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Nontarget host utilization of thistle species by introduced biological control agents and spatial prediction of non-target feeding habitats

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To the Graduate Council:

I am submitting herewith a dissertation written by Gregory J. Wiggins entitled "Nontarget host utilization of thistle species by introduced biological control agents and spatial prediction of non-target feeding habitats." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

Jerome F. Grant, Major Professor

We have read this dissertation and recommend its acceptance:

Paris L. Lambdin, Jack W. Ranney, John B. Wilkerson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Dissertation Presented for
The Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Gregory J. Wiggins

December 2009

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ABSTRACT

Rhinocyllus conicus Fröelich and *Trichosirocalus horridus* (Panzer) were introduced from Europe into North America as biological control agents of the exotic weed species *Carduus nutans* L. Concern exists over the feeding of these weevils on at least 25 species of native *Cirsium* thistles. Research was conducted to 1) estimate phenological synchrony of the eight thistle species in Tennessee with *R. conicus* and *T. horridus*, 2) investigate naturally-occurring populations of the five native *Cirsium* thistle species for non-target activity by *R. conicus* and *T. horridus*, 3) quantify the impacts to plants of each thistle species to feeding of *R. conicus* and *T. horridus*, and 4) identify potential areas of non-target feeding by the weevils using spatial analysis. Phenologies of two native species, *C. carolinianum* and *C. horridulum*, are synchronous with *R. conicus* reproduction, and all eight thistle species are phenologically synchronous with the reproduction of *T. horridus*. No non-target activity by *R. conicus* was observed in naturally-occurring populations of *Cirsium* thistles, but adults of *T. horridus* were documented for the first time on the native species *C. carolinianum, C. horridulum*, and *C. muticum*. In caged plant studies, larvae of *R. conicus* completed development in heads of *C. carolinianum* and *C. horridulum* and reduced seed numbers of both native species. Basal meristems of all eight thistle species exposed to *T. horridus* were damaged at varying levels in caged plant studies, but no other impacts to plants were observed. Spatial analyses showed associations between Mahalanobis distance values and plant counts of *Carduus nutans* and *Cirsium carolinianum* in predicted habitats, and the occurrence of *Carduus nutans* was associated with the occurrence of both weevil species in these habitats. About 12% of the total study area consisted of habitats where *C. nutans*

and *Cirsium carolinianum* overlap. The potential exists for these weevils to utilize native *Cirsium* species found in Tennessee as plant hosts. The spatial model developed during this study not only allows potential monitoring of populations of *C. carolinianum* to be more targeted, but also may be modified to apply to other systems involving interactions among introduced and native species.

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Overview of Invasive Species

Throughout natural history, cycles of species immigrations have resulted in fluctuations in species diversity at any given time (Elton 1958, Brown and Lomolino 1998). However, recent human population growth and subsequent global commerce activities, in combination with wide-spread environmental modifications by humans, have promoted many introductions of species from their native ranges into new areas (Elton 1958, Davis 2003, Perrings et al. 2005a). Accordingly, one of the major threats to global biological diversity and contributors to global environmental change is human-induced introductions of non-native (i.e., “introduced” or “exotic”) species into new areas (Vitousek et al. 1997, Davis 2003).

Whether these introductions are intentional or accidental, a fraction of the newly-introduced species may become problematic, and these problematic introduced species are often referred to as “invasive” species. Because many species that are prone to becoming an invasive species are disturbance-adapted (with traits such as broad diets or tolerances, rapid dispersal, and high reproduction rates), the alteration and modification of areas by human activities provide a source of establishment for these introduced species, while simultaneously discouraging endemic or native species (Elton 1958, McKinney and Lockwood 1999). These characteristics, coupled with the lack of biotic and abiotic factors that may suppress population levels in their native ranges, allow invasive species to rapidly increase in population size, disperse into new areas, and negatively impact economically important or ecologically sensitive species.

Although many episodes of natural species introductions have occurred throughout history, they were usually sporadic events separated by long periods of time (thousands of years). However, human activities, such as global trade, provide a continuous means to transfer species from one area to another, so that many more species are introduced to new areas than would usually occur (Sauer 1998, Ewel et al. 1999). An estimated 50,000 species have been introduced into the U.S. (Pimentel et al. 2000). The current rates of species introductions are comparable to rarely-occurring events in natural history. An example of one such event is the Great American Interchange when the Isthmus of Panama emerged as a land bridge linking the North and South American continents, allowing once-isolated species to disperse into new areas en masse (Brown and Sax 2004). Therefore, invasive species and their effects on human health and activities, as well as natural areas, are the subject of increasing scrutiny and study (Vitousek et al. 1997, Christian 2001, Brown and Sax 2004, Lockwood et al. 2007). The concern over invasive species prompted the issuing of Executive Order 13112, which outlined the actions of Federal agencies to prevent and manage invasive species, formed the Invasive Species Council to address current and future issues related to invasive species, and mandated a National Invasive Species Management Plan to provide a uniform set of guidelines and recommendations related to invasive species for Federal agencies (Clinton 1999).

Economic Impacts of Invasive Species

While species introductions can become problematic, not all introduced species are invasive, become harmful, or are viewed as detrimental. Over 98% of the human food supply comes from species, such as wheat, rice, corn, cattle, and poultry, which have been moved from their initial native ranges and introduced throughout the globe specifically for food production (Ewel et al. 1999, Pimentel et al. 2001). Invasive species can have detrimental economic effects on these agricultural species, as well as many other species, in areas where they become established.

The total monetary costs associated with invasive species, however, can be difficult to ascertain. Often, the economic impacts of invasive species are estimated based on the damage inflicted to products or commodities by invasive species or the cost of management and control efforts (Perrings et al. 2000, Perrings et al. 2005a). Estimates that combine damage and control costs to quantify total yearly losses in the U.S. due to introduced species have ranged from $1.1 billion to more than $130 billion annually, with the variations in estimates due to inclusion of differing numbers of species and variable damage and control costs for some of the species included in the cost estimate (US OTA 1993, Pimentel et al. 2000, Costello and McAusland 2003). In New Zealand, which has the greatest number of introduced mammals and the second greatest number of introduced bird species of any nation in the world, economic impacts of introduced pests that include economic losses, in addition to control and prevention costs, are estimated at more than $420 million annually (Williams and Timmins 2002). Invasive species that directly impact important commodities or industries lend themselves to these types of impact estimates, because market values and/or management expenditures are well documented. For example, introduced insect pests are estimated to cost crop producers in the U.S. ca $14.4 billion annually, based on the known number of introduced insect pests, losses in crop production, and costs of control measures (Pimentel et al. 2001). However, because estimates of this sort depend heavily on invasive species that are pests of commodities, criticism has arisen that ecological impacts are overlooked based on their difficulty to valuate (Perrings et al. 2005a).

Attempts to include indirect or non-production related costs in estimates of economic impacts of invasive species are underway. For example, both agricultural and non-agricultural values were used to estimate the total cost of impact of star thistle, *Centaurea solstitialis* L., in Idaho rangelands (Julia et al. 2007). This estimate calculated the reduction of the livestock carrying capacity of a pasture (agricultural) and the reduction in wildlife habitat and water quality (non-agricultural) in relation to infestations of star thistle. The total economic impact was calculated at $12.7 million per year, with 79% attributed to agricultural impacts and 21% attributed to non-agricultural impacts (Julia et al. 2007).

Nevertheless, calculating economic impacts of invasive species associated with natural or low-economic value systems can be challenging, because it is difficult to place a value on species that are not of economic importance (Perrings et al. 2000). One option is to quantify the products, functions, or services of the system affected by invasive species (Heywood 1995, Daily et al. 1997). Turpie and Heydenrych (2000) categorized the total economic value of natural resources found in Fynbos, a species-diverse biome in the Cape Floral Kingdom of southwestern South Africa. By placing monetary values on naturally-occurring “products”, such as herbaceous plants collected from Fynbos (ca. $1.4 million), in addition to ecosystem functions and services, such as pollination services by native bees (ca. $69 million) and contributions of native plants to soil stabilization and water quality and availability (ca. $22 million), potential economic impacts to non-production natural areas by invasive plants could be estimated (Turpie and Heydenrych 2000). One important consideration is that while the valuation of ecological functions and processes may be useful in economically evaluating the impacts of invasive species and increase the importance of invasive species to policy makers and/or the general public, ecological impacts are themselves important, whether or not they can be assigned a monetary value (Perrings et al. 2005a).

Ecological Impacts of Invasive Species

The impact of invasive species on natural systems can range greatly. The initial impacts of invasive species occur on individuals at the population level. While competition among invasive and native species may reduce native populations, factors such as herbivory and predation are much more common in reducing or eliminating localized populations (Davis 2003). Excessive predation or herbivory, especially to species that have low numbers or limited populations, may result in extinction.

Invasive species are a leading cause of extinctions of birds, fish, and mammals (Clavero and Garcia-Berthou 2005). Invasive species are suspected to have contributed to the extinction of 78 endemic pollinating insects in Hawaii (Cox and Elmqvist 2000). The brown tree snake, *Boiga irregularis* (Merrem), which was accidentally introduced in Guam shortly after World War II, is a classic example of the impact of an introduced predator on an island. Since its introduction, 10 of 13 native bird species and two of the three native mammals have become extinct on the island (Rodda et al. 1997, Fritts and Rodda 1998, Wiles et al. 2003).

Less obvious are extinctions through hybridization and/or introgression. Hybridization is interbreeding of individuals of genetically distinct populations, while introgression is mixing of gene pools of populations that have hybridized by hybrids backcrossing to one or more parental population. For example, the endemic Florida mottled duck, *Anas fulvigula fulvigula* Ridgway, may become genetically extinct through introgression due to breeding with the introduced mallard duck, *A. platyrhynchos* L. (Rhymer and Simberloff 1996). The Pecos pupfish, *Cyprinodon pecosensis* Echelle and Echelle, has become genetically extinct through hybridization due to the release of minnows used as bait fishes by fishermen in the U.S. (Echelle and Connor 1989, Mack et al. 2000).

Although extinction is the ultimate impact one species can have on another, invasive species also can affect the structure and composition of localized animal and plant communities. Before the invasion of the Argentine ant, *Linepithema humile* (Mayr), into areas of northern California, native ant communities were spatially and ecologically segregated. The introduction of *L. humile* into these communities acted as a disturbance factor and disassembled the community structure of the native ants, making them less segregated and more randomly distributed in areas where *L. humile* was established (Sanders et al. 2003). In the northeastern U.S., the introduced Norway maple, *Acer platanoides* L., is gradually replacing native hardwoods in some forest stands. In the Drew University Forest Reserve, New Jersey, Norway maple accounted for 98% of woody seedlings in study plots, while the native species (sugar maple, *A. saccharum* Marshall, and American beech, *Fagus grandifolia* Ehrhart) only comprised 2.00% and 0.05%, respectively (Wyckoff and Webb 1996, Sauer 1998).

In some cases, changes caused by an invasive species can grow beyond a local community and expand to impact entire ecosystems. For example, the hemlock woolly adelgid, *Adelges tsugae* Annand, is an insect of Asian origin that was first documented in the eastern U.S. in 1951 (Cheah et al. 2004). Feeding by this invasive insect on eastern hemlock, *Tsuga canadensis* (L.) Carierre, eventually results in the death of the tree, usually in three to ten years (McClure 2001, Orwig 2002). Initially, deaths of individual trees were in small localized areas. However, the hemlock woolly adelgid continues to spread and currently infests hemlock in over 50% of its range, causing mass mortality of this tree species across large areas (Cheah et al. 2004). These mass mortalities impact the forest ecosystems associated with eastern hemlock by changing forest composition, soil chemistry, and stream systems that flow through these forests (Orwig and Foster 1998, Snyder et al. 2002, Stadler et al. 2005, Roberts 2006).

Management of Invasive Species

The first line of defense against invasive species is guarding against the import of unwanted species. Accordingly, many countries have established regulations that restrict the importation of various invasive species. Members of the World Trade Organization (WTO) must adhere to the Sanitary and Phytosanitary Agreement (SPS), which establishes measures to protect human, animal, and plant health (WTO 1995, Smith 2003). In the U.S., the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) oversees many of the regulatory efforts related to agriculture and natural resources, such as inspection of imported materials, monitoring for quarantined and other pests of interest, enforcing import or quarantine violations, etc. (Kreith and Golino 2003). The monitoring efforts of APHIS are of great importance, as early detection of an invasive species may prevent its establishment or facilitate management against it (Wittenberg and Cock 2005).

While these regulations are useful, concerns regarding their effectiveness exist. First, to gain compliance with the SPS, any restriction on the importation of a potential commodity must be justified by a risk assessment, or the evaluation of a species' potential to cause damage (Smith 2003). The SPS requires that a species be proven invasive by the importing country, rather than requiring the exporter to prove that it is safe to export (Smith 2003, Simberloff 2005). In some cases, if the species is of great economic value and the risk assessment does not conclusively show that it is invasive, the importing country is required to allow the species to be traded (Perrings et al. 2005b, Simberloff 2006). So, while trade regulations such as these provide some framework for protection against invasive species, they may be limited in their regulatory power if the species of interest is of great economic value, thereby allowing questionable species to be imported and potentially to become invasive.

When species do enter a country, either purposely or accidentally, often there is no action taken to manage an introduced species when it is detected in a new area (Simberloff 2002). This inaction may be justified, because not all introduced species that establish in an area become invasive (Williamson 1996, Lockwood et al. 2007). So, even if an introduced species is detected in an area, no management action may be taken, because it does not exhibit invasive characteristics. By managing only those species that exhibit invasive traits or have a history of invading other areas, management funds and resources may be directed towards those species known to be problematic, to prevent their spread or reduce their impact (Sauer 1998).

For some populations of invasive species, eradication, or the elimination of the species from the infested area, may be a feasible management option. Many eradication programs have involved the elimination of human or animal diseases, such as *Anopheles gambiae* (Giles), a vector of human malaria, from Brazil, smallpox worldwide and primary screw-worm, *Cochliomyia hominivorax* (Coquerel), from the U.S. (Simberloff 2003). Eradication programs against pests of natural areas, such as the red imported fire ant, *Solenopsis invicta* Buren, from New Zealand and Taurian thistle, *Onopordum tauricum* Willdenow, from Victoria, Australia, also have been successful (Simberloff 2003, Sarty 2007, Liebhold and Tobin 2008). However, eradication efforts are often perceived as too costly and have a tarnished reputation due to some failed programs, such as the ineffective and costly ($200 million) attempt to eradicate *S. invicta* in the southeastern U.S. (Mack et al. 2000, Simberloff 2002). If eradication efforts are not implemented or are attempted but not effective, then invasive species must be managed to reduce their population levels as low as possible.

Chemical pesticides are commonly used to manage introduced agricultural pests, as well as invasive species in some natural areas (Sauer 1998, Mack et al. 2000). Although chemical pesticides may effectively control an invasive species, the high cost of materials and necessity of repeated treatments make chemical applications against invasive species across large natural areas impractical (Mack et al. 2000, Wittenberg and Cock 2005). Resistance to pesticides also can occur, facilitating more applications, which increases costs, further making chemical treatments less feasible (Wittenberg and Cock 2005). Despite these disadvantages, chemical treatments have been effective against some invasive species, such as applications of the herbicide 2, 4-D in combination with the physical removal (i.e., mechanical control) of the introduced aquatic weed water hyacinth, *Eichhornia crassipes* (Martius), in Florida (Simberloff 2002, Wittenberg and Cock 2005).

Mechanical control methods can range from removal of individuals by hand to the use of machines to harvest or remove large numbers of individuals. The great advantage of mechanical control is that, in most cases, it is highly specific, and impacts on non-target species are minimized (Simberloff 2002, Wittenberg and Cock 2005). However, mechanical controls are often labor intensive and costly, especially across large areas. Also, as in the case of many plants, mechanical controls must be repeated often to exert a long-term impact on populations of invasive species (Mack et al. 2000, Wittenberg and Cock 2005). The use of mechanical controls is, therefore, often restricted to confined infestations or areas where populations of invasive species are relatively accessible (Mack et al. 2000).

The drawbacks of chemical and mechanical control methods enhance the appeal of biological control to manage invasive species. Biological control is the suppression of the population of a species to levels at which it would not otherwise be by other living organisms (Eilenberg et al. 2001, Hajek 2004). In natural systems, this population suppression occurs through predator-prey and/or disease-host interactions. These interactions may be manipulated to be used in pest management scenarios. Accordingly, organisms used in biological control are often natural enemies of the pest species, such as predators, parasites, parasitoids, and herbivores, that feed on the pest species or microorganisms, such as bacteria, fungi, and viruses, that can cause disease in the pest species (Eilenberg et al. 2001, Coombs and Coombs 2003). The benefits of using natural enemies to manage pests are that, following release, populations of natural enemies may grow and expand with host populations and can grow and disperse across large areas (Mack et al. 2000). Additionally, established biological control agents are often self sustaining by fluctuating with host populations, potentially providing long-term control of pests (Bellows 2001).

Classical Biological Control and Invasive Species

While biological control can be implemented using several strategies, classical biological control, or the intentional introduction of an exotic biological control organism to permanently establish and control the pest long-term, is a strategy that has been used extensively (Eilenberg et al. 2001, Hajek 2004). An invasive species introduced into new areas may thrive there, in part, due to lack of natural enemies present in its original range (Bellows 2001, Liu and Stiling 2006). Classical biological control attempts to re-construct part of the associated natural enemy complex that helps to regulate populations of the introduced species in its native home range by introducing biological control organisms from the pest's region of origin (Hoddle 2004). Two of the best examples of successful classical biological control programs were the introduction and establishment of the vedalia beetle, *Rodolia cardinalis* (Mulsant), against the cottony cushion scale, *Icerya purchasi* Maskell, in California beginning in the 1880s and the release of *Cactoblastis cactorum* (Bergroth) (a herbivorous moth) against prickly pear cactus (*Opuntia* spp.) in Australia in the 1920s (Gurr et al. 2000). Both of these programs were successful, in that the biological control organisms established and have reduced and maintained pest populations at low levels since their initial introductions (Bellows and Hassell 1999).

Although numerous examples of successful classical biological control introductions exist (McFadyen 1998, Goeden and Andres 1999, Gurr and Wratten 2000, Bellows 2001), overall success rates of biological control releases are low. For example, 33.5% of 5,576 introductions against insect pests from 1880 to 2001 became established, and 11.2% resulted in complete control of the target species (Gurr et al. 2000, Hajek 2004). Likewise, 66.5% of 806 introductions against weeds established, and 26.7% resulted in complete control. The greater success of weed biological control agents is attributed, in part, to more extensive evaluation of the biological control agent prior to release (Hajek 2004). Thus, extensive pre-release testing of biological control agents, as well as selecting organisms that are suitable targets for biological control programs have become increasingly emphasized (Ehler 1990, Bellows and Fisher 1999, Pemberton 2002, Walter 2003, Hajek 2004).

Perhaps the most serious drawback of biological control is the phenomenon of unintended attacks of biological control agents on native, non-target species (Follet and Duan 2000). By attacking non-target species, biological control agents themselves can become pests of native species. Ironically, the traits that make biological control desirable as a management tactic (ability to permanently establish, disperse, find new hosts, sustain population numbers, etc.) also make it virtually irreversible if non-target activity occurs (Howarth 1983, 1991). In Hawaii, 8.6% of 175 established insect biological control agents also attacked native non-target species (Simberloff 1992). Some introductions of biological control agents have led to extinctions of native species, such as the predatory snail *Euglandina rosea* (Ferussac), a biological control agent of the giant African snail, *Achatina fulica* (Ferussac), causing the localized extinction of seven endemic snail species in Tahiti (Clarke et al. 1984, Murray et al. 1988, Simberloff and Stiling 1996). Despite these concerns, many critics of biological control in its current form still support its use as a management tactic against invasive species, provided that regulations regarding research and specificity testing are improved to be more rigorous and greater accountability for control programs is instituted (Howarth 1983, Simberloff and Stiling 1996, Ewel et al. 1999, Follet and Duan 2000, Mack et al. 2000).

The issues associated with the beneficial and detrimental aspects of the use of biological control as a management strategy can complicate the management of invasive species. One example of a classical biological control program that illustrates both positive and negative impacts of introduced biological control agents is the introduction of natural enemies against musk thistle, *Carduus nutans* L.

A Model System: Musk Thistle and Introduced Biological Control Agents

Overview of Musk Thistle Biological Control Program in North America

Musk thistle is a biennial herbaceous species in the family Asteraceae (subfamily Carduoideae, tribe Cardueae) native to Europe, western Asia, and the Mediterranean (Hodgson and Rees 1976, Zwölfer and Harris 1984, Garcias-Jacas et al. 2002, Judd et al. 2002). It was first introduced into North America in the mid-1800s (Stuckey and Forsyth 1971) and is now recorded from 45 states and at least five Canadian provinces (McCarty 1978, Desrochers et al. 1988, USDA 2008). Musk thistle often grows in disturbed or overgrazed pastures and rangelands, along roadsides and right-of-ways, and other waste areas (Kok 2001, Gassmann and Kok 2002). Individual plants can produce from ca. 10,000 to 120,000 wind-dispersed seeds that can remain viable up to 20 years (Trumble and Kok 1982, Kok 2001), providing abundant propagules for colonization of disturbed habitats.

The introduction, establishment, and spread of this exotic invasive species throughout the U.S. have had both economic and environmental ramifications. Its establishment in areas leads to increased management costs and reduced land productivity. Musk thistle can reduce pasture yield by 23% at a density of one plant per 1.49 m^2 but can reach densities over 150,000 plants/ha (Gassmann and Kok 2002). In the U.S., musk thistle is quarantined, regulated or listed as a noxious weed in 23 states (USDA 2008). In Tennessee, musk thistle is listed as a “Rank 2 – Significant Threat” by the Tennessee Exotic Pest Plant Council (TNEPPC 2008), and its seed is listed as a “Restricted Noxious Weed” (TDA Seed Regulations 1999).

Although chemical application provides some measure of weed control, populations of this invasive exotic weed persists despite repeated applications of herbicides. After treatment with herbicides, many standing plants of the target weeds and other plants in the proximity of the chemical application are killed. Because musk thistle thrives in open, disturbed habitats and has a large seed reservoir in the soil, it is often the first plant to re-grow following herbicide sprays (Kok 2001). Mowing and other mechanical controls or physical disturbances to the soil also can contribute to spreading seedheads and seeds, and disturbance to the ground can further facilitate the spread of the plant. Additionally, chemical and mechanical methods require multiple treatments each season, and these actions are usually restricted to readily accessible areas (Trumble and Kok 1982). Because it was introduced into the U.S., the complement of natural enemies that impacted and maintained lower populations of musk thistle in its native European habitats were not present, allowing this invasive plant to persist and reproduce without the subduing impact of important biotic factors. Thus, the introduction of exotic natural enemies was promoted and projected to provide a long-term, sustainable approach to reducing populations.

Investigations for suitable biological control insects were initiated in Europe and Asia in the early 1960s (Frick 1978b), and two weevil species [*Rhinocyllus conicus* Fröelich (the head weevil) and *Trichosirocalus horridus* (Panzer) (the rosette weevil) (Coleoptera: Curculionidae)] emerged as two promising biological control agents (Fig 1A and 1B). These weevils share similar home ranges with *C. nutans* and feed primarily on thistles in the genera *Carduus*, *Cirsium*, *Silybum*, and *Onopordum* (Zwölfer and Harris 1984). The biologies of these two weevil species complement one another in regards to the reduction of the reproductive capability of musk thistle. While the adults of both species feed casually on the foliage, larvae of *R. conicus* feed within the developing flowerheads on the receptacle and developing achenes, greatly decreasing the plant's reproductive capability (Shorthouse and LaLonde 1984, Kok 2001). The larvae of *T. horridus* feed on the meristems of the developing rosettes, causing necrosis of the meristem leading to plant deformities or even death (Fig. 1D) (Ward et al. 1974, Grant et al. 1990).

In the southeastern U.S., overwintered adults of *R. conicus* emerge in late-April and begin mating and ovipositing on the involucral bracts of the thistle heads. Eggs hatch in 6 to 7 days, the larvae mine into the developing head to feed for ca. 25 days, and pupate for 8 to 14 days. By mid-June most adults have emerged; they then aestivate and overwinter (Surles et al. 1974). Adults of *T. horridus* emerge in mid-May through mid-

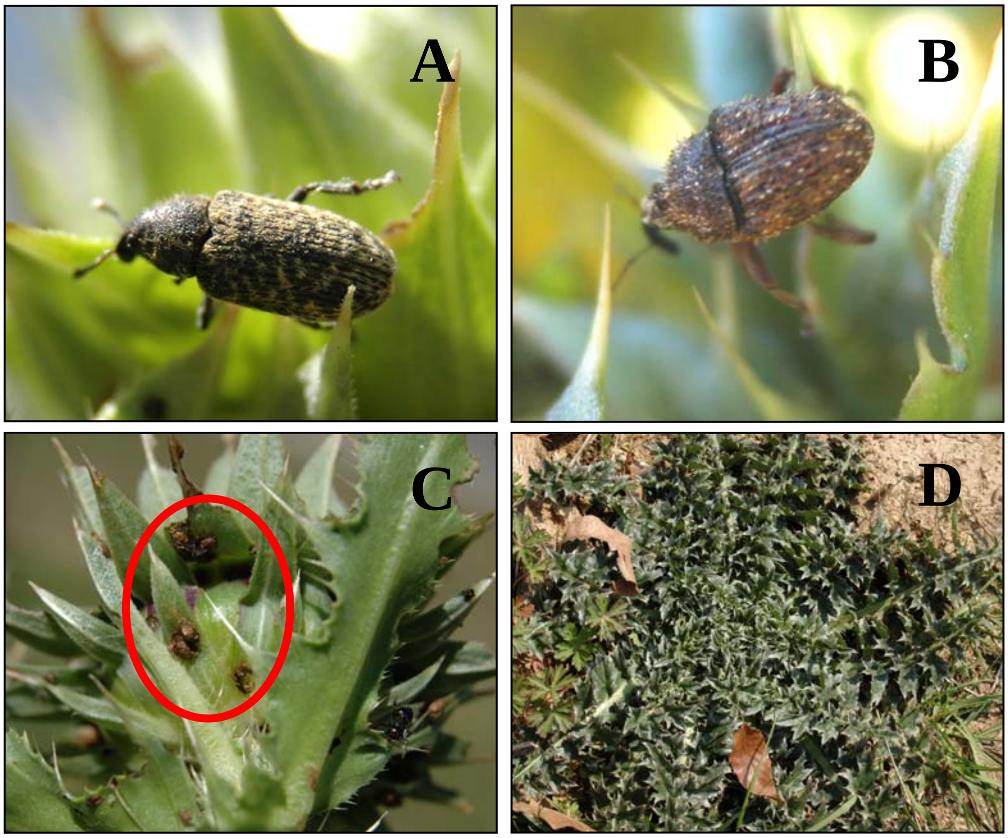

Figure 1. Weevils introduced as biological control agents of *Carduus nutans*: A) *Rhinocyllus conicus* adult (ca. 5 mm), B) *Trichosirocalus horridus* adult (ca. 4 mm), C) eggs of *R. conicus* on bud of *C. nutans* (encircled), D) rosette of *C. nutans* (in which larvae of *T. horridus* feed).

June from pupae that have developed beneath the soil surface. These adults aestivate from early July through the fall and begin ovipositing on the midribs of rosette leaves in late December through early March. Larvae bore into plant tissues and move to the meristem of the rosette, where they feed for ca. 2 months and drop to the ground to pupate (Trumble and Kok 1979).

Releases of *R. conicus* were first made in Canada in 1968 (Frick 1978b), and were followed in 1969 by releases in California (Goeden 1978), Montana (Hodgson and Rees 1976), and Virginia (Surles et al. 1974). Releases of *T. horridus* were first made in Virginia in 1974 (Ward et al. 1974, Trumble and Kok 1979), followed by releases in Canada in 1976 (Dunn 1978). Upon establishment of these weevils and subsequent control of musk thistle (ca. 95% mean reduction of plants in study plots in Virginia attributed to *R. conicu*s; Kok and Surles 1975), both *R. conicus* and *T. horridus* were widely redistributed as part of musk thistle control programs throughout the continental U.S. (Trumble and Kok 1982, Townsend et al. 1991, Lambdin and Grant 1992, Buntin et al. 1993, McDonald et al. 1994, Gassmann and Kok 2002).

Musk thistle biological control programs were considered a success in many areas where they were initiated. These weevils have reduced musk thistle populations in several areas across North America, including Montana (Rees 1977, 1978) and Saskatchewan (Harris 1984). In study plots where weevils were released in Tennessee, musk thistle densities were reduced by ca. 94% over a six-year period (Lambdin and Grant 1996). Musk thistle is considered to be adequately controlled in numerous areas of weevil release, with some plant populations showing long-term reductions (from 13 plants per m^2 to <1 plant per m^2) over two decades after release (Kok 2001).

While releases of these two weevil species were made throughout Tennessee from 1989 through the early 1990s, limited follow-up studies have been conducted to fully assess establishment, spread, or impact of these biological control agents (Lambdin and Grant 1989, Lambdin and Grant 1992, Grant and Lambdin 1993). Research has focused primarily on assessing insect species composition on musk thistle (Powell et al. 1996), investigating impact of native insect species on musk thistle (Powell et al. 1992, Landau et al. 1996, Grant et al. 1998), and assessing distribution of selected exotic species, such as Canada thistle, *Cirsium arvense* (L.) Scopoli (Sudbrink et al. 2001).

Non-target Feeding of Weevils on Native Thistles in North America

Recent documentation of non-target impacts of *R. conicus* on native thistles in the north central and western U.S., however, illustrate the importance of assessing the impact of these weevils on target and non-target thistles in areas in which they were released (Table 1). The first record of *R. conicus* feeding on native North American thistles in the genus *Cirsium* was in Montana and Canada on *Cirsium undulatum* (Nuttall) Sprengel and *Cirsium flodmannii* (Rydberg) Arthur (Rees 1977, 1978, Zwölfer and Harris 1984). Insect surveys of native thistles in California documented *R. conicus* feeding on natural populations of 13 native *Cirsium* species, one of which (*Cirsium fontinale* (Greene) Jepson var *obispoense* J. T. Howell) is listed as Federally endangered (Turner and Herr 1996), and three of which [*Cirsium campylon* Sharsmith, *Cirsium ciliolatum* (Henderson) J. T. Howell, and *Cirsium hydrophilum* (Greene) Jepson var *vaseyi* (Gray) J. T. Howell] are potential candidates to be listed as Federally threatened or endangered (Goeden and Ricker 1986, 1987, Turner et al. 1987). While the extension of the host range of

Table 1. Native North American *Cirsium* species utilized by *Rhinocyllus conicus* as an alternate host plant (modified from Pemberton 2000).

