initial root volume: Initial root diameter	62.10	0.02
Initial root depth: Initial root volume: Initial root diameter	-3.72	0.03
Intercept	283.03	<0.01

GDD stands for growing degree days (in Celsius) and is a measure of emergence time. There are 550 degrees of freedom.

Table 2.7: Model parameters for field-grown Canada thistle shoot weight.

Predictor	Dry Shoot Weight	p value
Initial root depth	15.60	0.12
Initial root length	3.34	0.50
Initial root diameter	39.18	0.02
Initial root depth: Initial root length	-1.32	0.03
Intercept	-39.26	<0.01

There are 308 degrees of freedom. Dry shoot weight is in grams.

Table 2.8: Model parameters for Canada thistle plant height and average canopy diameter over time in a fallow field.

Predictor	Height	Canopy Diameter
Initial root depth	-2.04	-1.45
Initial root weight	0.80	-
Intercept	8.91	14.90

Height and canopy diameter are in cm.

Table 2.9: Temperature and precipitation for the Arthur Post Research Farm near Bozeman, MT.

	LTA*	2007**	2008**
Temperature (°C)	Max/Min/Mean	Max/Min/Mean	Max/Min/Mean
March	7.8/-5.3/1.3	20.7/-11.6/5.0	11.7/-10.7/0.5
April	13.2/-1.2/6.0	27.7/-3.1/7.7	22.8/-11.7/3.2
May	18.3/3.2/10.7	29.7/2.8/13.5	28.0/-1.3/9.6
June	22.8/6.7/14.8	34.4/6.3/18.3	32.5/0.9/14.2
Precipitation (cm)			
Yearly	41.4	43.3	-
Jan-Sept.	34.1	33.4	30.7

LTA is long term average of years 1966-2007. Temperature is given in maximum and minimum; precipitation is given in yearly totals.
*Western Regional Climate Center 2008
**AgriMet: Weather & crop water use charts 2008

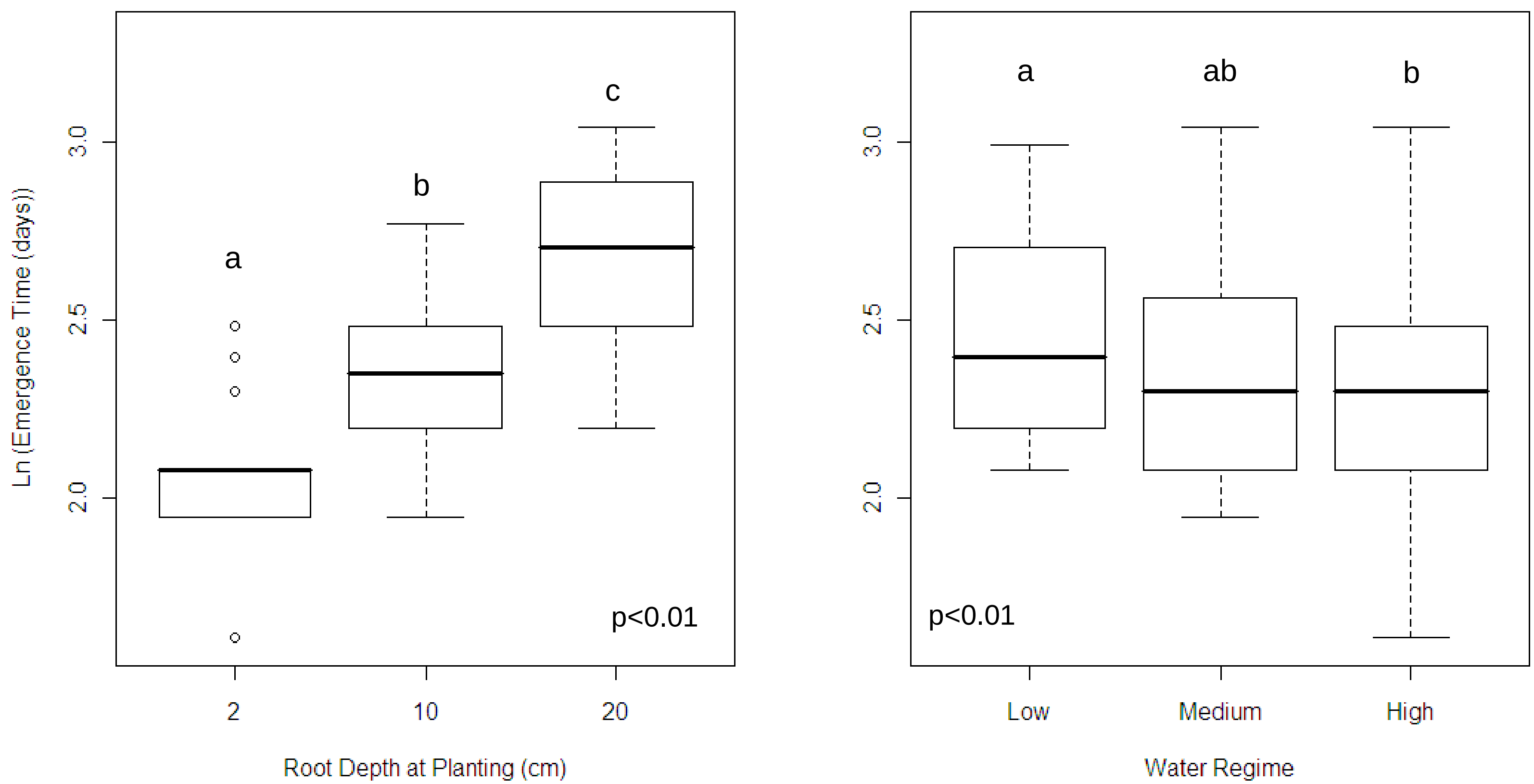

Figure 2.1: Effect of burial depth (left) and water (right) on greenhouse-grown Canada thistle emergence time. Water regime levels correspond to the values given in Table 2.1. The horizontal lines on the box plots, from bottom to top, represent the lowest value that is not an outlier, the first quartile, the median, the third quartile, and the largest value that is not an outlier. Any points outside the range of whiskers are outliers. Lower case letters represent significant differences in mean emergence time based on a linear model analyzed with ANCOVA.

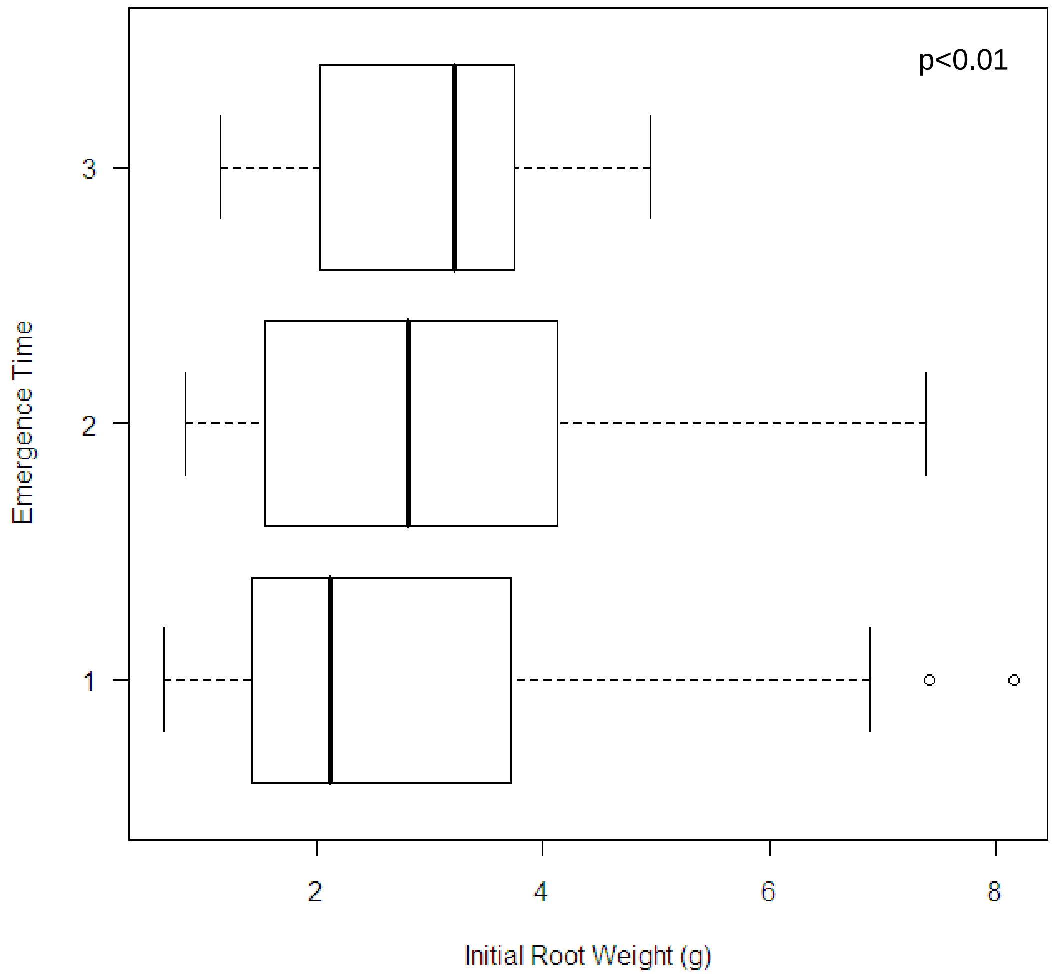

Figure 2.2: Emergence time as a function of initial root weight for Canada thistle grown in a greenhouse. An emergence time of 1 is 5-10 days, 2 is 11-15 days, and 3 is the longest at 16-21 days. The whiskers on either side of the box represent the lowest and highest values, respectively, that are not outliers. The vertical lines (in bold) running through the middle of the boxes represent the median. The vertical lines on either side of the median represent the first quartile (left of the median) and the third quartile (right of the median). Any points outside the range of whiskers are outliers.

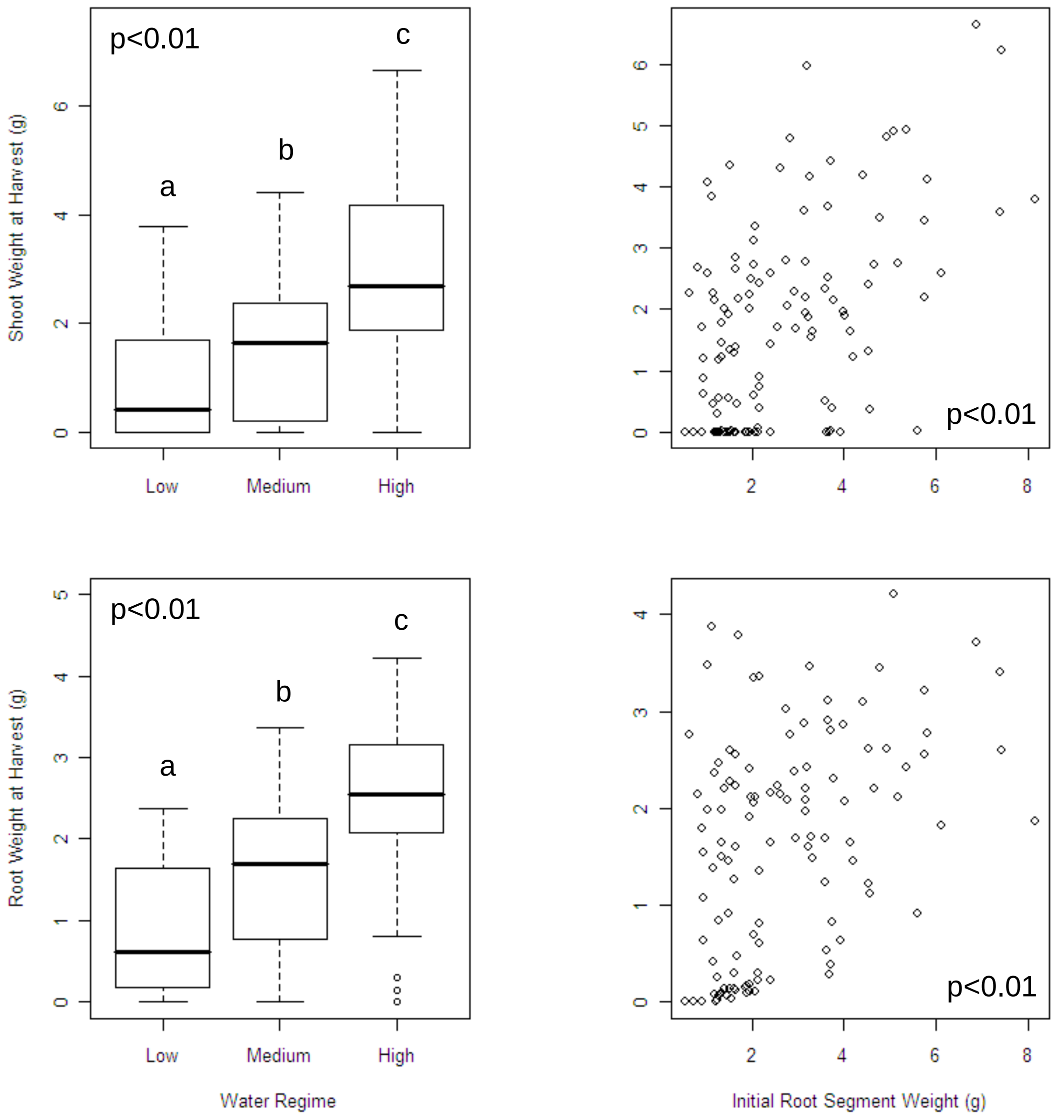

Figure 2.3: Effect of water regime on Canada thistle dry shoot (upper left) and root (lower left) weight and scatterplots of initial Canada thistle root segment weight's effect on final dry shoot (upper right) and root (lower right) biomass in a greenhouse. Water regime levels correspond to the values given in Table 2.1. The horizontal lines on the box plots, from bottom to top, represent the lowest value that is not an outlier, the first quartile, the median, the third quartile, and the largest value that is not an outlier. Any points outside the range of whiskers are outliers. Lower case letters represent significant differences in mean shoot and root weight based on a linear model analyzed with ANCOVA.

CHAPTER 3

COMBINED IMPACTS OF *CEUTORHYNCHUS LITURA* AND HERBICIDE TREATMENTS FOR CANADA THISTLE SUPPRESSION

Abstract

In repeated greenhouse experiments, we investigated the potential for suppressing Canada thistle, *Cirsium arvense*, using a combination of the stem-mining weevil *Ceutorhynchus litura* (now *Hadroplontus litura*) and late-bud to early-flower stage application of one of three herbicides: 2,4-D, glyphosate or clopyralid. We hypothesized that weevil damage combined with a subsequent herbicide application would synergistically reduce Canada thistle biomass by reducing the ability of thistle plants to compensate for herbivory. We found that the effects of weevil attack and herbicide treatment varied considerably across the experimental trials. Contrary to our hypothesis, the effects of weevil attack and herbicide application were additive on a natural log scale, indicating independent, non-synergistic effects of the two factors. In one of the three experimental trials, however, neither weevil attack nor glyphosate treatment alone significantly affected thistle shoot biomass but the combination of the two factors reduced shoot biomass considerably. Overall, combining weevil attack with clopyralid led to the most consistent and greatest suppression of root biomass of Canada thistle.

Introduction

Canada thistle (*Cirsium arvense* (L.) Scop.) is a deep-rooted perennial plant that is considered a weed in crops, pastures, rangelands and natural areas throughout North America (Moore, 1975). In 2000, Canada thistle ranked first among the most frequently listed noxious weeds in U.S. states and Canadian provinces (Skinner *et al.,* 2000). Canada thistle is highly competitive and may reduce plant species diversity (Stachion and Zimdahl, 1980), seed yields in alfalfa (Moyer *et al.,* 1991) and barley (O'Sullivan *et al.,* 1982), and forage production in pastures (Reece and Wilson, 1983).

Potential tools for managing Canada thistle include: herbicides (e.g., Haggar *et al.,* 1986, Donald, 1990), tillage (Donald, 1990), competition (Wilson and Kachman, 1999), mowing plus herbicides (Beck and Sebastian, 2000) and biological control (Coombs *et al.,* 2004). When used alone, these tools largely provide limited, short-term suppression of Canada thistle infestations. Liebman and Gallandt (1997) stressed the idea of "many little hammers" in weed management. Each "hammer" may be only marginally effective alone but when combined may provide significant suppression of weed infestations. This idea, of course, is the crux of integrated weed management.

The Canada thistle stem-mining weevil *Ceutorhynchus litura* E. may fit the description of a "little hammer." The weevil was intentionally introduced into Canada and the United States from Europe in the 1960s for biological control of Canada thistle (Piper and Andres, 1995). Adult weevils feed on thistle rosettes in early spring (Zwölfer and Harris, 1966). When the thistle plants bolt in the late spring, females lay eggs in holes chewed into the leaf midribs (Zwölfer and Harris, 1966). The larvae tunnel into the main stem, where they feed on parenchymal tissue (Peschken and Wilkinson, 1981). Later, the larvae tunnel from the stem into the root crown, chew one or more holes, exit the root crown and pupate in the soil. Adults emerge later in the summer and overwinter in the leaf litter (Zwölfer and Harris, 1966).

Over the past forty years, *C. litura* appears to have provided little to mixed success in suppressing Canada thistle infestations on its own. Rees (1990) found significant declines in Canada thistle stem density following release of *C. litura*. However, Peschken and Wilkinson (1981) attributed no suppression of Canada thistle to *C. litura*, and Reed *et al.*, (2006) found that stem length, stem density, flower production and winter survival were similar in areas with high densities of *C. litura* compared to areas with low densities of *C. litura*.