Cirsium species	Location[1]	Source[2]
C. andersonii (Gray) Petrak	CA	Turner et al. 1987
C. brevistylum Cronquist	CA	Turner et al. 1987
C. californicum Gray	CA	Goeden and Ricker 1986
C. callilepis (Greene) Jepson	CA	Turner et al. 1987
C. campylon Sharsmith	CA	Turner et al. 1987
C. canescens Nuttall	NE	Louda et al. 1997
	WY	Pemberton 2000
C. centaureae (Rydberg) K. Schumann	CO	Louda et al. 1997
C. ciliolatum (Henderson) J.T. Howell	CA	Turner et al. 1987
C. cymosum (Greene) J.T. Howell	CA	Turner et al. 1987
C. douglasii de Candolle	CA	Turner et al. 1987
C. edule Nuttall	CO	Pemberton 2000
C. flodmanii (Rydberg) Arthur	SK	Zwölfer and Harris 1984
	WY	Pemberton 2000
C. fontinale var *obispoense* J.T. Howell	CA	Turner and Herr 1996
C. hillii (Canby) Fernald	WI	Sauer and Bradley 2008
C. hydrophilum var *vaseyi* (Gray) J.T. Howell	CA	Turner et al. 1987
C. occidentale (Nuttall) Jepson	CA	Goeden and Ricker 1987
C. owenbyi Welsh	CO	Pemberton 2000
C. pastoris J.T. Howell	CA	Turner et al. 1987
C. proteanum J.T. Howell	CA	Goeden and Ricker 1986, Turner et al. 1987
C. pulchellum (Greene) Wooton & Standley	CO	Louda et al. 1997
C. quercetorum (Gray) Jepson	CA	Pemberton 2000
C. scariosum Nuttall	WY	Pemberton 2000
C. tioganum (Congiaux) Petrak	CA	Turner et al. 1987
C. tweedyi (Rydberg) Petrak	CO	Louda et al. 1997
C. undulatum (Nuttall) Sprengel	CO, NE, SD	Louda et al. 1997
	MT	Rees 1977
	WY	Pemberton 2000

1 – Locations in U.S.: CA-California; CO-Colorado; NE-Nebraska; MT-Montana; SD-South Dakota; WI-Wisconsin; WY-Wyoming. Locations in Canada: SK-Saskatchewan.

2 – Sources listed in "Literature Cited".

R. conicus seems considerable, the importance and impact of these new host associations is not yet known.

Although it is not one of the first observations of its type, the feeding of *R. conicus* on Platte thistle, *Cirsium canescens* Nuttall, is the most well documented case of non-target feeding by this weevil on native thistles. Larvae of *R. conicus* were first documented feeding in the seedheads of *C. canescens* in Nebraska in 1993 (Louda et al. 1997, Louda 1998). This thistle shares the same budding and flowering period with musk thistle in Nebraska and, therefore, is well synchronized with the period of oviposition of *R. conicus*. Within five years populations of *R. conicus* grew to infest over 25% of observed *C. canescens* plants, and the average number of *R. conicus* developing per plant increased to 40 (Louda 1998, Louda 2000a). Additionally, feeding of *R. conicus* in seedheads reduced the number of viable seeds in infested heads by 86%, which is predicted to decrease population numbers and densities of *C. canescens* (Louda 2000a). While these initial non-target observations occurred in isolation from any known musk thistle infestations, later surveys showed a positive relationship between proximity to and density of musk thistle populations and intensity of non-target feeding on native thistles (Rand et al. 2004). Feeding of *R. conicus* on *C. canescens* is of particular interest, because this thistle is a close relative of Pitcher's thistle, *Cirsium pitcheri* (Torrey) Torrey and Gray, a Federally listed threatened species (Pavlovic et al. 1992, Louda 1998, Louda 2000b). Concern exists that *R. conicus* will continue its host range expansion and utilize Pitcher's thistle, increasing its likelihood of extinction (Louda 1998, Louda 2000b).

The impact of the released weevils on non-target thistle species in the southern Appalachians is poorly known. While there have been no published reports on non-target activity of *R. conicus* on native thistles in this region, some research has been conducted on host specificity of *T. horridus*. The species included in non-target testing were primarily economically important crops (Ward et al. 1974, Kok 1975), but *T. horridus* was documented in field surveys to feed on 6% of *Cirsium discolor* (Muhlenberg ex Willdenow) Sprengel, field thistle, observed between 1981-85 in Virginia (McAvoy et al. 1987). Although not documented in the southern Appalachians, *T. horridus* was observed to infest rosettes of tall thistle, *C. altissimum* (L.) Sprengel, a species that also occurs in Tennessee, at similar levels to the introduced bull thistle, *C. vulgare* (Savi) Ten., in Nebraska (Takahashi et al. 2009).

Infestation of native thistles by *R. conicus* is not totally unexpected. Host specificity tests conducted as part of the release protocol for North America demonstrated the range of species *R. conicus* was able to utilize as a host for reproduction (Zwölfer and Harris 1984). Early host specificity tests and host data from its native range suggested that this insect species could feed and develop on several genera and species of thistles in the tribe Cardueae (Asteraceae). In European laboratory studies, the host suitability of 45 species of thistles in 29 genera in four subtribes of Cardueae and in eight other tribes of Asteraceae to *R. conicus* was evaluated. *R. conicus* consistently fed on four species of *Carduus* and on five species of *Cirsium* and adults oviposited and larvae developed successfully on two species of *Carduus* and two species of *Cirsium*. In European field studies designed to evaluate the host specificity of thistles to *R. conicus*, 70 species in 18 genera in four subtribes of tribe Cardueae were sampled. Eggs and larvae were found in flowerheads of five of the nine species of the target genus *Carduus*, in four of 17 species of *Cirsium*, in *Silybum marianum* (L.) Gaertner, and in *Onopordum acanthium* L. (all members of subtribe Carduinae). Although the host range of *R. conicus* was not restricted to a species or genus, the projected benefits of its release as a biological control agent outweighed the potential limitations and it was approved for release into the U.S. (Boldt 1997).

Native Thistles in Tennessee

Currently, 15 species in the Asteraceae tribe Cardueae have been documented in Tennessee (Table 2). Of these, five are native species [*Cirsium altissimum*, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, *C. discolor*, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux] (TENN 2008). In Tennessee, *C. carolinianum* and *C. horridulum* bloom in the spring, with *C. carolinianum* blooming from May through July (Wofford 1989). A native species in the Coastal Piedmont regions of eastern U.S. where it blooms from May through August (Gleason and Cronquist 1991), *C. horridulum* has recently expanded its range into the river valleys of Tennessee (Cronquist 1980). The remaining native thistles (*C. altissimum*, *C. discolor*, and *C. muticum*) bloom later in the year, all species bloom approximately from August through October (Wofford 1989). These native species are widely distributed throughout the eastern U.S., each occurring in at least 15 states in addition to Tennessee (USDA 2008). The other ten species in the tribe Cardueae are exotics of southern European/western Asian origin. Although not commonly known as a "thistle," lesser burdock, *Arctium minus* Bernhardi, is within the same tribe (Cardueae) and subtribe (Carduinae) as *Carduus* and *Cirsium* (Zwölfer and Harris 1984, Judd et al. 2002, Story 2002). Likewise, most species of *Centaurea* are not commonly called "thistles" but are in Cardueae (subtribe Centaureinae, as is *Cnicus benedictus* L.) (Story 2002).

Table 2. "Thistles"[1] and related species (Asteraceae: Cardueae) documented in Tennessee (courtesy of the University of Tennessee Herbarium) and their known associations with *Rhinocyllus conicus* and *Trichosirocalus horridus*.

Species	Counties[2]	Known host of [3]:	
		R. conicus	*T. horridus*
Subtribe Carduinae			
Arctium minus Bernhardi*	24	Unk.	Unk.
Carduus nutans L.*	33	Yes	Yes
Cirsium altissimum (L.) Sprengel	24	Unk.	Unk.
C. arvense (L.) Scopoli*	6	Yes	Yes
C. carolinianum (Walter) Fernald & Schubert	16	Unk.	Unk.
C. discolor (Muhlenberg ex Willdenow) Sprengel	33	Unk.	Yes
C. horridulum Michaux	7	Unk.	Unk.
C. muticum Michaux	12	Unk.	Unk.
C. vulgare (Savi) Tenore*	27	Yes	Yes
Silybum marianum (L.) Gaertner*	1	Yes	Unk.
Subtribe Centaureinae			
Centaurea cyanus L.*	27	No	Unk.
C. diffusa Lamark*	1	Unk.	Unk.
C. solstitialis L.*	1	Unk.	Unk.
C. stoebe L.*	30	No	No
Cnicus benedictus L. *	5	Unk.	Unk.

1 – Other species referred to as "thistles", such as species in the genus *Sonchus* (Asteraceae) and *Salsola tragus* (Chenopodaceae) are found in Tennessee. Only species in the genera *Carduus* and *Cirsium* recorded from Tennessee were included in this study.

2 – Number of counties in which each species has been documented.

3 – Yes = weevil has been found on this plant species; No = weevil has not been found on this species; Unk. = no data available for this plant species in Tennessee.

* – Introduced species.

Conversely, other species in Tennessee are known by their common name as "thistles" but are in different tribes of Asteraceae or different plant families. The genus *Sonchus* (species of which are commonly known as "sow thistles") is in the Asteraceae tribe Lactuceae (Judd et al. 2002, Kim et al. 2004). Prickly Russian thistle, *Salsola tragus* L., is in the family Chenopodiaceae. Because these groups are not closely related to true thistles they are not of interest to this study. Only those species in the genera *Carduus* or *Cirsium* that are documented in Tennessee were included in this research (Table 2).

Predicting Habitats Using Spatial Analysis

Because the distribution of populations of introduced thistles and weevils may influence the frequency of non-target impacts on native thistles (Rand et al. 2004), the use of spatial analysis via a geographic information systems (GIS) could be useful in analyzing the significance of these spatial relationships. A GIS is a computer system that accumulates, manipulates, analyzes, and displays geospatial data. Components of GIS include satellites, GPS receivers, georeferenced datasets, geospatial software applications and trained users and professionals (Chang 2008). The use of GIS facilitates the analysis and visualization of complex spatial and temporal relationships among multiple spatial components (Hartkamp et al. 1999). Although GIS has existed in limited forms since the late 1960s, recent advances in computer technology and spatial analytical software for personal computers have expanded its appeal to a broad range of researchers. In conjunction with advancements in software and computer technology, a variety of data (elevation, land cover, hydrology, climatic, infrastructure, etc.) is increasingly available for use from government agencies (U.S. Geological Survey, USDA Natural Resources

Conservation Service, USDA Forest Service, U.S. Department of Commerce National Oceanic and Atmospheric Administration, etc.) and organizations (Southern Appalachian Man and the Biosphere, The Nature Conservancy, etc). The combination of relevant spatial data and data collected in the field can enhance research by detecting associations that may not be obvious using conventional statistical models.

In disciplines that possess inherently spatial characteristics, such as natural resource management and agriculture, spatial analysis has become a useful tool in research. For example, Jensen et al. (2001) utilized spatial analysis to develop a potential vegetation map for the Little Missouri National Grasslands of North Dakota. The habitat-type raster map produced provided a base map for land use planning because it can be used to describe the land's potential for resource use, indicate plant community succession pathways, and reference conditions associated within minimally altered ecosystem states. In the context of interactions among native and introduced thistles and introduced biological control agents in Tennessee, development of spatial models could provide estimates of habitat characteristics to enable predictions of suitable habitats for each exotic and native thistle species. Populations of native thistles that are adjacent or close to exotic thistles may be more likely to encounter non-target feeding by the introduced biological control agents. An effective method to generate these habitat models is Mahalanobis distance.

Mahalanobis distance (D^2) is a multivariate statistic that estimates levels of dissimilarity using the mean and variance of the predictor variables and the covariance of all variables (Mahalanobis 1936, Rao 1952, 1973). This statistic defines a set of conditions as 'ideal' based on a sample of the data (i.e., observed data) and calculates dissimilarity from the ideal conditions for the remainder of the data (Rao 1952, Clark et al. 1993). So, conditions that are most similar to those of the observed locations will have relatively low D^2 values, and D^2 values increase with dissimilarity (Clark et al. 1993, Jenness 2003). Originally developed for use in anthropometric applications, the D^2 statistic has been used with success to predict suitable habitats for wildlife (Clark et al. 1993, Browning et al. 2005, Rotenberry et al. 2006, Thatcher et al. 2006, Watrous et al. 2006) and plants (Boetsch et al. 2003, van Manen et al. 2005, Thompson et al. 2006) in the U.S.

Mahalanobis distance uses presence only data unlike other habitat analyses, such as logistic regression, that require presence and absence locations, thus avoiding the pitfalls of how to classify habitats where the species of interest is absent (Clark et al. 1993, van Manen et al. 2005, Watrous et al. 2006). Despite the widespread distribution of both *Carduus nutans* and *Cirsium discolor*, other native *Cirsium* species in Tennessee are not as ubiquitous or obvious to detect, and determining the significance of their absence in a habitat would be problematic (i.e., is a species absent from a site because it is unsuitable or are other factors responsible?). Thus, Mahalanobis distance analysis provides a means to predict habitats for thistles that occur less frequently by using only their known localities.

The use of spatial analysis to develop habitat models, combined with research on non-target activity of the introduced biological control agents of musk thistle on native thistles found in Tennessee, provide opportunities to gain unique insights about these species. Most of the native thistles in Tennessee have not been included in previous non-target studies of *R. conicus* or *T. horridus*, and host utilization of these thistles by either introduced weevil is poorly known. So, the opportunity exits to document and evaluate the first occurrences of novel associations among these weevils and native thistles. The use of spatial analysis to develop habitat models for selected thistle species could be important, because it would facilitate more targeted management practices in areas where conservation of native plants is a priority. However, the development of these models may not only be useful to land managers in Tennessee, but also may assist land managers in other states with the prediction of suitable habitats for thistles of interest. While none of the native thistle species discussed above is listed as rare, threatened, or endangered (RTE) in Tennessee or Federally, three thistles found in Tennessee are listed RTE in other states (*C. carolinianum* - state listed as rare in Indiana and threatened in Ohio; *C. horridulum* - state listed as endangered in Connecticut, New Hampshire, and Pennsylvania; *C. muticum* - state listed as threatened in Arkansas) (USDA 2009). The techniques used to develop spatial models that characterize habitats for any of these thistles in Tennessee could be modified for other states where they are listed as protected species and help identify areas vulnerable to herbivory by either of the introduced weevils. As a result, this research in Tennessee could provide new insights to weevil-thistle interactions, as well as aid land managers and scientists in other states in managing and protecting native species.

Objectives of Research

The overall goal of this research is to document the host utilization of target and non-target thistle species of two European herbivorous insects introduced to manage musk thistle and predict where non-target host utilization may occur based on habitat predictions of selected thistle species using spatial analysis in a region where several native and exotic thistle species exist and both exotic insect species are widely established. Specific questions that need to be addressed towards reaching this goal include: 1) are these released weevils synchronized with native thistle phenologies in this region, 2) do these exotic weevils feed on these thistle species and do they negatively impact the plant, and 3) can a useful spatial model be developed to predict thistle habitats and areas where herbivory by either of these two weevils is likely? By addressing these questions, information on the biologies of both the plant and weevil species may be better understood.

Therefore, the objectives of this study are to: 1) assess the synchrony of phenologies of the three exotic and five native thistles in Tennessee with the phenologies of *R. conicus* and *T. horridus*, 2) document the incidence of the introduced weevils in natural populations of these eight thistle species, 3) quantify the impacts on plant reproduction or growth responses of each thistle species due to feeding of *R. conicus* and *T. horridus*, and 4) predict the occurrence of two native (*C. carolinianum* and *C. discolor*) and two exotic (*C. vulgare* and *Carduus nutans*) thistle species (model species) and identify potential habitats across a study area for these thistle species using spatial analysis. Interactions among introduced and native species can range from subtle to complex. Completion of these objectives will provide information useful in the management of native and introduced species, and greater knowledge of the relationships among the invasive musk thistle, the weevils introduced for its control, and native thistle species.

CHAPTER II. PHENOLOGIES OF NATIVE AND INTRODUCED THISTLE SPECIES AND THEIR SYNCHRONY WITH INTRODUCED BIOLOGICAL CONTROL AGENTS OF *CARDUUS NUTANS*

Introduction

Biological control of pest plants by insects has been studied and implemented throughout the world (Goeden and Andres 1999, Gurr et al. 2000, Hajek 2004). One important attribute of an effective biological control agent is its biological synchrony with the plant pest. The occurrence of the appropriate life stages of the plant during the time at which the biological control agent requires host material is important in the establishment and growth of insect populations against their weed hosts (Bellows and Headrick 1999). The importance of this synchrony can be overlooked when a natural enemy is evaluated for release in new areas, as it is one of a suite of characteristics that contributes to an herbivore's ability to impact a plant. Additionally, other factors, such as host specificity, plant impacts, likelihood of establishment and dispersal, etc., are the primary foci of natural enemy evaluations (Bellows and Headrick 1999, Hajek 2004). However, phenological synchrony between an introduced herbivore and native plants may facilitate host range expansion of the herbivore, especially if the herbivore has a wide host range in its region of origin. One such instance of the increasing importance of phenological synchrony in introduced insect-native plant interactions can be found in the biological control program against musk thistle, *Carduus nutans* L.

Eurasian thistles in the genus *Carduus* are introduced weed species that can become problematic in pastures, grasslands, and rangelands in North America (Frick 1978a, Van Driesche et al. 2002). Musk thistle is the most widely distributed of these introduced species, occurring throughout much of the U.S. and Canada (USDA 2008). A biological control program against *Carduus* thistles, with *C. nutans* as the primary focus, was initiated in 1968 and included the releases of two Eurasian weevil species, *Rhinocyllus conicus* Fröelich and *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae) (Frick 1978a, Zwölfer and Harris 1984, Kok 2001). Of these two weevils, *R. conicus* has been more widely promoted as a biological control agent, as larval feeding in the receptacle directly impacts the number of seeds produced by *C. nutans* (Surles and Kok 1977, Rowe and Kok 1984, Zwölfer and Harris 1984).

The ability of *R. conicus* to reduce seed numbers of thistles is directly related to its phenological synchrony with the host plants. Greater population establishment and infestation of seedheads by *R. conicus* were observed on *C. nutans* than *C. acanthoides* L., which was attributed to *C. acanthoides* blooming later in the season when *R. conicus* had completed oviposition (Surles et al. 1974, Surles and Kok 1977). Only after *R. conicus* exhibited a delay in oviposition in some populations of *C. acanthoides* did the weevil have an effect on seed production (Rowe and Kok 1984). Similarly, the effect of *R. conicus* on seed numbers of some populations of *C. pychnocephalus* L. in California was limited, due to flower and seed production continuing for one month after larvae had ceased feeding, allowing plant populations to remain stable (Goeden and Ricker 1985).

Although the ability of *R. conicus* to feed on a range of introduced thistle species was initially seen as a positive attribute, this weevil was able to survive and reproduce using other thistle species in the genus *Cirsium* that are native to North America as host plants. Rees (1977) first observed low levels of *R. conicus* feeding on *C. undulatum* (Nuttall) Sprengel in Montana, and *R. conicus* has now been documented on at least 25 of the ca. 96 species of *Cirsium* in North America (Zwölfer and Harris 1984, Goeden and Ricker 1986, Turner et al. 1987, Turner and Herr 1996, Louda et al. 1997, Pemberton 2000, Sauer and Bradley 2008, USDA 2008). The native *C. canescens* Nuttall buds and flowers synchronously with the oviposition period of *R. conicus*. This thistle was infested at higher levels than the less synchronous *C. undulatum*, and levels of weevil infestation were influenced more by variations in weevil phenology (due to abiotic factors) than variation in the plant's phenology (Louda 1998, 2000a, Russell and Louda 2004). So, the host range expansion of *R. conicus* can be better understood and predicted if the phenologies of native thistles are synchronous with the development of the weevils.

Phenologies of both *R. conicus* and *T. horridus* are known from previous release and establishment studies in the southeastern U.S. and Tennessee. The plant-damaging larvae of *R. conicus* are active beginning in early May through mid June (Surles et al. 1974, Surles and Kok 1976, Lambdin and Grant 1989). The biology of *R. conicus* is well synchronized with the observed development of buds and flowers of *C. nutans* in Tennessee (Powell et al. 1996). Larvae are also the damaging stage of *T. horridus* and are found in the rosettes beginning in late December through early May (Trumble and Kok 1979, Sieburth et al. 1983). Because most thistles overwinter as rosettes in regions with winter climates, *T. horridus* has the potential to impact a wider range of species. However, little non-target activity by *T. horridus* (when compared to *R. conicus*) has been documented (Ward et al. 1974, Kok 1975, McAvoy et al. 1987, Takahashi et al. 2009).

To better assess the potential non-target hosts of these weevils in Tennessee, a study was initiated to compare the phenologies of thistles that occur in the state with the phenologies of the introduced weevils. The objectives of this study were to 1) estimate the phenologies of thistle species that occur in Tennessee using literature sources and herbarium records, 2) compare thistle phenologies in the herbarium and literature with field observations, 3) confirm the phenologies of *R. conicus* and *T. horridus* through field observations, and 4) compare phenologies of thistles with weevil phenologies.

Materials and Methods

Specimen records of *C. nutans* and all species known to be host plants of *R. conicus* and *T. horridus* [in the genera *Carduus*, *Cirsium*, *Silybum*, *Onopordum*; tribe Cardueae (Asteraceae)] (Zwölfer and Harris 1984) and recorded from Tennessee were examined at the University of Tennessee Herbarium. For each specimen record, the collection date and numbers of buds, flowers, and seedheads were recorded. The average numbers each of buds, flowerheads, and seedheads per plant were calculated for each month the stages were present to compare with field observations. Herbarium records were periodically reviewed through 2008 for any updated thistle specimen records.

In 2008, monitoring of populations of *Carduus nutans* and thistles in the genus *Cirsium* for comparison with herbarium data began in late April and continued through early September, and sampling duration varied for each thistle species depending on its biology (Table 3). General areas in which to search for populations of each thistle species were based on locality records from plant specimens in the University of Tennessee Herbarium. Site reconnaissance for these populations was conducted between 2005 through 2007 as part of other studies. For each of the 25 populations, 20 plants were

Table 3. Thistle species and populations monitored for phenology in Tennessee, 2008.

Species	Populations Sampled	Total Observations	Observation Period
*Carduus nutans**	6	240	6 May to 12 June
Cirsium altissimum	3	120	22 June to 7 September
*Cirsium arvense**	2	120	6 May to 2 June
C. carolinianum	3	240	2 May to 18 June
C. discolor	4	120	7 May to 7 September
C. horridulum	3	120	29 April to 7 June
C. muticum	1	140	8 May to 6 September
*C. vulgare**	3	120	8 May to 6 September

* – Introduced species.

observed, and collection date and number of buds, flowers and seedheads were recorded. Each population was monitored from one to four times depending on when the population was located or available during the sampling season. Population monitoring ceased soon after initiation of seedheads. Included in this study were observations from 10-20 plants of each thistle species that were in use for other studies during the 2008 field season (control plants exposed to normal environmental conditions).

General phenologies for each thistle species were compiled from the literature (Cronquist 1980, Wofford 1989, Carman 2001, Weakley 2008). These sources were selected because they specifically addressed plants from Tennessee (Carman 2001) or specifically included Tennessee within the topic region (Cronquist 1980, Wofford 1989, Weakley 2008). Additional sources were considered but not utilized because they did not specifically consider plant seasonality in Tennessee (i.e., Radford et al. 1968, Gleason and Cronquist 1991). The selected literature sources list general flowering periods rather than specific timeframes of each developmental stage, and these flowering periods were charted from each literature source. Based on field observations in 2008, as well as data collected during the other studies conducted between 2005 through 2007, the period of each developmental stage was determined and charted for comparison with literature sources. The initiation of each developmental stage, from bud through seed release, was designated by the initial of each stage (B-bud, F-flower, S-seed) for charted phenologies based on field observations.

Weevil phenologies were determined by counting numbers of eggs and adults of *R. conicus* and adult *T. horridus* present on buds and flowers of the plants examined during the thistle phenology survey. These developmental stages were monitored in the field, because the eggs of *R. conicus* and both adult weevils are more easily detected and counted on the plant, whereas the larvae and pupae of these weevils require plant dissection to evaluate. Although these weevils are known to be associated with *Carduus nutans*, all thistle species were examined for the presence of these weevils. Numbers of adults of both weevils observed on *C. nutans* were averaged per plant for each weekly sampling, from the week of 4 May through the week of 8 June 2008 to estimate levels of activity. The average numbers of eggs of *R. conicus* per head (buds and flowers combined) also were calculated for each weekly sampling period listed previously. The total number of eggs of *R. conicus* and both adult weevils observed on other thistle species during this study were tallied per thistle species for the period between 29 April (when *R. conicus* generally become active) and 18 June 2008 (when *R. conicus* activity has lessened).

To confirm periods of adult activity of *T. horridus* during the winter with the literature (Ward et al. 1974, Trumble and Kok 1979), additional observations were made in 2009. A leaf blower (Husqvarna model 225B E-tech) modified after Harper and Guynn (1998) was used to collect weekly vacuum samples from populations of *C. nutans* in Knox, Cumberland, and Bradley Counties from 4 January through 29 March 2009. At each site, five random vacuum samples were taken in a 2 m x 2 m area containing at least four rosettes of *C. nutans*. In each sample, the rosettes and soil immediately surrounding them were vacuumed, and each of the five samples were stored in an individual cloth mesh bag, taken to the laboratory and examined for presence of adult weevils.

To test for statistical significance between plant records from herbarium and field observations, as well as plant and weevil data collected during 2008, analysis of variance was performed and significance ($P \leq 0.05$) among means was determined using least significant differences. The average numbers of buds, flowers, and seedheads per month of each of the eight thistle species found in Tennessee were compared to determine significant differences between means of herbarium and field-observed plants, and data were normalized using rank transformation. Additionally, the average number of eggs of *R. conicus* on heads of *C. nutans*, as well as the average number of buds and flowers present on plants of *C. nutans*, soft thistle, *Cirsium carolinianum* (Walter) Fernald & Schubert, and yellow thistle, *C. horridulum* Michaux, were compared each week over a five-week period during weevil oviposition to test for significance among weekly means. These data were normalized using log transformation, and all analyses were performed in SAS 9.2 (SAS Institute, Cary, North Carolina).

Results and Discussion

Comparison of Herbarium and Field Phenologies

Potential host plants of *R. conicus* and *T. horridus* from Tennessee were documented. A single record of *Silybum marianum* (L.) Gaertner is known from Loudon County, Tennessee, and there are no records of any species in the genus *Onopordum* from Tennessee (Chester et al. 1997, TENN 2008). The only *Carduus* species that occurs in Tennessee is *C. nutans* (Chester et al. 1997, TENN 2008). Five native [tall thistle, *Cirsium altissimum* (L.) Sprengel, *C. carolinianum*, field thistle, *C. discolor*, *C. horridulum*, and swamp thistle, *C. muticum* Michaux] and two introduced [bull thistle, *C. vulgare* (Savi) Tenore and Canada thistle, *C. arvense* (L.) Scopoli] *Cirsium* species are known to occur in natural populations in Tennessee (Chester et al. 1997, TENN 2008)

(Table 2). These records of *Carduus nutans* and the seven *Cirsium* species show that these species occur readily in Tennessee.

When only the source (i.e., herbarium or field observations) of the plant data was considered, significantly more buds per plant consistently were documented from field-observed plants of *Carduus nutans* (*F* = 37.02; df = 1, 292; *P* = <0.0001) and *Cirsium discolor* (*F* = 40.96; df = 1, 173; *P* = <0.0001) than herbarium specimens. Conversely, significantly more buds (*F* = 8.12; df = 1, 157; *P* = <0.0050) and flowers (*F* = 18.15; df = 1, 158; *P* = <0.0001) per plant consistently were documented from herbarium specimens of *C. arvense* than field observations.

When interactions between the source and collection month were considered, several thistle species exhibited significant interactions. Significantly more flowers per plant of *Carduus nutans* were observed from herbarium specimens than field-observed plants in May, while significantly more seedheads per plant were documented from field observations than herbarium specimens in June. For plants of *Cirsium carolinianum*, significance in flowers per plant alternated, with significantly more flowers per plant from herbarium specimens than field observations in May and significantly more field-observed flowers per plant than herbarium specimens in June (Fig. 2, Table 4). No significant interactions between source and collection month were observed for any stage of *C. arvense*. Significantly more flowers per plant of *C. vulgare* were observed from herbarium specimens than field observations in June, and greater numbers of buds (August and September), flowers (July), and seedheads (July and September) per plant were recorded from field-observed plants than herbarium specimens (Fig. 3, Table 5). Significantly greater numbers of buds per plant were documented from field observations

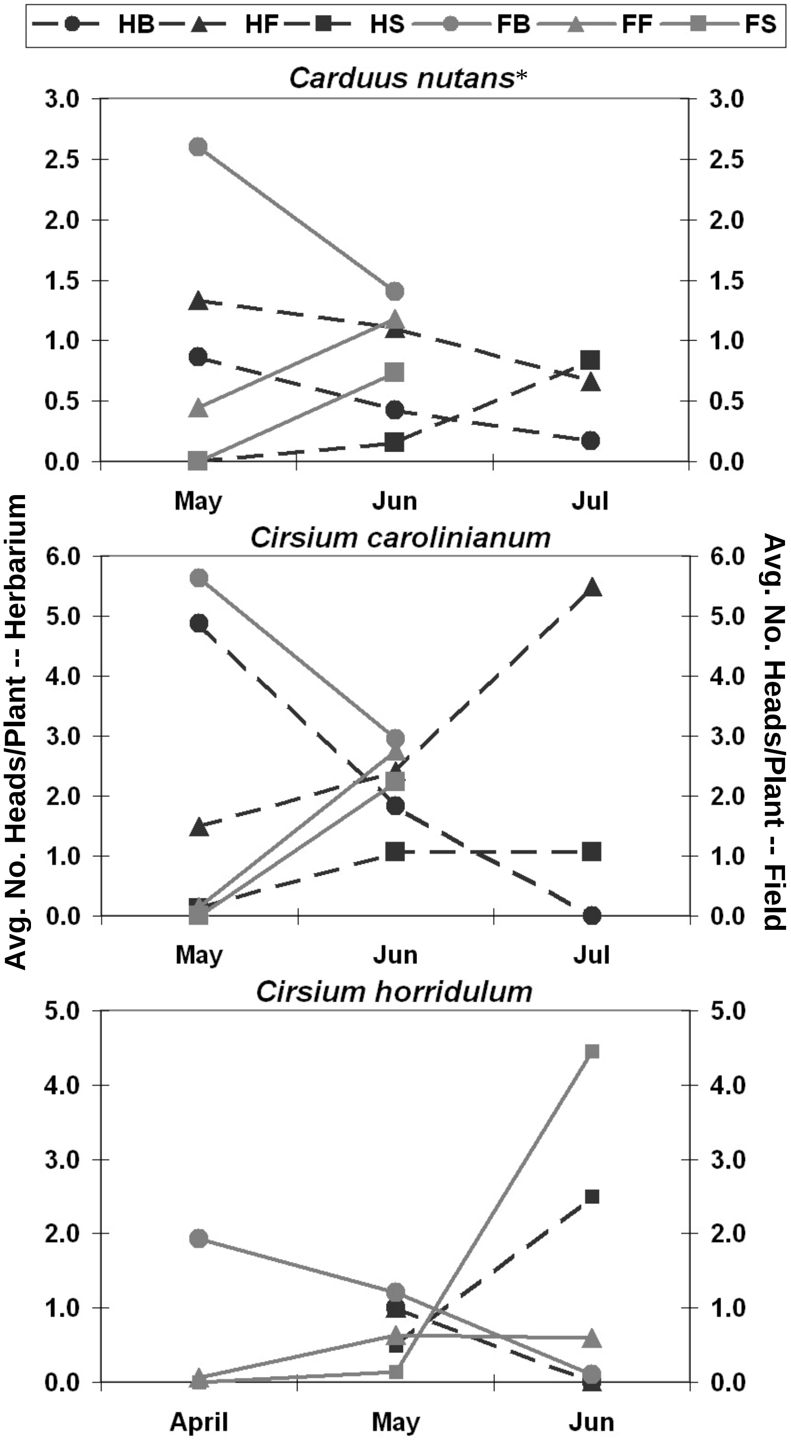

Figure 2. Average number of buds, flowers and seedheads per plant per month of spring-blooming thistles based on specimens from the University of Tennessee Herbarium (dashed lines; HB-buds, HF-flowers, HS-seeds) and field observations (solid lines; FB-buds, FF-flowers, FS-seeds) ('*' – denotes introduced species), 2008. Significance between monthly averages of plant parts from herbarium and field observations is reported in Table 4.

Table 4. Monthly means of buds, flowers, and seedheads recorded from plants of *Carduus nutans*, *Cirsium carolinianum* and *C. horridulum* either housed in the University of Tennessee Herbarium (‘TENN’) or observed in field surveys (‘Field’). Significant ($P \leq 0.05$) interactions between the source of plant data and the collection month were determined using analysis of variance.

Species	Stage	April		May		June		July		ANOVA[1]		
		Field	TENN	Field	TENN	Field	TENN	Field	TENN	*F*	df	*P*
*Carduus nutans**	Bud	.[2]	.	2.60	0.87	1.40	0.42	.	0.17	1.95	1, 292	0.1634
	Flower	.	.	0.45b[3]	1.33a	1.18	1.11	.	0.67	13.48	1, 292	0.0003
	Seedhead	.	.	0	0	0.73a	0.16b	.	0.83	14.98	1, 292	0.0001
Cirsium carolinianum	Bud	.	.	5.63	4.88	2.95	1.82	.	0	0.06	1, 270	0.8047
	Flower	.	.	0.16b	1.50a	2.75a	2.41b	.	5.5	16.50	1, 270	0.0001
	Seedhead	.	.	0	0.13	2.23	1.06	.	1.06	2.15	1, 270	0.1438
Cirsium horridulum	Bud	1.93	.	1.20	1.00	0.10	0	.	.	0.01	1, 109	0.9369
	Flower	0.07	.	0.63	1.00	0.60	0	.	.	2.98	1, 109	0.0869
	Seedhead	0	.	0.13	0.50	4.45	2.50	.	.	0.77	1, 109	0.3820

1 – Analysis of variance *F*-values, degrees of freedom, and levels of significance.

2 – No data were collected for these plant parts during these months.

3 – Means for plant parts from different plant data sources with different letters within the same month exhibited significant interactions between the source of plant data and the collection month, and only significant interactions are labeled with letters.

* – Introduced species.

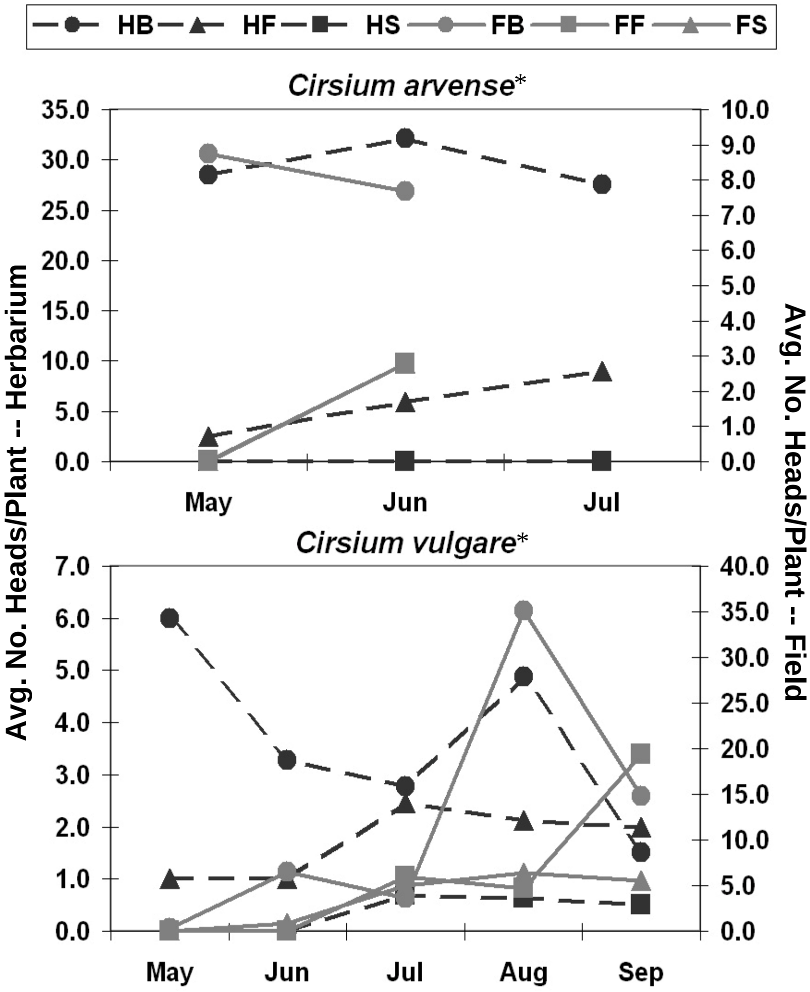

Figure 3. Average number of buds, flowers and seedheads per plant per month of summer-blooming thistles based on specimens from the University of Tennessee Herbarium (dashed lines; HB-buds, HF-flowers, HS-seeds) and field observations (solid lines; FB-buds, FF-flowers, FS-seeds) ('*' – denotes introduced species), 2008. Significance between monthly averages of plant parts from herbarium and field observations is reported in Table 5.

Table 5. Monthly means of buds, flowers, and seedheads recorded from plants of *Cirsium arvense* and *C. vulgare* either housed in the University of Tennessee Herbarium (‘TENN’) or observed in field surveys (‘Field’). Significant ($P \leq 0.05$) interactions between the source of plant data and the collection month were determined using analysis of variance.

Species	Stage	May		June		July		August		September		ANOVA[1]		
		Field	TENN	Field	TENN	Field	TENN	Field	TENN	Field	TENN	*F*	df	*P*
*Cirsium arvense**	Bud	8.75	28.50	7.68	32.13	.[2]	27.50	.	.	.	.	0.03	1, 158	0.8534
	Flower	0.02	2.50	2.78	5.89	.	9.00	.	.	.	.	0.14	1, 158	0.7091
	Seedhead	0	0	2.78	0	.	0	.	.	.	.	2.93	1, 158	0.0890
*Cirsium vulgare**	Bud	0.35	6.00[3]	6.49	3.27	3.64	2.77	35.08a[4]	4.88b	14.78a	1.50b	7.16	4, 180	0.0001
	Flower	0	1.00[3]	0.86b	1.00b	5.05a	2.46b	6.39	2.13	5.54	2.00	5.54	4, 180	0.0003
	Seedhead	0	0	0	0.25	5.92a	0.69b	4.63	0.63	19.41a	0.50b	11.77	4, 180	0.0001

1 – Analysis of variance *F*-values, degrees of freedom, and levels of significance.

2 – No data were collected for these plant parts during these months.

3 – Significance not reported due to no mean calculated, as only one record of *C. vulgare* in May was available from TENN.

4 – Means for plant parts from different plant data sources with different letters within the same month exhibited significant interactions between the source of plant data and the collection month, and only significant interactions are labeled with letters.

* – Introduced species.

of *C. altissimum* than herbarium specimens, and significantly greater flowers per plant were documented from herbarium specimens than field observations in August (Fig. 4, Table 6). Numbers of flowers per plant of *C. discolor* from herbarium specimens were significantly greater than field observations in July and August, but significantly more flowers were documented from field observations than herbarium specimens in September (Fig. 4, Table 6). Finally, significantly more flowers per plant of *C. muticum* were documented from herbarium specimens than field collections in August (Fig. 4, Table 6).

Although differences in mean number of plant parts may occur in each species at some point throughout the year, general plant phenologies from herbarium records and field observations followed similar trends for spring-, summer-, and fall-blooming thistles (Figs. 2-4). The discrepancy in monthly mean numbers of plant parts between field observations and herbarium specimens may reflect a collection bias for plant specimens that are suitable and practical for display and storage. Thistle species found in Tennessee have flowerheads ranging in size from ca. 10 to 30 mm in diameter, and the number of heads can range from less than 10 to more than 100 on a single plant. Species that possess larger heads or produce many buds and flowers may be impractical to mount and/or display, so specimens with fewer heads may be preferred by plant collectors to represent the species in collections.

Additionally, several specimen species (*C. altissimum*, *C. carolinianum, C. discolor*, *C. vulgare,* and *Carduus nutans*) from the herbarium showed a significantly higher number of blooms in months at the start of the blooming period than field-observed data (Tables 4, 5, and 6). Again, plants that are in bloom may be preferred by

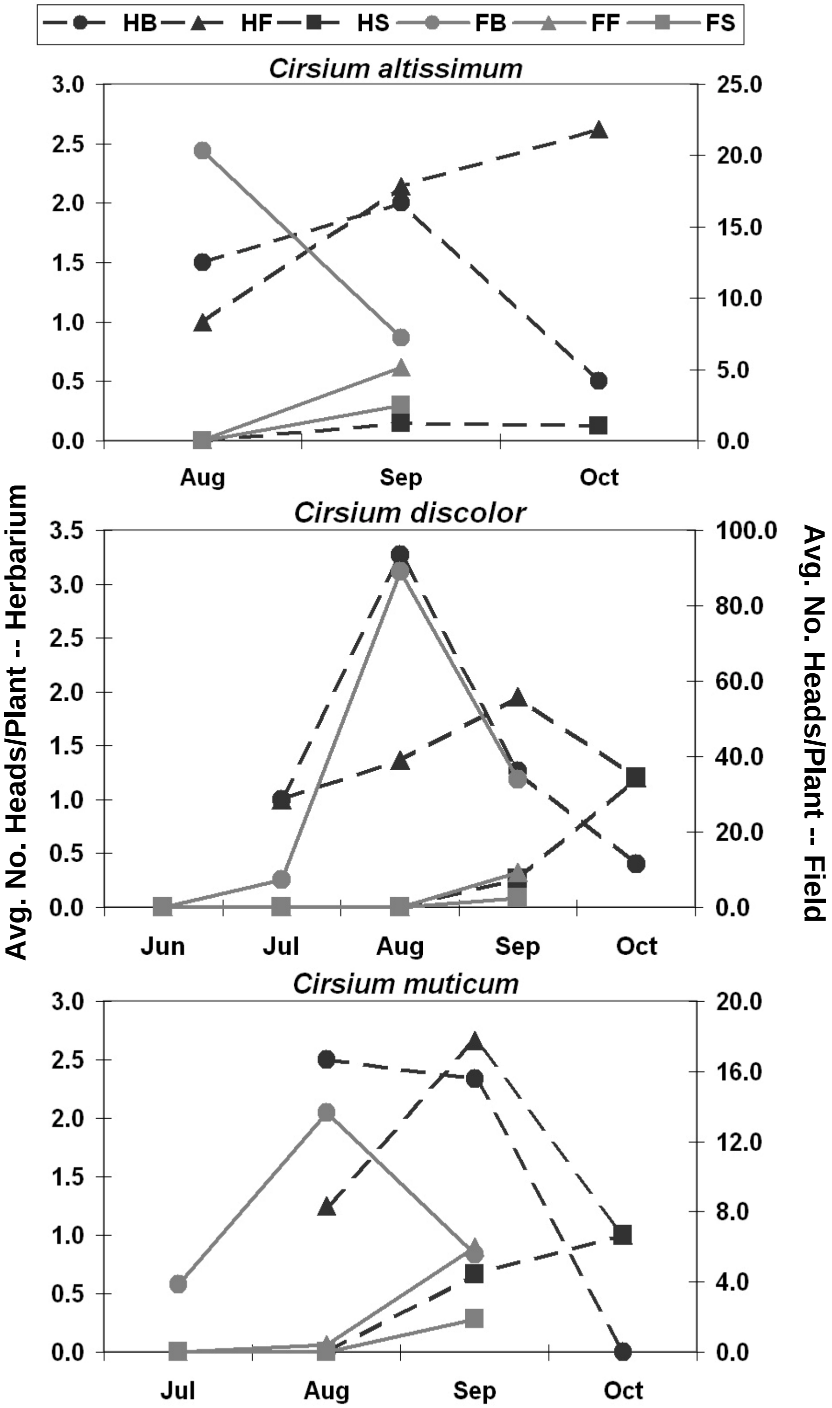

Figure 4. Average number of buds, flowers and seedheads per plant per month of fall-blooming thistles based on specimens from the University of Tennessee Herbarium (dashed lines; HB-buds, HF-flowers, HS-seeds) and field observations (solid lines; FB-buds, FF-flowers, FS-seeds), 2008. Significance between monthly averages of plant parts from herbarium and field observations is reported in Table 6.

Table 6. Monthly means of buds, flowers, and seedheads recorded from plants of *Cirsium altissimum*, *C. discolor*, and *C. muticum* either housed in the University of Tennessee Herbarium ('TENN') or observed in field surveys ('Field'). Significant ($P \leq 0.05$) interactions between the source of plant data and the collection month were determined using analysis of variance.

Species	Stage	July		August		September		October		ANOVA[1]		
		Field	TENN	Field	TENN	Field	TENN	Field	TENN	*F*	df	*P*
Cirsium altissimum	Bud	.[2]	.	20.30a[3]	1.50b	8.19a	2.00b	.	0.50	6.00	1, 147	0.0155
	Flower	.	.	0.05b	1.00a	3.68	2.14	.	2.63	6.29	1, 147	0.0132
	Seedhead	.	.	0	0	1.26	0.14	.	0.13	1.10	1, 147	0.2951
Cirsium discolor	Bud	7.35	1.00	89.05	3.27	33.79	1.26	.	0.40	0.39	1, 173	0.6755
	Flower	0b	1.00a	0.10b	1.36a	9.35a	1.96b	.	1.20	9.70	1, 173	0.0001
	Seedhead	0	0	0	0	2.53	0.26	.	1.20	1.30	1, 173	0.2747
Cirsium muticum	Bud	3.83	.	13.65	2.50	5.57	2.33	.	0	2.96	1, 157	0.0873
	Flower	0	.	0.40b	1.25b	6.00	2.67	.	1.00	23.08	1, 157	0.0001
	Seedhead	0	.	0	0	1.83	0.67	.	1.00	0.23	1, 157	0.6289

1 – Analysis of variance with corresponding *F*-values, degrees of freedom, and levels of significance.

2 – No data were collected for these plant parts during these months.

3 – Means for plant parts from different plant data sources with different letters within the same month exhibited significant interactions between the source of plant data and the collection month, and only significant interactions are labeled with letters.

collectors for herbarium specimens (i.e., displaying the critical reproductive stage and primary traits used to identify species) instead of those that are in a vegetative or pre-bloom stage. Therefore, the differences between bloom numbers may be due to selective collection rather than a developmental difference between the two groups.

Herbarium specimens are valuable records of a facet of natural history within a region, and can serve as reference materials for plant identification and general plant biology within that region. When working with field populations of plants, herbarium records could be used to obtain general localities of plant populations, estimate when surveys could be conducted, ascertain a general timeframe of when a specific plant stage may be present, and provide an example of the appearance of specific stages of plant development. However, statistical differences between the two sources of plant material from each collection month listed above illustrate that if abundance of specific plant parts (such as flowers) or other plant growth characteristics during a specific time period is an important factor for studies, surveys, etc., monitoring of naturally-occurring populations should be implemented to properly estimate plant growth characteristics in the field.

Comparison of Literature and Field Phenologies

As with the herbarium specimens, field-observed phenologies generally coincided with the flowering periods listed in the literature (Fig. 5). Cronquist (1980) and Carman (2001) coincided with field observations of *C. horridulum*, although both list the flowering period throughout the summer. All sources reflected the field-observed phenology of *C. carolinianum*. However, Weakley (2008) listed the initiation of flowering earlier in the year for both *C. horridulum* and *C. carolinianum*. Cronquist (1980) and Wofford (1989) listed the flowering period of the introduced thistles (*Carduus*

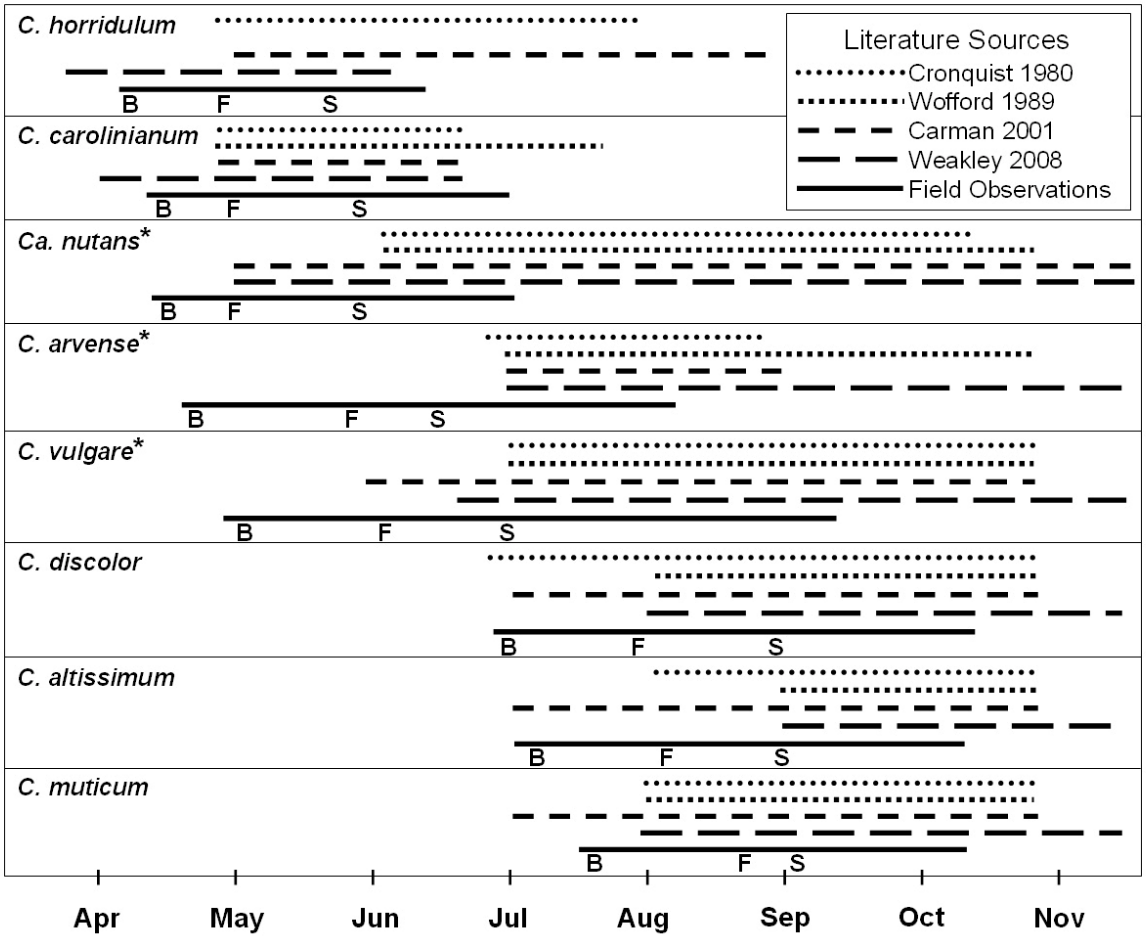

Figure 5. Comparative thistle phenologies based on literature and field observations. All phenologies based on literature sources display only the general flowering period. Phenologies based on field observations depict the initiation of each stage of plant development (B=bud, F=flower, S=seed), 2005-2008.

nutans, *Cirsium arvense*, and *C. vulgare*) later in the year than field observations, while Carman (2001) and Weakley (2008) were closer to field observed data for *Carduus nutans* and *C. vulgare*. The flowering period listed for *C. discolor* by Cronquist (1980) and Carman (2001) was earlier than observed in the field, and Carman (2001) listed flowering times for *C. altissimum* and *C. muticum* earlier as well. Wofford (1989) and Weakley (2008) list the flowering period for *C. altissimum* later than observed, and all sources list the flowering period for *C. muticum* earlier than observed (Fig. 5).

Literature sources list general flowering periods, because variation in flowering period may occur based on weather, elevation, soil and other site conditions. Also, several literature sources (Cronquist 1980, Wofford 1989, Weakley 2008) estimated plant phenologies in their broadest terms, as plant data for these sources were incorporated from across a broad geographic region (i.e., several states) rather than in one particular state or physiographic province. Therefore, exact agreement with literature sources is not expected with all thistle species.

The marked differences between literature and field observations for the introduced thistle species are curious. The flowering periods of *C. arvense*, *C. vulgare*, and *Carduus nutans* were listed as initiating about one month after what was observed in field popultations by at least two authors. It is unclear why these differences were observed. Flowers were present on herbarium specimens of each of these three species during the months flowering initiated in field-observed populations (Figs. 2 and 3, Tables 4 and 5). Additionally, field-observed data in this study support observations by Sudbrink et al. (2001), in that populations of *C. arvense* observed during 1997-1998 showed that in eastern Tennessee plants began flowering in late May, began initiating seedheads in mid June, were producing seed or senescing in late August and mostly senescing or vegetative by October. The earlier flowering of *C. arvense* observed by Sudbrink et al. (2001) and the flowering period of all three introduced thistle species documented during this study increase the likelihood of exposure of these thistle introduced species to infestation by *R. conicus*.

Despite the discrepancies among the herbarium, literature, and field-collected data, the phenologies of the thistles species in Tennessee have little variation over time (herbarium records date from 1898 to present) and reflect what is present in the current literature. As with comparisons of herbarium and field-observed data, literature sources provide valuable information on general plant phenologies, but field monitoring should be incorporated into any study involving natural populations to properly estimate phenologies of locally-occurring plants.

Although field observations for these studies were conducted in eastern Tennessee (from Van Buren County eastward), plant populations were surveyed across a range of elevations and latitudes that occur within this region. The range of conditions found across eastern Tennessee may emulate climatic conditions in other areas of the state and influence plant phenologies similarly. Accordingly, plant phenologies in field populations in other areas of Tennessee are not expected to be drastically different from what was observed during this study.

Weevil Phenologies and Incidence

Weevil phenologies were estimated using the seasonal infestation levels of *R. conicus* and *T. horridus* on *Carduus nutans*. The average number of adult *R. conicus* per plant peaked during the week of 11 May, while the average number of eggs of *R. conicus* per head peaked the following week (18 May) (Fig. 6). Adult *T. horridus* also were found in spring plant surveys at low levels in early May and decreased through June (Fig. 7). Vacuum samples of *C. nutans* rosettes from January through March 2009 documented the presence of *T. horridus* (between 2.0 and 11.6 adults per sample) throughout this study period (Fig. 7). These data correspond to previous phenologies of both weevils observed in Virginia (Surles et al. 1974, Surles and Kok 1976, Trumble and Kok 1979, Sieburth et al. 1983) and Tennessee (Grant et al. 1990, Powell et al. 1996).

Both species of weevil were documented from *Cirsium* thistles during this study. Eggs of *R. conicus* were documented on *C. arvense*, and adults were documented from *C. arvense* and *C. vulgare* (Fig. 8). Both of these thistle species are introduced and considered invasive. During this study, adult *T. horridus* were documented from five *Cirsium* species, including three native thistles (*C. discolor*, *C. horridulum*, and *C. muticum*). Although *R. conicus* was not documented on the native *C. carolinianum* or *C. horridulum*, and neither species has been recorded as a host for *R. conicus* in other areas of the U.S., these two species may be at the greatest risk of non-target feeding by this weevil in Tennessee based on their phenologies. However, the infestation levels of these two introduced weevils on *Cirsium* thistles are much lower than the levels at which they infest *Carduus nutans*. For example, a total of 56 *T. horridus* were observed on *C. vulgare* (Fig. 8) from 8 May through 18 June, much less than the 219 adults of *T. horridus* observed on *Carduus nutans* (the target host) for the same time period.

Comparison of Weevil and Thistle Phenologies

Based on these observations, several thistle species in Tennessee are synchronous with the development of either *R. conicus* or *T. horridus* or both. Adult *R. conicus*

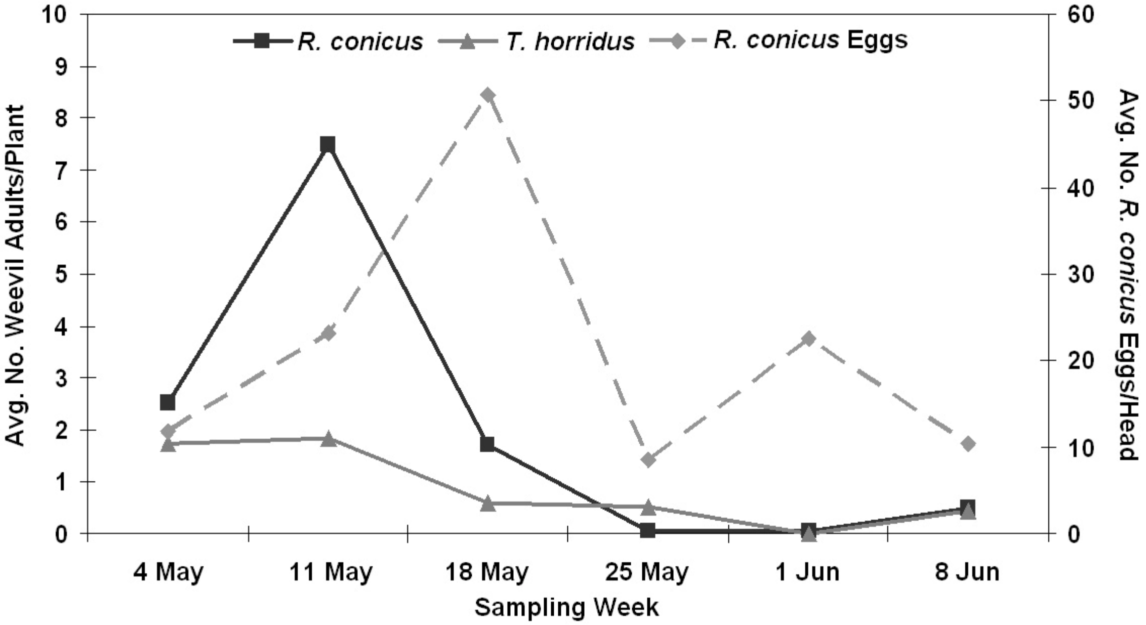

Figure 6. Average number of adult *Rhinocyllus conicus* and *Trichosirocalus horridus* per *Carduus nutans* plant and average number of eggs of *R. conicus* per head per each sampling week, 2008.

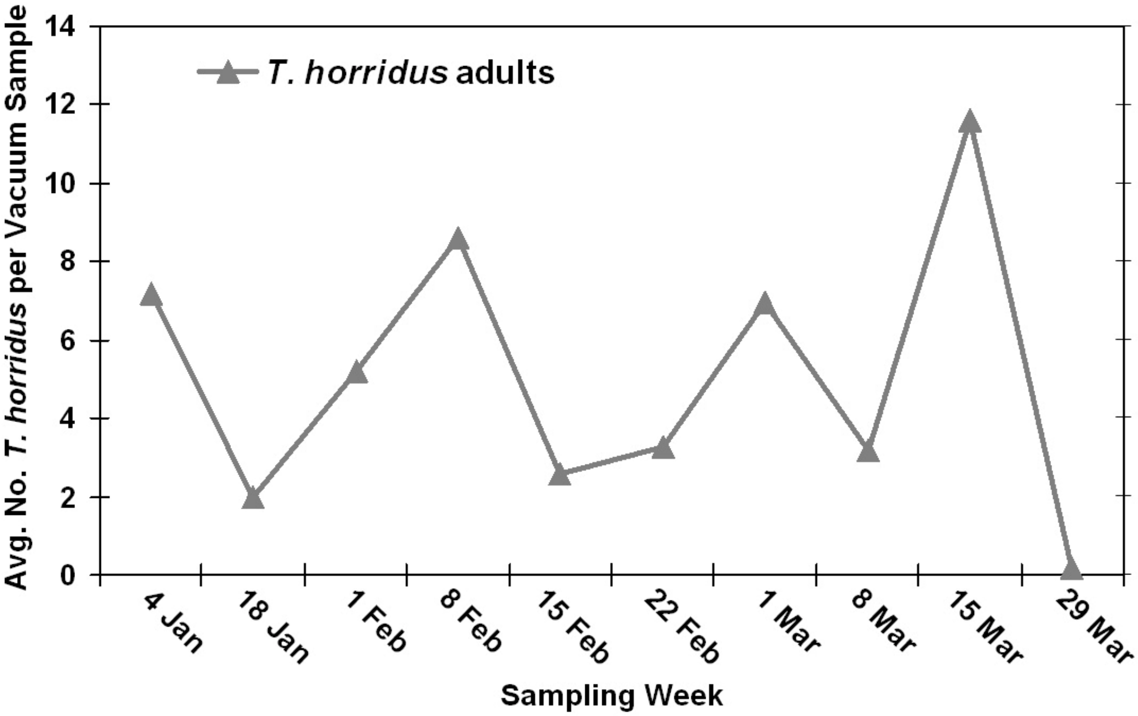

Figure 7. Average number of *Trichosirocalus horridus* per vacuum sample (2 m x 2 m area) of rosettes of *Carduus nutans*, January through March, 2009.

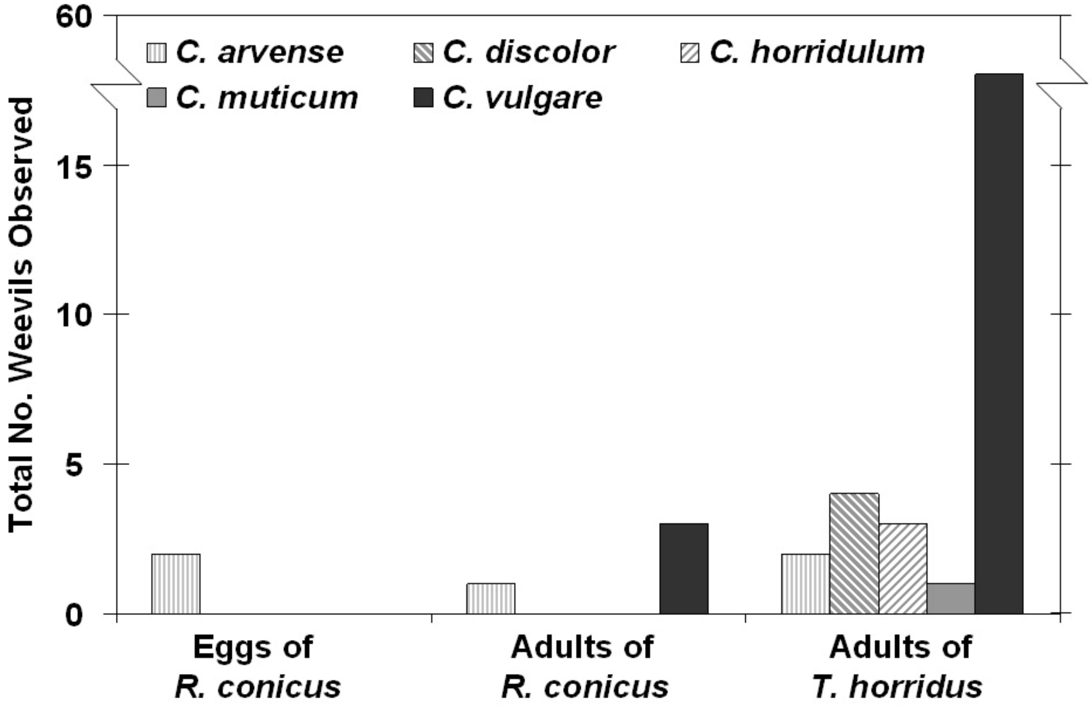

Figure 8. Total number of *Rhinocyllus conicus* (eggs and adults) and *Trichosirocalus horridus* (adults) observed on *Cirsium* thistles in field surveys, 2008.

become active and begin laying eggs in late April/early May when buds and/or flowers of *C. nutans*, *Cirsium arvense*, *C. carolinianum*, *C. horridulum*, and *C. vulgare* are present (Figs. 2, 3, 6, and 7). In plant and weevil surveys conducted in 2008, significantly ($P \leq 0.05$) more buds per plant of *C. horridulum* were observed the week of 4 May than any following week. Significantly ($P \leq 0.05$) more buds per plant of *C. carolinianum* were documented during the weeks of 11 May and 18 May than during the weeks of 4 May and 25 May. Concurrently, egg levels of *R. conicus* (observed on *Carduus nutans*) were greater than 20 eggs per bud of *C. nutans* during the first three weeks of May (Fig. 9). Significantly ($P \leq 0.05$) more flowers per plant of *Cirsium carolinianum* were observed the weeks of 25 May and 1 June than the three previous weeks, and eggs of *R. conicus* were observed at densities of 29.75 eggs per flower of *Carduus nutans* during the week of 1 June (Fig. 10). Significantly ($P \leq 0.05$) more flowers per plant of *Cirsium horridulum* were observed during the week of 25 May than any other week, and eggs of *R. conicus* were observed at densities of 10.16 eggs per flower of *Carduus nutans* during this week (Fig. 10). Flowering of *C. horridulum* may have increased during the week of 18 May, but no observations of *C. horridulum* were made during that week.