The problem may be that Canada thistle compensates over the summer for damage caused by *C. litura* in the spring. Peschken and Derby (1992) found that, in June, thistle plants attacked by weevils showed a 36-53% reduction in the carbohydrate content of the roots. However, by early August, root carbohydrate reserves had recovered to levels in unattacked plants. Hein and Wilson (2004) similarly found that, in the summer, levels of fructans and free sugars were reduced in the roots of thistle plants damaged by *C. litura*. However, later in the season, fructan and free-sugar levels in the roots recovered completely.

Peschken and Derby (1992) suggested that post-damage stress in the form of drought might explain the differences between their results and the results of Rees (1990). Peschken and Derby (1992) found weak to non-existent impacts of *C. litura* at sites in British Canada, where summer rains were common. Rees (1990) observed considerable impact of *C. litura* in Montana, where summer rainfall was low. Hein and Wilson (2004) have also suggested that stress imposed after weevil feeding might lead to improved suppression of Canada thistle. An integrated approach to managing Canada thistle infestations might therefore combine attack by the weevil with additional sources of stress, e.g., herbicide application.

In general, the integration of weed management strategies requires that the different strategies are not antagonistic. Antagonism between chemical herbicides and insect biological control agents might arise if the herbicide impacts the agent directly through toxicity or indirectly through a reduction in plant quality (Messersmith and Adkins, 1995). Although insect biological control agents may be resistant to herbicide-induced mortality (e.g., Trumble and Kok, 1980, Lindgren *et al.*, 1998), some studies have shown direct antagonism between herbicides and biological control agents (e.g., Paynter, 2003; Boydston and Williams, 2004, Story and Stougaard, 2006). Herbicide timing relative to the phenology of the agent can be crucial in limiting antagonism between herbicides and biological control (Story *et al.*, 1988, Paynter, 2003; Story and Stougaard, 2006).

When antagonism between management strategies does not occur, the strategies may be characterized as either additive or synergistic. Additivity indicates that the different factors have independent effects on weed growth and/or mortality. Synergy occurs when the factors cause greater suppression of weed infestations in combination than would be expected from their independent, additive effects. Synergism between herbicides and biological control might arise if weed biological control agents actually benefit from the herbicide through increased plant quality (Messersmith and Adkins, 1995; see also Wilson *et al.*, 2004) or if attack by the biological control agent enhances herbicide uptake (Nelson and Lym, 2003). Very few studies, however, have explicitly evaluated additive versus synergistic impacts of weed biological control agents and herbicides (Boydston and Williams, 2004).

Additivity (non-antagonism) can justify integrating herbicides and biological control agents for weed management but synergy is clearly even more desirable. How might integrative approaches be designed *a priori* to limit antagonism and achieve synergy? In developing an approach for integrating herbicides and biological control for Canada thistle management, we sought to time herbicide applications to occur after the larval development of *C. litura*, when thistle carbohydrate reserves are known to be lowest during the season (Peschken and Derby, 1992, Hein and Wilson, 2004) and the weevils are no longer feeding on the plant (Zwölfer and Harris, 1966). To our knowledge, there are no published studies of integrating *C. litura* and herbicide applications for Canada thistle suppression. We conducted greenhouse studies to evaluate the impact of attack by *C. litura* followed by application of one of three herbicides: 2, 4-D, glyphosate or clopyralid. We hypothesized that weevil attack combined with a subsequent herbicide application would synergistically reduce Canada thistle biomass by reducing the ability of thistle plants to compensate for herbivory.

Materials and Methods

The experimental design was a randomized, complete factorial arrangement of herbicide (none, 2, 4-D, clopyralid, or glyphosate) and biological control (*C. litura* or none) in three different experimental trials. A replicate consisted of a single Canada thistle ramet in a 7.9 L PVC column. Each treatment was typically replicated in four columns in each of the three experimental trials, although 8 replicates of glyphosate treatments were conducted in one experimental trial (Wyoming 2006) and one replicate of the 2, 4-D plus weevil treatment was lost in one of the experimental trials (Montana 2006).

Canada thistle roots were collected from the top 30 cm of soil in March 2005 (for the 2005 experimental trials) and September 2005 (for the 2006 experiment trials) from an upland grassland site near Cheyenne, WY. Roots were planted into flats (52 cm x 26 cm x 6 cm) in 2005 or 10.6 L pots (Steuwe and Sons, Corvallis, OR) in 2006 containing topsoil collected in Laramie, WY. Thistle plants were maintained in a temperature-controlled greenhouse at the University of Wyoming Greenhouse Facility until transplanted for experiments. In 2005 and 2006, experimental trials were set up at the University of Wyoming greenhouses. In 2006, roots were shipped overnight to Bozeman, MT for an experimental trial at Montana State University.

Lateral roots of approximately uniform diameter were removed from the flats or pots, divided into 10 cm sections and planted singly into the PVC columns (15 cm diameter by 45 cm height). Prior to planting, each column was lined with a clear plastic sleeve that was knotted at the bottom to hold the soil in place. The liners were perforated at the bottom to allow drainage. The growth medium for the Wyoming 2005 and 2006 trials was 50% sand and 50% Canadian sphagnum peat by volume. For the Montana 2006 trial, the growth medium was equal parts loam soil, sand and Canadian sphagnum peat moss by volume. In addition, AquaGro 2000 G wetting agent was blended into the mixture at a rate of 5.9 kg/L. The mixture was then steam pasteurized at 80° C for 45 min. Pots were watered daily and fertilized weekly.

Adult *C. litura*, collected near Bozeman, Montana in late April to early May, were purchased from a commercial source (K. Winward, Bozeman, MT). In the Wyoming experimental trials, weevils were placed on thistles 2-3 days after collection. In the Montana experimental trial, two separate collections of weevils (a week apart) were maintained on Canada thistle foliage until experimental plants had well established rosettes, 2-9 days after the weevils were collected. At the time weevils were placed on the thistle plants, rosettes were 5-10 cm in diameter. Five adult *C. litura* were added to each column receiving weevils. All columns were individually caged with 40 cm wide x 40 cm long mesh sleeves made from mosquito netting (Rockywoods Outdoor Fabrics, Loveland, CO). Weevils were allowed to feed and presumably oviposit for one week, at which point all weevils and cages were removed. Successful development of *C. litura* larvae was observed; newly emerged second-generation weevils were removed from the plants.

Herbicide treatments were timed to correspond with the late-bud to early-flower stage of Canada thistle phenology. Herbicide treatments were: none, 2,4-D [(2,4-Dichlorophenoxy) acetic acid], glyphosate [N-(phosphonomethyl) glycine] or clopyralid [3, 6-dichloro-2-pyridinecarboxylic acid]. In all experimental trials, the rates for 2,4-D and clopyralid corresponded to 0.98 kg ae/ha and 0.28 kg ae/ha, respectively. A non-ionic surfactant was included with the clopyralid treatment at 0.25% v/v. Different formulations of glyphosate were inadvertently used in the Wyoming versus Montana experimental trials, resulting in different amounts of active ingredient applied per unit area: 2.52 kg ae/ha (Wyoming) versus 3.78 kg ae/ha (Montana). In the Wyoming experimental trials, herbicides were applied in a spray chamber with a single 8002 even flat fan nozzle at a total spray volume of 187 l/ha at 276 kPa pressure. In the Montana experimental trial, herbicides were applied outside the greenhouse with a single flat fan 8001 nozzle at a total spray volume of 93.5 l/ha at 276 kPa pressure. Herbicide applications were made on July 10, 2005, and July 15, 2006 for the Wyoming experimental trials, and July 8, 2006 for the Montana experimental trial.

Plants were destructively sampled approximately 90 days after herbicide application. Shoots were clipped at the soil surface and roots were sieved and washed on a 1-mm wire screen. Roots and shoots were air dried in separate bags for 72 hours at 65^{o}C and weighed.

Final root and shoot biomass were analyzed for each of the three herbicides separately, initially combining the three experimental trials using three-factor, factorial ANOVA (with or without herbicide, with or without weevils, and the three different experimental trials). Because of numerous trial effects and trial-by-treatment interactions (analyses not shown), the three experimental trials were then analyzed separately for each of the three herbicides using two-factor ANOVA (with or without herbicide, and with or without weevils).

A statistically significant herbicide-by-weevil interaction represents evidence for either synergy or antagonism between weevil attack and herbicide application depending on whether the combined impact of the two factors are greater (synergy) or lower (antagonism) than would be expected from the independent effects of the factors. All biomass data were natural logarithm transformed prior to analysis. Using a simple population dynamic model, Rees and Brown (1992) argued that factors additively affecting plant biomass should be additive on a natural log scale rather than a linear scale. Therefore, synergy should be evaluated on a natural log scale (Rees and Brown, 1992). Natural log transformation also equalized variances among the treatments. Qualitatively, the results were the same for non-transformed data.

Results

Root Impacts

The effects of both the weevil and herbicides on root biomass varied across the experimental trials. Significant weevil impact occurred in only one of the three trials (Wyoming 2005), where the weevil alone reduced root biomass by 54% (Table 3.1, Fig. 3.1). Clopyralid had the greatest and most consistent effect, reducing root biomass by 50%, 48% and 65% in the Wyoming 2005, Wyoming 2006 and Montana 2006 trials, respectively (Fig. 3.1). In the Wyoming 2005 and 2006 experimental trials, glyphosate reduced root biomass by 20% and 24%, respectively; the latter was statistically significant (Table 3.1). In the Montana 2006 experimental trial, glyphosate significantly reduced root biomass, by 65% (Fig. 3.1). Finally, application of 2,4-D had relatively little impact on roots, significantly reducing root biomass in only the Montana 2006 trial (by 34%).

For root biomass, interactions between herbicide treatment and weevil attack were not statistically significant for any of the three herbicides in any of the experimental trials (Table 3.1). This suggests that the combined impacts of the weevil and the herbicides on root biomass were additive instead of synergistic or antagonistic. Because of their additive effects, the combination of weevils and herbicide produced greater suppression of Canada thistle root biomass than the single factors alone (Fig. 3.1). In particular, weevils plus clopyralid reduced root biomass by 72-92% across the three experimental trials.

Shoot Impacts

Statistical significance of weevil impacts (Table 3.2) on shoot biomass depended on the herbicide used because of differences in the magnitude of the error variance in the ANOVA's. Impacts of the weevil on shoot biomass were limited (Fig. 3.2). Shoot biomass was reduced by glyphosate in all three experimental trials, and by 2,4-D in two of the three experimental trials. There was no living above-ground biomass in any of the clopyralid-treated columns in the 2005 Wyoming experimental trial, i.e., no variance, so no statistical test could be conducted for this trial. Clopyralid reduced shoot biomass in the 2005 and 2006 Wyoming experimental trial but not the 2006 Montana trial (Table 3.2).

Of the eight 2x2 ANOVAs involving shoot biomass, one analysis showed an interaction between weevil attack and herbicide (Table 3.2). In the 2006 Montana trial, neither weevil nor glyphosate treatment appeared to affect thistle shoot biomass (2% and 0% reduction, respectively) but the combination of the two factors reduced shoot biomass by 45%. This appears as a statistically significant weevil-by-herbicide interaction ($p = 0.037$; Table 3.2).

Discussion

The impact of weevil attack and herbicide application on Canada thistle varied considerably across the experimental trials. Differences in the results may have reflected the somewhat different conditions or methods used in the three experiments. Likely sources of variation include: the different soil media used in the Wyoming versus Montana trials, differences in greenhouse conditions between the years and sites, and differences in the condition of the weevils at the time they were put on the plants. For 2,4-D and clopyralid, differences in the impact of these herbicides in the Montana versus Wyoming trials may have reflected the different spray volumes used in the experiments, although this seems unlikely because the amount of active ingredient applied per plant was the same. The different amounts of active ingredient of glyphosate applied per plant in the Wyoming and Montana trials probably explains the variation in the impact of this herbicide in these trials.

Despite the variation in the impact of the weevils and herbicides across the experimental trials, there was consistency in the lack of synergy of weevil attack and herbicide treatments. In one case, *C. litura* and glyphosate appeared to have synergistic, negative effects on shoot biomass. Apart from this one example, the impacts of the weevil and herbicides were additive and therefore independent. Overall, the results stand in contrast to our hypothesis that synergy between herbicide application and biological control would result if weevil-attacked thistles were treated with herbicide.

We also found no evidence of antagonism in the impacts of weevil attack and any of the herbicide treatments. Weevil attack did not apparently reduce the efficacy of the herbicides nor was the impact of the weevils reduced by herbicide application. In our experiments, we attempted to avoid herbicide impacts on the weevil by applying the herbicide after the weevil larvae had left the plant. In other systems, timing herbicide applications to reduce antagonism may not be possible because the appropriate timing for herbicides directly coincides with the presence of the larval stage of the agent in or on the plant (e.g., Story and Stougaard, 2006)

The general lack of research on synergistic impacts of herbicides and weed biological control agents may at least partly reflect the technical problem of containing insects in appropriate plots in the field. Field studies integrating herbicides and biological control typically have not included insect-free plots, which are needed to evaluate the independent versus combined impacts of biological control agents and herbicide applications. Two studies that used cages to contain insects in the field did not statistically evaluate the synergistic effects of herbicides and biological control agents *per se* (Lym and Nelson, 2002, Henne *et al.*, 2005).

In the greenhouse, the presence or absence of biological control agents can be manipulated more easily than in the field to evaluate interactions between the impacts of herbicides and biological control agents. Impacts on root growth can also be more easily evaluated in the greenhouse than in the field. In a study similar to our own, Boydston and

Williams (2004) investigated the combined impact of the gall mite *Aceria malherbae* and either 2,4-D or glyphosate on root and shoot biomass of potted field bindweed plants. These authors also found no evidence of synergism between the mite and herbicide treatments. Boydston and Williams (2004) did find direct negative impacts of glyphosate on the mite (number of galls) but these effects did not translate into antagonism between the impacts of the mite and glyphosate. It is possible that stresses present in the field and/or multi-year impacts of insects and herbicides might lead to synergy that would be absent in short-term greenhouse studies such as Boydston and Williams (2004) and our own.

Even if synergy is not observed in experiments conducted in the greenhouse, it may be worthwhile to conduct further testing and/or apply integrative strategies in the field. First, synergy may actually be more likely to occur under field conditions, where there may be additional sources of plant stress. Second, although synergistic impacts are clearly desirable, a lack of antagonism between herbicides and biological control is the most important consideration in integrating weed management strategies. Finally, although herbicides and weevils did not produce synergistic impacts on Canada thistle in our study, combining weevils with clopyralid led to the most consistent and greatest suppression of Canada thistle root biomass overall. *Ceutorhynchus litura* may indeed represent a little hammer that could contribute to Canada thistle suppression when integrated with clopyralid applications.

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Wyoming 2005

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	3.25 n.s.	4.14 n.s.	20.43***
Weevils	34.38****	28.54****	26.65****
interaction	0.04 n.s.	0.02 n.s.	0.23 n.s
d.f.	1,16	1,16	1,16

Wyoming 2006

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	2.31 n.s.	12.39**	67.22****
Weevils	1.09 n.s.	1.15 n.s.	2.05 n.s.
interaction	0.48 n.s.	0.06 n.s.	1.13 n.s.
d.f.	1,16	1,20	1,16

Montana 2006

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	14.02**	33.57****	84.59****
Weevils	2.26 n.s.	1.09 n.s.	3.98 n.s.
interaction	0.12 n.s.	0 n.s.	0.18 n.s.
d.f.	1,15	1,16	1,16

Table 3.1. Results of root biomass statistical analyses. Shown are the F-values from two-by-two factorial ANOVA's analyzing the effects of *Ceutorhynchus litura* and one of three herbicides (2,4-D, glyphosate or clopyralid) on Canada thistle root biomass in three different greenhouse trials. Statistical significance is indicated as follows: n.s.: not significant at $\alpha = 0.05$; *: $p<0.05$, **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$. Also shown are the degrees of freedom (d.f.) for the F-tests.

Wyoming 2005

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	6.71*	12.32**	--
Weevils	6.05*	4.22 n.s.	--
interaction	0.09 n.s.	0.48 n.s.	--
d.f.	1,16	1,16	--

Wyoming 2006

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	0.27 n.s.	14.62**	28.78****
Weevils	0.02 n.s.	0.20 n.s.	1.82 n.s.
interaction	0.01 n.s.	0.06 n.s.	2.47 n.s.
d.f.	1,16	1,20	1,16

Montana 2006

Treatment	2,4-D	Glyphosate	Clopyralid
Herbicide	12.40 **	5.31 *	3.22 n.s.
Weevils	2.04 n.s.	6.02 *	1.62 n.s.
interaction	1.20 n.s.	5.19 *	1.19 n.s.
d.f.	1,15	1,16	1,16

Table 3.2. Results of shoot biomass statistical analyses. Shown are the F-values from two-by-two factorial ANOVA's anayzing the effects of *Ceutorhynchus litura* and one of three herbicides (2,4-D, Glyphosate or Clopyralid) on Canada thistle shoot biomass in three different greenhouse trials. Statistical significance is indicated as follows: n.s.: not significant at $\alpha = 0.05$; *: $p<0.05$, **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$. Also shown are the degrees of freedom (d.f.) for the F-tests. Results for Clopyralid treatments in 2005 because of zero variance in shoot biomass in this year; no living shoots were present.