The budding and flowering of *C. carolinianum* and *C. horridulum* during the period of mating and oviposition of *R. conicus* provides the appropriate conditions in which the weevil could expand its host range to include these two native thistles. So, while no *R. conicus* were observed on non-target native thistle species during this study period, the potential for non-target activity exists due to the synchronous phenologies of these thistles and the weevil. Non-target activity of *R. conicus* on *C. canescens* was not observed until 21 years after releases of the weevil for biological control of *Carduus*

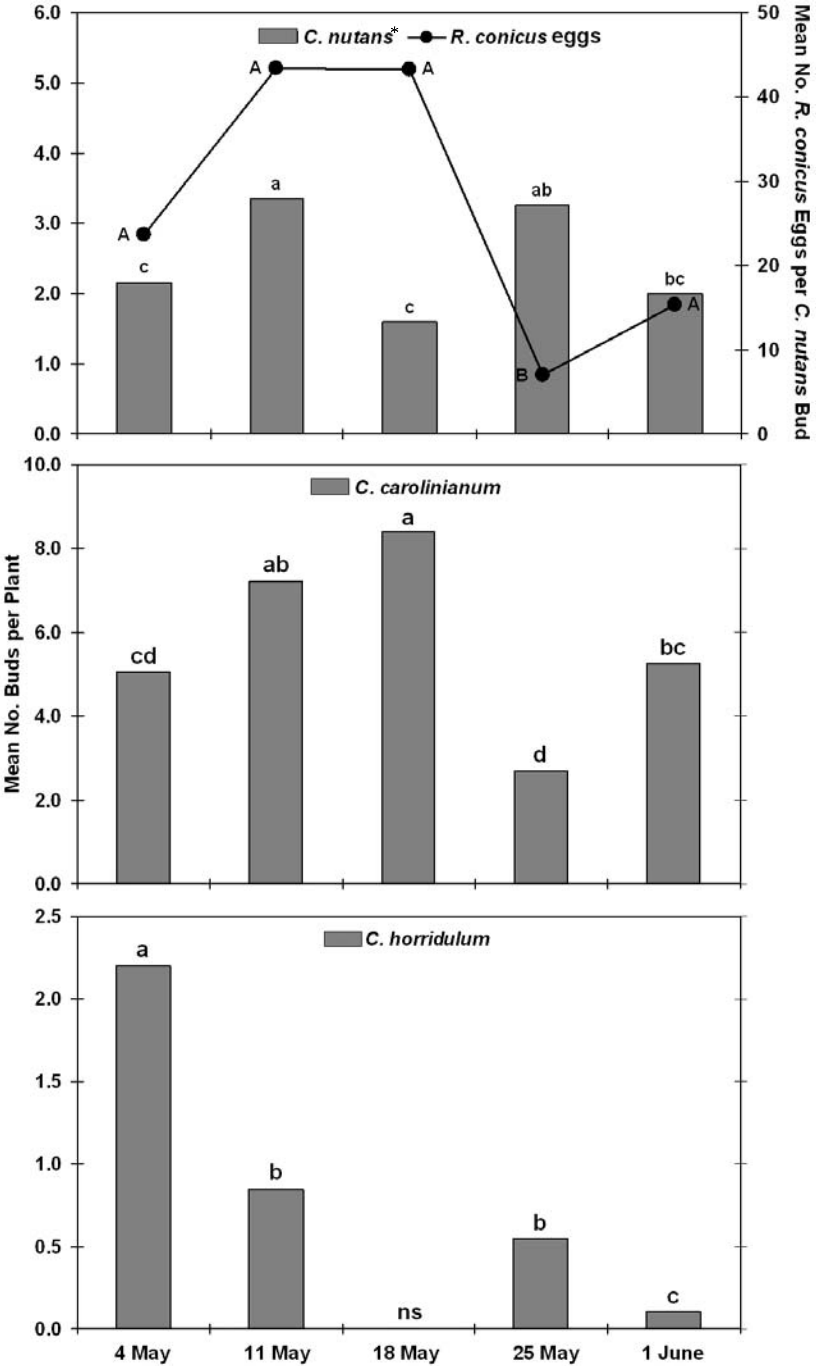

Figure 9. Weekly average numbers of eggs of *Rhinocyllus conicus* on buds of *Carduus nutans* and weekly average numbers of buds per plant of *C. nutans*, *Cirsium carolinianum*, and *C. horridulum*, 2008 (‘*’ denotes introduced thistle species). Columns and data points with different letters denote significant ($P \leq 0.05$) differences within each species, and ‘ns’ denotes no samples collected during that date for *C. horridulum*.

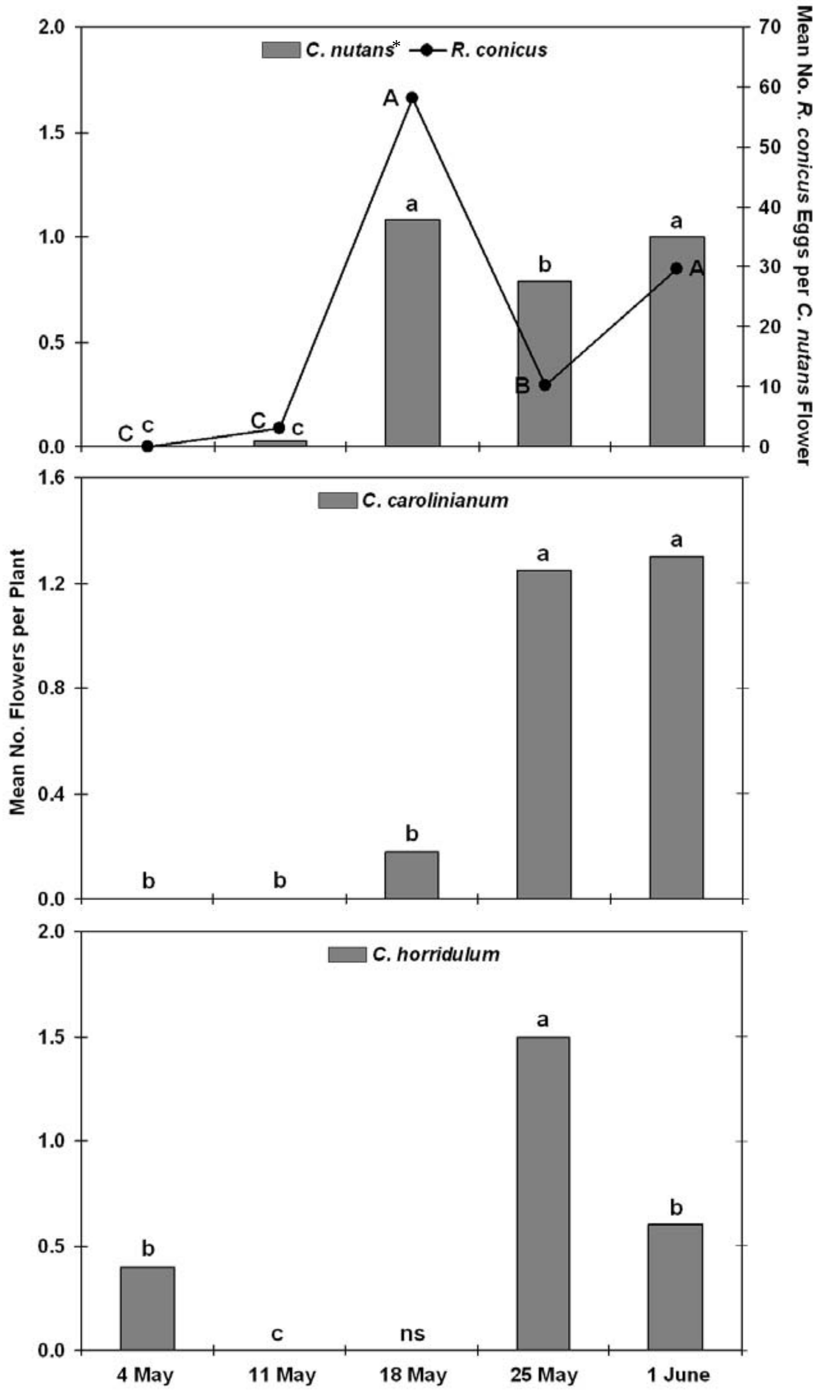

Figure 10. Weekly average numbers of eggs of *Rhinocyllus conicus* on flowers of *Carduus nutans* and weekly average numbers of flowers per plant of *C. nutans, Cirsium carolinianum*, and *C. horridulum*, 2008 ('*' denotes introduced thistle species). Columns and data points with different letters denote significant ($P \leq 0.05$) differences within each species, and 'ns' denotes no samples collected during that date for *C. horridulum*.

nutans in Nebraska in 1972 (Louda 1998). Additionally, *R. conicus* was released in low numbers in Wisconsin in 1975 and only recently was observed utilizing the state-threatened *C. hillii* (Canby) Fernald as a host (Sauer and Bradley 2008). Although the preference of *R. conicus* to feed on *Carduus nutans* at greater levels than other thistle species is well documented (Surles and Kok 1977, Rees 1978, Zwölfer and Harris 1984, Arnett and Louda 2002), non-target feeding of *R. conicus* on native *Cirsium* continues to occur throughout much of the U.S. Therefore, it is uncertain if or when *R. conicus* may expand its host range to include native *Cirsium* thistles in Tennessee.

Because all of the thistles in Tennessee overwinter as rosettes, all thistles may be at potential risk of infestation by *T. horridus*. The consistent presence of adult *T. horridus* over a three-month period when all thistle species in Tennessee are in the rosette stage demonstrates the opportunity that is present for this weevil to feed on non-target thistle species (Fig. 7). Although adults of *T. horridus* were observed on some non-target thistle species, this occurrence does not necessarily signify a host range expansion. The harmful stage of *T. horridus* is the larval stage, and the adult weevils may vicariously feed on leaf tissues of a wide array of plants until they aestivate during the summer. However, should *T. horridus* begin to oviposit on native thistles and larvae utilize them as hosts, it is difficult to estimate the impact larval feeding may have on native thistles or anticipate which thistles may become new hosts. Species of thistle that produce small rosettes, such as *C. carolinianum* and *C. arvense*, may be at a reduced risk of infestation, as Sieburth and Kok (1982) observed that *T. horridus* oviposited and developed at higher levels on larger rosettes of *Carduus nutans* and *C. acanthoides* in Virginia. Additionally, few observations of non-target feeding by *T. horridus* have been documented. McAvoy et al.

(1987) found that rosettes of introduced thistle species in Virginia were infested at much greater levels (*C. nutans* – 54%, *C. acanthoides* and *Cirsium vulgare* – 20%) with larvae of *T. horridus* than the native *C. discolor* (ca. 6%), and only recently were *T. horridus* documented to infest *C. altissimum* in Nebraska (Takahashi et al. 2009). Finally, the infestation of native thistles by *T. horridus* may not have any significant impact on overall plant reproduction, as seed numbers of *Carduus nutans* were not reduced when rosettes were infested with low numbers of *T. horridus* larvae (<20 per plant) (Milbrath and Nechols 2004a).

Several species of native and exotic thistles may be potentially susceptible to infestation by either *R. conicus* or *T. horridus* or both. However, the weevils may not prefer or be able to reproduce on some of these species due to many unknown factors. As the size of rosettes may limit the utilization of some thistles by *T. horridus*, the smaller size of flowerheads of some native thistles relative to *C. nutans* may be less preferable to *R. conicus*. For example, flowerheads of *Cirsium carolinianum* are similar in size to those of *C. arvense*, both of which are much smaller than flowerheads of *C. nutans* (ca. 8-10 mm vs. ca. 30 mm, respectively). Although *C. arvense* flowers later in the year (which may effect frequency of infestation), buds are present when *R. conicus* is ovipositing. This smaller head size may not be utilized or preferred by *R. conicus*, as infestation rates of *C. arvense* by *R. conicus* in eastern Tennessee are low (Fig. 7; unpublished data).

While general seasonal information on native *Cirsium* thistles and the introduced weevils exists in the literature, this study synthesizes these existing data with the current conditions of phenological co-occurrence of each weevil species and their potential plant hosts. The knowledge gained should allow agencies or institutions that are interested in conservation of native plants to consider the development of programs to monitor for species of interest to them. Additional studies to document the specific impacts of each weevil species on each thistle species are crucial to understanding the current and potential interactions in this insect-plant species complex. This study not only provides specific insights into the potential risks of non-target host utilization by introduced weevils in Tennessee, but also adds to the greater knowledge of how introduced species become integrated into the ecology of new areas.

CHAPTER III. NON-TARGET ACTIVITY OF *RHINOCYLLUS CONICUS* AND *TRICHOSIROCALUS HORRIDUS* ON NATIVE *CIRSIUM* POPULATIONS IN TENNESSEE

Introduction

The biennial herbaceous plant musk thistle, *Carduus nutans* L., has become a prominent weed species since its introduction into North America from Europe in the mid-1800s (Stuckey and Forsyth 1971). Since that time, musk thistle has spread to all but three states in the continental U.S., where it is considered a noxious weed species in 25 states (USDA 2008). As part of a classical biological control program against musk thistle, two weevils [*Rhinocyllus conicus* Fröelich and *Trichosirocalus horridus* (Panzer) (Coleoptera: Curculionidae)] were introduced from Europe and first released in the U.S. in Virginia in 1969 (Surles et al. 1974, Ward et al. 1974). In many areas where these weevils have been released, musk thistle populations have decreased dramatically (Rees 1977, Lambdin and Grant 1996, Kok 2001).

Although weevils reduced musk thistle populations in many areas where released, non-target feeding on native thistles in the genus *Cirsium* has been documented for both *R. conicus* and, to a lesser extent *T. horridus*. Non-target feeding of *R. conicus* has been documented on at least 25 species of native *Cirsium*, mostly in the central and western U.S. (Rees 1977, Zwölfer and Harris 1984, Goeden and Ricker 1986, Goeden and Ricker 1987, Turner et al. 1987, Turner and Herr 1996, Louda et al. 1997, Pemberton 2000, Sauer and Bradley 2008). Documentation of non-target feeding by *T. horridus* on native *Cirsium* has been observed only on field thistle, *C. discolor* (Muhlenberg ex Willdenow) Sprengel (McAvoy et al. 1987) and tall thistle, *C. altissimum* (L.) Sprengel (Takahashi et al. 2009).

While the taxonomy of *R. conicus* has remained stable since the initiation of the biological control program, *T. horridus* recently was divided into a complex of three species: *T. horridus*, and two sibling species, *T. briesei* Alonso-Zarazaga and Sanchez-Ruiz and *T. mortadelo* Alonso-Zarazaga and Sanchez-Ruiz (Alonso-Zarazaga and Sanchez-Ruiz 2002). It is believed that the weevils released in North America are either *T. mortadelo*, *T. horridus*, or a mixture of both species (Alonso-Zarazaga and Sanchez-Ruiz 2002). It is unclear which species are present in Tennessee. Adults of both *T. mortadelo* and *T. horridus* feed on the foliage of the plant and damage the midribs of the leaves of the rosettes during oviposition, which has little impact on plant health (Trumble and Kok 1979). Larvae feed on the meristems of the developing rosettes, causing necrosis of the meristem leading to plant deformities and/or sometimes death (Ward et al. 1974, Grant et al. 1990).

Upon establishment in areas of release, both weevils became widely distributed as part of musk thistle biological control programs throughout the continental U.S. (Trumble and Kok 1982, McDonald et al. 1994, Kok 2001, Gassmann and Kok 2002). Initial releases of *Trichosirocalus* spp. in Tennessee were made on a small scale in 1978 with weevils obtained from P. Dunn, United States Department of Agriculture, Albany CA. Large-scale releases of both weevils were made in 1989 in 11 counties as part of a multi-year research program funded by the Tennessee Department of Transportation and the Federal Highway Administration with weevils obtained from L. Kok, Virginia Polytechnic Institute and State University, VA (Lambdin and Grant 1989, Grant et al.1990). Following the establishment of these weevils on musk thistle in several release sites, additional releases were made throughout eastern and middle Tennessee throughout the mid-to-late 1990s as part of a multi-year regional program funded by the Sustainable Agriculture Research and Education/Agriculture in Concert with the Environment (SARE/ACE) granting program. Populations of *Trichosirocalus* spp. also were established in areas of eastern Tennessee prior to these releases, dispersing into Tennessee from release areas in Virginia (Ward et al. 1974, McAvoy et al.1987). However, follow-up studies to fully assess establishment, spread, or impact of these biological control agents in Tennessee have been limited (Lambdin and Grant 1989, Lambdin and Grant 1992, Grant and Lambdin 1993). In 2005, surveys were initiated to investigate the incidence of both *R. conicus* and *T. horridus* on native thistles in Tennessee.

Materials and Methods

Naturally-occurring populations of *Cirsium* thistles were surveyed in 16 counties in eastern Tennessee (Fig. 11). These native species included *C. altissimum*, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, *C. discolor*, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux From each population, 10-20 plants, depending on total population numbers, were collected and examined for the presence of adult weevils (255 plants examined in 2005, 543 plants in 2006, and 425 plants in 2008) (Table 7). Adult weevils were collected and identified using Arnett et al. (2002) and genitalia characters presented in Alonso-Zarazaga and Sanchez-Ruiz (2002). Species identification using male and female adults was confirmed by C. O'Brien, Green Valley, AZ, and R. Anderson, Canadian Museum of Nature, Ottawa, Ontario, Canada.

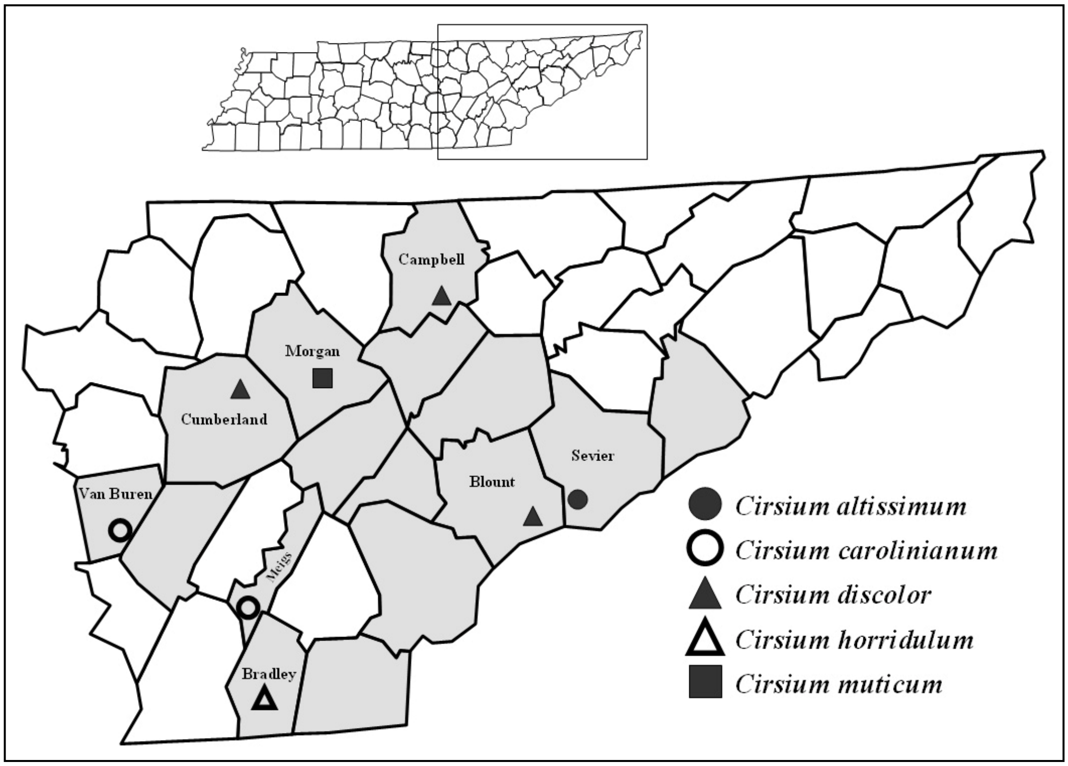

Figure 11. Counties surveyed for non-target feeding by introduced weevils on native thistles (gray), and sites where non-target feeding by *Trichosirocalus horridus* was documented.

Table 7. Levels of infestation of native *Cirsium* thistles in Tennessee by *Trichosirocalus horridus*, 2005-2008*.

Thistle species	% (no.) plants infested			Total weevils/total plants				Mean weevils/ plant
	2005	2006	2008	2005	2006	2008	Total	
Cirsium altissimum	25.00 (5)	1.33 (1)	0	6/20	1/75	0/80	7/175	0.040
C. carolinianum	2.50 (2)	0	0	2/80	0/60	0/100	2/240	0.008
C. discolor	3.75 (3)	5.64 (23)	5.00 (4)	13/80	69/408	7/80	89/568	0.157
C. horridulum	0	n/a	5.00 (4)	0/75	n/a	4/80	4/155	0.026
C. muticum	n/a	n/a	1.77 (1)	n/a	n/a	1/85	1/85	0.012

* Thistle populations were not examined for weevil activity during 2007.

Results and Discussion

Of 1,223 plants examined from 2005 through 2008, no non-target activity of *R. conicus* was observed. However, adult *Trichosirocalus* spp. were documented occurring on all five native *Cirsium* species, and all were determined to be *T. horridus* (Table 7, Fig. 11). It is important to note that low numbers of adults were observed on the leaves of mature *C. carolinianum* (0.008 per plant) and *C. horridulum* (0.026 per plant), and only one adult was observed from 85 plants of *C. muticum* (0.012 per plant). These weevils were probably feeding opportunistically on the leaf tissues of *C. carolinianum* and *C. horridulum*, as the plants exhibited feeding damage similar to that seen on the foliage of musk thistle, yet the meristems exhibited no damage. These adults may have developed and matured in nearby musk thistle populations and dispersed into the native thistle populations upon emergence in spring. Although only one *T. horridus* adult was documented from *C. muticum*, two other rosettes examined in that population had damaged meristems. It is unclear if this damage was caused by larvae of *T. horridus* or one of the native weevil species (*Baris* spp., *Conotrachelus* spp., *Listroderes* spp., or *Rhodobaenus* spp.) that have been documented on native thistles in this area. Field-collected larvae died in the laboratory, and identification could not be confirmed. Larvae could have died due to improper rearing conditions in the laboratory, the age of the larvae (perhaps too young to complete development) within plant tissues, or other factors.

Adults of *T. horridus* were observed in the greatest numbers and on the greatest percentage of plants of *C. altissimum* and *C. discolor*. However, the percentage of infested *C. altissimum* during 2005 was greater than percentages observed in subsequent years due to the relatively low number of total plants sampled, coupled with the relatively high numbers of plants infested with *T. horridus* (Table 7). When data were combined over the three-year period, *T. horridus* was documented from 3.43% of all *C. altissimum* plants (*n* = 6 of 175). Therefore, infestation levels observed in 2006 and 2008 may be more typical of infestation of *C. altissimum* by *T. horridus*. Nonetheless, this weevil may be utilizing these species as a reproductive host, as some plants exhibited meristematic damage in conjunction with scars along the midribs of some leaves, possibly indicating oviposition.

These occurrences are the first reports of *T. horridus* feeding to any degree on *C. carolinianum*, *C. horridulum*, or *C. muticum* in the U.S. and only the second record of non-target feeding on *C. altissimum* (Piper and Coombs 2004, Takahashi et al. 2009). Furthermore, the infested populations of *C. altissimum* in Sevier Co. and *C. discolor* in Blount Co. are both within the borders of the Great Smoky Mountains National Park (GRSM). Releases of *T. horridus* were not made in GRSM, and this finding is the first record of *T. horridus* in the GRSM. The occurrence of *T. horridus* in GRSM probably resulted from the natural dispersal of this weevil into the area from pastures and roadsides infested with musk thistle outside its borders.

The presence of *T. horridus* on these native thistle species is not unexpected, as it feeds and develops within the rosettes of several introduced European thistle species (*Carduus nutans*, *C. acanthoides* L., *Cirsium vulgare* and others) in the U.S. (Ward et al. 1974, Kok 1975, Sieburth and Kok 1982, McAvoy et al.1987). Because all *Cirsium* species exhibit a rosette stage as part of their development, the likelihood exists that *T. horridus* would eventually utilize new hosts for reproduction.

Conversely, the absence of *R. conicus* on any plants is somewhat unexpected. Although none of the *Cirsium* species from which *R. conicus* has been documented in other regions of the U.S. occur within Tennessee, the seemingly broad host range of *R. conicus* would imply that non-target feeding would occur in Tennessee as well. Continued monitoring of native *Cirsium* species in Tennessee is needed to more fully understand their relationship with this introduced weevil.

These observations of *T. horridus* infesting native thistle species indicate that prolonged and continued exposure increases the potential for non-target interactions among native and introduced species with synchronous biologies. However, the impact of feeding by larvae of *T. horridus* on the reproductive potential of native plants is uncertain. While larval feeding in the rosettes of musk thistle is believed to cause reduced fitness and contribute to reduction in thistle populations, plants rarely die or are prevented from producing seed, even with high larval infestations (ca. 40-50 larvae/rosette) (Sieburth et al. 1983, Milbrath and Nechols 2004a, b). Also, several native insect species feed within the meristematic tissues of native thistles, either in the rosette stage or after the plant has begun to elongate, with marginal impact on plant seed production (unpublished data). Further research and monitoring will elucidate the relationship between these introduced weevils and native thistle species.

CHAPTER IV. IMPACTS OF LARVAL FEEDING OF *RHINOCYLLUS CONICUS* ON PLANT REPRODUCTION AND LEVELS OF HOST UTILIZATION ON FIELD-CAGED TARGET AND NON-TARGET THISTLES IN TENNESSEE

Introduction

Musk thistle, *Carduus nutans* L., is a biennial herbaceous species native to Europe, western Asia, and the Mediterranean (Hodgson and Rees 1976, Zwölfer and Harris 1984). It was first introduced into North America in the mid-1800s (Stuckey and Forsyth 1971) and is now recorded from 45 U.S. states and at least five Canadian provinces (McCarty 1978, Desrochers et al. 1988, USDA 2008). The introduction of exotic natural enemies was promoted and projected to provide a long-term, sustainable approach to reducing populations of musk thistle. Releases of *Rhinocyllus conicus* Fröelich were first made in Canada in 1968 (Frick 1978b), and were followed in 1969 by releases in California (Goeden 1978), Montana (Hodgson and Rees 1976), and Virginia (Surles et al. 1974). Upon establishment, *R. conicus* was widely redistributed as part of musk thistle biological control programs throughout the continental U.S. (Trumble and Kok 1982, Townsend et al. 1991, Lambdin and Grant 1992, Buntin et al. 1993, Gassmann and Kok 2002). Releases of *R. conicus* were made throughout Tennessee from 1989 through the 1990s, with populations of musk thistle reduced by 94% in some areas (Lambdin and Grant 1992, Grant and Lambdin 1993, Lambdin and Grant 1996).

While early host specificity tests and host data from its native range suggested that *R. conicus* could feed and develop on several genera and species of thistles in the tribe Cardueae (Asteraceae), the projected benefits of its release as a biological control agent outweighed the potential limitations (Zwölfer and Harris 1984, Boldt 1997). *R.*

conicus subsequently was approved for release into the U.S. (Surles et al. 1974, Frick 1978a). Recent documentation of non-target impacts of *R. conicus* on native thistles in the north central and western U.S., however, illustrates the importance of assessing the impact of these weevils on target and non-target thistles in areas where they were released. The first record of *R. conicus* feeding on native North American thistles in the genus *Cirsium* was in Montana and Canada on *Cirsium undulatum* (Nuttall) Sprengel and *Cirsium flodmannii* (Rydberg) Arthur (Rees 1977, 1978, Zwölfer and Harris 1984). Insect surveys of native thistles in California documented larvae of *R. conicus*, which is the developmental stage of the weevil most damaging to plants, feeding on natural populations of 13 native *Cirsium* species (Turner et al. 1987). One of these species [*Cirsium fontinale* (Greene) Jepson var *obispoense* J. T. Howell] is listed as Federally endangered (Turner and Herr 1996), and three [*Cirsium campylon* Sharsmith, *Cirsium ciliolatum* (Henderson) J. T. Howell, and *Cirsium hydrophilum* (Greene) Jepson var *vaseyi* (Gray) J. T. Howell] are potential candidates to be listed as Federally threatened or endangered (Goeden and Ricker 1986, 1987, Turner et al. 1987).

While the significance and impact of *R. conicus* on many of these native species are not yet known, studies of the feeding of *R. conicus* on Platte thistle, *C. canescens* Nuttall, have documented significant impacts due to larval feeding within flowerheads. Larvae of *R. conicus* were first documented feeding in the flowerheads of *C. canescens* in Nebraska in 1993, and since that time *R. conicus* populations have grown to infest over 25% of observed *C. canescens* plants (Louda et al. 1997, Louda 1998, Louda 2000a). Additionally, feeding of *R. conicus* in seedheads reduced the number of viable seeds in infested heads by 86%, which is predicted to decrease population numbers of *C.*

canescens (Louda 2000a). Concern exists that *R. conicus* will continue its host range expansion and utilize Pitcher's thistle [*Cirsium pitcheri* (Torrey) Torrey and Gray], a Federally listed threatened species that is closely related to *C. canescens* (Pavlovic et al. 1992, Louda 1998, Louda 2000b).

The impact of *R. conicus* on non-target thistle species in the southern Appalachians is poorly known. Five native species of thistles in the genus *Cirsium* are found in Tennessee [tall thistle, *C. altissimum* (L.) Sprengel, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, field thistle, *C. discolor* (Muhlenberg ex Willdenow) Sprengel, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux] (Fig. 12) (TENN 2008). *C. carolinianum* and *C. horridulum* bloom in the spring (May through July), with the remaining native thistles (*C. altissimum*, *C. discolor*, and *C. muticum*) blooming later in the year (approximately from August through October) (Wofford 1989, Gleason and Cronquist 1991). These native species are widely distributed throughout the eastern U.S., each occurring in at least 15 states in addition to Tennessee (USDA 2008). No non-target activity of *R. conicus* on native thistles has been reported in this region.

Two introduced *Cirsium* species [Canada thistle, *C. arvense* (L.) Scopoli, and bull thistle, *C. vulgare* (Savi) Tenore] also occur in Tennessee (TENN 2008), and both species are considered invasive in the state (Fig. 12) (TNEPPC 2008). Both *C. arvense* and *C. vulgare* have wide flowering periods and can bloom from late June through October in the southern Appalachians (Wofford 1989). These two introduced species can serve as hosts of *R. conicus*, but at lower levels than *Carduus nutans*.

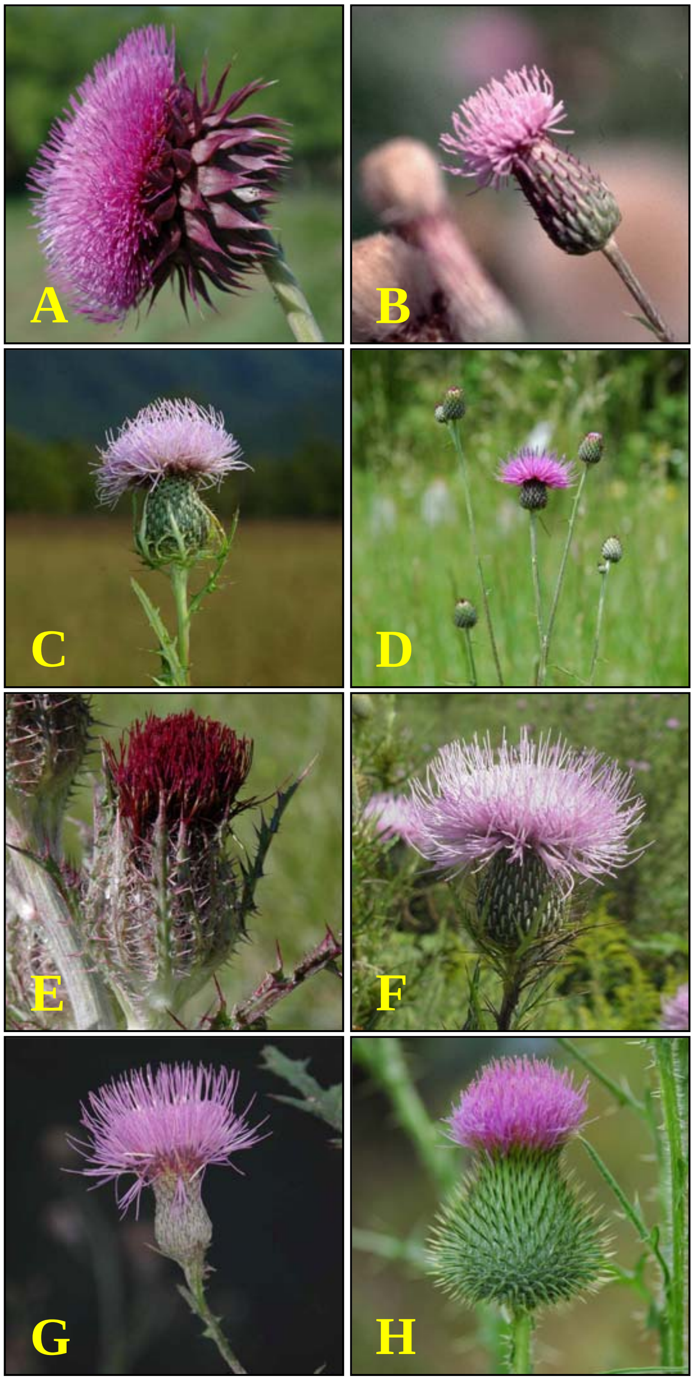

Figure 12. Native and introduced thistle species occurring in Tennessee: A) *Carduus nutans, B) *Cirsium arvense**, C) *C. altissimum*, D) *C. carolinianum*, E) *C. horridulum*, F) *C. discolor*, G) *C. muticum*, and H) *C. vulgare** ('*' denotes introduced species). Photo of *C. arvense* courtesy of Integrated Pest Management and Biological Control Laboratory, The University of Tennessee.**

In surveys conducted from 2004 through 2008, no *R. conicus* were observed on non-target native thistles (Chapter 3). However, controlled studies are necessary to quantify levels of impact (tissue damage, decreased seed numbers, etc.) these weevils may have on plants, should they begin utilizing native thistles. Caged plant studies are an effective way to restrict herbivory to specific areas of plants, allowing quantification of the impacts of a known number of weevils on a known number of plant parts. Therefore, a study was initiated in 2008 using field-caged plants to evaluate the impact of larval feeding of *R. conicus* on plant reproduction and assess levels of host utilization of native and introduced thistles in eastern Tennessee.

Materials and Methods

Adults of *R. conicus* were collected from field populations of *C. nutans* in Knox and Cumberland Counties, Tennessee, for use in this study. Beginning 22 April 2008, adult *R. conicus* were shaken from bolting stems of *C. nutans* into sweep nets. Adult weevils were then placed in a clear plastic container (31 x 31 x 41 cm with four 12-cm screened holes for ventilation) with bouquets of clipped *C. nutans* and taken to the laboratory, where they were held and observed for mating activity. Two pairs of copulating *R. conicus* were placed in one 29.6 ml cup (two male and two female weevils per cup) with a moistened cotton ball which was then sealed with a plastic lid and held in a growth chamber at 15 °C until weevils were placed on caged plants in the field. Adult weevils were contained in cups no longer than four days prior to use in study.

From 24 April to 7 September 2008, one to two populations each of *C. nutans* and the seven *Cirsium* species that occur in Tennessee were selected, and plants from each population were caged to study the impact of introduced weevils (Table 8). To enclose *R. conicus* adults on plants, one nylon mesh bag (45 x 50 cm; Delnet® pollination bags) was placed on the mainstem of each plant, and the opening of the bag was bunched together around the plant stem and tied with plastic flagging. In each study population, one of three treatments was applied to each plant (10-20 plants per treatment per population, 60 plants per thistle species; Table 8): terminal heads of mainstems enclosed in mesh bags with four field-collected *R. conicus* adults (two male, two female), terminal heads of mainstems enclosed in mesh bags with no weevils (caged control), or plants flagged, but left uncaged as an open control. Treatments were left on the plants for four weeks. After this time, plants of *C. nutans*, *Cirsium arvense*, *C. carolinianum*, and *C. horridulum* were flowering and/or initiating seeding and, therefore, were clipped at ground level, placed in a plastic bag, and taken to the laboratory for processing. Weevils were removed from the bags of *C. altissimum*, *C. discolor*, *C. muticum*, and *C. vulgare* after four weeks of exposure to weevils, and the cages were left on these species until collected from the field in mid-summer to fall to prevent other herbivorous insects from accessing the caged plant parts (Table 8). In the laboratory, the head width of buds, flowers, and seedheads per plant, and numbers of seeds per flowerhead were measured and recorded. Also, the number of eggs, larvae, pupae and adults of *R. conicus* were recorded from all buds, flowers, and seedheads.

This caged plant study was modified before it was repeated in 2009 (Table 9). Treatments of *R. conicus* were restricted to *Carduus nutans*, *Cirsium arvense*, *C. carolinianum*, *C. horridulum*, and *C. vulgare*, as *C. altissimum*, *C. discolor*, and *C. muticum* do not bud or flower during the time the weevil larvae require those plant

Table 8. Site information for *Carduus nutans* and seven *Cirsium* species at 13 field populations used to study the effects of *Rhinocyllus conicus* on caged plants, 2008.

Thistle species[1]	County	Duration[2]
*Carduus nutans**	Knox	30 Apr – 27 May
	Roane	21 May – 10 June
Cirsium altissimum	Polk	24 Apr – 18 August
*C. arvense**	Knox	6 May – 13 June
	Anderson	27 May – 23 June
C. carolinianum	Bledsoe	2 May – 30 May
	Cumberland	7 May – 3 June
C. discolor	Cumberland	21 May – 7 September
	Cumberland	21 May – 7 September
C. horridulum	Bradley	29 April – 27 May
	Bradley	29 April – 27 May
C. muticum	Morgan	13 May – 20 August
*C. vulgare**	Knox	8 May – 17 July

1 – One population containing 60 plants each of *C. altissimum*, *C. muticum* and *C. vulgare* were used during the study. Two populations containing 30 plants each were used for all other species included in this study. Treatments (caging with adult *R. conicus*, caging without adult *R. conicus*, and uncaged plants) were divided equally among the plants within a population.

2 – Duration = the time period that treatments were applied to plants before they were taken to the laboratory for processing.

* – Introduced species.

Table 9. Site information for *Carduus nutans* and four *Cirsium* species at nine field populations[1] (30 plants per population) used to study the effects of *Rhinocyllus conicus* on caged plants, 2009.

Thistle species	County	Duration[2]
*Carduus nutans**	Roane	29 April – 29 May
	Knox	29 April – 6 June
*Cirsium arvense**	Knox	19 May – 30 June
	Anderson	19 May – 14 June
C. carolinianum	Bledsoe	8 May – 7 June
	Cumberland	8 May – 6 June
C. horridulum	Bradley	28 April – 1 June
	Bradley	28 April – 1 June
*C. vulgare**	Roane	28 May – 22 June

1 – One population containing 30 plants of *C. vulgare* was used during the study. Two populations containing 30 plants each were used for all other species included in this study. Treatments (caging with adult *R. conicus*, caging without adult *R. conicus*, and uncaged control plants) were divided equally among the plants within each population.

2 – Duration = the time period that treatments were applied to plants before they were taken to the laboratory for processing.

* – Introduced species.

structures to be available. Beginning 26 April 2009, adult *R. conicus* were collected from field populations of *C. nutans* in Knox and Cumberland Counties, Tennessee, and were processed for use in caged plant studies in the same manner implemented in 2008. As in 2008, weevils used in the field study were retained in cups for no longer than four days prior to use in the study. From 28 April to 30 June 2009, treatments were applied to plants from two populations (30 plants per population) of each thistle species, except for a single population of *Cirsium vulgare* (no other suitable population was located) (Table 9). At each population, one of three treatments was applied to each of 30 plants (10 plants per treatment): apical buds/flowers of mainstems enclosed in mesh bags with four field-collected *R. conicus* adults (two male, two female), apical buds/flowers of mainstems enclosed in mesh bags with no weevils (caged control), or plants flagged, but left uncaged as an open control (Table 9). Treatments remained in the field until a majority (ca. >50 %) of the caged heads initiated flowering, after which plants were clipped at ground level, placed in a plastic bag, and taken to the laboratory for processing.

In the laboratory, plant height, the head width diameter of buds, flowers, and seedheads per plant were measured, and the number of eggs of *R. conicus* were recorded. Unlike 2008, where heads of plants were immediately dissected, heads of all plants were retained in a growth chamber (26 °C) for two to four weeks following removal from the field to allow maturation of immature *R. conicus* to adulthood. Following this period, number of larvae, pupae and adults of *R. conicus* from all buds, flowers, and seedheads, and numbers of seeds per flowerhead were recorded. Body length of adult *R. conicus* has been used as a measure of weevil fitness, with smaller adults believed to be less fit to mate and/or produce fewer offspring. Body size also is used as an indicator of host suitability, with host plants that produce larger weevils considered more suitable than those that produce smaller adults (Rowe and Kok 1984, Turner et al. 1987). The lengths (distance between the anterior edge of the eyes to posterior tip of elytra in mm) of all adult *R. conicus* recovered from plants caged with *R. conicus* of each thistle species were measured using a Ziess® Stemi SVG microscope with an ocular micrometer calibrated with a Mini-scale® measuring scale (0.1mm increments) and recorded.

Analysis of variance was performed to compare the number of seeds produced by control plants with seed production of weevil-treated plants. For cage studies conducted in 2008, significance ($P \leq 0.05$) among the mean numbers of seeds per flower for each treatment and each thistle species were compared using least significant differences. Data for each species were normalized using either log transformation (*Cirsium altissimum*, *C. carolinianum*, *C. discolor*, *C. horridulum*, and *C. muticum*) or rank transformation (*C. arvense*, *C. vulgare*, *Carduus nutans*). For cage studies conducted in 2009, data for all species were rank transformed, and significant ($P \leq 0.05$) differences among mean seed numbers per flower for each treatment and each thistle species were compared using least significant differences.

Analysis of variance also was performed on plants caged with *R. conicus* to compare measures of utilization of thistle species by *R. conicus* and to estimate the influence of each thistle species on adult weevil fitness. Ovipositional activity on each thistle species was estimated by comparing significance ($P \leq 0.05$) among the mean number of eggs of *R. conicus* per head (bud, flower and seedhead) using least significant differences. As a general comparison of the levels of host utilization of each thistle species, significance ($P \leq 0.05$) among mean number of *R. conicus* (total larvae, pupae, and adults) per head was determined using least significant differences. Data for egg numbers and *R. conicus* per head were normalized using log transformation. As a standardized measure of host utilization, significance ($P \leq 0.05$) among the mean number of *R. conicus* per centimeter of head width diameter of each thistle species was estimated using least significant differences, and data were normalized using rank transformation. To estimate the influence of thistle species on adult weevil fitness based on body length, the least significant differences test was used to determine significant ($P \leq 0.05$) differences among the mean body lengths of adult *R. conicus* reared from weevil-treated plants of the different thistle species in 2009, and data were normalized using rank transformation. The mean length of adult *R. conicus* recovered from caged and uncaged plants of *C. nutans* were compared separately to test for cage effects on length of *R. conicus* using least significant differences and rank-transformed data. All analyses were conducted using SAS 9.2 (SAS Institute, Cary, North Carolina).

Results and Discussion

This study documented several significant interactions among *R. conicus* and thistle species in Tennessee. Significantly ($P \leq 0.05$) more seeds per flower were produced by caged and uncaged control plants of *Cirsium carolinianum* and *C. vulgare* and caged control plants of *C. arvense* and *C. horridulum*, when compared to plants caged with four adult *R. conicus* in 2008 (Fig. 13A). Compared to caged control plants, both spring-blooming native thistle species showed reductions (by ca. 85% in *C. carolinianum* and ca. 35% in *C. horridulum*) in seed numbers due to larval feeding of *R. conicus* (Fig. 13A). No significant ($P \leq 0.05$) differences were observed in seeds per

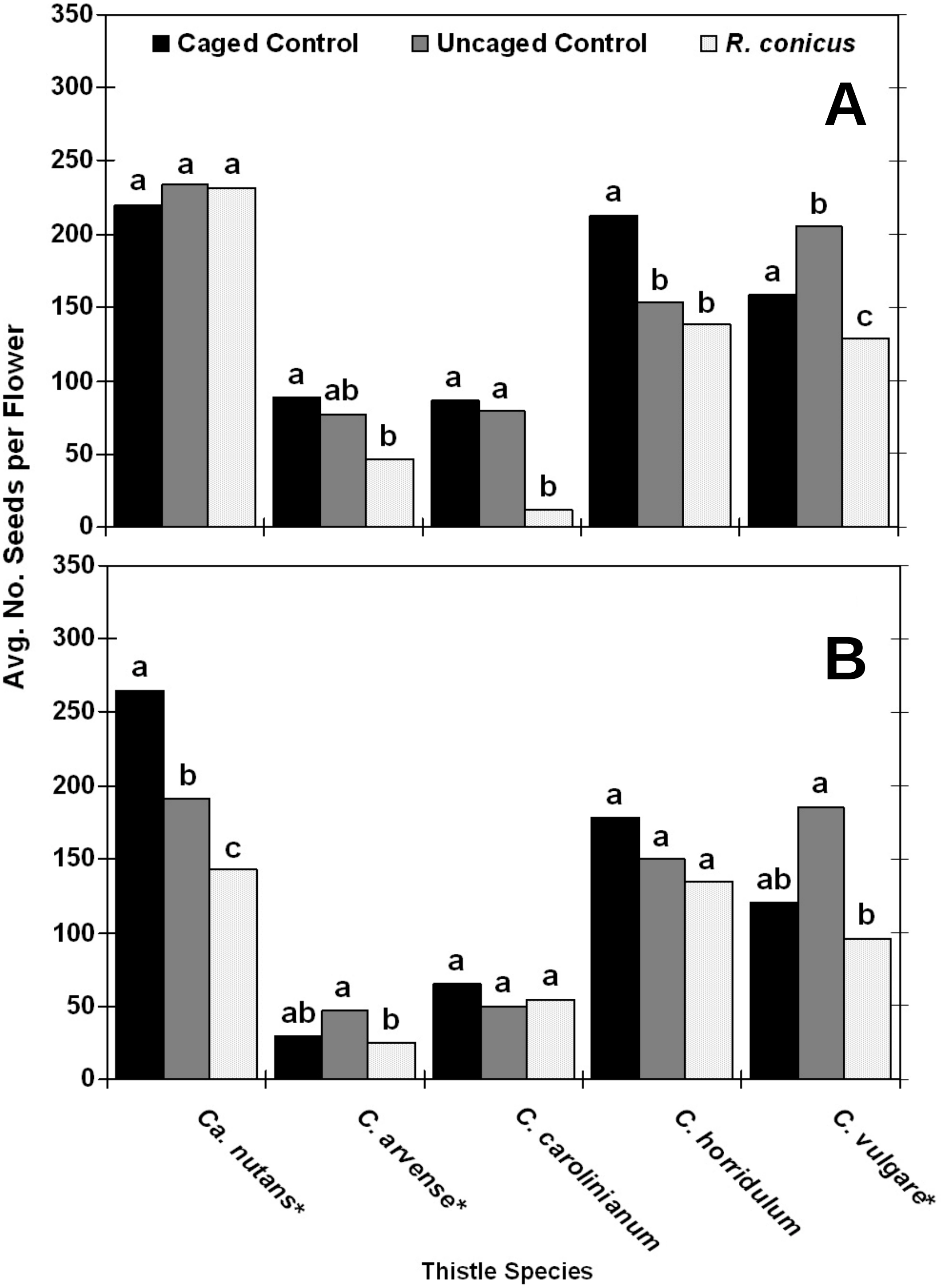

Figure 13. Average number of seeds per flower of each thistle species among plants caged with *Rhinocyllus conicus* and caged and uncaged control (no *R. conicus* applied) plants, A) 2008 and B) 2009 ('*' denotes introduced thistle species). Columns with different letters within species denote significant (*P* $\leq$ 0.05) differences.

flower among treatments in fall-blooming thistles (*C. altissimum*, *C. discolor*, and *C. muticum*, not shown). In 2009, significantly ($P \leq 0.05$) more seeds were produced by uncaged control plants of *C. arvense* and *C. vulgare* when compared to plants caged with four adult *R. conicus*, and although not significant ($P \leq 0.05$), seed production of native thistles caged with *R. conicus* was reduced (by ca. 16% in *C. carolinianum* and ca. 24% in *C. horridulum*) when compared to caged controls (Fig. 13B). Although there were no significant ($P \leq 0.05$) differences in seeds per flower among treatments on plants of *Carduus nutans* in 2008, significantly ($P \leq 0.05$) fewer seeds were produced by plants caged with *R. conicus* (ca. 46% reduction compared to caged control) than by caged and uncaged control plants in 2009 (Fig. 13).

The differences observed among levels of feeding on these *Cirsium* thistles over the two-year study period may be indicative of the preference of *R. conicus* to feed on *Carduus nutans*. Levels of feeding by *R. conicus* (i.e., none) were as expected on the fall-blooming species in 2008, as these species do not develop buds necessary for larval development during the time *R. conicus* is reproductively active in the spring. Despite larval feeding and development in heads of spring- and summer-blooming *Cirsium* species enclosed in cages with *R. conicus*, only low levels of *R. conicus* feeding have been documented on *Cirsium arvense* and *C. vulgare* in naturally-occurring populations, and no non-target feeding has been documented on *C. carolinianum* or *C. horridulum* (see Chapters 2 and 3). It is important to note that all larvae in caged plants treated with *R. conicus* were the offspring of two females per plant, so perhaps low levels of non-target feeding of *R. conicus* are all that is required to negatively impact seed production of some thistle species. Although the possibility of feeding by *R. conicus* on the non-target native species in this study exists, the extent to which it may occur in natural populations, if ever, remains unclear.

While the impact of *R. conicus* on seed numbers of *C. nutans* was as expected in 2009, the lack of differences among control plants and plants exposed to *R. conicus* in 2008 is not indicative of these plant/insect interactions in Tennessee. Only flowerheads of each species were included in the analyses, because it is difficult to accurately determine potential seed numbers in buds or general seed numbers in seedheads that have begun to senesce and lose seeds. However, many buds and seedheads were present on plants of *C. nutans* when the plants were collected from the field in 2008, and statistical estimates were made on a small number of flowerheads of *C. nutans* ($n = 12$). When seedheads were also included in the analyses (total heads analyzed = 48), significantly fewer seeds were observed in open control plants (mean = 119.97; $t = 5.66$; df = 47; $P < 0.0001$) than heads caged with *R. conicus* (mean = 199.41; $t = 10.44$; df = 47; $P < 0.0001$) or caged control plants (mean = 279.20; $t = 8.54$; df = 47; $P < 0.001$), and significant differences were observed between heads caged with *R. conicus* and caged control plants. The provision of allowing more than ca. 50% of heads to begin flowering in 2009 instead of leaving plants in the field for a set amount of time allowed for more uniform seed counts from greater numbers ($n = 120$) of flowerheads of *C. nutans* in 2009.

On heads caged with *R. conicus*, significantly ($P \leq 0.05$) more eggs per head were recorded on *C. nutans*, *Cirsium carolinianum*, and *C. horridulum* than on *C. arvense* and *C. vulgare* in 2008 (Fig. 14A). In 2009, significantly ($P \leq 0.05$) more eggs were recorded on *Carduus nutans* and *Cirsium horridulum* than *C. arvense*, *C. carolinianum*, and *C. vulgare* (Fig. 14B). Both *C. arvense* and *C. vulgare* bud and bloom later in the year than

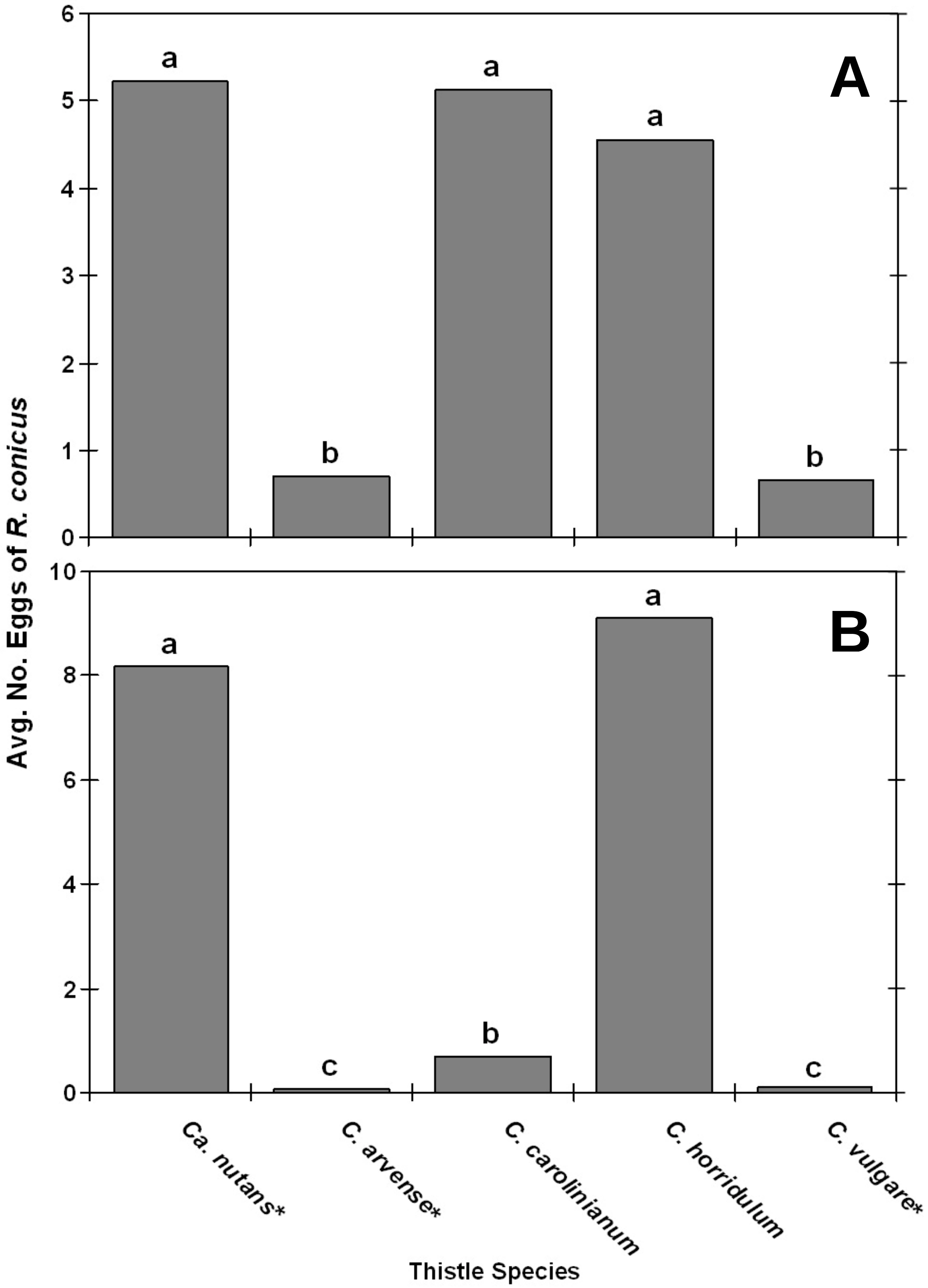

Figure 14. Average number of eggs of *Rhinocyllus conicus* per head for each thistle species caged with *R. conicus*, A) 2008 and B) 2009 (‘*’ denotes introduced thistle species). Columns with different letters among species denote significant ($P \leq 0.05$) differences.

does *Carduus nutans*, and are not utilized as frequently as host plants by *R. conicus* (personal observation). Therefore, lower levels of oviposition are expected on these species when compared to *C. nutans*. Plants of both *Cirsium carolinianum* and *Cirsium horridulum* exhibited levels of oviposition comparable to those observed on *Carduus nutans* in 2008. While in 2009 similar levels of oviposition of *R. conicus* were observed on *C. nutans* and *Cirsium horridulum*, *R. conicus* oviposited on *C. carolinianum* at much lower levels than the previous year. The reason for differences in egg numbers on *C. carolinianum* between years is unclear, but oviposition of *R. conicus* on this species in both years indicates suitability as a potential host species.

Further examination of heads caged with *R. conicus* documented significantly ($P \leq 0.05$) more *R. conicus* recorded per head of *C. nutans* than from any other thistle species in 2008 and 2009 (Fig. 15). However, significantly ($P \leq 0.05$) more *R. conicus* per centimeter of plant head (bud, flower, or seedhead) width were observed in *Cirsium carolinianum* than other species in 2008, with significantly ($P \leq 0.05$) more *R. conicus* per centimeter head width observed in *C. nutans* in 2009 (Fig. 15B). Host utilization (total weevils/head) also was significantly ($P \leq 0.05$) greater in *C. horridulum* than *C. vulgare* in 2008 and *C. arvense* in both years. The higher levels of infestation of both native thistles (*C. carolinianum* and *C. horridulum*) compared to *C. arvense* and *C. vulgare* (both introduced thistles) indicate that these native species may be at least as suitable a host species, although no naturally-occurring non-target activity has been observed in either species (Chapters 2 and 3, Fig. 15).

While the mean number of *R. conicus* per head can be influenced by the head width of the plant (i.e., larger heads provide greater area for more larvae), the mean

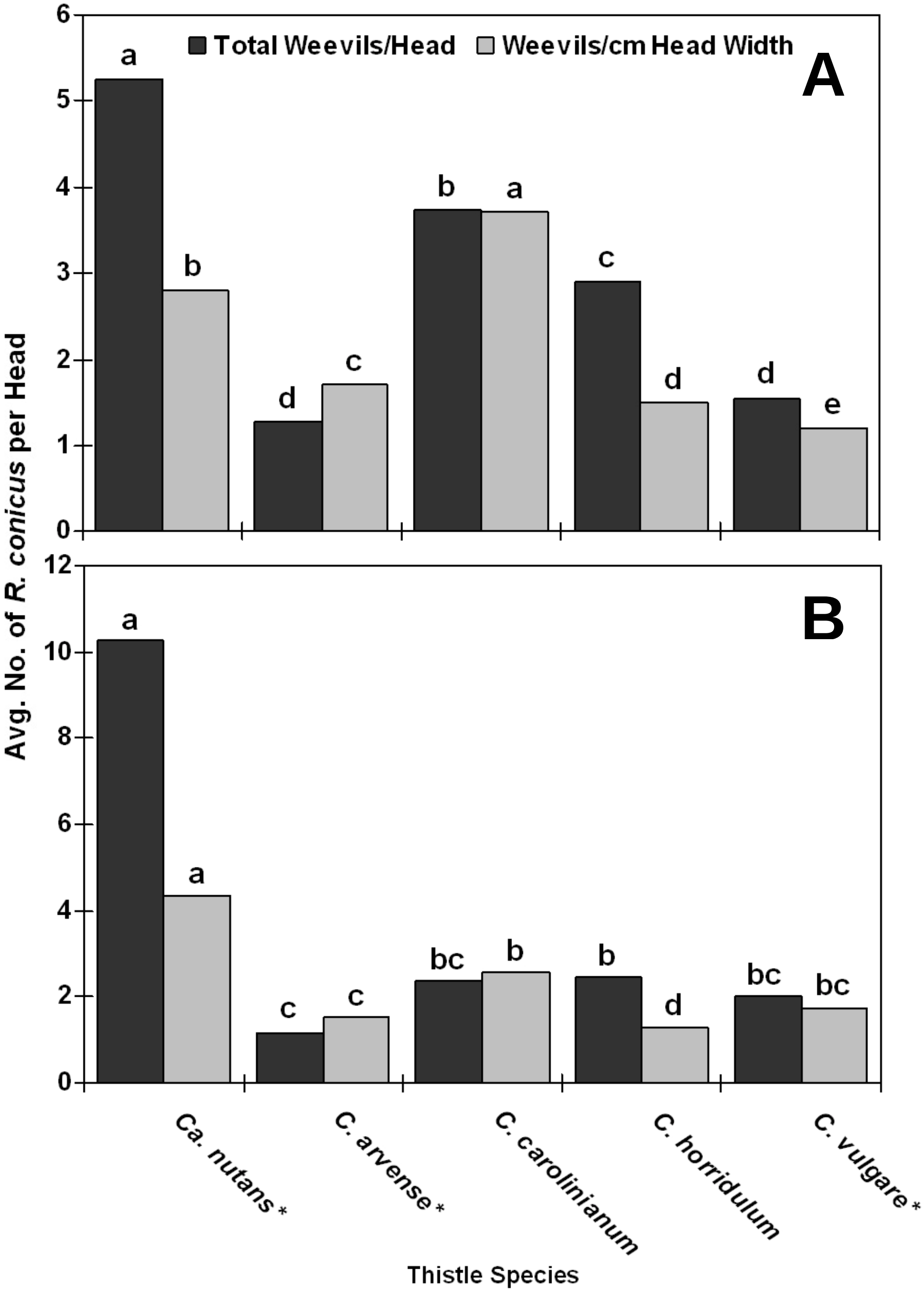

Figure 15. Average number of *Rhinocyllus conicus* (larvae, pupae, and adults) per head (dark column) and average number of *R. conicus* per centimeter of plant head width diameter (light column) for each thistle species caged with *R. conicus*, A) 2008 and B) 2009 ('*' denotes introduced thistle species). Columns of different shades with different letters among species denote significant ($P \leq 0.05$) differences.