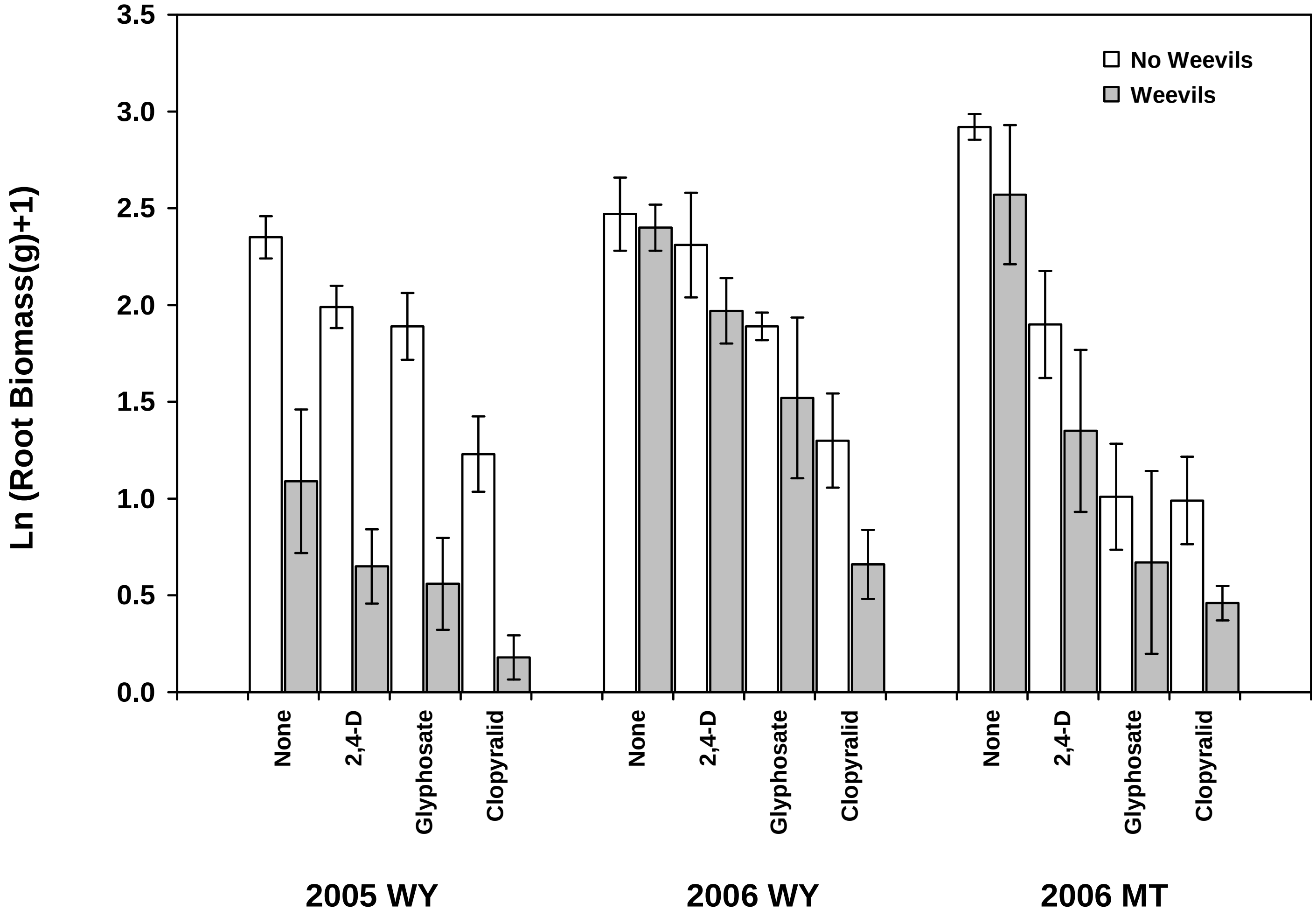

Figure 3.1. Impacts on Canada thistle root biomass of *Ceutorhynchus litura* and one of three herbicides (2,4-D, glyphosate or clopyralid) in three different greenhouse trials.

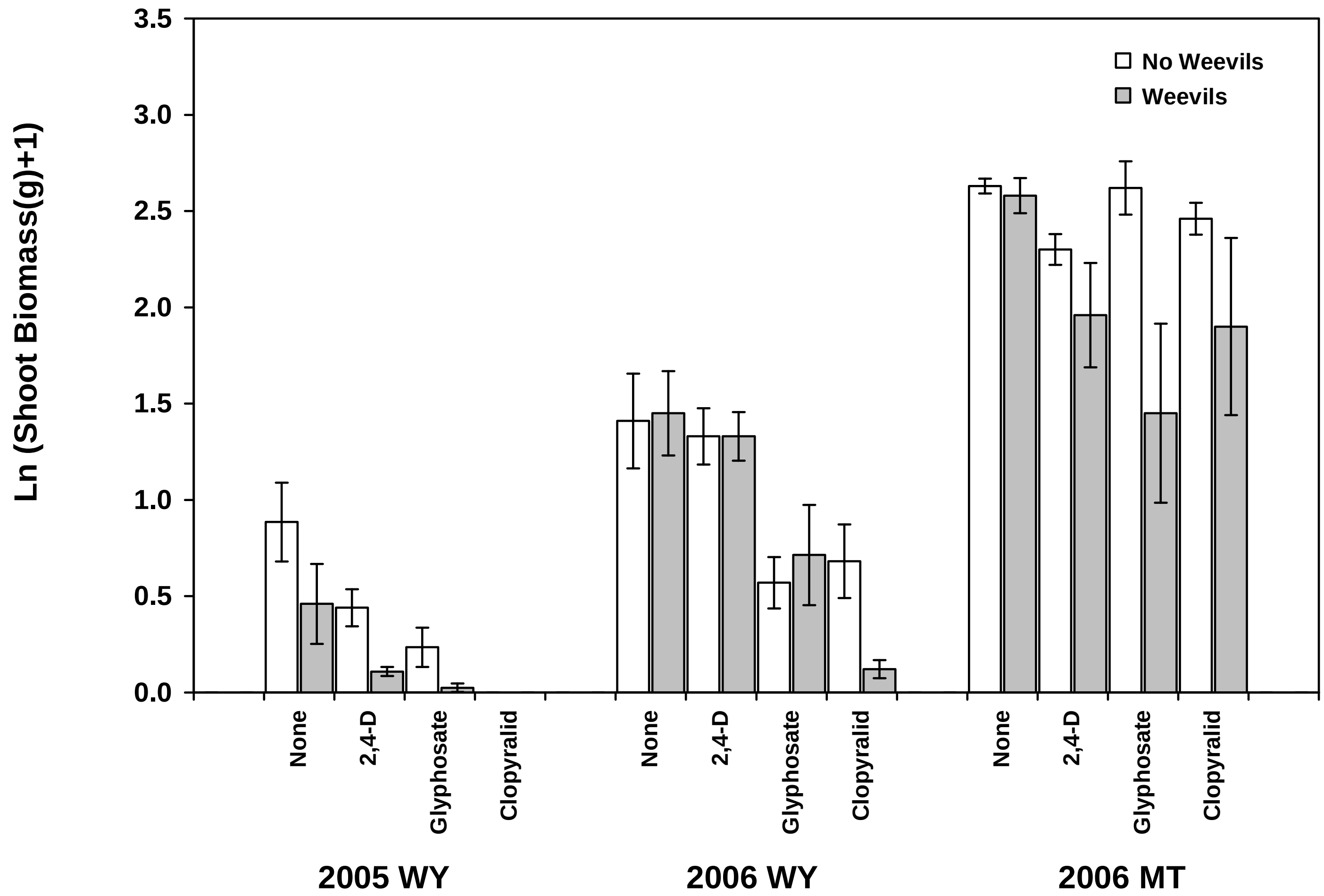

Figure 3.2. Impacts on Canada thistle shoot biomass of *Ceutorhynchus litura* and one of three herbicides (2,4-D, glyphosate or clopyralid) in three different greenhouse trials.

References Cited

Beck, K.G., Sebastian, J.R., 2000. Combining mowing and fall applied herbicides to control Canada thistle (*Cirsium arvense*). Weed Technol. 14, 351-356.

Boydston, R.A., Williams, M.M., 2004. Combined effects of *Aceria malherbae* and herbicides on field bindweed (*Convolvulus arvensis*) growth. Weed Sci. 52, 297-301.

Coombs, E.M., Clark, J.K., Piper, G.L., Cofrancesco, A., Jr., 2004. Biological Control of Invasive Plants in the United States. Oregon State University Press, Corvallis OR.

Donald, W.W., 1990. Management and control of Canada thistle (*Cirsium arvense*). Rev. Weed Sci. 5, 193-250.

Haggar, R.J., Oswald, A.K., Richardson, W.G., 1986. A review of the impact and control of creeping thistle (*Cirsium arvense* L.) in grassland. Crop Prot. 5, 73-76.

Hein, G.L.,Wilson, R.G., 2004. Impact of *Ceutorhynchus litura* feeding on root carbohydrate levels in Canada thistle (*Cirsium arvense*). Weed Sci. 52, 628-633.

Henne, D.C., Lindgren, C.J., Gabor, T.S., Murkin, H.R., Roughly, R.E., 2005. An integrated management strategy for the control of purple loosestrife *Lythrum salicaria* L. (Lythraceae) in the Netley-Libau Marsh, southern Manitoba. Biol. Control 32, 319-325.

Liebman, M., Gallandt, E., 1997. Many little hammers: ecological approaches for the management of crop-weed interactions. In: Jackson L.E. (Ed.), Ecology in Agriculture. Academic Press, pp. 291-343.

Lindgren, C.J., Gabor, T.S., Murkin, H.R., 1998. Impact of Tricopyr Amine on *Gallerucella calamarensis* L. (Coleoptera: Chrysomelidae) and a step toward integrated management of purple loosestrife *Lythrum salicaria* L. Biol. Control 12, 14-19.

Lym, R.G., Nelson, J.A., 2002. Integration of *Aphthona* spp. flea beetles and herbicides for leafy spurge (*Euphorbia esula*) control. Weed Sci. 50, 812-819.

Messersmith, C.G., Adkins, S.W., 1995. Integrating weed-feeding insects and herbicides for weed control. Weed Technol. 9, 199-208.

Moore, R.J., 1975. The biology of Canadian weeds. 13. *Cirsium arvense* (L.) Scop. Can. J. Plant Sci. 55, 1033-1048.

Moyer, J.R., Schaalje, G.B., Bergen, P., 1991. Alfalfa (*Medicago sativa*) seed yield loss due to Canada thistle (*Cirsium arvense*). Weed Technol. 5, 723-728.

Nelson, J.A., Lym, R.G., 2003. Interactive effects of *Aphthona nigriscutis* and picloram plus 2,4-D in leafy spurge (*Euphorbia esula*). Weed Sci. 51, 118-124.

O'Sullivan, P.A., Kossatz, V.C., Weiss, G.M., Drew, D.A., 1982. An approach to estimating yield loss of barley due to Canada thistle. Can. J. Plant Sci. 62, 725-731.

Paynter, Q., 2003. Integrated weed management: effect of herbicide choice and timing of application on the survival of a biological control agent of the tropical wetland weed, *Mimosa pigra*. Biol. Control 26, 162-167.

Peschken, D.P., Derby, J.L., 1992. Effect of *Urophora cardui* (L.) (Diptera: Tephritidae) and *Ceutorhynchus litura* (E.) (Coleoptera: Curculionidae) on the weed Canada thistle, *Cirsium arvense* (L.) Scop. Can. Entomol. 124 (1), 145-150.

Peschken, D.P., Wilkinson, D.P., 1981. Biocontrol of Canada thistle *Cirsium arvense*: releases and effectiveness of *Ceutorhynchus litura* (Coleoptera: Curculionidae) in Canada. Can. Entomol. 107, 1101-1110.

Piper, G.L., Andres, L.A., 1995. 63. Canada Thistle. In: J. R. Nechols (Ed.), Biological Control in the Western United States. Publication 3361. University of California, Oakland, California, pp. 233-236.

Reece, P.E., Wilson, R.G., 1983. Effect of Canada thistle (*Cirsium arvense*) and musk thistle (*Carduus nutans*) control on grass herbage. Weed Sci. 31, 488-492.

Reed, C.C., Larson, D.L, Larson, J.L., 2006. Canada thistle biological control agents on two South Dakota wildlife refuges. Nat. Areas J. 26, 47-52.

Rees, N.E., 1990. Establishment, dispersal and influence of *Ceutorhynchus litura* on Canada thistle (*Cirsium* arvense) in the Gallatin valley of Montana. Weed Sci. 38, 198-200.

Rees, M., Brown, V.K., 1992. Interactions between invertebrate herbivores and plant competition. J. Ecol. 80, 353-360.

Skinner, K., Smith, L., Rice, P., 2000. Using noxious weed lists to prioritize targets for developing weed management strategies. Weed Sci. 48, 640–644.

Stachion, W.J., Zimdahl, R.L., 1980. Allelopathic activity of Canada thistle (*Cirsium arvense*) in Colorado. Weed Sci. 28, 83-86.

Story, J.M., Stougaard, R.N., 2006. Compatibility of two herbicides with *Cyphocleonus achates* (Coleoptera: Curculionidae) and *Agapeta zoegana* (Lepidoptera: Tortricidae), two root insects introduced for biological control of spotted knapweed. Environ. Entomol. 35(2), 373-378.

Story, J.M., Boggs, K.W., Good, W.R., 1988. Optimal timing of 2,4-D applications for compatibility with *Urophora affinis* and *U. quadrifasciata* (Diptera: Tephritidae) for control of spotted knapweed. Environ. Entomol. 17(5), 911-914.

Trumble, J.T., Kok, L.T., 1980. Impact of 2,4-D on *Ceuthorhynchidius horridus* (Coleoptera: Curculionidae) and their compatibility for integrated control of *Carduus* thistles. Weed Res. 20, 73-75.

Wilson, R.G., Kachman, S.D., 1999. Effect of perennial grasses on Canada thistle (*Cirsium arvense*) control. Weed Technol. 13, 83-87.

Wilson, R., Beck, K.G., and Westra, P., 2004. Combined effects of herbicides and *Sphenoptera jugoslavica* on diffuse knapweed (*Centaurea diffusa*) population dynamics. Weed Sci. 52, 418-423.

Zwölfer, H., Harris, P., 1966. *Ceutorhynchus litura* (F.) (Col.: Curculionidae), a potential insect for the biological control of thistle, *Cirsium* arvense (L.) Scop., in Canada. Can. J. Zool. 44, 23-38.

CHAPTER 4

SINGLE AND COMBINED EFFECTS OF A PATHOGEN, INSECT, AND HERBICIDE ON CANADA THISTLE GROWTH

Introduction

Canada thistle (*Cirsium arvense*) control has a long and varied history (Friesen 1968, Donald 1990, Sullivan 2004). Many singular methods, the most common being herbicides, have been tried with limited success. Unfortunately, herbicides are not usually fully effective, allowing other weeds to invade by weakening but not eliminating Canada thistle, or injuring the surrounding crop or native vegetation (Tipping 2001, Grekul et al. 2005, Mesbah and Miller 2005). Also, although many insects have been tested as biological agents for Canada thistle control, results are far from promising due to failure of establishment at high enough numbers to impact Canada thistle stands (Reed et al. 2006) or due to their feeding preferences on native thistles (Louda et al. 1997, Louda and O'Brien 2002). Pathogens such as *Pseudomonas syringae* pv. *tagetis* (PST) or the rust fungus *Puccinia punctiformis* have also been tested for Canada thistle control but showed less than satisfactory results (Frantzen 1994, Gronwald et al. 2002).

The realization that individual control practices are not always effective at controlling Canada thistle infestations has spurred research into the integration of mechanical, cultural, biological, and chemical practices as an approach to manage this weed (Edwards et al. 2000, Friedli & Bacher 2001a, Friedli and Bacher 2001b, Hoeft et al. 2001, Kluth et al. 2003, Travnicek et al. 2005, Graglia et al. 2006, Collier et al. 2007). A method of conceptualizing integrated weed management (IWM), introduced by Liebman and Gallandt (1997), is the idea of utilizing “many little hammers” to develop an ecologically-based weed reduction strategy. In this scenario, each little hammer refers to a weed control technique that may not be successful on its own, but may have a deleterious effect on the target organism. This approach to weed management, while not excluding direct controls such as herbicides, shifts its focus to indirect controls such as varied crop rotations, seed predation pressures, and enhanced crop competitive ability. It also includes the host of interactions among management practices with the goal of stressing weeds with multiple and varied tactics. If properly designed, IWM can provide a more sustainable, economic, and successful method of weed control that reduces or prevents shifts from benign to noxious weeds and decreases the dependence on external high energy inputs (Liebman and Gallandt 1997, Liebman et al. 2001, Masters and Sheley 2001). For example, in a combination of field studies and modeling exercises, Westerman et al. (2005) demonstrated that IWM could be successfully used to manage velvetleaf (*Abutilon theophrasti* Medik.) populations in row-crop systems.

In an IWM program, the interactions between practices can be synergistic, additive, complementary, or antagonistic (Messersmith and Adkins 1995, Simpson and Stoller 1996, Norris et al. 2001, Kluth et al. 2003, Abdollahi and Ghadiri 2004, Collier et al. 2007). Thus, in the development of an integrated approach to manage weeds, it is crucial to carefully document each effect singularly and in factorial combination with the other proposed tactics. For example, in a greenhouse study, Collier et al. (2007) established that although combining herbicides with a stem-boring weevil led to greater Canada thistle control, no synergistic interactions occurred among individual control practices. The goal of our greenhouse and field experiments was to test if patterns observed by Collier et al. (2007) applied when more than two control agents were combined in the development of an IWM strategy for Canada thistle. Specifically, we combined two rates of glyphosate with *Hadroplontus litura* weevils and a pathogen, PST, in greenhouse and field settings to evaluate the existence of additive or synergistic interactions among these three factors in reducing the growth of Canada thistle. We hypothesized that, first, while each treatment would have a negative effect on Canada thistle growth, the integrated strategies would have higher impact than individual control methods. Second, a PST application following weevil infestation would be more effective than one prior to it, as the damage caused by the insects would provide new vectors of infection for the bacteria, increasing damage to the plants.

Materials and Methods

Greenhouse

This study was conducted in a light and temperature controlled greenhouse at Montana State University, Bozeman, MT, from April to October 2006, 2007, and 2008. Canada thistle roots used in the 2006 and 2007 experiment were dug from the top 30 cm of soil in an upland grassland site near Cheyenne, WY. Roots used in the 2008 experiment were dug from the Arthur Post Research Farm near Bozeman. Before planting the 2006 experiment, the roots were stored in a plastic zip top bag with a damp paper towel at 4°C for approximately three weeks. In 2007, roots were stored in the same conditions for a maximum of three days before planting. In 2008, the roots were dug the previous fall and stored in peat moss at 4°C. Experimental units consisted of individual Canada thistle plants grown in 43-cm tall pots constructed with 15-cm diameter thin wall PVC plastic irrigation pipes. Each pot was lined with 0.1-mm thick plastic liner to create a bottom for the pot. Approximately 7.6 L of a soil blend was put into each pot. The soil blend consisted of a 1:1:1 ratio of mineral soil, Canadian Sphagnum peat moss, and washed concrete sand. A wetting agent, Aqua-Gro 2000G (Aquatrols Company), was added at a rate of 5.9 kg L^{-1}. The mix was aerated steam pasteurized using the Lindig soil treatment system for 45 min at 80°C. Before planting, soil was moistened to facilitate water infiltration and discourage heavy settling. After filling pots with soil, the plastic liner was perforated at the bottom to provide drainage. Canada thistle roots were planted 4-5 cm deep and watered regularly.

Greenhouse lights supplemented ambient light to provide a 16L:8D photoperiod to facilitate plant emergence and establishment. The bulbs used were 1000 watt GE Multi-Vapor MVR1000/C/U metal halide lamps. Temperature in the greenhouse ranged from a nightly minimum of 13-15° C to a daily maximum of 30-40° C. The experimental design was a randomized complete block design with glyphosate rate (none, one-sixth of the labeled rate – 0.63 kg ae/ha, or a full labeled rate – 3.78 kg ae/ha), insects (absent or present), and timing of PST application (none, early – before insect release, or late – after insect release) as the main factors. A total of three different trials were run. The 2006 and 2008 trials had five replicates per treatment, while the 2007 had three.

Adult *H. litura*, collected in late April to early May, were purchased from a commercial source (Katy Winward, Bozeman, MT) and maintained on Canada thistle foliage until release onto experimental plants. Two to nine days after collection, five adult weevils were placed on each experimental thistle receiving an insect treatment. Weevils were released when targeted Canada thistle plants achieved a 5-10 cm diameter, which occurred 22 d after planting (DAP) in 2006, 39 DAP in 2007, and 37 DAP in 2008. At the time of the weevil release, individual pots were caged with a 40 x 40 cm mesh sleeve made from mosquito netting (Rockywoods Outdoor Fabrics, Loveland, CO). Weevils were allowed to feed and oviposit on thistle plants for one week, after which all weevils and cages were removed. Newly emerged second-generation weevils were removed when appropriate.

The PST was grown in the laboratory on tryptic soy agar and suspended in a cryo-solution of 1% tryptic soy broth and 10% glycerol before storage in a freezer at -20°C. It was first thawed from this frozen state, then 100 mL of this solution were spread onto a Petri dish containing tryptic soy agar at 50% strength and incubated at 25° C for at least 48 hr. For each greenhouse trial, one plate of PST containing approximately 10^10 bacteria was mixed with 500 mL of water to create a solution that contained approximately 2 x 10^7 bacteria per ml. The organosilicone surfactant Silwet L-77® was added to the suspension at a rate of 0.4% final volume to facilitate spreading, wetting, and absorption. A solution of Silwet L-77® alone was sprayed on all control plants. The PST was applied at two different times: either one day before the insects were released on Canada thistle (early application) or one week after the insects were removed (late application). Early PST was applied 21 DAP in 2006, 28 DAP in 2007, and 36 DAP in 2008. Late PST was applied 36 DAP in 2006, 53 DAP in 2007, and 55 DAP in 2008.

Herbicide treatments were applied after the flowering stage of Canada thistle, 77 DAP in 2006, 160 DAP in 2007, and 84 DAP in 2008 as there is evidence that this stage is most susceptible to translocating chemicals (Wilson and Michiels 2003). Glyphosate (Roundup Original Max®) was used at either the full recommended rate of 3.78 kg ae/ha or a reduced rate of 0.63 kg ae/ha. In 2006, glyphosate was sprayed outside the greenhouse using an 8001 flat fan nozzle at a pressure of 276 kPa and a total spray volume of 93.5 Lha^{-1}. In 2007 and 2008, glyphosate was sprayed in a spray chamber with a single 8002 even flat fat nozzle at a pressure of 276 kPa and a total spray volume of 131 Lha^{-1}. Approximately 90 d after herbicide application, all Canada thistle plants were harvested and weighed. Shoots were clipped at the soil surface and dried in a 65°C oven for at least 72 hours; roots were gently washed before drying.

Field

The interactions between herbicide and pathogen were further evaluated in a reduced study conducted in field conditions during the summer and fall of 2007 at the Montana State University Arthur H. Post Agronomy Farm in Bozeman, MT, 45º 40’29”

N, 11º 09'14" W, 1423 m elevation. Mean annual precipitation for the last 5 yr was 38 cm. Mean temperature for May through October for the last 5 yr was 14° C. Soil in the area is classified as an Amsterdam-Quagle Silt Loam (Brooker 2002). Canada thistle roots were dug from the farm in May 2007 and planted 24 May 2007 in a field that had been fallow for 3 yr prior to the start of this study. During these three years, weeds were managed using a disc cultivator as needed. The experiment followed a randomized complete block design with four 30 x 10 m replicates and a total of 252 roots per replicate (experimental units). Individual root length, diameter, and weight were measured before planting. Individual roots were planted approximately one meter apart at either 2, 10, or 20 cm deep.

Only the plants from the 10-cm burial depth emerged in sufficiently high numbers to conduct this study. Twenty plants from each replicate were randomly selected and assigned to either control, reduced rate of glyphosate (1/6 the labeled rate, 0.63 kg ae/ha), recommended rate of glyphosate (3.78 kg ae/ha), or the combination of pathogen (PST) and reduced rate of glyphosate. *H. litura* was not available at the time of treatment and was not included in the study.

The PST spray solution was made with three cultured plates of PST containing approximately 10^10 bacteria each. Bacteria from the plates were suspended with enough water to make pourable, then mixed with 3 L of a solution of 0.4% Silwet L-77® in water to yield approximately 10^7 bacteria per ml. Sixty eight days after planting, PST was applied until runoff with a backpack sprayer, as it has been found to be the most effective way of PST application in the field (Gronwald et al. 2002).

Glyphosate was applied 107 d after planting using a calibrated backpack sprayer with a flat spray nozzle at either the labeled rate or 1/6 the labeled rate. Near the end of the growing season (9 Sept. 2007, or 120 d after planting), aboveground biomass was clipped at the soil surface, dried at 65°C for at least 72 hr and weighed to the nearest 0.01 g. The next spring, emergence for the plants treated in 2007 was monitored daily for eight weeks and noted when the first shoot was visible.

Data Analysis

Greenhouse

Final root and shoot biomass as well as shoot number were analyzed in SAS statistical software (version 9.1 for Unix) using the Proc Mixed command with glyphosate, pathogen, and insects as fixed factors and year, and rep nested in year, as random effects. Root weight was natural log transformed to meet normality and variance assumptions for ANOVA. Significant main effects were further analyzed for differences among levels using a Tukey multiple comparisons test. Significant interactions between main effects ($p<0.05$) would suggest synergism or antagonism (Collier et al. 2007), while lack of significant interactions would suggest that the control methods were additive. Significant interactions were further sliced and analyzed with a Bonferroni multiple comparisons procedure to determine differences among levels (Littell et al. 1996). The data from 2006 and 2008 were pooled together for all analyses since year was not significant. In 2007, a combination of poor emergence and surfactant injury led to skewed data which was not included in the analysis.

Field

For the field study, Canada thistle growth and suppression were compared based on treatments. Canada thistle growth was determined using dry shoot biomass at harvest. A one way ANOVA using R statistical software (version 2.7.2) analyzed differences in shoot weight among treatments. Canada thistle suppression was determined the growing season after treatments were applied, based on the inverse of percent emergence. Since every plant in the control treatment emerged, the data for this treatment generated no variability. A generalized linear model using a binomial error distribution and a logit link function (R statistical software, version 2.7.2) was used to analyze differences in Canada thistle suppression among treatments.

Results

Year was not a significant random effect for shoot weight ($p=0.34$), root weight ($p=0.35$), or shoot number ($p=0.39$) in greenhouse conditions. Rep was insignificant in the model ($p<0.98$) and, as the estimate for its coefficient was so close to zero, it was removed from the model. There were no significant interactions among random effects (year, rep nested in year) and the fixed effects (glyphosate, PST, or insects) for any response variable analyzed.

In greenhouse conditions, individual management practices impacted Canada thistle shoot weight, root weight, and shoot number (Table 4.1). Glyphosate, *H. litura*, and PST significantly affected Canada thistle shoot weight and root weight; *H. litura* and PST affected shoot number. Significant interactions, suggesting antagonism, were only found between glyphosate and PST for shoot weight.

A post-hoc analysis on the main effect of insects showed that presence of *H. litura* significantly decreased shoot weight (Fig. 4.1). Glyphosate and PST main effects were not analyzed further because they exhibited a significant interaction. The slicing of the data across herbicide treatments indicated that in the absence of glyphosate and when glyphosate was applied at the full rate, the early and late applications of PST reduced Canada thistle shoot weight (Fig. 4.2). We failed to detect a significant reduction in shoot weight when early and late applications of PST were combined with a reduced rate of glyphosate. When data were sliced across pathogen applications, results indicted that in the absence of PST, there were no significant differences in shoot weight when either a reduced or a full rate of glyphosate were applied, with both treatment biomasses lower than control (Fig. 4.3). For early and late applications of PST, both rates of glyphosate were equivalent to the control.

Root weight was affected by all three individual control practices. A full rate of glyphosate reduced root weight more than a reduced rate of glyphosate and both were significantly lower than the control (Fig. 4.4). Presence of insects significantly decreased mean root weight. Early and late applications of PST reduced mean root weight, with the early application producing weights significantly lower than the late application.

The presence of both *H. litura* and PST affected shoot number. For both control practices, their presence was associated with a lower number of shoots per plant. However, early and late applications of PST were not different from each other (Fig. 4.5).

In the field study, reduced rate of glyphosate, full rate of glyphosate, and reduced rate of glyphosate plus PST were all equivalent in suppressing Canada thistle emergence by about 80% the spring following treatment (Fig. 4.6, $p<0.05$). Shoot weight was similar for all four treatments, including the control ($p=0.21$).

Discussion

In greenhouse settings and in accordance with Collier et al (2007), we detected additive but not synergistic interactions among management tactics aimed at controlling Canada thistle, contradicting our first hypothesis. For shoots, the interaction between glyphosate and pathogen was an antagonistic one, as the two agents interfered with one another at both glyphosate application rates. It is possible that glyphosate could have reduced PST's efficacy by killing shoot tissue. Meanwhile, PST could have interfered with glyphosate's activity by perhaps metabolizing or degrading some of the herbicide's active ingredient. Boyette et al. (2008) suggests a similar mechanism to explain antagonistic effects between glyphosate and the fungus *Colletotrichum truncatum*. Glyphosate and the tagetitoxin that PST produces both inhibit production of chlorophyll in meristematic tissue. This could explain how the two agents were able to interfere with each other. A growth regulating herbicide such as clopyralid could potentially avoid this complication and actually encourage synergism by forcing the plant to grow much faster while the tagitoxin inhibits chloroplast formation.

The nature of the relationship among control tactics is critical in any integrated weed management plan (Messersmith and Adkins 1995). It is important to determine that tactics interact positively in order to prevent management hindrance or even failure. In a cage experiment, Kruess (2002) found that ovipositing leaf beetle (*Cassida rubiginosa*) preferred Canada thistle plants that were not infected with the necrotrophic fungus *Phoma destructiva*, suggesting that joint use of these biological agents could be counterproductive. Kluth et al. (2001) found that infection of Canada thistle by the rust fungus *Puccinia punctiformis* created a range of insect responses, with some insects avoiding infected thistles, others preferring them, and most having no preference. In this study, the existence of glyphosate, PST, and insect main effects suggests an additive relationship among these management practices. However, we also observed an interaction between glyphosate and PST that suggested antagonism, demonstrating that it is crucial to know how individual tactics can react with one another by measuring a variety of plant responses.

Although it has been suggested that insect injury to Canada thistle can provide vectors for pathogen infection (Friedli and Bacher 2001b, Kluth et al. 2001), our results did not fully support this. We hypothesized that a late application of PST would do more damage to Canada thistle than an early application. However, early and late PST applications had the same effect on shoot biomass and shoot number, and an early application of PST was more deleterious to Canada thistle root weight than a late application. The impact of the relative time of pathogen application on Canada thistle root could be due to the fact that PST is only active on newly forming tissue in the plant (Mathews and Durbin 1990, Steinberg et al. 1990, Johnson et al. 1996). Since the early application was sprayed on young, developing shoots, the debilitating effects of the infection could have been more pronounced. Root reserves would be forced to replenish shoot tissue in order to keep photosynthesis going. It is possible that by the time of the late application, the plants were large enough to overcome PST's negative effects. While there is plenty of information on PST's infection mechanism in the shoots, more work needs to be done to determine how root tissue responds to infection.

In accordance with previous studies assessing the applicability of reduced herbicide rates for weed control (Popp et al. 2000, Zhang et al. 2000, Beckie and Kirkland 2003, Williams et al. 2004), our results showed that compared to the full rate, one-sixth the recommended labeled rate of glyphosate provided equivalent decreases in shoot weight. However, a full rate was more effective at decreasing root weight, and glyphosate in general was not effective in decreasing shoot number. In practice, growers can be adverse to the risk that reduced herbicide rates bring, such as lack of control, selection for metabolic resistance, and increasing the weed seed bank (Doyle and Stypa 2004). Also, applying a reduced rate of herbicide multiple times can be more effective than applying the same herbicide at the full rate only once (Lockhart and Howatt 2004).Conversely, with herbicides now comprising 20-30% of input costs in the northern Great Plains (Derksen et al. 2002), growers are becoming concerned about the negative effects of such a high degree of reliance on these chemicals (Zoschke 1994). Low commodity prices, crop injury, herbicide carryover, resistance, and effects on the environment and human health are other compelling reasons to rethink this reliance and develop integrated weed management strategies (Blackshaw et al. 2006).

Canada thistle's capacity for extensive vegetative proliferation is the major obstacle in its successful control (Hunter 1996). For control with herbicides such as glyphosate, translocation is the key to affecting this root system. However, according to Donald (1990), individual control measures applied at only one part in Canada thistle's life cycle are never completely effective. As demonstrated in this study, pairing such a herbicide with other control practices, including insect and pathogen damage, can provide continuous stress on both roots and shoots. It is possible that in our experiments, while glyphosate acted as the translocating agent, insects and the pathogen damaged the shoot, forcing the root to expend energy reserves to replace damaged shoot tissue.

Treatments applied to fallow field-grown Canada thistle plants excelled at suppressing emergence the following spring. This suggests that when caught early in its lifetime and treated appropriately, Canada thistle can be successfully managed. It also indicates that even one sixth of the recommended labeled rate of glyphosate can do equally well in suppressing Canada thistle, whether it is paired with PST or not.

However, only one year of this experiment was done, and caution should be taken with the conclusions made for this experiment. In addition, at the end of the first growing season, no difference was evident in shoot weight between treatments.

While our hypotheses were not always proven correct, several conclusions can be drawn from this work. First, this study set a precedent of the importance of testing the existence of interactions between several control practices. Although previous studies have evaluated the combined used of two singular control methods for Canada thistle control, (Ferrero-Serrano et al. 2008, Travnicek et al. 2005, Kluth et al. 2003) the literature is lacking in detailing interactions between insects and herbicides and interactions between more than two control practices, especially an insect, herbicide, and pathogen.

Second, this work adds to the growing body of research that emphasizes the use of integrated management over individual tactics (Swanton and Weise 1991, Elmore 1996, Buhler 2002). Our results show that integrated methods work equal to or more effectively than the best individual management practices. A cost-benefit analysis would be a useful accompaniment to the effectiveness of the various tactics examined.

Finally, although these results did not display synergism, there is a possibility that synergistic interactions could still develop in field conditions due to the added abiotic and biotic stressors that plants face (Bostock et al. 2001, Martone and Wasson 2008). Conversely, it is also important to determine whether interactions are antagonistic in field conditions to prevent unsuccessful management. Further work should focus on interactions between individual control tactics on field-grown Canada thistle to determine how effectively IWM can control this weed.

Table 4.1: *F*-values and degrees of freedom (df) from ANOVA analyzing the effect of glyphosate, *Pseudomonas syringae* pv. *tagetis* (PST), and *Hadroplontus litura* weevils on Canada thistle shoot weight, root weight, and shoot number growing in greenhouse conditions.