R. conicus per cm of plant head width diameter is standardized across species. The relatively smaller average head size of *C. carolinianum* (ca. 10 mm diameter) may render it less preferable or attractive to *R. conicus* as a host plant compared to *C. nutans* (ca. 30 mm diameter), but also may enable greater damage by fewer weevils per head than *C. nutans* should non-target feeding occur in natural populations.

The number of *R. conicus* per head and per cm plant head width followed similar trends as egg loads of *R. conicus* for most thistle species during both years (Figs. 14 and 15). However, mean egg numbers per head on *C. horridulum* were not significantly ($P \leq 0.05$) different from *Carduus nutans* in either year, yet *R. conicus* per head and per head width on *Cirsium horridulum* were significantly fewer than *Carduus nutans* both years. These differences may indicate that while *R. conicus* may be capable of laying high numbers of eggs on *Cirsium horridulum*, few of those eggs hatch or result in larval development within the head. Unlike the other thistle species in this study, *C. horridulum* has leaf-like outer bracts that enclose the head (receptacle) (Fig. 12E). These bracts may provide an obstacle through which it is more difficult for newly-hatched *R. conicus* to maneuver as they attempt to move into the receptacle and account for lower levels of *R. conicus* per cm of plant head width despite high numbers of eggs per head.

Mean body lengths of adult *R. conicus* reared on different thistle species ranged from 5.38 mm (*Cirsium carolinianum*) to 5.74 mm (*C. vulgare*), but no significant differences in body lengths were documented (Fig. 16). Additionally, no significant ($F = 1.28$; df = 1, 407; $P > 0.2589$) differences were documented in body lengths of *R. conicus* from caged (5.5 mm) versus uncaged (5.63 mm) flowers of *Carduus nutans*. Because *R. conicus* were collected in large numbers from uncaged plants of only *C. nutans*, this test

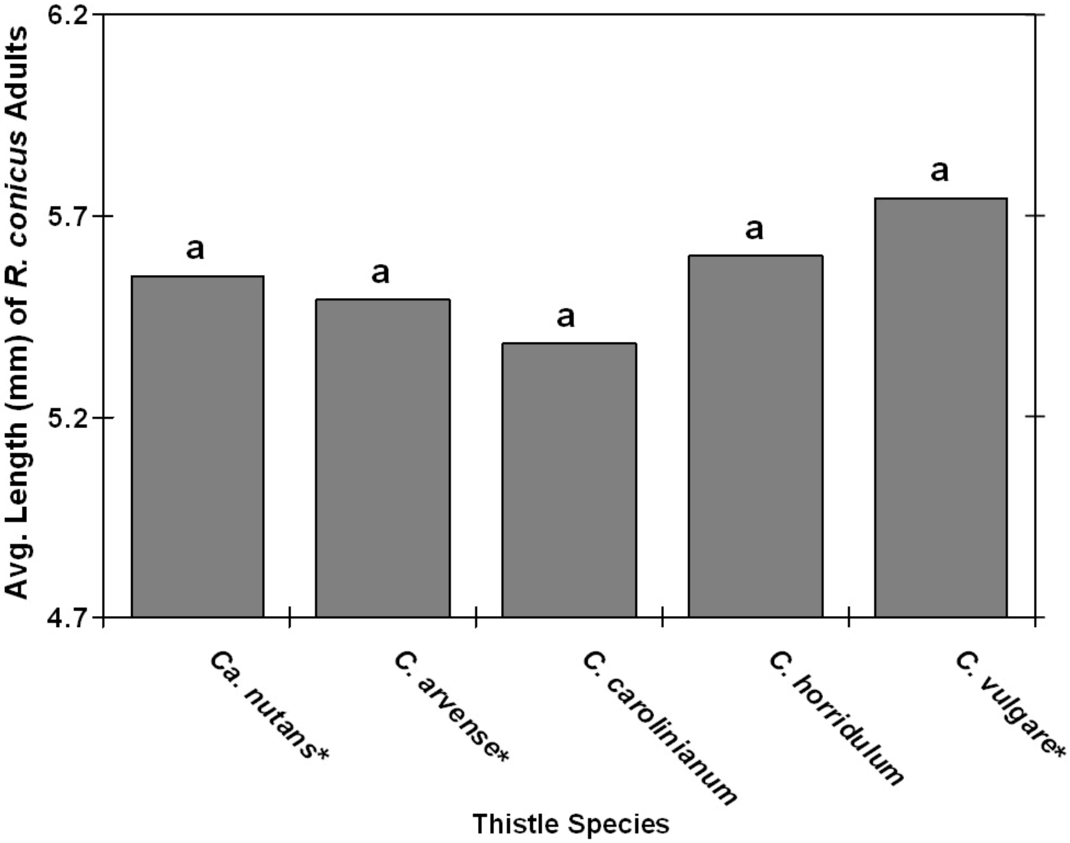

Figure 16. Average body lengths (mm) of adult F1 *Rhinocyllus conicus* reared and recovered from thistle species caged with *R. conicus*, 2009 (‘*’ denotes introduced thistle species). Columns with different letters among species denote significant (*P* ≤ 0.05) differences.

was performed only for plants of *C. nutans*. The lack of differences between *R. conicus* reared from caged and uncaged *C. nutans* demonstrates the lack of impact of caging on the development of the weevils inside of flowerheads. Because body length of adult *R. conicus* has been used as a measure of weevil fitness and an indicator of host suitability (Rowe and Kok 1984, Turner et al. 1987), the similarity in body lengths of *R. conicus* reared from different thistle species indicates that each of these species is a potential suitable host.

Other studies have confirmed the host preference of *R. conicus* for *C. nutans* (Arnett and Louda 2002) and have demonstrated its ability to reduce seed production long after initial releases and subsequent establishment in an area (Kok 2001). *R. conicus* continues to expand its host range to include native *Cirsium* species (Pemberton 2000, Sauer and Bradley 2008). Future studies could be conducted on chemical or other cues that may be used by *R. conicus* to search for and/or determine suitable host plants. These studies may provide a better understanding of the factors that enable *R. conicus* to expand its host range to thistle species native to North America.

This study is the first documentation of the ability of *R. conicus* to develop, and accordingly reduce viable seed numbers, in heads of either *C. carolinianum* or *C. horridulum*. Results from this study demonstrate that *R. conicus* maintains an apparent preference for *C. nutans* as a reproductive host plant in naturally-occurring populations in this region. This weevil, however, has the ability to utilize the native thistles *Cirsium carolinianum* and *C. horridulum* as host species. Reductions in seed numbers of both native species were observed in both years of the study, with significant reductions occurring during 2008. *R. conicus* readily oviposited on both native thistles at similar levels to those observed on *Carduus nutans* in 2008, and both native thistles exhibited significantly greater egg loads than *Cirsium arvense* or *C. vulgare* both years. Also, infested heads of *C. carolinianum* contained proportionately more *R. conicus* per cm of plant head width than any introduced thistle species in 2008, and both native species contained proportionately greater numbers of *R. conicus* per head than *C. arvense* or *C. vulgare* both years of the study. This information, coupled with the lack of significant variation in body length between *R. conicus* reared on native thistles and its target host *Carduus nutans*, further signifies the suitability of *Cirsium carolinianum* and *C. horridulum* as host species.

The evidence of the potential for *R. conicus* to utilize these species is tempered by the fact that no non-target activity has been observed in naturally-occurring populations of either species. It is important to note, however, that *R. conicus* was present in Nebraska but not observed utilizing *C. canescens* in annual monitoring efforts for 16 years prior to its initial documentation on this native species (Louda 1998). Monitoring of *C. carolinianum* and *C. horridulum* should be considered in land-management areas where conservation of native species is a priority. These monitoring efforts could provide early detection of non-target feeding by *R. conicus* if it should occur on these native species and improve information on which to base appropriate management decisions.

CHAPTER V. PLANT RESPONSES TO *TRICHOSIROCALUS HORRIDUS* ON FIELD-CAGED NON-TARGET NATIVE THISTLES IN TENNESSEE

Introduction

Since its introduction from its native range in Europe into North America in the mid-1800s, musk thistle, *Carduus nutans* L., has become a prominent weed species and is now recorded from 45 U.S. states and at least five Canadian provinces (Stuckey and Forsyth 1971, McCarty 1978, Desrochers et al. 1988, USDA 2008). Because chemical and mechanical control methods can be costly, time consuming, and impractical to implement in some areas, the introduction of exotic natural enemies was promoted and projected to provide a long-term, sustainable approach to reducing populations of musk thistle. As part of the biological control program against musk thistle, releases of *Trichosirocalus horridus* (Panzer) were first made in Virginia in 1974 (Ward et al. 1974, Trumble and Kok 1979), followed by releases in Canada in 1976 (Dunn 1978). Adults of *T. horridus* lay eggs on the underside of the leaves in the midribs of the rosette stage of the plant, and the larvae move down through the midrib to the apical meristem of the rosette. Feeding of the larvae damages the meristem, causing plant deformities and sometimes death (Trumble and Kok 1982, Gassmann and Kok 2002). Upon establishment in areas of release, *T. horridus* was widely redistributed as part of musk thistle biological control programs throughout the continental U.S. (Trumble and Kok 1982, Grant et al. 1990, McDonald et al. 1994, Kok 2001, Gassmann and Kok 2002).

Recently, *T. horridus* was divided into a complex of three species: *T. horridus*, and two sibling species, *T. briesei* Alonso-Zarazaga and Sanchez-Ruiz and *T. mortadelo* Alonso-Zarazaga and Sanchez-Ruiz (Alonso-Zarazaga and Sanchez-Ruiz 2002). It is believed that the weevils released in North America are either *T. mortadelo*, *T. horridus*, or a mixture of both species (Alonso-Zarazaga and Sanchez-Ruiz 2002). To date, *T. horridus* is the only species that has been identified occurring in Tennessee (Wiggins et al. 2009).

While another weevil species [*Rhinocyllus conicus* (Fröelich)] released in tandem with *T. horridus* has been observed to feed on several native species of thistles in the genus *Cirsium*, relatively little non-target feeding by *T. horridus* has been observed. Low levels of non-target feeding of *T. horridus* on native thistles was first documented in field surveys of the native field thistle, *C. discolor* (Muhlenberg ex Willdenow) Sprengel, in Virginia (McAvoy et al. 1987). More recently, *T. horridus* has been documented to infest the native tall thistle, *C. altissimum* (L.) Sprengel, in Nebraska (Takahashi et al. 2009), and adult *T. horridus* were observed on all five native *Cirsium* species that occur in Tennessee (Chapter 3, Wiggins et al. 2009). The significance and impact of these new host associations between *T. horridus* and native thistles are concerning but are not yet fully understood.

Because adult *T. horridus* only recently have been observed on native *Cirsium* species in Tennessee, potential impact on non-target thistle species in the southern Appalachians is poorly known. Five native species in the genus *Cirsium* are found in Tennessee [*C. altissimum*, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, *C. discolor*, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux] (TENN 2008). These native species are widely distributed throughout the eastern U.S., each occurring in at least 15 states in addition to Tennessee (USDA 2008). These native species bloom at different times of the year, but all species form rosettes prior to bolting and blooming as part of their development (Wofford 1989, Gleason and Cronquist 1991).

Two introduced *Cirsium* species [Canada thistle, *C. arvense* (L.) Scopoli, and bull thistle, *C. vulgare* (Savi) Tenore] also occur in Tennessee (TENN 2008), and both species are considered invasive in the state (TNEPPC 2008). Both *C. arvense* and *C. vulgare* have wide flowering periods and can bloom from late June through October in the southern Appalachians (Wofford 1989). As with the native *Cirsium* species, these two introduced thistles form rosettes before bolting and blooming.

Releases of *T. horridus* were made throughout Tennessee from 1989 through the early 1990s, but limited follow-up studies have been conducted to fully assess establishment, spread, or impact (Lambdin and Grant 1989, Grant and Lambdin 1993, Lambdin and Grant 1996). An effective way to allow the weevils to feed on the plants to determine plant responses is the use of mesh bags or cages. These cages can restrict weevils to specific plants and allow the response of the plant to exposure to a specific herbivore to be quantified. Therefore, a study was initiated in 2009 to evaluate the plant responses in height, seed production, bud and flower production, and the size of flowerheads of native and introduced thistles to feeding of *T. horridus* on field caged plants.

Materials and Methods

Beginning 11 February through 18 March 2009, *T. horridus* were collected in vacuum samples from populations of at least 30 plants of *Carduus nutans* in Knox (*n* = 2), Cumberland (*n* = 1), and Bradley (*n* = 1) Counties for use in this cage study. While *T.*

horridus are present on plants during this time of year, the small size of weevils and the close proximity of rosettes to the ground slow collection efforts of weevils. To facilitate collection of adult *T. horridus* for use in the study, a leaf blower (Husqvarna model 225B E-tech) modified after Harper and Guynn (1998) was used to collect adults of *T. horridus*. At each collection population, the rosettes and soil immediately surrounding them were vacuumed, and each sample was stored in an individual cloth mesh bag, taken to the laboratory and examined for presence of adult weevils. In the laboratory, adult weevils were placed in a clear plastic container (31 x 31 x 41 cm with four 12-cm screened holes for ventilation) with bouquets of clipped *C. nutans*, where they were held and observed for mating activity. Two copulating pairs of *T. horridus* were placed in one cup (29.6 ml) with a moistened cotton ball which was then sealed with a plastic lid and held in a growth chamber at 7 °C until placed on caged plants in the field. Adult weevils were contained in cups no longer than four days prior to application onto study plants.

From 13 February through 20 March 2009, treatments were applied to rosettes from two populations of each thistle species, except for a single population of *Cirsium muticum* (adequate numbers of rosettes for use in the study were present at only one of three areas surveyed) (Table 10). At each population, 30 thistle rosettes were selected, and one of three treatments was applied to each rosette: four field-collected *T. horridus* adults (two male, two female) were caged on one rosette, one rosette was caged with no *T. horridus* (caged control), or rosette was flagged and left open (uncaged control). Caging of the rosettes was performed by digging a four-cm deep trench around the rosette prior to placement of weevils, placing one nylon mesh bag (45 x 50 cm; Delnet® pollination bags) over the rosette immediately following application of weevils onto

Table 10. Site information for *Carduus nutans* and seven *Cirsium* species at 15 field populations (30 plants per population) used to study the effects of *Trichosirocalus horridus* on caged plants, 2009.

Thistle species[1]	County	Duration[2]
*Carduus nutans**	Roane	25 Feb – 29 May
	Knox	20 March – 6 June
Cirsium altissimum	Polk	4 March – 5 September
	Polk	8 March – 5 September
*C. arvense**	Knox	25 February – 30 June
	Anderson	13 March – 14 June
C. carolinianum	Bledsoe	7 March – 7 June
	Cumberland	11 March – 6 June
C. discolor	Cumberland	11 March – 7 September
	Cumberland	15 March – 7 September
C. horridulum	Bradley	3 March – 1 June
	Bradley	8 March – 1 June
C. muticum	Morgan	15 February – 13 September
*C. vulgare**	Knox	13 February – 10 August
	Cumberland	17 March – 7 September

1 – One population containing 30 plants of *C. muticum* was used during the study. Two populations containing 30 plants each were used for all other species included in this study. Treatments (caging with adult *T. horridus*, caging without adult *T. horridus*, and uncaged control plants) were divided equally among the plants within each population.

2 – Duration = the time period that treatments were applied to plants before they were taken to the laboratory for processing.

* – Introduced species.

rosettes, and burying the base of the bag in the trench to fully enclose the rosette. Weevils were left on the plants approximately four to six weeks, depending on the thistle species (when bolting occurs) and date of initial treatment. As plant stems began to elongate past the height limitations of the cage, the base of the cages were removed from the ground, the apical meristems/buds/flowers of the mainstem of plants were enclosed in a 45 x 50 cm cage, and the opening of the bag was bunched together around the plant stem and tied with plastic flagging. Plant populations were monitored every seven to 21 days, and damage to basal meristems (i.e., meristem of rosette) and plant mortality, when applicable, were recorded. During this population monitoring, cages also were adjusted as plants grew to allow for plant development. When a majority (ca. >50%) of the heads initiated flowering, plants were clipped at ground level, placed in a plastic bag, and taken to the laboratory for processing. Because all plants were bagged early in the year during the rosette stage, it was difficult to determine if bolting would occur. Plants that remained rosettes throughout the study were left in the field, and damage to the meristem and plant mortality were recorded when applicable. In the laboratory, the head width of each flowerhead and the height of each plant were measured, and the number of buds, flowers, and seedheads per plant and numbers of seeds per flowerhead were recorded for all species.

Several variables were analyzed to estimate the response of plants to exposure to *T. horridus.* Chi-square analysis using the Fisher's Exact test was performed for all thistle species to test for significant ($P \leq 0.01$) associations among treatments and damage to basal meristems, as well as associations among treatments and plant death, using SPSS 14.0 (SPSS Inc., Chicago, Illinois). Because comparisons of treatments were performed by species, multiple Chi-square tests were performed, which increased the likelihood of Type I (finding significance when there is none) errors. Therefore, a conservative criterion alpha of $P \leq 0.01$ was used to decrease the likelihood of Type I errors. Average seed numbers per flower for plants caged with *T. horridus* and control plants, as well as head widths of flowerheads among treatments, was compared using least significant differences ($P \leq 0.05$), and data were normalized using rank transformation. No analyses were performed for seed numbers or flowerhead widths of *C. vulgare*, because no flowers were present on these plants when collected from the field. Due to an accidental lapse in monitoring activities for sites of *C. vulgare*, plants had either gone to seed, or remained rosettes throughout the study. Plants of *C. vulgare* are included in the following analyses, as the variables examined still could be measured for this species. Average number of heads per plant, as well as the average plant height among treatments, were compared using least significant differences ($P \leq 0.05$), and data were normalized using log transformation. All mean separation analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina).

Results and Discussion

Damage to basal meristems was documented for each of the eight thistle species included in this study, but no treatment had a significant ($P \leq 0.01$) effect on levels of damage (Table 11). *Carduus nutans*, *Cirsium altissimum*, and *C. discolor* exhibited damaged basal meristems in all three treatments, while *C. carolinianum* and *C. vulgare* exhibited damage in uncaged control plants and plants caged with *T. horridus*. Plants of

Table 11. Chi-square[1] analysis using Fisher's Exact test to document associations among treatments and damage to basal meristems of *Carduus nutans* and seven *Cirsium* species during a study of *Trichosirocalus horridus* on caged plants, 2009.

Thistle species	No. Plants with Damaged Meristems			Fisher's[2]	Probability
	CC[3]	UC	Th		
*Carduus nutans**	3	11	8	7.101	0.039
Cirsium altissimum	2	2	4	1.113	0.714
*C. arvense**	0	0	2	5.070	0.037
C. carolinianum	0	3	3	3.550	0.232
C. discolor	2	2	3	0.454	1.000
C. horridulum	0	0	1	1.851	1.000
C. muticum	0	0	4	6.876	0.025
*C. vulgare**	0	2	6	7.449	0.023

1 – Criterion alpha of $P \leq 0.01$ was used to decrease likelihood of Type I errors.

2 – Fisher's Exact coefficient.

3 – Treatments: CC – caged control, UC – uncaged control, Th – caged with *T. horridus.*

* – Introduced species.

C. arvense, C. horridulum, and *C. muticum* only exhibited damage to basal meristems on plants caged with *T. horridus*. While the levels of damage are similar within each *Cirsium* species, more damaged meristems of *Carduus nutans* were observed on plants caged with *T. horridus* and uncaged control plants than caged control plants. The higher (but not significant) level of damage to meristems of *C. nutans* is expected, as it is the target host of *T. horridus*.

Damaged basal meristems of caged control plants of *Carduus nutans*, *Cirsium altissimum*, and *C. discolor* were probably due to the presence of eggs or larvae of herbivorous insects at the time of initial caging of the rosettes. Although rosettes were inspected in an attempt to utilize only uninfested plants, the signs of oviposition or meristematic feeding were not detected on these rosettes during initial caging. Damaged basal meristems observed in all treatments of *Carduus nutans* and in plants caged with *T. horridus* for all *Cirsium* species may be due to larval feeding of *T. horridus.* However, native insects, such as weevil species in the genus *Baris*, feed on meristems of native plants (personal observation). Because there was no effect of treatment on the levels of damage, in combination with both native herbivores and adults of *T. horridus* possibly present at low levels (Chapter 3, Wiggins et al. 2009), damaged basal meristems of *Cirsium* species, especially uncaged control plants, could be due to feeding by either native insect herbivores or by *T. horridus*. Insecticides have been used previously in studies of plant-feeding weevils to exclude unwanted herbivores from whole plants (Bevill et al. 1999) or specific plant parts (Cartwright and Kok 1985). In future studies, plants could be treated with a prophylactic treatment of insecticide prior to the study to decrease the likelihood of presence of unintended herbivores.

Chi-square analysis found no significant ($P \leq 0.01$) effect of treatments on levels of plant mortality during this study (Table 12). While plant death was observed at some level for most thistle species, no plant mortality was observed in populations of *C. arvense* or *C. muticum*. Higher levels of plant mortality occurred in populations of *C. altissimum* and *C. vulgare* than other populations, but most of this mortality was observed on either caged control plants or plants caged with *T. horridus*. Additionally, most of the plant mortality of these two species occurred in one of their two populations used in the study. Both sites where higher mortality occurred were located in open woodlands, and the combination of the conditions at these population sites and the caging of the plants may have restricted light or influenced other factors to levels that effected plant mortality. In general, thistles often grow in ruderal or disturbed habitats, and are exposed to a wide variety of stresses and herbivory. Although some plant death observed during this study may have been due to feeding by *T. horridus*, death in caged and uncaged control plants may be due to herbivores, either vertebrate or arthropod, weather damage, accidental physical damage from humans or animals, or other factors.

Significantly ($P \leq 0.05$) fewer seeds were produced by uncaged control plants of *Carduus nutans* than plants of either caged treatment, with no other thistle species showing significant differences among treatments (Fig. 17). Because *T. horridus* does not feed within the buds or flowers of plants, negative impacts on seed numbers are not expected to occur unless *T. horridus* infest rosettes in large numbers (ca. 40-50 larvae/rosette) (Sieburth et al.1983, Milbrath and Nechols 2004a, b). The reduced numbers of seeds observed from uncaged control plants of *C. nutans* are probably due to feeding of *R. conicus*, which were present in these populations and readily feed within

Table 12. Chi-square[1] analysis using Fisher's Exact test to document associations among treatments and mortality of plants of *Carduus nutans* and five *Cirsium* species[2] during a study of *Trichosirocalus horridus* on caged plants, 2009.

Thistle species	No. Dead Plants			Fisher's[3]	Probability
	CC[4]	UC	Th		
*Carduus nutans**	0	1	0	1.851	1.000
Cirsium altissimum	6	1	8	7.366	0.023
C. carolinianum	2	0	4	4.239	0.156
C. discolor	2	0	4	4.239	0.156
C. horridulum	0	2	1	1.921	0.771
*C. vulgare**	7	3	4	4.410	0.134

1 – Criterion alpha of $P \leq 0.01$ was used to decrease likelihood of Type I errors.

2 – No Chi-square test was performed for *Cirsium arvense* or *C. muticum*, because no plant mortality was observed in these populations during the study.

3 – Fisher's Exact coefficient.

4 – Treatments: CC – caged control, UC – uncaged control, Th – caged with *T. horridus*.

* – Introduced species.

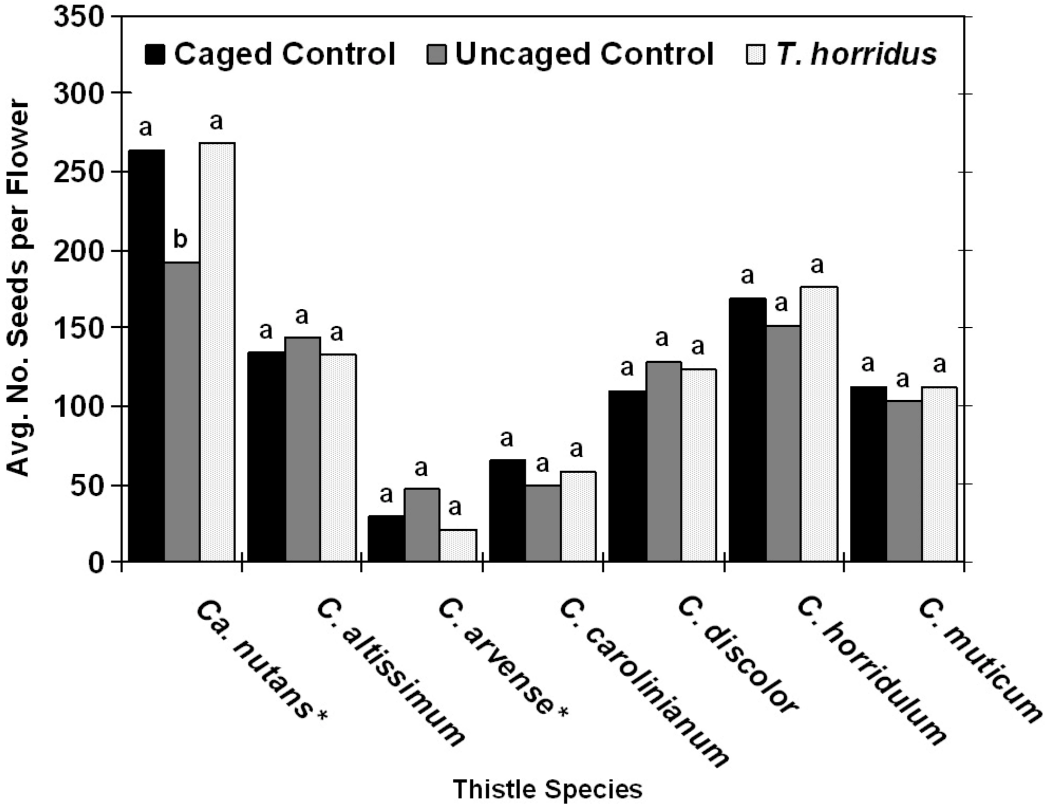

Figure 17. Average number of seeds per flower of each thistle species among plants caged with *Trichosirocalus horridus* and caged and uncaged control (no *T. horridus*) plants, 2009 (‘*’ denotes introduced thistle species). Columns with different letters within species denote significant ($P \leq 0.05$) differences.

the heads of these plants. Future studies could include the treatment of uncaged control plants with insecticide prior to exposure to the herbivore of interest to decrease the presence of *R. conicus* and other herbivorous insects.

When the effect of exposure to *T. horridus* on the head width of thistle flowerheads was examined, significantly ($P \leq 0.05$) greater flowerhead widths were observed on uncaged plants of *Carduus nutans* and *Cirsium discolor* compared to both caged control plants and plants caged with *T. horridus*, with no other species showing significant differences among treatments (Fig. 18). As observed with seed numbers per flower, large numbers of larvae of *T. horridus* may be necessary to influence the size of flowerhead widths. Differences in uncaged flowerhead widths compared to caged plants observed from *Carduus nutans* and *Cirsium discolor* may be due to the cages deterring development of the heads to grow to their full potential.

Significantly ($P \leq 0.05$) more heads (i.e., buds, flowers, and seedheads) were produced by uncaged control plants of *C. altissimum* and *C. arvense* and caged and uncaged control plants of *C. discolor* than plants caged with *T. horridus* (Fig. 19). Also, significantly ($P \leq 0.05$) more heads were observed on uncaged plants of *C. muticum* than on caged control plants (Fig. 19). The number of heads per plant reflects the potential total seed production. Despite the lack of effect on seeds per flowerhead, lower numbers of heads on plants exposed to *T. horridus* observed in *C. discolor* may indicate the ability of *T. horridus* to reduce potential seed production for the whole plant (fewer heads, fewer total seeds produced). Both *C. altissimum* and *C. arvense* exhibited more heads from uncaged control plants than those caged with *T. horridus*, so caging the plants may have limited the ability to form as many heads as uncaged plants.

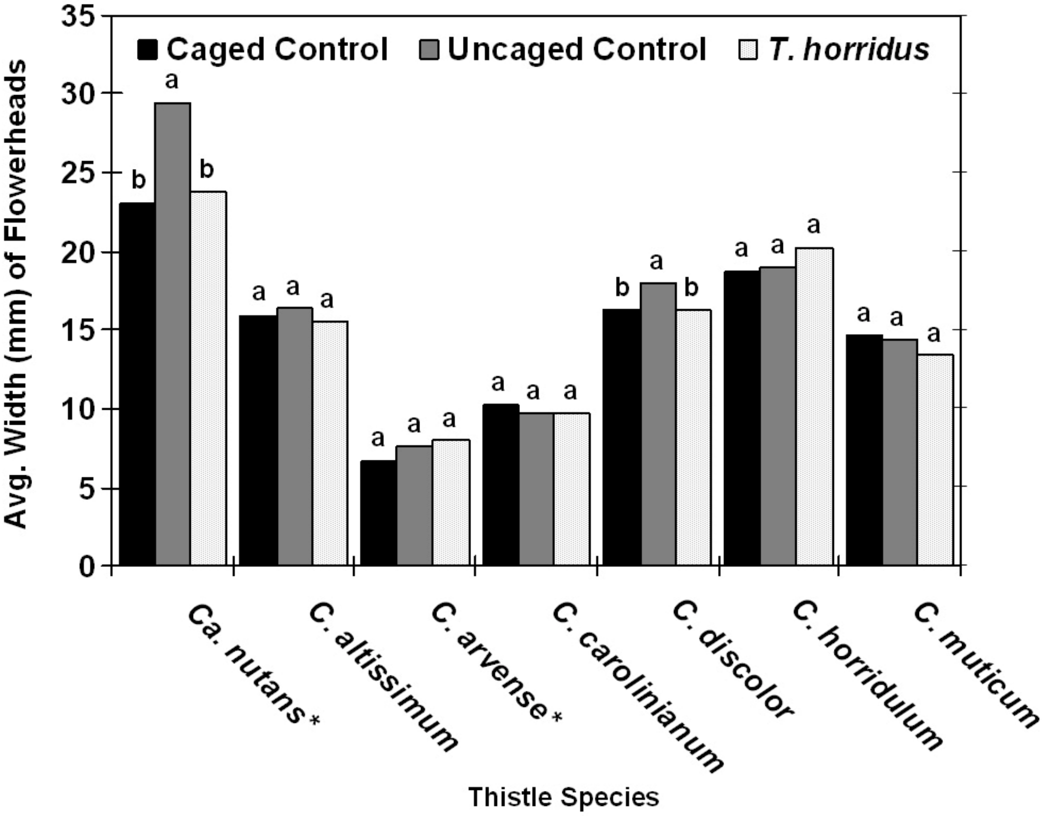

Figure 18. Average flowerhead width (mm) of each thistle species among plants caged with *Trichosirocalus horridus* and caged and uncaged control (no *T. horridus*) plants, 2009 ('*' denotes introduced thistle species). Columns with different letters within species denote significant ($P \leq 0.05$) differences.