Factor	Shoot Biomass	Root Biomass	Shoot Number	df
Glyphosate	10.01**	26.36**	2.91$^{n.s.}$	2, 171
H. litura	35.25**	29.18**	19.53**	1, 171
PST	26.55**	29.87**	15.03**	2, 171
Glyphosate : *H. litura*	0.11$^{n.s.}$	1.10$^{n.s.}$	0.71$^{n.s.}$	2, 171
Glyphosate : PST	3.26*	0.56$^{n.s.}$	0.76$^{n.s.}$	4, 171
H. litura : PST	0.49$^{n.s.}$	1.71$^{n.s.}$	0.56$^{n.s.}$	2, 171
Glyphosate : *H. litura* : PST	1.48$^{n.s.}$	1.84$^{n.s.}$	0.80$^{n.s.}$	4, 171

Statistical significance is indicated as follows: n.s., not significant at a=0.05; *p<0.05; **p<0.001

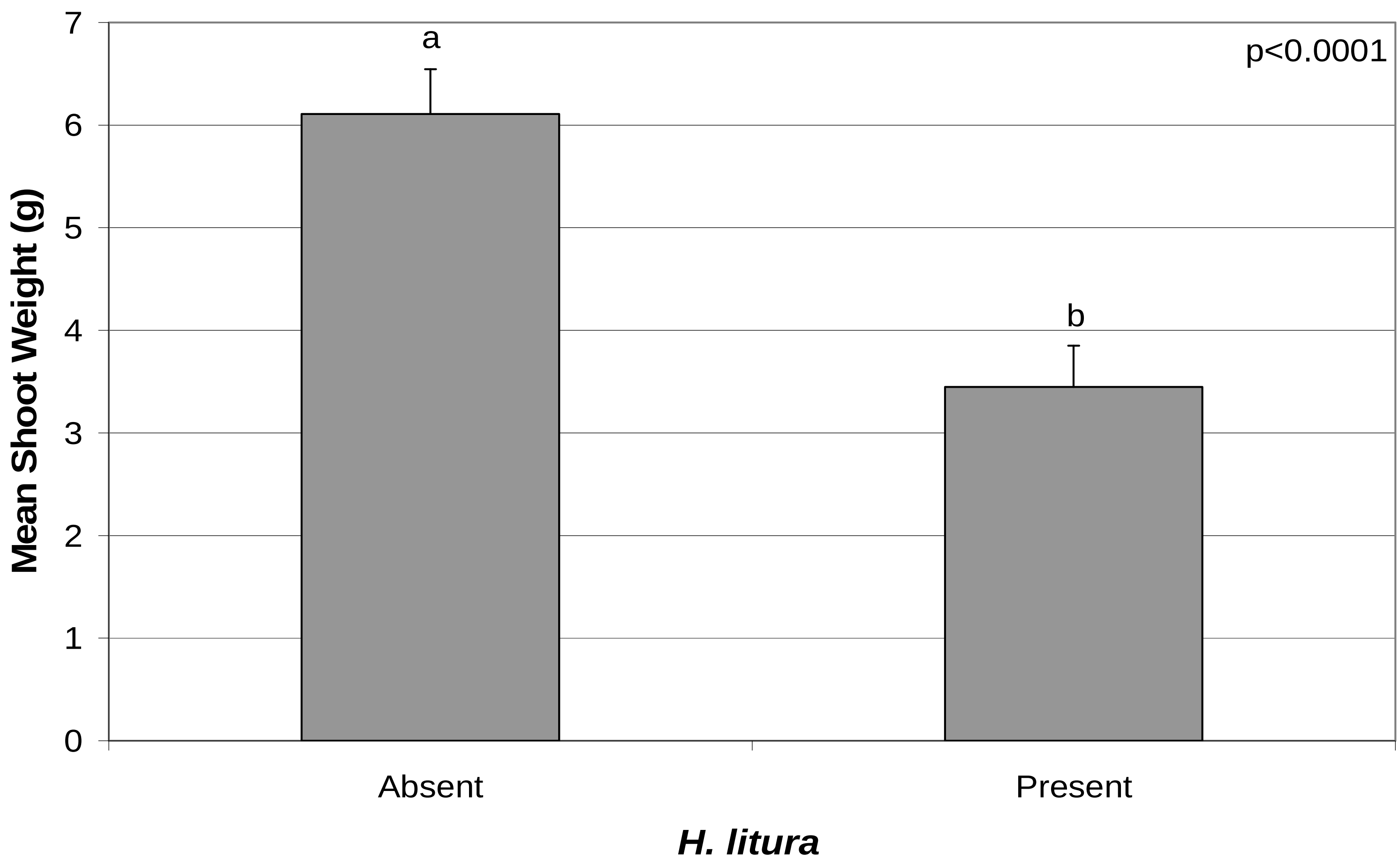

Figure 4.1: Impact of the stem boring weevil, *Hadroplontus litura*, on Canada thistle dry shoot weight in greenhouse conditions. Bars represent mean values plus standard error of the mean, and letters represent values that are different at the $p=0.05$ level.

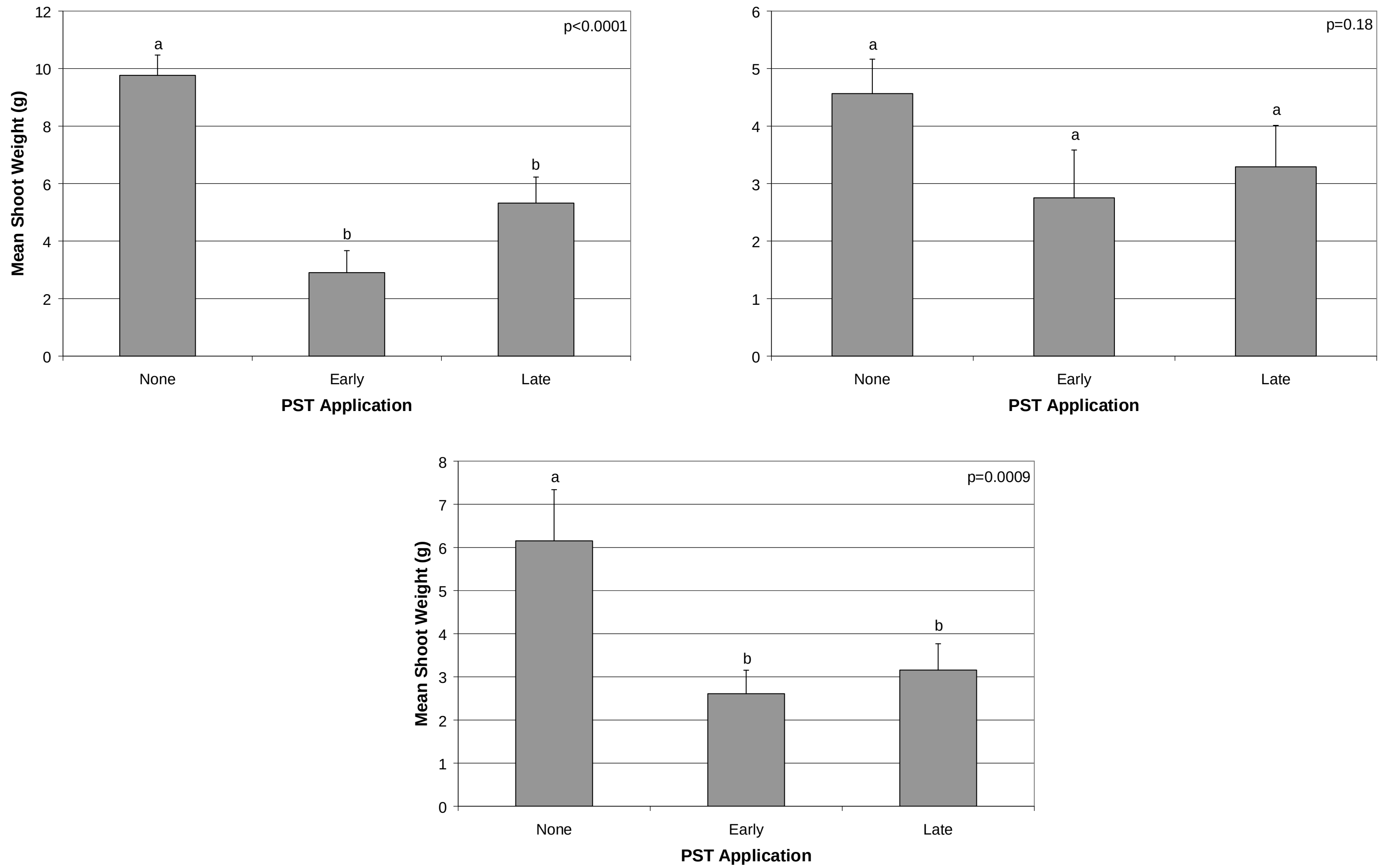

Figure 4.2: Impact of *Pseudomonas syringae* pv. *tagetis* (PST) on Canada thistle dry shoot weight in greenhouse conditions when no glyphosate is applied (top left), a reduced rate of glyphosate is applied (top right), and a full rate of glyphosate is applied (bottom). Bars indicate mean values plus standard error of the mean. Letters indicate values that are different at the p=0.05 level.

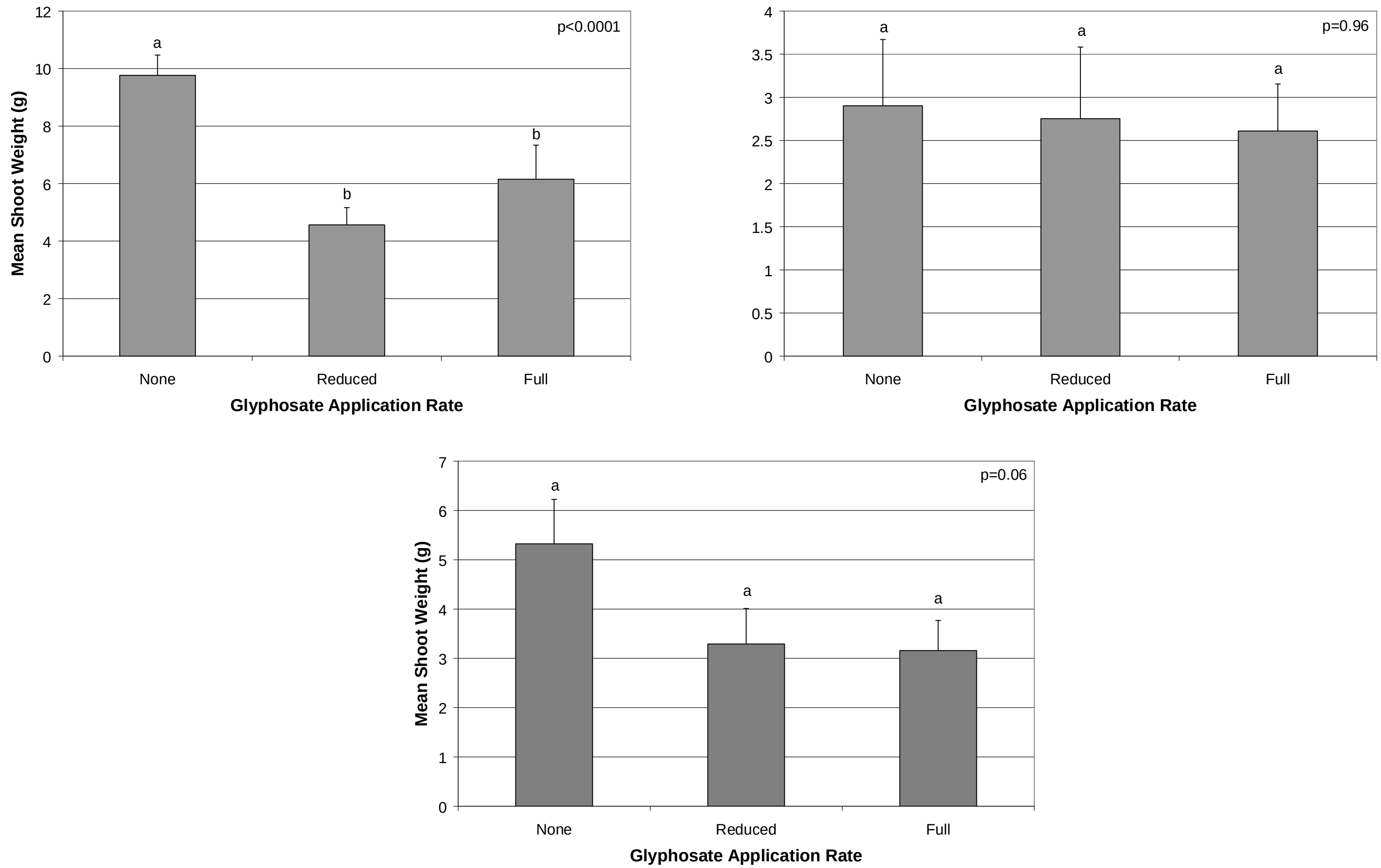

Figure 4.3: Impact of glyphosate on Canada thistle dry shoot weight in greenhouse conditions when no *Pseudomonas syringae* pv. *tagetis* (PST) is applied (top left), early PST is applied (top right), and late PST is applied (bottom). Bars indicate mean values plus standard error of the mean. Letters indicate values that are different at the p=0.05 level.

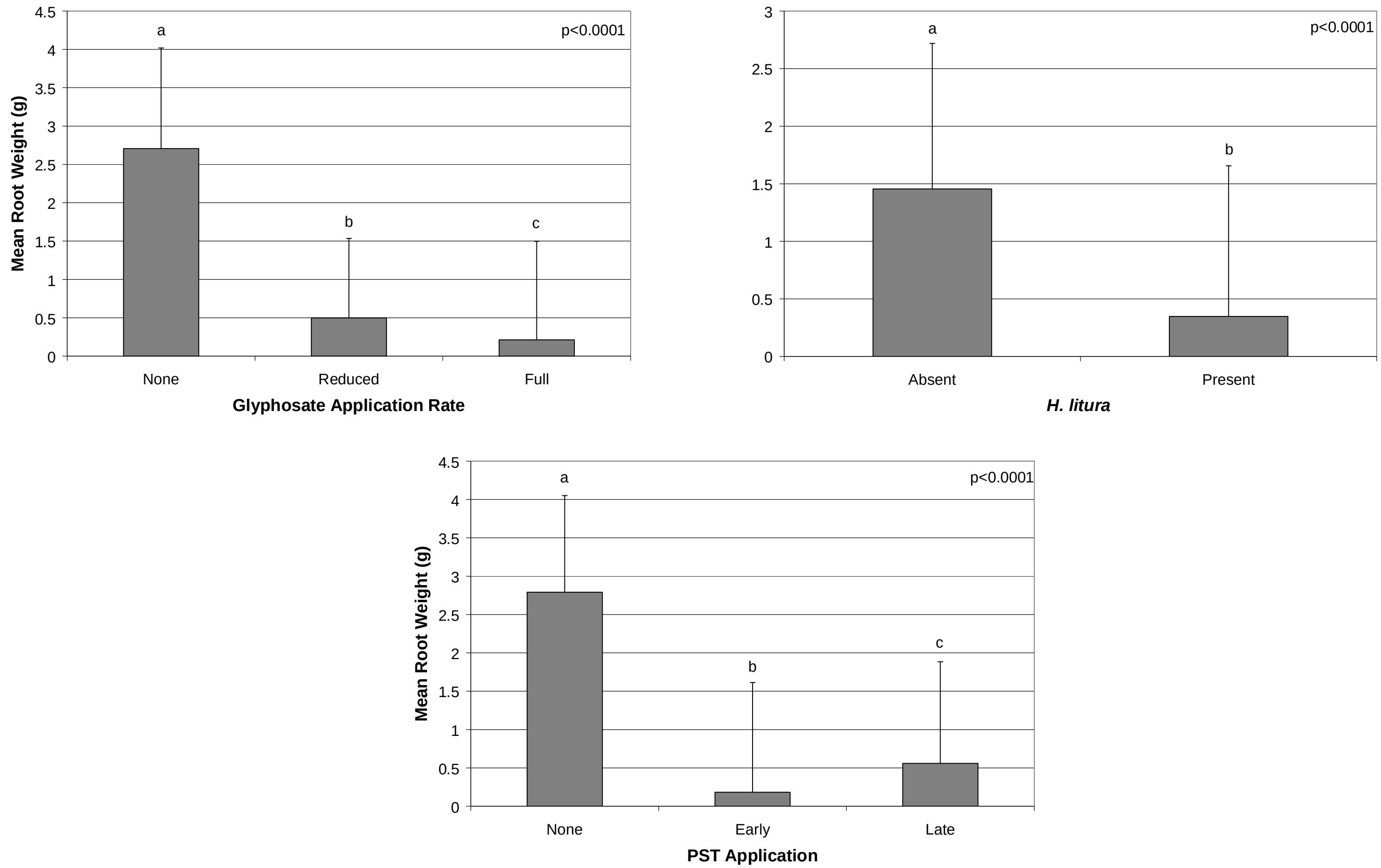

Figure 4.4: Impact of glyphosate (top left), *Hadroplontus litura* (top right) and *Pseudomonas syringae* pv. *tagetis* (PST) (bottom) on Canada thistle dry root weight in greenhouse conditions. Bars indicate mean values plus standard error of the mean. Letters indicate values that are different at the p=0.05 level. While all calculations were done using natural log transformed values, means and standard errors presented in the graphs have been backtransformed.