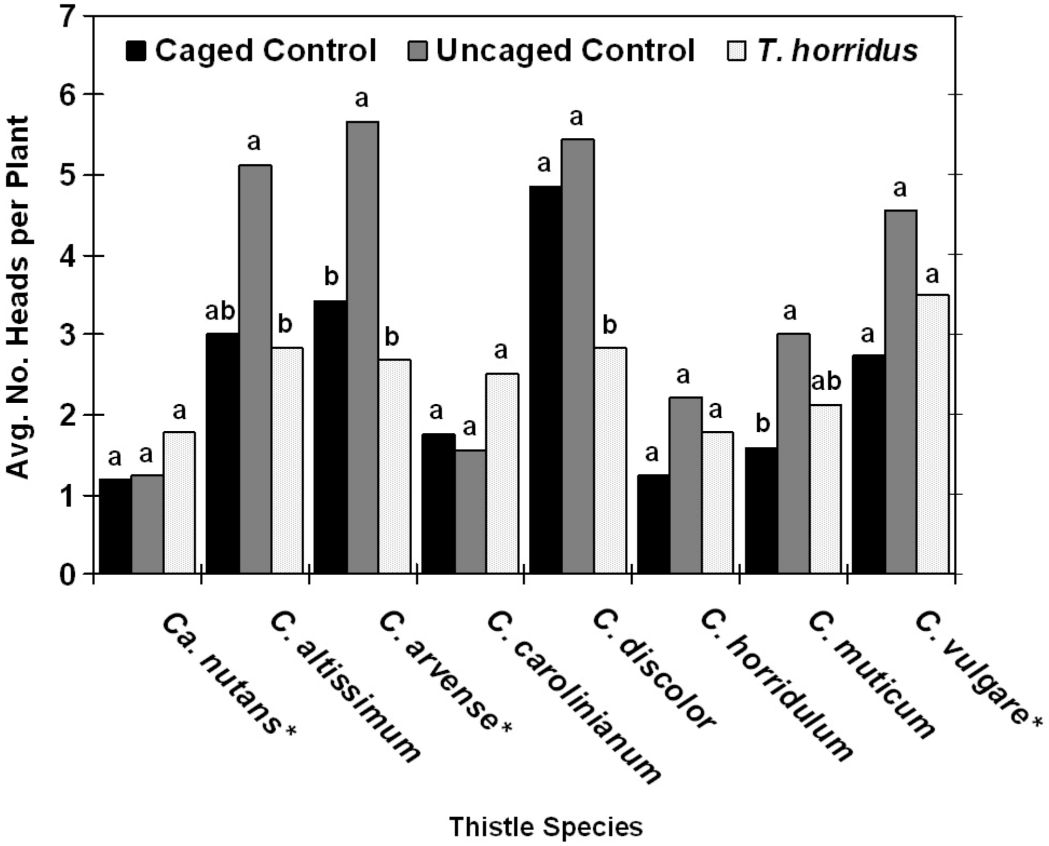

Figure 19. Average number of heads (buds, flowers, and seedheads) per plant of each thistle species among plants caged with *Trichosirocalus horridus* and caged and uncaged control (no *T. horridus*) plants, 2009 (‘*’ denotes introduced thistle species). Columns with different letters within species denote significant (*P* $\leq$ 0.05) differences.

When effect of exposure to *T. horridus* on the height of thistle plants was examined, uncaged control plants of *Carduus nutans*, *Cirsium arvense*, *C. carolinianum*, *C. discolor*, and *C. muticum* were significantly ($P \leq 0.05$) taller than caged control plants or plants caged with *T. horridus* (Fig. 20). These differences between heights of caged and uncaged plants are probably due to a limiting effect of cages on apical growth. Although cages were periodically adjusted throughout the season, they may still have been restrictive enough to deter growth.

To mitigate some of the potential cage effects for future studies, populations could be monitored more often, and cages adjusted more frequently, to better account for plant growth. Also, larger cages could be applied in the same manner as in this study (i.e., caging the terminal head of the plant in a larger bag-like cage), potentially allowing more room for plant development. Finally, instead of applying cages to the terminal heads of the plant, cages could be constructed to enclose the whole plant. Although these cages may be more difficult to construct and/or erect in the field, whole-plant cages may allow thistles to grow in a more natural manner.

The overall lack of differences in levels of damage, levels of mortality, seed production and number of heads among treatments signifies that *T. horridus* does not have a noticeable effect on plants at the levels of exposure of this study. Since larvae of *T. horridus* feed within developing meristems and not directly on the seeds or receptacle where seeds are formed, plants only may be weakened by feeding of *T. horridus* and less able to withstand future feeding within seedheads by *R. conicus* (in the case of *C. nutans*) (Kok 2001) or other seed feeders. Little reduction in seed numbers of *C. nutans* has been documented when rosettes were infested with low numbers of *T. horridus* larvae (<20 per

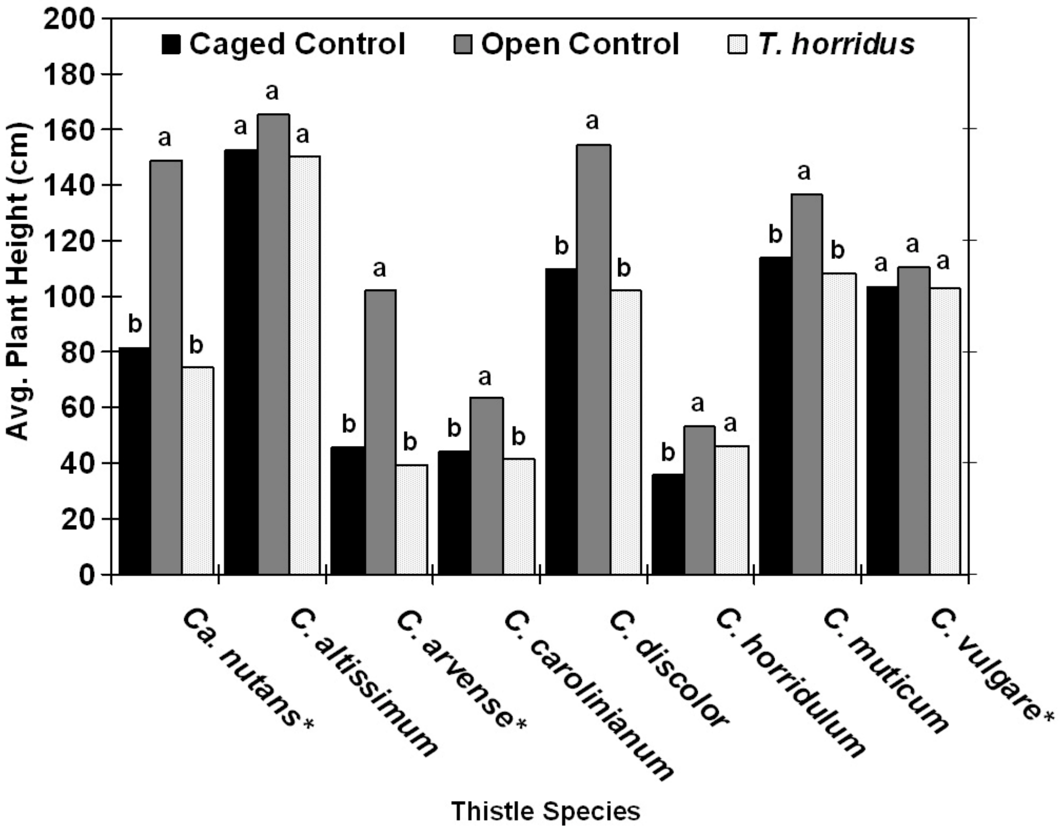

Figure 20. Average height (cm) per plant of each thistle species among plants caged with *Trichosirocalus horridus* and caged and uncaged control (no *T. horridus*) plants, 2009 ('*' denotes introduced thistle species). Columns with different letters within species denote significant ($P \leq 0.05$) differences.

plant), and seed reduction was only accomplished with infestation levels >60 larvae per plant (Milbrath and Nechols 2004a, b). During this study, two mated females of *T. horridus* were exposed to each plant caged with weevils. Because female *T. horridus* can lay an average of ca. 800 eggs (Kok 2001), it was believed that oviposition and larval development would occur at levels great enough to impact plants. However, caging of these weevils may have affected the level of oviposition, through restriction of oviposition to one plant, or by physical factors, such as reduced light and air-flow, increased humidity, or other factors, and, thus, diminished larval numbers feeding on rosettes.

Additionally, larval infestation rates were not evaluated during this study, as plant dissection and destruction are required to obtain larvae. Although damage to basal meristems of each thistle species was documented in plants caged with *T. horridus*, larvae were not present in meristems when plants were examined in the laboratory after collection from the field (Fig. 21). Larvae of *T. horridus* pupate in the soil in mid- to late-May, and pupation lasts 14 to 25 days (Trumble and Kok 1979, Kok 2001). Most of the plants in this study began bolting during this period, so when the base of the cages were removed from the soil and adjusted to enclose the bolting stem, any larvae that had developed in plants were pupating in the soil. Therefore, it was difficult to ascertain the level of infestation, or if infestation had occurred, other than by observing damaged meristems. Other studies of impacts of *T. horridus* on *C. nutans* obtained first-instar larvae, which were directly placed on the plant (Ward et al. 1974, Kok 1975, Cartwright and Kok 1985, and Milbrath and Nechols 2004a, b).

Figure 21. Stems (a) and damaged basal meristem (b) of *Cirsium carolinianum* caged with *Trichosirocalus horridus*.

In addition to application of prophylactic treatment of plants with insecticide to reduce unwanted feeding by other insect herbivores and the use of whole-plant cages to help mitigate cage effects, other modifications could be incorporated in future studies to provide more definitive results. Greenhouse-grown plants or plants grown in field plots could be used for more control over unintended herbivores, and the uniform growth conditions of these plants would help mitigate unintended site effects observed in field populations. Application of larvae of *T. horridus* directly to basal meristems would allow more accurate evaluation of impacts of larval feeding on plants, and varying levels of larvae could be applied to document threshold levels of larvae required to detrimentally impact plants. In order to incorporate applications of larvae into future studies, either collections of larvae from infested plants in the field must be made, or colonies of *T. horridus* must be maintained. Although either of these efforts would be both labor- and time-intensive, each would enhance the ability to determine plant responses to feeding of larvae of *T. horridus*. Additionally, mesh screening could be installed around the base of plants to prevent larvae from burrowing in the soil to pupate. If monitored at regular intervals, larvae could be counted as they leave the plant to pupate, and larval survival rates could be calculated. If larvae were allowed to enter the soil to pupate, the soil surrounding the base of the plant could be caged after the plant was clipped for processing and monitored for emergence of adult *T. horridus* from the soil. Documenting adult emergence would improve knowledge of the biology of *T. horridus* and help determine plant host suitability by allowing estimates of weevil survival throughout its lifecycle on specific hosts. These modifications would enhance future studies and enable a greater understanding of the impacts of larval feeding of *T. horridus* on native thistles.

The degree to which *T. horridus* may utilize these native *Cirsium* species in naturally-occurring populations remains unclear. The observation of some degree of damaged basal meristems of plants of all species caged with *T. horridus* implies that *T. horridus* has the ability to feed at some level on these thistle species. However, the lack of differences in seeds per flowerhead and head numbers per plant observed in this study, combined with the low infestation levels observed on naturally-occurring populations of the five native *Cirsium* species in Tennessee (Chapter 3, Wiggins et al. 2009), indicate that *T. horridus* currently does not impact plants at significant levels. Levels of adults and larvae of *T. horridus* observed on *C. discolor* in Virginia (ca. 6%) (McAvoy et al. 1987) were similar to those observed more recently in Tennessee, but there have been no significant reductions in populations of *C. discolor* in Virginia attributed to feeding of *T. horridus* since that time. Monitoring of native *Cirsium* species should be encouraged in natural areas to determine if *T. horridus* are present in those populations and, if so, their infestation levels. This monitoring would increase our understanding of interactions among *T. horridus* and native thistles, and provide an early warning system for management actions should they be required.

CHAPTER VI. SPATIAL PREDICTION OF HABITAT OVERLAP OF INTRODUCED AND NATIVE THISTLES TO IDENTIFY POTENTIAL AREAS OF NON-TARGET ACTIVITY OF BIOLOGICAL CONTROL AGENTS

Introduction

Biological control has received increased scrutiny in recent years, largely because of documentation of non-target feeding of biological control agents on native species (e.g., Clarke et al. 1984, Simberloff and Stiling 1996, Ewel et al. 1999, Follet and Duan 2000, Pemberton 2000, Louda and Stiling 2004). An important factor in considering interactions among introduced biological control agents, their target hosts, and potential native hosts is the degree to which habitats of introduced pests and related native species overlap (Barratt et al. 2000). Additionally, herbivorous insects introduced to control exotic, invasive weed species may pose a substantial threat to native plant species that are taxonomically related to the target weed species and may share similar habitats (Pemberton 2000). The importance of these spatial and taxonomic factors in non-target activity is evident in the case of the introduced weed musk thistle, *Carduus nutans* L., and native thistles in the genus *Cirsium* in North America.

Musk thistle, a biennial herbaceous species native to Europe, was first introduced into North America in the mid-1800s and was considered a major plant pest across much of the U.S. by the1960s (Stuckey and Forsyth 1971, Dunn 1976). Two weevil species [*Rhinocyllus conicus* Fröelich and *Trichosirocalus horridus* (Panzer)] native to Europe were approved for release in the U.S. and Canada as biological control agents of musk thistle in the late 1960s and early 1970s (Kok 1975, 2001). Prior to these releases, non-target testing was conducted on species of European thistles in the genera *Cirsium*, *Carduus*, *Silybum*, and *Onopordum*, but few species native to North America were included (Rees 1977, Zwölfer and Harris 1984).

Prior to its release in North America, host specificity testing revealed that *R. conicus* fed on several European species of *Cirsium* (Zwölfer and Harris 1984). In the years following its release *R. conicus* has been documented from flowerheads of at least 25 native species of *Cirsium* in the western and central U.S. (Goeden and Ricker 1986, Goeden and Ricker 1987, Turner et al. 1987, Rees 1992, Turner and Herr 1996, Louda et al. 1997, Pemberton 2000, Sauer and Bradley 2008). Non-target feeding of *R. conicus* on the Federally Endangered species *Cirsium fontinale* (Greene) Jepson var *obispoense* J. T. Howell has been documented (Turner and Herr 1996, USDA 2009) and, due to its increasing host-range expansion to *Cirsium* species native to North America, *R. conicus* may begin to use other species listed as Federally Rare, Threatened or Endangered (RTE), such as Pitcher's thistle, *C. pitcheri* (Torrey) Torrey and Gray (Pavlovic et al. 1992, Louda 1998). Whereas documented non-target activity of *T. horridus* has not been as extensive as that of *R. conicus*, Takahashi et al. (2009) observed rosettes of tall thistle, *C. altissimum* (L.) Sprengel, infested by *T. horridus* at similar frequencies and abundances as bull thistle, *C. vulgare* (Savi) Tenore, in Nebraska. McAvoy et al. (1987) reported limited feeding by *T. horridus* on field thistle, *C. discolor* (Muhlenberg ex Willdenow) Sprengel, in Virginia.

Rhinocyllus conicus and *T. horridus* were released throughout Tennessee from 1989 through the 1990s, and both weevils have subsequently become established in much of the state (Lambdin and Grant 1992, Grant and Lambdin 1993, Lambdin and Grant 1996). Currently, seven *Cirsium* species occur in Tennessee: five native species [*Cirsium* *altissimum*, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, *C. discolor*, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux] and two introduced species [Canada thistle, *C. arvense* (L.) Scopoli, and *C. vulgare*]. Adult *T. horridus* recently have been documented in low numbers on all native thistle species in Tennessee (Wiggins et al. 2009) but there have been no published reports of non-target feeding of *R. conicus* on native thistles in this region. Therefore, the impact of the released weevils on non-target thistle species in Tennessee is poorly known.

Although the proximity of native thistles to populations of musk thistle varies where non-target impacts have been observed, Rand et al. (2004) documented greater non-target infestation of the native *C. undulatum* (Nuttall) Sprengel by *R. conicus* within stands of *Carduus nutans* than on native plants 30 to 100 m from stands. Additionally, egg densities of *R. conicus* on *C. undulatum* and *C. flodmanii* (Rydberg) Arthur (also native) were four times greater in landscapes heavily infested with *Carduus nutans* than in landscapes with low densities of the exotic thistle (Rand and Louda 2004). These studies show that proximity of *C. nutans* to native thistles can influence non-target feeding on both local and landscape scales.

Modeling the habitats in which native and exotic thistles occur could be a useful technique to identify areas where potential non-target feeding of *R. conicus* and *T. horridus* may occur. Habitat modeling can provide unbiased predictions of species occurrences and distribution across a large spatial area (Boetsch et al. 2003). Although it has been used extensively to study indigenous and introduced vertebrate species (Scott et al. 2002), habitat modeling is increasingly used in association with invasive plant species (Nielsen et al. 2008, Williams et al. 2008, Ibanez et al. 2009). Because thistles in the genera *Carduus* and *Cirsium* are known to be necessary for the reproduction of *R. conicus* and *T. horridus*, characterizing suitable habitats for the known and potential plant hosts of these weevils would also identify habitats where these weevils may occur.

In Spring 2005, a study was initiated to characterize habitats where native and exotic thistles occur and to identify areas where these thistle species may overlap. The objectives of this study were to: 1) predict habitat areas of native and exotic thistles, 2) quantify associations of *R. conicus* and *T. horridus* with introduced and native thistle species, and 3) identify areas where native and non-native thistle species overlap. This project was designed to predict where habitats of native and non-native thistle species may overlap and, thus where non-target feeding by the two introduced weevils may occur.

Materials and Methods

Study Area

Four counties (Bledsoe, Cumberland, Morgan, and Van Buren; ca. 4,812 km^2) in eastern Tennessee were selected to develop site suitability indices and predict potential thistle habitats (Fig. 22). This four-county area is mostly forested (69.3% of the total area), but also has a range of land cover types (i.e., 12.8% pasture, 8.2% grassland, 6.1% developed, 1.9% scrub or barren, and 1.0% crop land) in which thistle species may occur. This study area was selected because the four thistle species chosen as model species [two introduced (*Carduus nutans* and *Cirsium vulgare*) and two native (*C. carolinianum*, *C. discolor*)] occur throughout these counties based on specimen records from the University of Tennessee Herbarium (TENN).

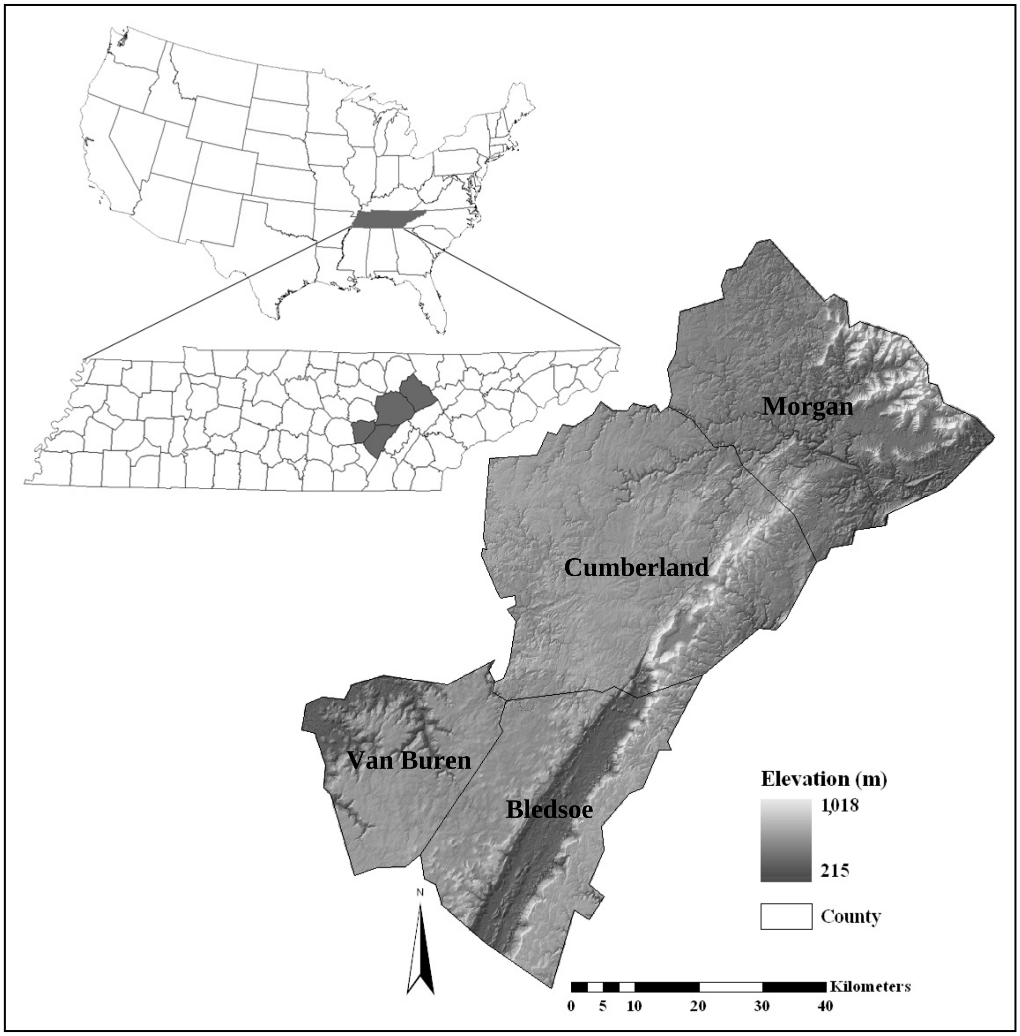

Figure 22. Map of study area in Tennessee (Bledsoe, Cumberland, Morgan, and Van Buren Counties), USA, used to generate Mahalanobis distance models of thistle habitat.

During Spring and Summer 2005 through 2007, site reconnaissance was conducted to locate populations of the four thistle species in the study area. Thistle populations were detected visually, and plant identification was confirmed using Wofford (1989) and based on comparisons of field-collected plants with thistle specimens in TENN. Populations of each thistle species (42 of *Carduus nutans*, 35 of *Cirsium carolinianum*, 76 of *C. discolor*, and 39 of *C. vulgare*) were georeferenced using a Trimble® GeoExplorer CE GPS unit (Trimble Navigation Limited, Sunnyvale, California) and entered into a GIS database in (ArcMap® version 9.2.1, ESRI, Redlands, California).

Habitat Attributes

Predicting species occurrence requires knowledge of the resource conditions that contribute to use of a habitat by a particular species. The habitat attributes selected for the model represent the basic physiographic, soil quality/chemistry, light, and moisture requirements that contribute to thistle growth. Eight habitat attributes were used to generate habitat models for each thistle species (Table 13). Digital elevation models (30-m pixels) were obtained from the U.S. Geological Survey (USGS 2009). Slope was calculated from the digital elevation model (Boetsch et al. 2003, Thompson et al. 2006). Two unitless indices were used as habitat attributes. The topographic relative moisture index (TRMI) is a measure of potential soil moisture, and the solar insolation index is an estimate of solar radiation potential that takes topography into account. The Soil Data Viewer was used to extract soil data from the Soil Survey Geographic database (NRCS 2009; Table 13). The specific soils data (i.e., percent clay, percent organic matter, percent sand and pH) were selected because they are general indicators of soil quality. To

Table 13. Spatial data used to generate attributes used in Mahalanobis distance (D^2) models to predict occurrence of *Carduus nutans, Cirsium carolinianum, C. discolor,* and *C. vulgare* in Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009.

Attribute[1]	Description	Value range	Source
Elevation	Elevation (m)	215-1,018	U.S. Geological Survey 30-m resolution digital elevation model (USGS 2009)
Slope	Slope (degrees)	0-67.4	Calculated from Elevation
Soils Data[2]:			
– % Clay	Percent of clay in soils	0-43.3	National Resources Conservation Service Soil Survey Geographic Database (1:20,000) (NRCS 2009)
– % Organic	Percent of organic matter in soils	0-3.0	National Resources Conservation Service Soil Survey Geographic Database (1:20,000) (NRCS 2009)
– % Sand	Percent of sand in soils	0-68.7	National Resources Conservation Service Soil Survey Geographic Database (1:20,000) (NRCS 2009)
– pH	Soil acidity	4.6-7.0	National Resources Conservation Service Soil Survey Geographic Database (1:20,000) (NRCS 2009)
Solar insolation	Unitless index of exposure to sunlight; approximated for the solar equinox	1-227	Calculated after Thompson et al. (2006)
Topographic relative moisture index	Unitless index of moisture considering the effects of slope position, aspect, and elevation	0-60	Calculated after Boetsch et al. (2003)

1 – Elevation data were accessed 29 March 2006. Soils data for Bledsoe, Cumberland and Morgan Counties were accessed 8 November 2006. Soils data for Van Buren County were accessed 14 June 2007.

2 – All soils data were converted from vector data to 30 x 30 m raster grid data.

correspond to the raster data, soils data were converted from vector to raster data with a pixel size of 30 m. The mean and standard deviation were calculated for each of the eight model attributes for each thistle species to document the relative contribution of each attribute to the model.

Predicting Habitats

Mahalanobis distance (D^2) was used as the method to characterize plant habitats and predict species occurrence. Mahalanobis distance is a multivariate measure of dissimilarity and uses the mean, variance, and covariance of habitat characteristics associated with the sample locations (Mahalanobis 1936, Clark et al. 1993, van Manen et al. 2002):

$$D^2 = (\underline{x} - \underline{\hat{u}})' \, \Sigma^{-1} \, (\underline{x} - \underline{\hat{u}})$$

where $\underline{x}$ is the vector of the habitat characteristics based on the GIS data layers of the 8 habitat variables, $\underline{\hat{u}}$ is the mean vector of habitat characteristics of the sample sites, and Σ^{-1} is the inverse variance-covariance matrix calculated from the sampling sites.

Mahalanobis distance is the standard squared distance between a set of sample variates, $\underline{x}$, and "ideal" habitats defined by the sampling sites and represented by $\underline{\hat{u}}$. Low D^2 values indicate conditions that are most similar to those of the sampling sites (i.e., observed locations), and D^2 values increase with greater dissimilarity. Conventional habitat modeling techniques, such as logistic regression, discriminant function analysis, or classification tree analysis, require one dataset that describes habitat suitability and a corresponding dataset that reflects unsuitable sites where the species does not occur (Boetsch et al. 2003). Mahalanobis distance was selected for use in this study because, unlike those other techniques, it requires only presence data. Despite the widespread distribution of *Carduus nutans*, *Cirsium discolor*, and *C. vulgare*, *C. carolinianum* is not as ubiquitous or easily detected. Using D^2 analysis to predict habitats eliminates the need to survey sites where thistle species do not occur. Additional advantages of this technique are that correlations among habitat variables are accounted for and multivariate normality is only required to rescale Mahalanobis distance to a probability distribution (Clark et al. 1993, van Manen et al. 2005). In addition to predicting wildlife habitats (i.e., Clark et al. 1993, Knick and Rotenberry 1998, Browning et al. 2005, Rotenberry et al. 2006, Wartrous et al. 2006, Thatcher et al. 2006), Mahalanobis distance has been used in plant-related systems throughout the world, including predicting distributions of plant communities (DeVries 2005), enhancing plant conservation and restoration efforts (Boetsch et al. 2003, van Manen et al. 2005, Thompson et al. 2006), and identifying areas vulnerable to the spread of invasive weeds (Rouget et al. 2004). This technique is not believed to have been used previously to identify potential habitat overlap of target and non-target species of introduced biological control agents.

Calculations of $\underline{\hat{u}}$ and Σ^{-1} were conducted in SAS (Version 9.2, SAS Institute, Cary, North Carolina) to develop the habitat models using the habitat characteristics of 192 populations of the four thistle species based on the eight habitat attributes. These values were used to calculate D^2 values for each pixel in the study area using Arc/Info GRID® (ESRI, Redlands, California).

Cumulative frequency graphs were generated for each thistle species to delineate habitat from non-habitat areas by comparing the D^2 values of the observed localities against D^2 values if populations were randomly distributed (null model, $n = 366$). The D^2

values for the thistle and null model localities of each thistle species were extracted from the respective habitat models based on Mahalanobis distance and the percent cumulative frequency of each locality was graphed against the corresponding D^2 value. Increasing difference between the two cumulative frequency curves indicates a greater ability of the model to identify habitat areas different from those available within the study area. The greatest difference between the observed and null model curves is the threshold D^2 value below which the greatest percentage of plant locations occurs within the smallest percentage of the study area, thus providing a meaningful measure to delineate habitat (Pereira and Itami 1991, van Manen et al. 2005). Accordingly, areas with D^2 values below the threshold value were delineated as habitat areas for each species and areas above the threshold value were not considered habitat (van Manen et al. 2005, Thatcher et al. 2006).

Model Testing

The models were tested using an independent set of 200 test locations to determine the association between D^2 values and the presence of thistle species in predicted habitats. To insure sufficient observations across the range of D^2 values, 50 sites were stratified for each thistle species according to the distribution of D^2 values within the study area (van Manen et al. 2002, 2005, Boetsch et al. 2003):

100 (percent of all pixels) = $n + 2n + 4n + 8n + 16n$

where n is a constant percentage of the pixels sampled within a stratum (i.e., area).

This geometric equation doubles the area of each of five successive strata, with the first strata containing n (3.23%) pixels with the lowest range of D^2 values. Each successive stratum includes twice the area from which to sample (i.e., stratum 2 contained 6.46% of the pixels with the next lowest range of D^2 values, stratum 3 contained 12.92% of the pixels and so on). For each species, coordinates for ten survey sites were generated for each of the five strata (i.e., 50 test sites per species; 200 test sites total). Although 50 test sites were generated specifically for each thistle species, D^2 values were calculated for all thistle species for all 200 sites. Thus, stratified sampling was conducted for 50 sites per thistle species, and the remaining 150 sites contained random levels of D^2 values.

The 200 sites were surveyed for the presence of each thistle species during May and June 2009. The four thistle species grow in open habitats (Wofford 1989, Carman 2001, Weakley 2008). Therefore, land-cover types from the 2001 National Land Cover Data (i.e., development, barren, shrub, grassland, pasture, crops) were combined to delineate open land cover types (USGS 2009). This open type was used as a filter to restrict surveys of predicted habitats to non-forested sites (Thompson et al. 2006). Survey sites were located using a Garmin® GPS map 60CSx WAAS-enabled GPS unit (Garmin International, Olathe, Kansas). Although predictions were made for 30- x 30-m pixels, a larger survey area was used to account for potential effects of GPS and GIS errors. At each test site, a 50- x 50-m area centered on the sampling position was surveyed to determine the presence or absence of each thistle species.

Data Analysis

Count regression (Proc Countreg, SAS 9.2, SAS Institute, Cary, North Carolina) was used to test if D^2 values were associated with the number of plants observed in the 200 test sites. Because of the possibility of zero-inflation (large number of zeros) and overdispersion (variance exceeding the mean) relative to the Poisson distribution, all dependent variables were first fit with zero-inflated, negative binomial models (Erdman et al. 2008). These models were then tested for zero-inflation and the presence of overdispersion. For thistle species that showed a significant association between the number of thistle plants and D^2 values, predictive habitat maps were generated in ArcMap® to determine areas where habitat overlap occurred.