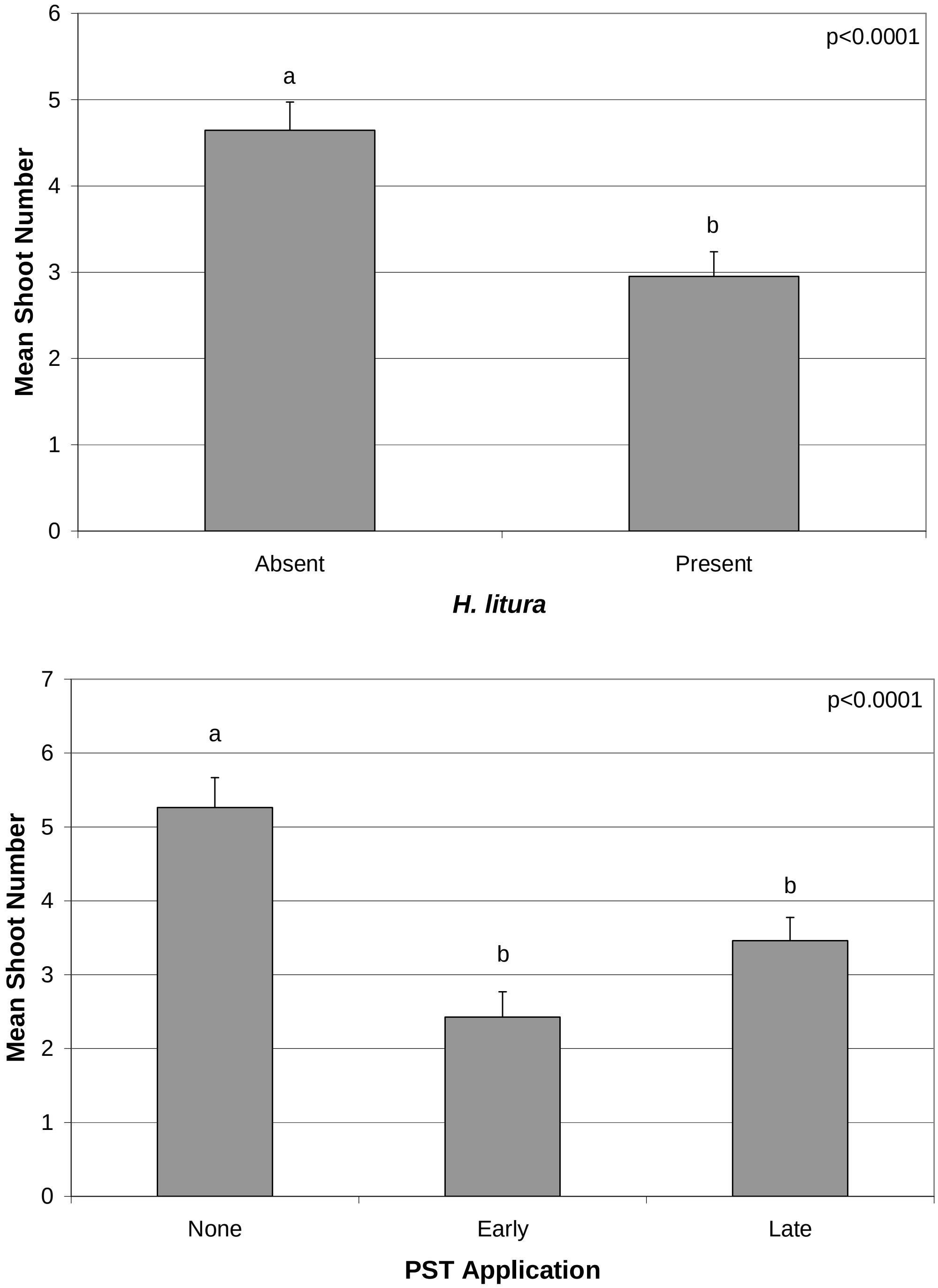

Figure 4.5: Impact of *H. litura* (top) and *Pseudomonas syringae* pv. *tagetis* (PST) (bottom) on Canada thistle shoot number in greenhouse conditions. Bars indicate mean values plus standard error of the mean. Letters indicate values that are different at the p=0.05 level.

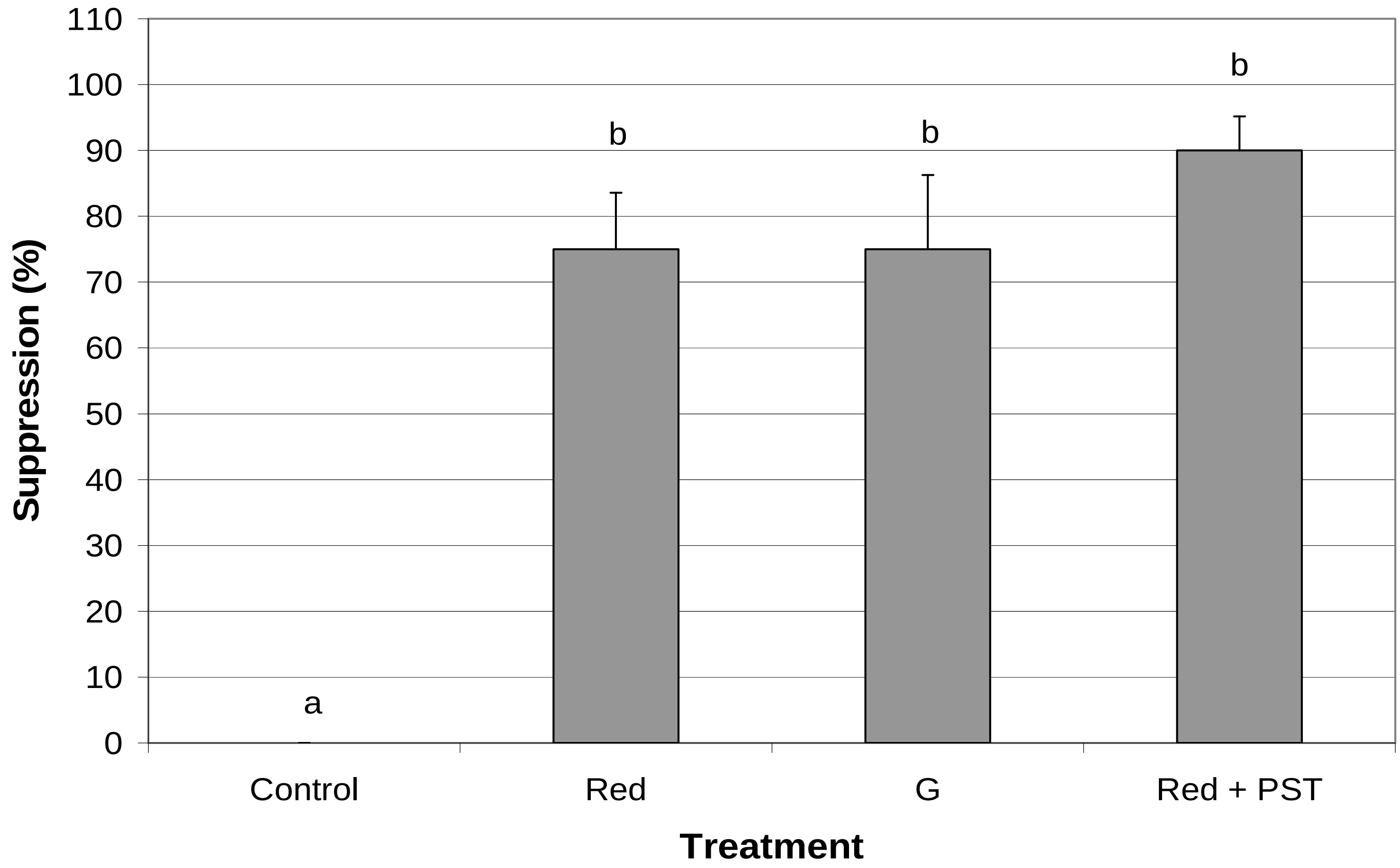

Figure 4.6: Impact of single and combined management practices on Canada thistle percent suppression determined by emergence the spring after treatments were applied in a fallow field. Red refers to reduced rate of glyphosate, G refers to full rate of glyphosate, and PST refers to *Pseudomonas syringae* pv. *tagetis*, the pathogen. Bars indicate mean values plus standard error of the mean. Letters indicate values that are different at the p=0.05 level.

CHAPTER 5

SUMMARY OF FINDINGS / FUTURE RESEARCH

While most published work has focused either on Canada thistle's deleterious effects or on ways to control it, this work evaluated its biology and integrated management, concepts that are often overlooked. To enhance our understanding of this creeping weed, we measured the impact of several factors including root size, depth of burial, soil moisture, herbicides, and insect and pathogen interference on its emergence and growth. We determined that root size, moisture availability, and burial depth affected the resulting plant's emergence likelihood and time, as well as overall biomass and growth. Canada thistle develops more root and shoot biomass and higher numbers of shoots with a large, heavy root, high water availability, and a 10-cm burial depth. In addition, we showed that results from greenhouse studies did not correlate strongly with patterns observed in field situations.

Integrated weed management is an important strategy for controlling invasive perennial weeds such as Canada thistle. Although much work has focused on individual tactics, our work concurs with the growing body of literature that advocates a multi-faceted approach to weed management. First, our results indicated that combining insects with each of three different herbicides improved Canada thistle control over herbicide alone. Second, we demonstrated increased impact of integrating two biological control agents with one herbicide versus singular tactics on Canada thistle growth. Combinations of glyphosate, PST, and *H. litura* always resulted in lower shoot and root biomass and lower number of shoots than the control and often provided greater Canada thistle suppression than individual tactics. While we did not find synergistic interactions in either study, a lack of antagonism between singular agents encourages the development of integrated weed management strategies as, in our experiments, no agent interfered with the efficacy of any other agent.

We investigated the effects of a myriad of factors on individual Canada thistle plants. Further work should focus on testing how these factors affect Canada thistle's population dynamics and invasiveness. For example, does higher water availability make invasion easier? How do biological controls and herbicides affect a patch or population of Canada thistle? The answers to questions such as these would give a more detailed understanding of Canada thistle's properties as a weed in a broader context to facilitate management. Predictive models can help bridge the gap between fundamental biological processes and management recommendations (Maxwell and Sheley 1997). So far, models have been developed for other weeds that take treatment and environment into account when predicting plant population dynamics (Rees and Hill 2001), but little work has been done concerning Canada thistle. Since depth, water availability, root size and biomass, and treatment all affect individual plant emergence and growth, it stands to reason that these variables should affect Canada thistle short-, mid-, and long-term dynamics. It is important that these effects be studied to provide a more informative picture of Canada thistle biology and ecology.

While our results are applicable in today's conditions, the future remains unknown. Global changes in temperature are leading to changes in biological, geological, and atmospheric dynamics (Cameron and Scheel 2001, Iverson and Prasad 2001, Williams et al. 2003, Thuiller et al. 2005) and changes in abiotic patterns (Boardman and Favis-Mortlock 1993, Hulme et al. 1993, Wu et al. 2008), which pose serious consequences for invasive weed management (Hellmann et al. 2008). Further research could focus on the reactions of Canada thistle to changes in atmospheric carbon dioxide and how they can directly and indirectly impact its emergence, growth, reproduction, and management. For example, Canada thistle biomass, photosynthetic rate, and number of spines increase with increasing atmospheric carbon dioxide levels (Ziska 2002). In fact, out of six common invasive species tested, Canada thistle growth responded the most to elevated levels of CO_2 (Ziska 2003a). Specifically, an increase in carbon dioxide increases Canada thistle root biomass more than shoot biomass, elevating its root:shoot ratio. This could cause a dilution effect when the plant is sprayed with glyphosate, as Canada thistle growing in elevated CO_2 conditions does not exhibit a change in shoot biomass when sprayed (Ziska et al. 2004). This could mean that elevated CO_2 levels in the future could encourage Canada thistle to become an even more aggressive competitor in agronomic situations, especially since this response does not appear to be affected by nutrients such as nitrogen (Ziska 2003b).

This study represents another building block in the foundation of integrated weed management. We have determined that biological and abiotic factors can impact the emergence and growth of Canada thistle, while also elucidating some of the fundamental growth mechanisms of such a highly competitive weed. Additionally, we assessed Canada thistle responses to treatments that could be useful for managers. Overall, the knowledge gathered here plus the current body of work in weed science and Canada thistle biology and ecology should be used to develop an informed management paradigm for this noxious weed. It is our hope that in the future, enough knowledge will be gained to successfully manage Canada thistle.

REFERENCES CITED

Abbas, H. K., D. R. Johnson, B. J. Johnson and D. L. Wyse. 1995. Effect of *Pseudomonas syringae* pv. *tagetis* on common cocklebur (*Xanthium strumarium* L.). Phytopathology 85:1130.

Abdollahi, F. and H. Ghadiri. 2004. Effect of separate and combined applications of herbicides on weed control and yield of sugar beet. Weed Technology 18:968-976.

AgriMet: Weather & crop water use charts. 2008. The Great Plains Cooperative Agricultural Weather Network. Available http://www.usbr.gov/gp/agrimet/index.cfm.

Amor, R. and R. Harris. 1974. Distribution and seed production of *Cirsium arvense* (L.) Scop. in Victoria, Australia. Weed Research 14: 317-323.

Amor, R. and R. Harris. 1975. Seedling establishment and vegetative spread of *Cirsium arvense* (L.) Scop. in Victoria, Australia. Weed Research 15: 407-411.

Ang, B. N., L. T. Kok, G. I. Holtzman and D. D. Wolf. 1994. Canada thistle (*Cirsium arvense*) response to simulated insect defoliation and plant competition. Weed Science 42:403-410.

Ang, B., L. Kok, G. Holtzman, and D. Wolf. 1995. Canada thistle (*Cirsium arvense* (L.) Scop.) response to density of *Cassida rubiginosa* Müller (Coleoptera: Chrysomelidae) and plant competition. Biological Control 5: 31-38.

Agrios, G. 1980. Insect involvement in the transmission of fungal pathogens. p. 293-324 *in* F. Harris and K. Maramorosch, editors. Vectors of plant pathogens. Academic Press, New York.

Bailey, K. L., S. M. Boyetchko, J. Derby, W. Hall, K. Sawchyn, T. Nelson and D. R. Johnson. 2000. Evaluation of fungal and bacterial agents for biological control of Canada thistle. Pages 203-208 *in* N. R. Spencer, editor. Proceedings of the X International Symposium on Biological Control of Weeds, Bozeman, MT.

Bakker, D. 1960. A comparative life-history study of *Cirsium arvense* (L.) Scop. and *Tussilago farfara* L., the most troublesome weeds in the newly reclaimed polders of the former Zuiderzee. p. 205-222 *in* J. L. Harper, editor. The biology of weeds. Blackwell Scientific Publications, Oxford.

Battaglin, W., E. Thurman, S. Kalkhoff, and S. Porter. 2003. Herbicides and transformation products in surface waters of the midwestern United States. Journal of the American Water Resources Association 39:743-756.

Beck, K. G. and J. R. Sebastian. 2000. Combining mowing and fall-applied herbicides to control Canada thistle (*Cirsium arvense*). Weed Technology 14:351-356.

Beckie, H. 2006. Herbicide-resistant weeds: management tactics and practices. Weed Technology 20:793-814.

Beckie, H. J. and K. J. Kirkland. 2003. Implication of reduced herbicide rates on resistance enrichment in wild oat (*Avena fatua*). Weed Technology 17:138-148.

Bhowmik, P. C. 1997. Weed biology: Importance to weed management. Weed Science 45:349-356.

Blackshaw, R. E., J. T. O'Donovan, K. N. Harker, G. W. Clayton and R. N. Stougaard. 2006. Reduced herbicide doses in field crops: A review. Weed Biology and Management 6:10-17.

Boardman, J. and D. T. Favis-Mortlock. 1993. Climate change and soil erosion in Britain. The Geographical Journal 159:179-183.

Boerboom, C. M. and D. L. Wyse. 1988. Response of Canada thistle (*Cirsium arvense*) and birdsfoot trefoil (*Lotus corniculatus*) to bentazon. Weed Science 36:250-253.

Bostock, R. M., R. Karban, J. S. Thaler, P. D. Weyman and D. Gilchrist. 2001. Signal interactions in induced resistance to pathogens and insect herbivores. European Journal of Plant Pathology 107:103-111.

Box, G. E. P. and D. R. Cox. 1964. An analysis of transformations. Journal of the Royal Statistical Society Series B 26:211-252.

Boyette, C. D., R. E. Hoagland and M. A. Weaver. 2008. Interaction of a bioherbicide and glyphosate for controlling hemp sesbania in glyphosate-resistant soybean. Weed Biology and Management 8:18-24.

Brooker, J. W. 2002. Soil survey of Gallatin County area, Montana Natural Resources Conservation Service.

Buhler, D. J. 2002. Challenges and opportunities for integrated weed management. Weed Science 50:273-280.

Buhler, D. D., M. Liebman, and J. J. Obrycki. 2000. Theoretical and practical challenges to an IPM approach to weed management. Weed Science 48:274-280.

Burnside, O. C., R. G. Wilson, S. Weisberg and K. G. Hubbard. 1996. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Science 44:74-86.

Cameron, G. N. and D. Scheel. 2001. Getting warmer: Effect of global climate change on distribution of rodents in Texas. Journal of Mammalogy 82:652-680.

Carlson, S. J. and W. W. Donald. 1988. Fall-applied glyphosate for Canada thistle (*Cirsium arvense*) control in spring wheat (*triticum aestivum*). Weed Technology 2:445-455.

Chao, W., D. Horvath, J. Anderson, and M. Foley. 2005. Potential model weeds to study genomics, ecology, and physiology in the 21st century. Weed Science 53: 929-937.

Collier, T. R., S. F. Enloe, J. K. Sciegienka and F. D. Menalled. 2007. Combined impacts of *Ceutorhynchus litura* and herbicide treatments for Canada thistle suppression. Biological Control 43:231-236.

Colonnelli, E. 2004. Catalogue of Ceutorhynchinae of the world, with a key to genera (Insecta: Coleoptera: Curculionidae). Argania Editio, S.C.P, Barcelona.

Darwent, A. L., L. Townley-Smith and L. P. Lefkovitch. 1994. Comparison of time and depth of last tillage on the growth of Canada thistle (*Cirsium arvense*) in summerfallow and its response to glyphosate. Canadian Journal of Plant Science 74:867-873.

Davis, A. 2006. When does it make sense to target the weed seed bank? Weed Science 54: 558-565.

De Bruijn, S. and E. Bork. 2006. Biological control of Canada thistle in temperate pastures using high density rotational cattle grazing. Biological Control 36: 305-315.

Derksen, D. A., R. L. Anderson, R. E. Blackshaw and B. Maxwell. 2002. Weed dynamics and management strategies for cropping systems in the northern Great Plains. Agronomy Journal 94:174-185.

Derscheid, L. A. 1962. Control of thistles and leafy spurge. Proceedings of the North Central Weed Control Conference 19:3-5.

Demers, A., D. Berner, and P. Backman. 2006. Enhancing incidence of *Puccinia punctiformis,* through mowing, to improve management of Canada thistle (*Cirsium arvense*). Biological Control 39: 481-488.

Detmers, F. 1927. Canada thistle, *Cirsium arvense*. Ohio Experimental Station Bulletin 414.

Dewey, H. L. 1901. Canada thistle. U. S. Department of Agriculture Bureau of Botany Circular 27. 14 pp.

Dietz, H. 2002. Plant invasion patches - reconstructing pattern and process by means of herb-chronology. Biological Invasions 4:211-222.

Dietz, H. and I. Ullmann. 1998. Ecological applications of 'herbchronology': Comparative stand age structure analyses of the invasive plant *Bunias orientalis* L. Annals of Botany 82:471-480.

Donald, W. W. 1990. Management and control of Canada thistle (*Cirsium arvense*). Reviews of Weed Science 5: 193-250.

Donald, W. W. 1994. The biology of Canada thistle (*Cirsium arvense*). Reviews of Weed Science 6:77-101.

Donald, W. 2000. A degree-day model of *Cirsium arvense* shoot emergence from adventitious root buds in spring. Weed Science 48:333-341.

Doyle, P. and M. Stypa. 2004. Reduced herbicide rates: A Canadian perspective. Weed Technology 18:1157-1165.

Duncan, C. A. and J. J. Jachetta. 2005. Introduction. *in* C. A. Duncan and J. K. Clark, editors. Invasive plants of range and wildlands and their environmental, economic, and societal impacts. Weed Science Society of America, Lawrence, KS.

Edwards, G. R., G. W. Bourdot and M. J. Crawley. 2000. Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grassland. Journal of Applied Ecology 37:321-334.

Elmore, C. L. 1996. A reintroduction to integrated weed management. Weed Science 44:409-412.

Ferrero-Serrano, A., T. R. Collier, A. L. Hild, B. A. Mealor and T. Smith. 2008. Combined impacts of native grass competition and introduced weevil herbivory on Canada thistle (*Cirsium arvense*). Rangeland Ecology and Management 61:529-534.

Forsberg, D. E. 1962. Another look at the Canada thistle root system. Proceedings of the annual meeting of the Canadian Society of Agronomy 8:94-97.

Foy, C. L. and Inderjit. 2001. Understanding the role of allelopathy in weed interference and declining plant diversity. Weed Technology 15:873-878.

Franklin, R. E., V. L. Quisenberry, B. J. Gossett and E. C. Murdock. 1994. Selection of herbicide alternatives based on probable leaching to groundwater. Weed Technology 8:6-16.

Frantzen, J. 1994. An epidemiological study of *Puccinia punctiformis* (Str). Röhl as a stepping-stone to the biological control of *Cirsium arvense* (L.) Scop. New Phytologist 127:147-154.

Friedli, J. and S. Bacher. 2001a. Direct and indirect effects of a shoot-base boring weevil and plant competition on the performance of creeping thistle, *Cirsium arvense*. Biological Control 22:219-226.

Friedli, J. and S. Bacher. 2001b. Mutualistic interaction between a weevil and a rust fungus, two parasites of the weed *Cirsium arvense*. Oecologia 129:571-576.

Friesen, H. A. 1968. Trends in Canadian research to control Canada thistle. Proceedings of the annual meeting of the Northeastern Weed Control Conference 22:27-36.

Gebhardt, M. R., T. C. Daniel, E. E. Schweizer and R. R. Allmaras. 1985. Conservation tillage. Science 230:625-630.

Glenn, S. and L. K. Heimer. 1994. Canada thistle (*Cirsium arvense*) control in no-tillage corn (*Zea mays*). Weed Technology 8:134-138.

Graglia, E., B. Melander and R. K. Jensen. 2006. Mechanical and cultural strategies to control *Cirsium arvense* in organic arable cropping systems. Weed Research 46:304-312.

Green, S. and K. Bailey. 2000. Effects of leaf maturity, infection site, and application rate of *Alternaria cirsinoxia* Conidia on infection of Canada thistle (*Cirsium arvense*). Biological Control 19: 167-174.

Grekul, C. W., D. E. Cole and E. W. Bork. 2005. Canada thistle (*Cirsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technology 19:298-306.

Grekul, C. and E. Bork. 2004. Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense)*. Weed Technology 18: 784-794.

Gronwald, J. W., K. L. Plaisance and B. A. Bailey. 2004. Effects of the fungal protein Nep1 and *Pseudomonas syringae* on growth of Canada thistle (*Cirsium arvense*), common ragweed (*Ambrosia artemisiifolia*), and common dandelion (*Taraxacum officinale*). Weed Science 52:98-104.

Gronwald, J., K. Plaisance, D. Ide, and D. Wyse. 2002. Assessment of *Pseudomonas syringae* pv. *tagetis* as a biocontrol agent for Canada thistle. Weed Science 50: 397–404.

Gulya, T. J., R. R. Urs and E. E. Banttari. 1981. Apical chlorosis of sunflower incited by *Pseudomonas-tagetis*. Phytopathology 71:221.

Gulya, T. J., R. Urs and E. E. Banttari. 1982. Apical chlorosis of sunflower caused by *Pseudomonas syringae* pv. *tagetis*. Plant Disease 66:598-600.

Gustavsson, A. 1997. Growth and regenerative capacity of plants of *Cirsium arvense.* Weed Research 37: 229-236.

Haderlie, L.C., S. Dewey, and D. Kidder. 1987. Canada thistle biology and control. Bulletin No. 666, University of Idaho Cooperative Extension Service. 7 p.

Hamdoun, A. M. 1970. The anatomy of subterranean structures of *Cirsium arvense* (L.) Scop. Weed Research 10:284-287.

Hamdoun, A. M. 1972. Regenerative capacity of root fragments of *Cirsium arvense* (L.) Scop. Weed Research 12:128-136.

Hansen, A. A. 1918. Canada thistle and methods of eradication. U. S. Department of Agriculture Farmers Bulletin 1002. 15 pp.

Harris, P. 1979. Cost of biological control of weeds by insects in Canada. Weed Science 27:242-250.

Hartley, M. J. and T. K. James. 1979. Cost benefit of selective control of Californian thistle in pasture. Proceedings of the New Zealand Weed and Pest Control Conference 32:245-249.

Hay, W. D. 1937. Canada thistle seed production and its occurrence in Montana seeds. Seed World 41:6-7.

Hayden, A. 1934. Distribution and reproduction of Canada thistle in Iowa. American Journal of Botany 21:355-373.

Heap, I. The international survey of herbicide resistant weeds. Online. Internet. October 15, 2008. Available www.weedscience.com.

Heimann, B. and G. Cussans. 1996. The importance of seeds and sexual reproduction in the population biology of *Cirsium arvense* – a literature review. Weed Research 36: 493-503.

Hein, G. L. and R. G. Wilson. 2004. Impact of *Ceutorhynchus litura* feeding on root carbohydrate levels in Canada thistle (*Cirsium arvense*). Weed Science 52:628-633.

Hellmann, J. J., J. E. Byers, B. G. Bierwagen and J. S. Dukes. 2008. Five potential consequences of climate change for invasive species. Conservation Biology 22:534-543.

Hodgson, J. M. 1958. Canada thistle (*Cirsium arvense* Scop.) control with cultivation, cropping, and chemical sprays. Weeds 6:1-11.

Hodgson, J. 1964. Variation in ecotypes of Canada thistle. Weeds 12: 167-170.

Hodgson, J. 1970. The response of Canada thistle ecotypes to 2,4-D, amitrole, and intensive cultivation. Weed Science 18: 253-255.

Hodgson, J. M. 1973. Lipid deposition on leaves of Canada thistle ecotypes. Weed Science 21:169-172.

Hoeft, E. V., N. Jordan, J. Zhang and D. L. Wyse. 2001. Integrated cultural and biological control of Canada thistle in conservation tillage soybean. Weed Science 49:642-646.

Holt, J. S. and H. M. Lebaron. 1990. Significance and distribution of herbicide resistance. Weed Technology 4:141-149.

Hulme, M., J. E. Hossell and M. L. Parry. 1993. Future climate change and land use in the United Kingdom. The Geographical Journal 159:131-147.

Hunter, J. H. 1995. Effect of bud vs rosette growth stage on translocation of ^{14}C-glyphosate in Canada thistle (*Cirsium arvense*). Weed Science 43:347-351.

Hunter, J. H. 1996. Control of Canada thistle (*Cirsium arvense*) with glyphosate applied at the bud vs rosette stage. Weed Science 44:934-938.

Hunter, J. H., A. I. Hsiao and G. I. McIntyre. 1985. Some effects of humidity on the growth and development of *Cirsium arvense*. Botanical Gazette 146:483-488.

Hunter, J. H. and L. W. Smith. 1972. Environment and herbicide effects on Canada thistle ecotypes. Weed Science 20:163-167.

Iverson, L. R. and A. M. Prasad. 2001. Potential changes in tree species richness and forest community types following climate change. Ecosystems 4:186-199.

Johnson, D. R. and D. L. Wyse. 1991. Use of *Pseudomonas syringae* pv. *tagetis* for control of Canada thistle. Proceedings of the North Central Weed Science Society 46:14-15.

Johnson, D. R. and D. L. Wyse. 1992. Biological control of weeds with *Pseudomonas syringae* pv. *tagetis*. Proceedings of the North Central Weed Science Society 47:16.

Johnson, D. R., D. L. Wyse and K. J. Jones. 1996. Controlling weeds with phytopathogenic bacteria. Weed Technology 10:621-624.

Julien, M. H. and M. W. Griffiths, editors. 1998. Biological control of weeds: A world catalogue of agents and their target weeds, 4th edition. CABI Publishing, Wallingford, UK.

Kluth, S., A. Kruess, and T. Tscharntke. 2001. Interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects on creeping thistle. Journal of Applied Ecology 38: 548-556.

Kluth, S., A. Kruess and T. Tscharntke. 2003. Influence of mechanical cutting and pathogen application on the performance and nutrient storage of *Cirsium arvense*. Journal of Applied Ecology 40:334-343.

Krueger-Mangold, J., R. L. Sheley and B. D. Roos. 2002. Maintaining plant community diversity in a waterfowl production area by controlling Canada thistle (*Cirsium arvense*) using glyphosate. Weed Technology 16:457-463.

Kruess, A. 2002. Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*. Oecologia 130:563-569.

Kumar, V. and D. Irvine. 1971. Germination of seeds of *Cirsium arvense* (L.) Scop. Weed Research 11: 200-203.

Kutner, M. H., C. J. Nachtsheim, J. Neter and W. Li. 2005. Applied linear statistical models, Fifth edition. McGraw-Hill/Irwin, New York.

Lalonde, R. and B. Roitberg. 1994. Mating system, life-history, and reproduction in Canada thistle (*Cirsium arvense*; Asteraceae). American Journal of Botany 81: 21-28.

Laubhan, M. and T. Shaffer. 2006. Seed germination of *Cirsium arvense* and *Lepidium latifolium*: implications for management of montane wetlands. Wetlands 26: 69-78.

Lauridson, T. C., R. G. Wilson and L. C. Haderlie. 1983. Effect of moisture stress on Canada thistle (*Cirsium arvense*) control. Weed Science 31: 674-680.

Leistra, M. and J. J. T. I. Boesten. 1989. Pesticide contamination of groundwater in western Europe. Agriculture, Ecosystems, & Environment 26:369-389.

Liebman, M. and E. R. Gallandt. 1997. Many little hammers: Ecological management of crop-weed interactions. p. 291-343 *in* L. E. Jackson, editor. Ecology in agriculture. Academic Press, San Diego.

Liebman, M., C. L. Mohler and C. P. Staver. 2001. Ecological management of agricultural weeds. Cambridge University Press, Cambridge.

Littell, R. C., G. A. Milliken, W. W. Stroup and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC, 633 pp.

Lockhart, S. J. and K. A. Howatt. 2004. Split applications of herbicides at reduced rates can effectively control wild oat (*Avena fatua*) in wheat. Weed Technology 18:369-374.

Louda, S., D. Kendall, J. Connor, D. Simberloff. 1997. Ecological effects of an insect introduced for the biological control of weeds. Science 277: 1088-1090.

Louda, S. M. and C. W. O'Brien. 2002. Unexpected ecological effects of distributing the exotic weevil, *Larinus planus* (F.), for the biological control of Canada thistle. Conservation Biology 16: 717-727.

Lym, R. and C. Duncan. 2005. Sunflower family: Asteraceae. Canada thistle, *Cirsium arvense* (L.) Scop. *In* C. Duncan and L. Clark, eds. Invasive Plants of Range and Wildlands and the Environmental, Economic, and Societal Impacts. Lawrence, KS: Weed Science Society of America, pp 69-83.

Mack, R. N. and M. Erneberg. 2002. The United States naturalized flora: Largely the product of deliberate introductions. Annals of the Missouri Botanical Garden 89:176-189.

Madsen, S. 1962. Germination of buried and dry stored seeds III, 1934-1960. Proceedings of the International Seed Testing Association 27: 920-928.

Mahn, E. G. and A. Kastner. 1985. Effects of herbicide stress on weed communities and soil nematodes in agroecosystems. Oikos 44:185-190.

Mamolos, A. and K. Kalburtji. 2001. Competition between Canada thistle and winter wheat. Weed Science 49: 755-759.

Martone, R. G. and K. Wasson. 2008. Impacts and interactions of multiple human perturbations in a California salt marsh. Oecologia 158:151-163.

Masters, R. A. and R. L. Sheley. 2001. Principles and practices for managing rangeland invasive plants. Journal of Range Management 54:502-517.

Mathews, D. E. and R. D. Durbin. 1990. Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and *Escherichia coli*. The Journal of Biological Chemistry 265:493-498.

Mathews, D. E. and R. D. Durbin. 1994. Mechanistic aspects of tagetitoxin inhibition of RNA polymerase from *Escherichia coli*. Biochemistry 33:11987-11992.

Maxwell, B. D. and R. L. Sheley. 1997. Noxious weed population dynamics education model. Weed Technology 11:182-188.

McAllister, R. S. and L. C. Haderlie. 1981. Canada thistle root anatomy and root bud dormancy. Proceedings of the North Central Weed Control Conference 36:157-160.

McMaster, G. S. and W. W. Wilhelm. 1997. Growing degree-days: One equation, two interpretations. Agricultural and Forest Meteorology 87:291-300.

McWhorter, C. G. and W. L. Barrentine. 1988. Research priorities in weed science. Weed Technology 2:2-11.

Mesbah, A. O. and S. D. Miller. 2005. Canada thistle (*Cirsium arvense*) control in established alfalfa (*Medicago sativa*) grown for seed production. Weed Technology 19: 1025-1029.

Messersmith, C. G. and S. W. Adkins. 1995. Integrating weed-feeding insects and herbicides for weed control. Weed Technology 9:199-208.

Miller, B. and R. Lym. 1998. Using the rosette technique for Canada thistle (Cirsium arvense) control in row crops. Weed Technology 12: 699-706.

Mokany, K. and J. Ash. 2008. Are traits measured on pot grown plants representative of those in natural communities? Journal of Vegetation Science 19:119-126.

Morishita, D. 1999. Canada thistle. p. 162-172 *in* R. Sheley and J. Petrov, editors. Biology and management of noxious rangeland weeds. Oregon State University Press, Corvallis, OR.

Moore, R. J. 1975. The biology of Canadian weeds: 13. *Cirsium arvense* (L.) Scop. Canadian Journal of Plant Science 55:1033-1048.

Moyer, J., G. Schaalje, and P. Bergen. 1991. Alfalfa (*Medicago sativa*) seed yield loss due to Canada thistle (*Cirsium arvense*). Weed Technology 5: 723-728.

Montana Department of Natural Resources & Conservation. 2004. Chapter 3 – The affected environment. Final programmatic environmental impact statement, 1-118 <http://dnrc.mt.gov/trust/programmatic/default.asp>.

Morishita, D. 1999. Canada thistle. p. 162-172 *in* R. Sheley and J. Petrov, editors. Biology and management of noxious rangeland weeds. Oregon State University Press, Corvallis, OR.

National Research Council Board on Agriculture. 1989. Alternative Agriculture. Washington, D.C., National Academy Press.

Nelson, H., and R. Jones. 1994. Potential regulatory problems associated with atrazine, cyanazine, and alachor in surface water source drinking water. Weed Technology 8:852-861.

Norris, J. L., D. R. Shaw and C. E. Snipes. 2001. Weed control from herbicide combinations with three formulations of glyphosate. Weed Technology 15:552-558.

Norris, R. F. and M. Kogan. 2000. Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. Weed Science 48:94-158.

Nuzzo, V. 1997. Element stewardship abstract for *Cirsium arvense*. The Nature Conservancy, Arlington, VA.

Parker R., and J. Krall. 1984. Weeds. *In* Alfalfa Seed Production and Pest Management. Western Regional Extension Publication 12.

Peschken, D. P. and R. W. Beecher. 1973. *Ceutorhynchus litura* (Coleoptera: Curculionidae): Biology and first releases for biological control of the weed Canada thistle (*Cirsium arvense*) in Ontario, Canada. The Canadian Entomologist 105:1489-1494.

Peschken, D. P. and A. T. S. Wilkinson. 1981. Biocontrol of Canada thistle (*Cirsium arvense*): Releases and effectiveness of *Ceutorhynchus litura* (Coleoptera: Curculionidae) in Canada. The Canadian Entomologist 113:777-785.

Peschken, D. P. and J. Derby. 1992. Effect of *Urophora cardui* (L) (Diptera, Tephritidae) and *Ceutorhynchus litura* (F) (Coleoptera, Curculionidae) on the weed Canada thistle, *Cirsium arvense* (L) Scop. The Canadian Entomologist 124: 145-150.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience 50: 53-65.

Popay, I. and R. Field. 1996. Grazing animals as weed control agents. Weed Technology 10:217-231.

Popp, M. P., L. R. Oliver, C. R. Dillon, T. C. Keisling and P. M. Manning. 2000. Evaluation of seedbed preparation, planting method, and herbicide alternatives for dryland soybean production. Agronomy Journal 92:1149-1155.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org.

Rask, A. and C. Andreasen. 2007. Influence of mechanical rhizome cutting, rhizome drying and burial at different developmental stages on the regrowth of *Calystegia sepium*. Weed Research 47: 84-93.

Reed, C., D. Larson, and J. Larson. 2006. Canada thistle biological control agents on two South Dakota wildlife refuges. Natural Areas Journal 26: 47-52.

Rees, N. 1990. Establishment, dispersal, and influence of *Ceutorhynchus litura* on Canada thistle (*Cirsium arvense)* in the Gallatin Valley of Montana. Weed Science 38:198-200.

Rees, M. and R. L. Hill. 2001. Large-scale disturbances, biological control and the dynamics of gorse populations. Journal of Applied Ecology 38:364-377.

Reever Morghan, K. J., T. R. Seastedt and P. J. Sinton. 2000. Frequent fire slows invasion of ungrazed tallgrass prairie by Canada thistle (Colorado). Ecological Restoration 18:194-195.

Rhodehamel, N. H. and R. D. Durbin. 1985. Host range of strains of *Pseudomonas syringae* pv. *tagetis*. Plant Disease 69:589-591.

Rice, P. 2008. Invaders database system. Univ. of Montana, Missoula, MT. Online. http://invader.dbs.umt.edu. Accessed: February 2008.

Ritter, W. F. 1990. Pesticide contamination of groundwater in the United States - a review. Journal of Environmental Science and Health B25:1-29.

Rodriguez, V., M. Thiruchelvam, and D. Cory-Slechta. 2005. Sustained exposure to the widely used herbicide atrazine: altered function and loss of neurons in brain monoamine systems. Environmental Health Perspectives 113:708-715.

Schreiber, M. M. 1967. Effect of density and control of Canada thistle on production and utilization of alfalfa pasture. Weed Science 15:138-142.

Schröder, D., H. Müller-Schärer, and C. Stinson. 1993. A European weed survey in 10 major crop systems to identify targets for biological control. Weed Research 33, 449–458.

Seifers, D. L. and W. D. Stegmeier. 1983. *Pseudomonas syringae* pv. *tagetis*, causal agent of apical chlorosis, isolated from sunflower in Kansas. Plant Disease 67:1290.

Shane, W. W. and J. S. Baumer. 1984. Apical chlorosis and leaf spot of Jerusalem artichoke incited by *Pseudomonas syringae* pv. *tagetis*. Plant Disease 68:257-260.

Sheikh, T., T. A. Wheeler, P. A. Dotray and J. C. Zak. 2001. Biological control of woollyleaf bursage (*Ambrosia grayi*) with *Pseudomonas syringae* pv. *tagetis*. Weed Technology 15:375-381.

Simpson, D. M. and E. W. Stoller. 1996. Physiological mechanisms in the synergism between thifensulfuron and imazethapyr in sulfonylurea-tolerant soybean (*Glycine max*). Weed Science 44:209-214.

Skinner, K., L. Smith, and P. Rice. 2000. Using noxious weed lists to prioritize targets for developing weed management strategies. Weed Science 48: 640-644.

Skinner, M. 2005. The Plants Database. Version 3.5. Baton Rouge, LA: National Plant Data Center. <plants.usda.gov>

Stachon, W. J. and R. L. Zimdahl. 1980. Allelopathic activity of Canada thistle (*Cirsium arvense*) in Colorado. Weed Science 28:83-86.

Steinberg, T. H., D. E. Mathews, R. D. Durbin and R. R. Burgess. 1990. Tagetitoxin: A new inhibitor of eukaryotic transcription by RNA polymerase III. The Journal of Biological Chemistry 265:499-505.

Strong, D. 1997. Fear no weevil? Science 277: 1058-1059.

Styer, D. J. and R. D. Durbin. 1982a. Isolation of *Pseudomonas syringae* pv. *tagetis* from sunflower in Wisconsin. Plant Disease 66:601.

Styer, D. J. and R. D. Durbin. 1982b. Common ragweed: A new host of *Pseudomonas syringae* pv. *tagetis*. Plant Disease 66:71.

Styer, D. J., G. L. Worf and R. D. Durbin. 1980. Occurrence in the United States of a marigold leaf spot incited by *Pseudomonas tagetis*. Plant Disease 64:101-102.

Sullivan, P. G. 2004. Thistle control alternatives. Pages 1-9 *in* N. S. A. I. Service, editor. National Sustainable Agriculture Information Service.

Swanton, C. J. and S. F. Weise. 1991. Integrated weed management: The rationale and approach. Weed Technology 5:657-663.

Teugels, J. L. and G. Vanroelen. 2004. Box-cox transformations and heavy-tailed distributions. Journal of Applied Probability 41:213-227.

Thill, D. C., J. M. Lish, R. H. Callihan, and E. J. Bechinski. 1991. Integrated weed management: A component of integrated pest management: A critical review. Weed Technology 5:648-656.

Thuiller, W., S. Lavorel, M. B. Araujo, M. T. Sykes, I. C. Prentice and H. A. Mooney. 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences of the United States of America 102:8245-8250.

Tichich, R. and J. D. Doll. 2006. Field-based evaluation of a novel approach for infecting Canada thistle (*Cirsium arvense*) with *Pseudomonas syringae* pv. *tagetis*. Weed Science 54:166-171.

Tipping, P. W. 2001. Canada thistle (*Cirsium arvense*) control with hexazinone in crown vetch (*Coronilla varia*). Weed Technology 15:559-563.

Travnicek, A., R. Lym, and C. Prosser. 2005. Fall-prescribed burn and spring-applied herbicide effects on Canada thistle control and soil seedbank in a northern mixed-grass prairie. Rangeland Ecology & Management 58: 413-422.

Trumble, J. and L. Kok. 1982. Integrated pest management techniques in thistle suppression in pastures of North America. Weed Research 22: 345-359.

Tworkoski, T. J., M. E. Engle and P. T. Kujawski. 1998. Effect of moisture stress and glyphosate on adventitious shoot growth of Canada thistle (*Cirsium arvense*). Weed Science 46:59-64.

Ward, M. H., J. Lubin, J. Giglierano, J. S. Colt, C. Wolter, N. Bekiroglu, D. Camann et al. 2006. Proximity to crops and residential exposure to agricultural herbicides in Iowa. Environmental Health Perspectives 114:893-897.

Warwick, S. 1991. Herbicide resistance in weedy plants: physiology and population biology. Annual Review of Ecology and Systematics 22: 95-114.

Weiner, J. 1982. A neighborhood model of annual-plant interference. Ecology 63:1237-1241.

Weiner, J., H.W. Griepentrog, and L. Kristensen. 2001. Suppression of weeds by spring wheat *Triticum aestivum* increases with crop density and spatial uniformity. Journal of Applied Ecology 38:784-790.

Westerman, P. R., M. Liebman, F. D. Menalled, A. H. Heggenstaller, R. G. Hartzler and P. M. Dixon. 2005. Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two- and four-year crop rotation systems. Weed Science 53:382-392.

Western Regional Climate Center. 2008. Available http://www.wrcc.dri.edu/index.html.

Whish, J.P.M., B.M. Sindel, R.S. Jessop, and W.L. Felton. 2002. The effect of row spacing and weed density on yield loss of chickpea. Australian Journal of Agricultural Research 53:1335-1340.

Whitson, T. D., L. C. Burrill, S. A. Dewey, D. W. Cudney, B. E. Nelson, R. D. Lee and R. Parker. 2004. Weeds of the west, 9th edition. Western Society of Weed Science, Jackson, WY.

Wiles, L.J., G.W. Oliver, A.C. York, H.J. Gold, and G.G. Wilkerson. 1992. Spatial-distribution of broadleaf weeds in North-Carolina soybean (*Glycine-Max*) fields. Weed Science 40:554-557.

Williams, II, M. M., D. B. Walsh and R. A. Boydston. 2004. Integrating arthropod herbivory and reduced herbicide use for weed management. Weed Science 52:1018-1025.

Williams, S. E., E. E. Bolitho and S. Fox. 2003. Climate change in Australian tropical rainforests: An impending environmental catastrophe. Proceedings: Biological Sciences 270:1887-1892.

Wilson, R.G. 1981. Effect of Canada thistle (*Cirsium arvense*) residue on growth of some crops. Weed Science 29:159-164.

Wilson, R. and A. Michiels. 2003. Fall herbicide treatments affect carbohydrate content in roots of Canada thistle (*Cirsium arvense*) and dandelion (*Taraxacum officinale*). *Weed Science* 51: 299-304.

Wilson, R. G. and S. D. Kachman. 1999. Effect of perennial grasses on Canada thistle (*Cirsium arvense*) control. Weed Technology 13:83-87.

Wrubel, R. P. and J. Gressel. 1994. Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technology 8:635-648.

Wu, S., L. J. Mickley, D. J. Jacob, D. Rind and D. G. Streets. 2008. Effects of 2000-2050 changes in climate and emissions on global tropospheric ozone and the policy-relevant background surface ozone in the United States. Journal of Geophysical Research 113: D18312, doi:10.1029/2007JD009639.

Zand, E. 2002. Response of two Canada thistle (*Cirsium arvense*) varieties to herbicides. Canadian journal of plant science 82: 625-8.

Zhang, J., S. E. Weaver and A. S. Hamill. 2000. Risks and reliability of using herbicides at below-labeled rates. Weed Technology 14:106-115.

Zidack, N. K. and P. A. Backman. 1996. Biological control of kudzu (*Pueraria lobata*) with the plant pathogen *Pseudomonas syringae* pv. *phaseolicola*. Weed Science 44:645-649.

Zidack, N. K., P. A. Backman and J. J. Shaw. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: Implications for biological weed control. Biological Control 2:111-117.

Zidack, N. K., B. J. Jacobsen and J. P. C. Quimby. 2000. Biological control of houndstongue (*Cynoglossum officinale*) with the plant pathogen *Pseudomonas syringae* pv. *tagetis* synergized with pelargonic acid. *in* N. Spencer, editor. Proceedings of the X International Symposium on Biological Control of Weeds.

Zimdahl, R. L. and G. Foster. 1993. Canada thistle (*Cirsium arvense*) control with disking and herbicides. Weed Technology 7:146-149.

Zimdahl, R., J. Lin, and A. Dall'Armellina. 1991. Effect of light, watering frequency, and chlorsulfuron on Canada thistle (*Cirsium arvense*). Weed Science 39: 590-594.

Ziska, L. H. 2002. Influence of rising atmospheric CO_2 since 1990 on early growth and photosynthetic response of a noxious invasive weed, Canada thistle (*Cirsium arvense*). Functional Plant Biology 29:1387-1392.

Ziska, L. H. 2003a. Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. Journal of Experimental Botany 54:395-404.

Ziska, L. H. 2003b. The impact of nitrogen supply on the potential response of a noxious, invasive weed, Canada thistle (*Cirsium arvense*) to recent increases in atmospheric carbon dioxide. Physiologia Plantarum 119:105-112.

Ziska, L. H., S. Faulkner and J. Lydon. 2004. Changes in biomass and root:shoot ratio of field-grown Canada thistle (*Cirsium arvense*), a noxious, invasive weed, with elevated CO_2: Implications for control with glyphosate. Weed Science 52:584-588.

Zoschke, A. 1994. Toward reduced herbicide rates and adapted weed management. Weed Technology 8:376-386.

Zwölfer, H. and P. Harris. 1966. *Ceutorhynchus litura* (F.) (Col. Curculionidae), a potential insect for the biological control of thistle, *Cirsium arvense* (L.) Scop., in Canada. Canadian Journal of Zoology 44:23-38.

APPENDICES

APPENDIX A

SUPPLEMENTAL MODEL FOR CANADA THISTLE EMERGENCE TIME

Table A.1: Model parameters to predict field-grown Canada thistle emergence time.

Predictor	Coefficient	p value
Initial root depth	0.47	0.05
Initial root volume	119.97	0.05
Initial root length	-7.22	0.27
Initial root diameter	173.66	0.01
Initial root weight	-73.77	0.71
Initial root depth: Initial root length	4.02	<0.01
Initial root volume: Initial root length	0.26	0.19
Initial root depth: Initial root diameter	-154.75	0.13
Initial root volume: Initial root diameter	-203.07	0.27
Initial root volume: Initial root weight	-11.26	0.15
Initial root length: Initial root weight	1.76	0.25
Initial root diameter: Initial root weight	126.91	0.06
Initial root depth: Initial root volume: Initial root length	-1.74	0.01
Initial root depth: Initial root volume: Initial root diameter	56.41	<0.01
Initial root volume: Initial root length: Initial root weight	0.27	0.15
Intercept	21.65	<0.01

The sum of predictors above represents the simplest model with the lowest AIC value. (AIC = 581.21)

APPENDIX B

CANADA THISTLE GROWN IN AN ALFALFA FIELD

Canada Thistle in Alfalfa Field Experiment

Introduction

A traditional method of Canada thistle control in agricultural lands is to outcompete it with a hardy crop. In an independent study I evaluated how Roundup Ready® alfalfa (*Medicago sativa* L.) and Canada thistle would affect each other. Roundup Ready® alfalfa was selected for this study because it is a perennial crop, it has a deep root system, is grown in MT, and it develops shoot tissue quickly. In addition, the market was moving toward Roundup Ready® alfalfa at that point in time, and we believed that Roundup Ready® technology would enable us to use herbicides without fear of damaging the alfalfa.

Materials and Methods

This experiment was evaluated in field conditions during 2006-7 at the Montana State University Arthur H. Post Agronomy Farm in Bozeman, MT. On 4 May 2006, soil was tilled before being broadcast with Dekalb brand Roudup Ready® alfalfa at 11 kg ha^{-1}. At the same time, a 50/50 mix of ammonium phosphate and potassium sulfate fertilizer was applied with a Fabro drill at a rate of 112 kg ha^{-1}. Finally, on 5 May, the field was rolled with a Bannerman roller to ensure adequate seed-to-soil contact.

Canada thistle roots were dug from the farm in Sept. 2006 and planted 3 Oct. 2006 in the alfalfa field. The experiment followed a split plot design with four 55 x 9 m replicates and a total of 864 roots per replicate. Each split plot contained eight 3 x 3 m subplots that received either 0, 3, or 9 roots per m^2. Individual roots were planted approximately 7-10 cm deep.

Discussion

We had planned to plant 3,456 roots in the field, but even that number was scaled down from an original number of 5,760 roots. Midway through planting in the fall, a collaborator informed us that any Canada thistle roots planted shallower than 30 cm deep would freeze and die over the winter. As a result, we stopped planting and planned to continue in the spring.

Although the alfalfa established readily and easily both years, the Canada thistle did not. We were expecting the Canada thistle to overtake the alfalfa, but the reverse happened. The alfalfa quickly formed a lush, dense canopy in the spring, shading out the already struggling Canada thistle plants. We initially had 11% Canada thistle emergence, but the alfalfa managed to outcompete the Canada thistle before the experiment even started, and we ended up with just 10 Canada thistle plants. We determined that planting any more Canada thistle would not be a good use of resources or time, since the alfalfa was so competitive.

This experiment failed because we underestimated the performance of alfalfa and overestimated the performance of Canada thistle. In retrospect, we should have established the Canada thistle first, then planted the alfalfa. A better option would have been to find a field infested with Canada thistle, then plant alfalfa into it. Although this experiment failed, it taught us a lot about Canada thistle establishment and growth in MT agricultural systems. While Canada thistle is a vigorously competitive weed once established, it seems that new seedlings and freshly buried roots need some time to become successful. This concurs with our research that found very young infestations to be fairly susceptible to treatments in fallow field conditions. Our recommendation is to manage Canada thistle infestations as early as possible. While a well-established root system would have probably made this experiment work, new infestations were not able to establish well and could probably be controlled rather easily.