Chi-square analysis was used to test the association between the presence of each thistle species in predicted habitats and the occurrence of both introduced weevil species. The presence of each thistle species at the 200 field-test sites was compared with the presence of *R. conicus* and *T. horridus* in SPSS 14.0 (SPSS Inc., Chicago, Illinois).

Results and Discussion

Mahalanobis Distance Model

Analyses using the D^2 statistic predicted habitats for all four thistle species. The mean D^2 values ranged from a low of 196.13 for *Carduus nutans* to 451.90 for *C. carolinianum*, and the greatest range of D^2 values was observed for *C. carolinianum* (Table 14). Mahalanobis distance compares the dissimilarity of every cell in the combined spatial dataset to the ideal set of characteristics derived from observed data (i.e., initial 192 model sites), so increasing D^2 values denote less suitable conditions.

Cumulative frequency graphs indicated that Mahalanobis distance models for each species effectively identified site conditions that contribute to the presence of the respective species and designated threshold D^2 values of all four thistle species (Table 14, Figs. 23 [native thistle species] and 24 [introduced thistle species]). For example, at a threshold D^2 value of 11.66, 94.29% of the 35 observed populations of *Cirsium*

Table 14. Mahalanobis distance (D^2) value ranges and means ($\underline{+}$ standard deviation), classification rate, and cumulative frequency of habitat models to determine habitat of native and introduced thistle species, Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009.

Thistle species	D^2 range	D^2 (Mean $\underline{+}$ SD)	Threshold D^2 [1]	% observed classified as habitat[2]	% random classified as habitat[3]
*Carduus nutans**	0.26–601.97	196.13$\underline{+}$147.14	11.90	92.86 (39/42)	72.95 (267/366)
Cirsium carolinianum	1.06–1,710.20	451.90$\underline{+}$352.63	11.66	94.29 (33/35)	37.43 (137/366)
Cirsium discolor	0.27–877.30	206.51$\underline{+}$134.55	18.35	98.68 (75/76)	74.86 (274/366)
*Cirsium vulgare**	0.65–1,441.09	435.79$\underline{+}$346.56	9.90	76.92 (30/39)	43.99 (161/366)

1 – D^2 value which delineates lesser values as habitat and greater values as non-habitat (habitat $D^2 \leq$ threshold $D^2 <$ non-habitat D^2).

2 – Percent of observed sites that were correctly classified as habitat (number of locations classified as habitat/total locations).

3 – Percent of random sites that were correctly classified as habitat (number of locations classified as habitat/total locations).

* – Introduced species.

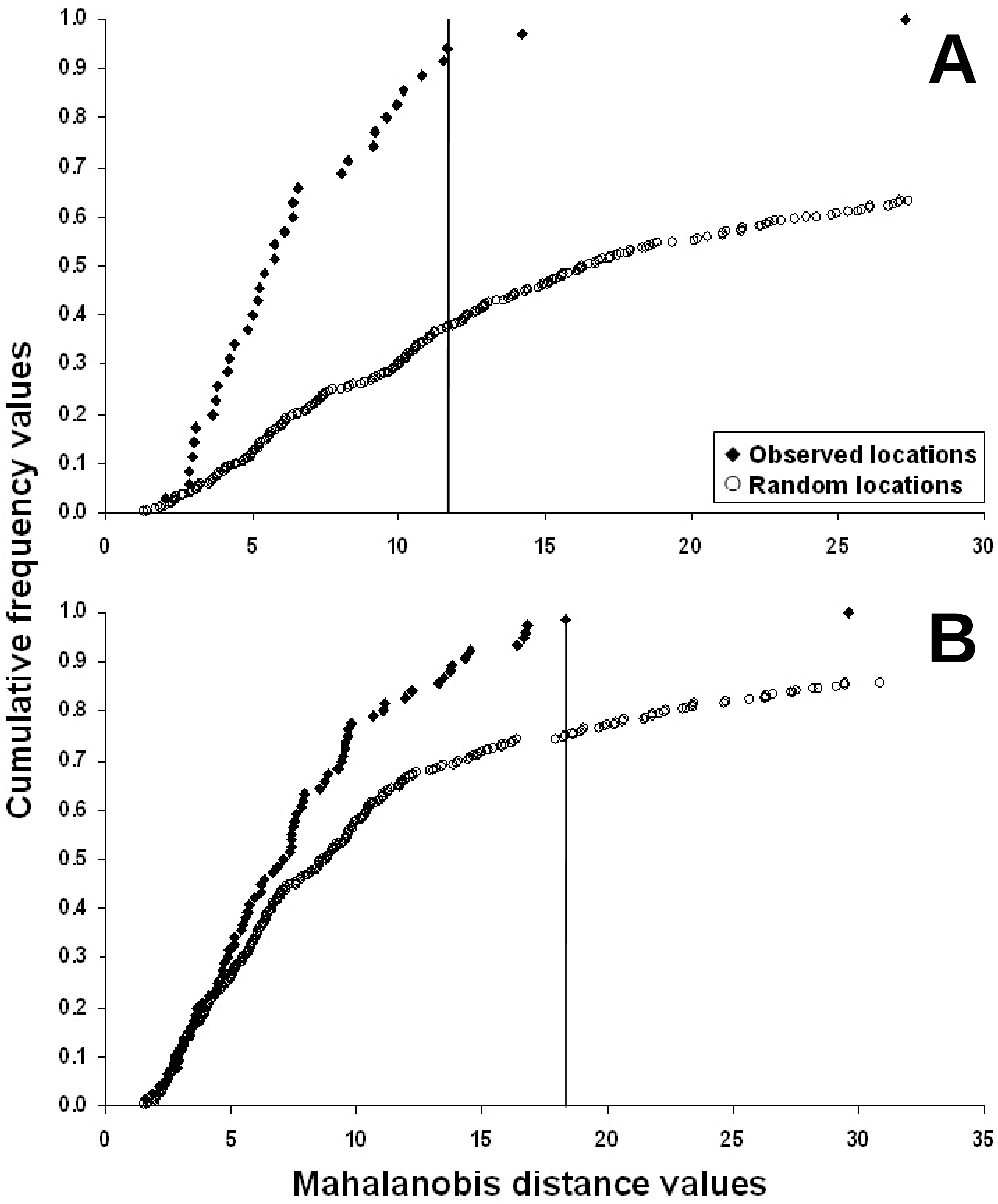

Figure 23. Cumulative frequencies of D^2 values for A) *Cirsium carolinianum* (*n* = 35 sites) and B) *Cirsium discolor* (*n* = 76 sites) and 366 null model locations in Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009. Vertical lines indicate D^2 threshold values (greatest differences between cumulative frequency for thistle locations and null model locations).

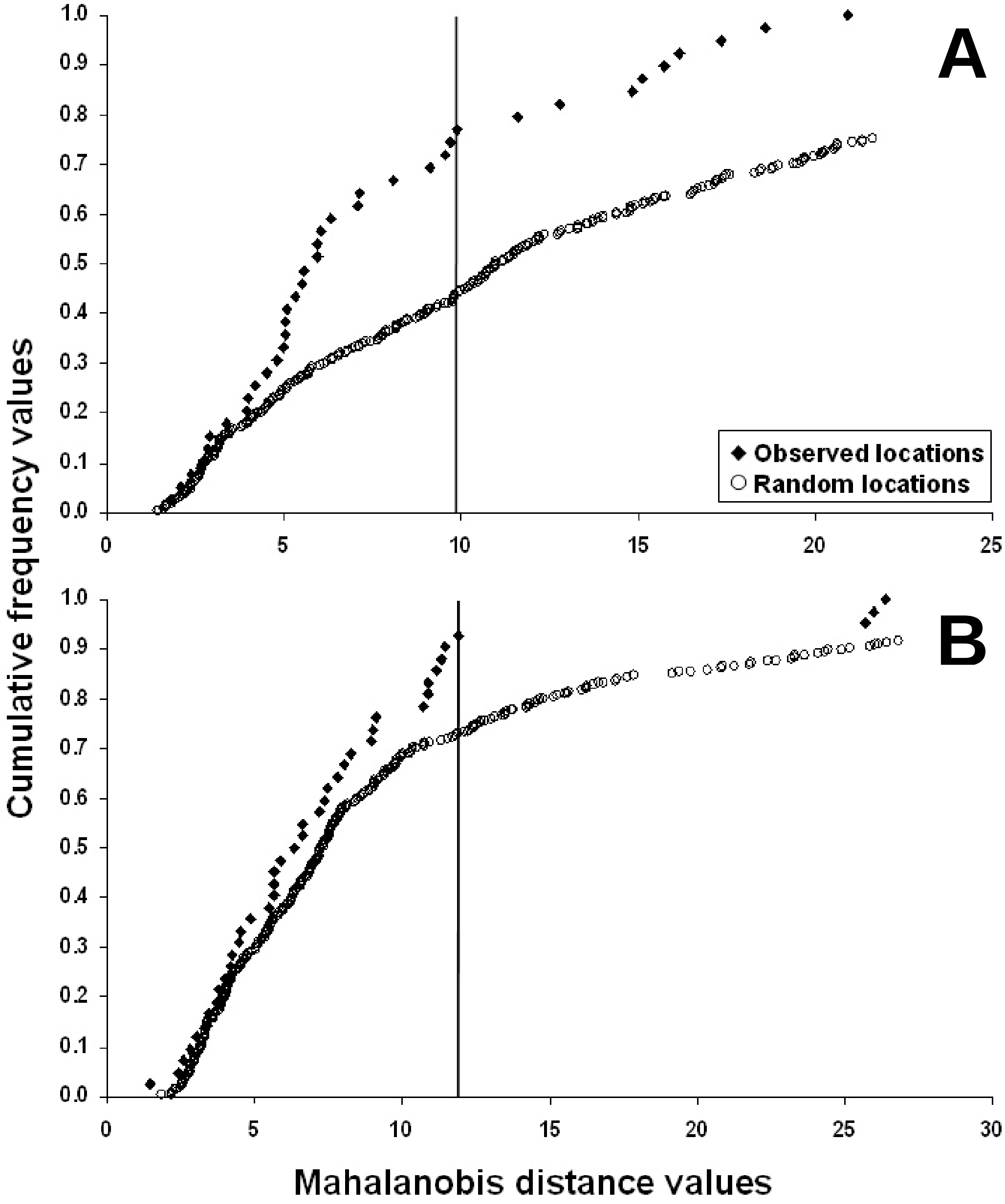

Figure 24. Cumulative frequencies of D^2 values for A) *Cirsium vulgare* (*n* = 39 sites) and B) *Carduus nutans* (*n* = 42 sites) and 366 null model locations in Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009. Vertical lines indicate D^2 threshold values (greatest differences between cumulative frequency for thistle locations and null model locations).

carolinianum were classified as habitats (Table 14, Fig. 23A). At that same D^2 value, only 37.43% of the 366 randomly distributed locations were classified as habitats. The difference in the number of locations classified as habitats between the observed and random data sets demonstrates that the model predicts the occurrence of each thistle species in its corresponding habitat at a greater level than what would randomly be observed.

In general, habitat models indicate that thistle habitats within the study area are primarily represented in areas of relatively high solar insolation at low-middle elevations, with gentle slopes and slightly acidic soils containing ca. 25% clay, ca. 35% sand, and ca. 0.75% organic matter (Table 15). For all thistle species, pH and solar insolation were highly influential attributes for habitats, as their standard deviations were relatively low (< 25% of the mean). Elevation and percent clay were highly influential attributes for all three *Cirsium* species, but were less influential for habitats of *Carduus nutans*. Percent sand and TRMI were influential habitat attributes for all thistle species, and percent organic matter and slope had the weakest influence on thistle habitats (Table 15).

Model Testing

The presence of thistles in field-test sites ranged from seven sites where *C. carolinianum* was present to 67 sites where *C. discolor* was present (Table 16). Whereas the zero-inflated model was not required for the four thistle species, overdispersion was observed in the data for *Cirsium discolor* and *C. vulgare*, and the negative binomial model was used. Poisson regression was sufficient for *C. carolinianum* and *Carduus nutans*, as neither zero-inflation nor overdispersion was a factor for these species. Poisson regression showed that plant counts at field-test sites increased with decreasing values of

Table 15. Comparison of each of the eight attributes used to generate the spatial models predicting habitats of *Carduus nutans*, *Cirsium carolinianum*, *C. discolor* and *C. vulgare*, Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009 (see Table 13 for description of each attribute).

Thistle species	Attributes (Mean ± Standard Deviation)							
	Elevation (m)	Slope (degrees)	% clay	% organic	% sand	pH	Solar[1]	TRMI[2]
*Carduus nutans**	417.33±128.33	5.86±6.13	25.74±6.71	0.76±0.48	38.03±10.55	5.02±0.44	176.60±12.48	30.07±8.84
Cirsium carolinianum	495.20±56.92	5.16±4.30	25.32±4.60	0.76±0.37	31.82±11.69	4.79±0.25	173.29±10.64	29.77±9.56
Cirsium discolor	444.74±105.03	4.84±4.07	25.31±5.95	0.76±0.39	39.15±9.27	4.94±0.35	174.61±10.04	30.49±9.96
*Cirsium vulgare**	485.67±95.44	5.33±4.57	25.37±4.67	0.71±0.34	38.56±8.38	4.82±0.28	174.95±11.16	29.54±10.40

1 – Solar insolation index (unitless).

2 – Topographic relative moisture index (unitless).

* – Introduced species.

Table 16. Count regression analysis to determine the association between Mahalanobis distance (D^2) values and occurrence and frequency of plants of *Carduus nutans, Cirsium carolinianum* (Poisson regression[1]), *C. discolor* and *C. vulgare* (negative binomial regression[2]) at 200 field-test sites, Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009.

Thistle Species	Frequency of Presence/ Absence	Variable	Parameter Estimate	Standard Error	t Value	Pr > |t|
*Carduus nutans**	46/154	Intercept	1.0040	0.0791	12.6800	<0.0001
		D^2	-0.0427	0.0139	-3.0700	0.0022
Cirsium carolinianum	7/193	Intercept	0.7154	0.3852	1.8600	0.0633
		D^2	-0.5778	0.1334	-4.3300	<0.0001
C. discolor	67/133	Intercept	0.6916	0.2780	2.4900	0.0129
		D^2	0.0027	0.0348	0.0800	0.9382
*C. vulgare**	45/155	Intercept	0.4289	0.3318	1.2900	0.1961
		D^2	-0.0033	0.0309	-0.1100	0.9156

1 – Poisson regression was used for data that fit the Poisson distribution and did not exhibit overdispersion.

2 – Negative binomial regression was used for data that fit the Poisson distribution and exhibited overdispersion.

* – Introduced species.

D^2 for *Carduus nutans* (-0.0427, $P = 0.0022$) and *Cirsium carolinianum* (-0.5778, $P < 0.0001$; Table 16). Negative binomial regression showed no association ($P \leq 0.05$) between D^2 values and plant counts for *C. discolor* or *C. vulgare* (Table 16). The lack of significance of *C. discolor* and *C. vulgare* in field-test sites may be due to the wider range of D^2 values at test sites where these species were present. Although *C. carolinianum* was found in the fewest sites, all those sites had relatively low D^2 values (1.57–4.76; all below the threshold value identified by cumulative frequency analysis). Conversely, D^2 values for test sites where *C. discolor* and *C. vulgare* were recorded ranged from 1.98–19.56 (two sites were greater than the cumulative frequency threshold value of 18.35) and 1.13–29.82 (six sites were greater than the cumulative frequency threshold value of 9.90), respectively. In previous studies (Boetsch et al. 2003, van Manen et al. 2005), a narrow range of habitats was identified for the model plant species. Because *C. discolor* and *C. vulgare* are both ruderal species, the habitat models reflect the generalist nature of these two species.

These spatial analyses were conducted using raster data with a pixel size of 30 m, but different resolution data perhaps would have yielded different results. Several factors, such as the size of the study area, the biology of the species studied, data availability, etc., must be considered when determining the most appropriate resolution for a study using spatial data. For this study the highest resolution data available for the study area were used, because thistle populations are localized in relatively small areas throughout a landscape and 30-m pixel data were appropriate for the size of the study area. Higher resolution data (i.e., 10-m pixel size) may have enabled greater delineation of habitats, as smaller pixels provide more precise data. Conversely, lower resolution data (i.e., 100-m pixel size) may have lessened the ability of the model to identify habitats. As pixel size increases, the data associated with each pixel must be averaged over a larger area. Future studies could be conducted using spatial data across a range of resolutions to test the specific impacts of data resolution on habitat prediction.

Chi-square tests showed associations between both weevil species and sites where *C. vulgare* and *Carduus nutans* were found (Table 17).   For example, both *C. nutans* and *R. conicus* were predicted to be present at 4.8 sites, but these two species were actually present at 21 sites (Table 17). Thus, the presence of either introduced thistle species in an area increases the probability of the introduced weevil species occurring there as well, reflecting previously reported associations among these species (Surles et al. 1974, Ward et al. 1974, Surles and Kok 1976, Frick 1978, Zwölfer and Harris 1984).   Whereas both weevil species were recorded from sites where introduced and native species were documented to co-occur, no significant associations were observed between either weevil species or either native thistle species (Table 17). This lack of association is expected, as no non-target activity of *R. conicus* was documented for either native thistle species, and only low levels of *T. horridus* have been observed on each species (McAvoy et al. 1987, Wiggins et al. 2009).

The significance of the D^2 model to predict habitats for *C. nutans* and *Cirsium carolinianum* justified generating maps of habitats of both thistle species using the threshold value determined by their respective cumulative frequency graphs (Table 14). No habitat maps were generated for *C. discolor* or *C. vulgare*, as the D^2 models were not predictive of the test data. Approximately 12% overlap of *Carduus nutans* and *Cirsium carolinianum* habitat was observed and non-target feeding of introduced weevils on

Table 17. Chi-square[1] tests for associations between the presence of each thistle species with the presence of each weevil species at each of 200 field-test sites of the Mahalanobis distance (D^2) habitat models, Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009.

Thistle Species	Weevil species	Pearson Chi-square	df	Asymptotic Significance	No. Observed/ Predicted
*Carduus nutans**	*Rhinocyllus conicus*	78.552	1	<0.001	21.0/4.8
	Trichosirocalus horridus	20.051	1	<0.001	13.0/4.8
Cirsium carolinianum	*R. conicus*	0.111	1	0.739	1.0/0.7
	T. horridus	0.851	1	0.356	0.0/0.7
C. discolor	*R. conicus*	0.175	1	0.675	8.0/7.1
	T. horridus	0.005	1	0.946	7.0/7.1
*C. vulgare**	*R. conicus*	5.861	1	0.015	15.0/9.8
	T. horridus	14.504	1	<0.001	18.0/9.8

1 – Criterion alpha of $P \leq 0.01$ was used to decrease likelihood of Type I errors.

* – Introduced species.

C. carolinianum may be most likely to occur there (Fig. 25). The presence of *Carduus nutans* was an important predictor of the occurrence of both weevil species (Table 17). Thus, the co-occurrence of *C. nutans* with *Cirsium carolinianum* increases the likelihood that the introduced weevils will be in close proximity to the native thistle, thus increasing the opportunity for non-target activity. Although the predictive model for *C. vulgare* was not significant, this thistle species was also a significant predictor of weevil occurrence so the distribution of *C. vulgare* populations also could be a contributing factor to non-target activity.

Whereas surveys of naturally-occurring populations of *C. carolinianum* in eastern Tennessee have yielded no observations of non-target feeding by *R. conicus*, caged-plant studies have documented oviposition, larval development, and adult emergence of *R. conicus* from buds and flowerheads of *C. carolinianum* (Wiggins 2009). Additionally, low numbers (2 weevils from 240 plants) of adult *T. horridus* have been documented on *C. carolinianum* in eastern Tennessee (Wiggins et al. 2009). Therefore, the potential exists for both weevil species to use *C. carolinianum* as a reproductive host.

This study may be the first to use Mahalanobis distance, in conjunction with the close associations known to occur between invasive weeds and insects introduced to control them, to predict where feeding may occur on native host plants. Protocols developed during this research may have important implications for future research of introduced and native species. On a general scale, these protocols could be modified to evaluate spatial associations among introduced and native species. More specifically, future host-testing efforts for potential biological control agents could incorporate plant

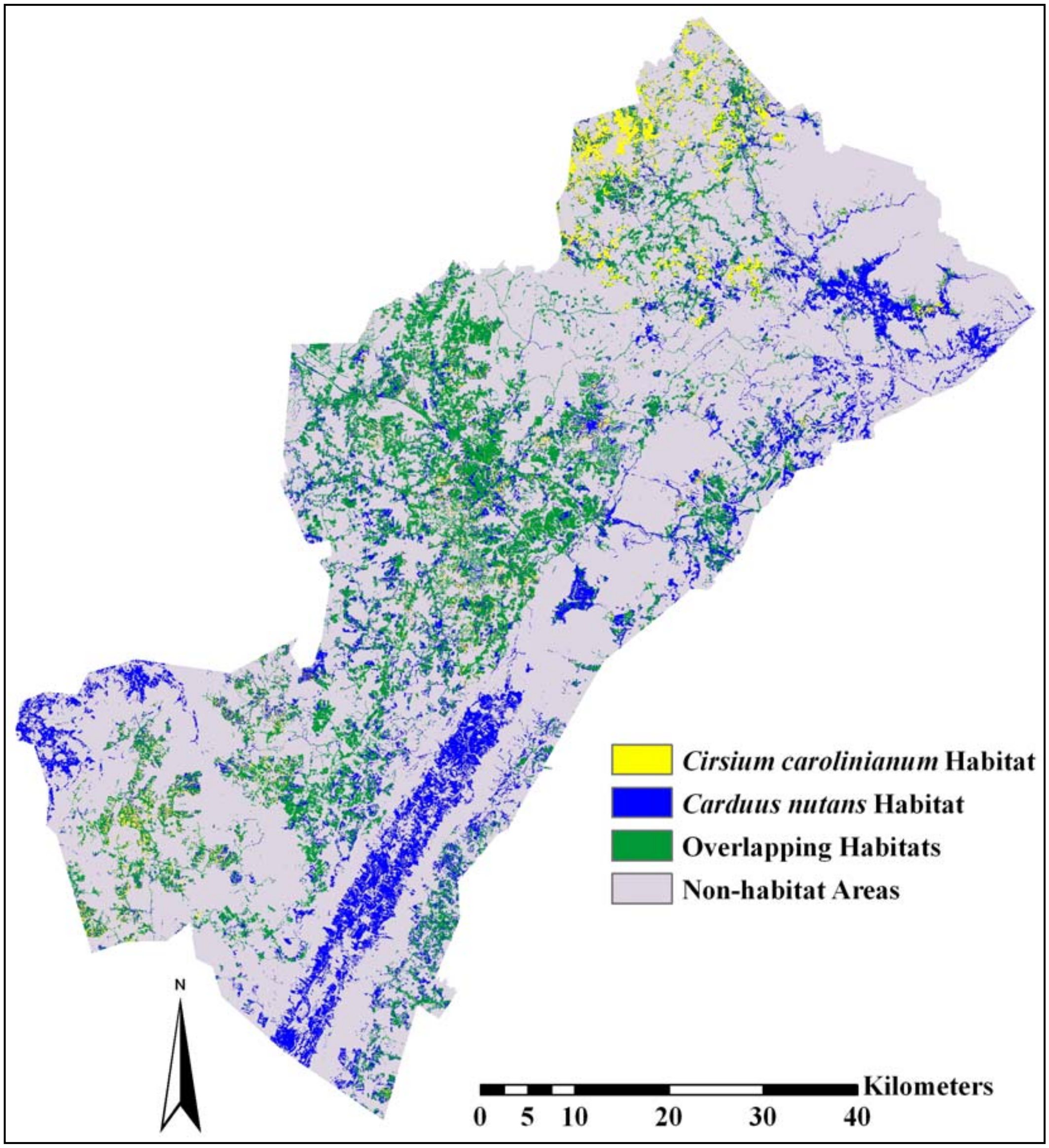

Figure 25. Habitat overlap of *Carduus nutans* and *Cirsium carolinianum* based on predictions from Mahalanobis distance models, Bledsoe, Cumberland, Morgan, and Van Buren Counties, Tennessee, 2005-2009. Non-habitat areas include forested areas and areas predicted as non-habitat by the model.

location data from herbaria or natural resource agencies to identify potential areas of habitat overlap, thus providing a type of spatial risk assessment of non-target feeding.

Results from this research also may have current management implications for areas where conservation of native *Cirsium* species is a concern. The well-documented non-target feeding of *R. conicus* in other areas of the U.S., in combination with the recent documentation of *T. horridus* occurring on the native thistle species *C. altissimum*, *C. carolinianum*, *C. horridulum*, and *C. muticum* (Takahashi et al. 2009, Wiggins et al. 2009), underscore the importance of detection and monitoring of native thistle populations, especially those listed as RTE species, for future conservation. Although no *Cirsium* species that occur in Tennessee are state or Federally listed, *C. carolinianum* is state listed as rare in Indiana and threatened in Ohio (USDA 2009). Land managers in these states could modify these spatial modeling protocols to identify suitable habitats for *C. carolinianum* and other native thistles to locate new populations and to monitor populations in areas where non-target feeding may be most likely. Monitoring efforts in those habitats could provide early detection of non-target activity of *R. conicus* or *T. horridus* and enable managers to make appropriate decisions regarding conservation of these native plants.

CHAPTER VII. CONCLUSIONS

Since its introduction from Europe in the mid-1800s, musk thistle, *Carduus nutans* L., has become a prominent weed species in North America. Because of its widespread distribution across most of North America, coupled with the time and expense associated with management of infested areas through chemical, mechanical, or other controls, biological control (i.e., the use of natural enemies to suppress pest populations) was promoted as an effective, sustainable management technique. In the late-1960s, a biological control program that included two European weevils, *Rhinocyllus conicus* Fröelich and *Trichosirocalus horridus* (Panzer), was initiated in the U.S. and Canada. Due to their complementary biologies, with larvae of *R. conicus* feeding within flowerheads and larvae of *T. horridus* feeding within meristems of rosettes, these weevils were credited with reducing population densities of musk thistle in many areas of release.

Feeding of *R. conicus,* and to a lesser extent *T. horridus,* on non-target native host plants in the genus *Cirsium* has prompted criticism of these biological control agents and raised concern over their effects on populations of native thistles. Across much of the central and western U.S., *R. conicus* has been documented feeding on at least 25 species of native thistles in the genus *Cirsium*. While the impacts on most thistle species by this feeding are unclear, significant reductions in seed numbers in *C. canescens* Nuttall generate concern that populations of this thistle species may begin to decline. Prior to this project, *T. horridus* had only been recorded as feeding on field thistle, *C. discolor* (Muhlenberg ex Willdenow) Sprengel. Because few records of non-target feeding of *T. horridus* exist, the impact this weevil may have on native thistle populations is uncertain.

No non-target feeding of either weevil species had been documented in Tennessee at the initiation of this study. However, five native [tall thistle, *Cirsium altissimum* (L.) Sprengel, soft thistle, *C. carolinianum* (Walter) Fernald & Schubert, *C. discolor*, yellow thistle, *C. horridulum* Michaux, and swamp thistle, *C. muticum* Michaux] and two introduced [Canada thistle *C. arvense* (L.) Scopoli, and bull thistle, *C. vulgare* (Savi) Tenore] *Cirsium* species are known to occur here. Because few studies have been conducted on these thistle species in Tennessee, little is known of the potential interactions of *R. conicus* or *T. horridus* with these species. Therefore, a project was begun to 1) assess the synchrony of phenologies of the three exotic and five native thistles in Tennessee with the phenologies of *R. conicus* and *T. horridus,* 2) document the incidence of the introduced weevils in natural populations of these eight thistle species, 3) quantify the impacts on plant reproduction or growth responses of each thistle species due to feeding of *R. conicus* and *T. horridus*, and 4) predict the occurrence of two native (*C. carolinianum* and *C. discolor*) and two exotic (*C. vulgare* and *Carduus nutans*) thistle species (model species) and identify potential habitats across a study area for these thistle species using spatial analysis.

Phenological synchrony is important when evaluating potential non-target hosts of herbivores introduced as biological control agents of weeds, as plant species with phenologies concurrent with the herbivores are most suited to become hosts. The phenologies of musk thistle and seven *Cirsium* thistles were estimated based on University of Tennessee Herbarium records and field observations made in 2008 and compared to general phenologies listed in selected literature sources. Phenologies of *R. conicus* and *T. horridus* were estimated by monitoring their occurrence in field populations of *Carduus nutans* in 2008 and 2009 (*T. horridus* only). Of the seven *Cirsium* thistles that occur in Tennessee, four species [*C. arvense* (introduced), *C. carolinianum* (native), *C. horridulum* (native), and *C. vulgare* (introduced)] either bud and/or bloom during the spring when adult *R. conicus* are ovipositing and the larvae feed within developing buds and flowers. Although differences in mean number of buds, flowers, and/or seedheads occurred in each species at some point throughout the year, general plant phenologies from herbarium records and field observations followed similar trends for spring-, summer-, and fall-blooming thistles. The discrepancy in monthly mean numbers of these plant parts between field observations and herbarium specimens may reflect a collection bias for herbarium plant specimens that are suitable and practical for display and storage, with smaller plant specimens or those possessing fewer heads possibly preferred by plant collectors to represent the species in collections. While field observations for these studies were conducted in eastern Tennessee, the range of elevations and latitudes at which plant populations were surveyed may emulate climatic conditions in other areas of the state and influence plant phenologies similarly. Therefore, plant phenologies in field populations in other areas of Tennessee are not expected to be drastically different from what was observed during this study. Differences in bud, flowerhead, and seedhead numbers between the herbarium and field-observed plants illustrate that if abundance of specific plant parts (such as flowers) or other plant growth characteristics during a specific time period is an important factor for studies, monitoring of naturally-occurring populations should be implemented to properly estimate plant growth characteristics in the field.

When phenologies of *C. carolinianum* and *C. horridulum* were compared to the phenology of *R. conicus*, significant levels of budding occurred with both native thistles during the period of oviposition of *R. conicus* on buds of *Carduus nutans*, and both native thistles were flowering when *R. conicus* was ovipositing on flowers of *C. nutans*. As the larvae of *T. horridus* feed within rosettes of musk thistle beginning in late December throughout early May, and all eight thistles recorded from Tennessee overwinter as rosettes, all thistle species may be susceptible to infestation by this weevil. Therefore, the potential exists for these weevils to utilize native *Cirsium* species found in Tennessee as host plants.

In surveys conducted to investigate non-target feeding of *R. conicus* and *T. horridus* on native *Cirsium* thistle species in naturally-occurring populations, no non-target feeding by *R. conicus* was observed, but adults of *T. horridus* were observed on all five native *Cirsium* thistles. These adult occurrences are the first documentation of *T. horridus* occurring on *C. carolinianum, C. horridulum*, and *C. muticum*, and the first record of *T. horridus* occurring in the Great Smoky Mountains National Park. While *C. carolinianum* and *C. horridulum* did not show symptoms of larval feeding in the meristematic tissues, *C. altissimum*, *C. discolor*, and *C. muticum* all had damaged meristems and possible oviposition scars on the midribs of the leaves. However, the impact of feeding by larvae of *T. horridus* on the reproductive potential of native plants is uncertain, because even in the target species (musk thistle and other introduced *Cirsium* species) plant death rarely occurs, and seed production continues.

Studies isolating adults of *R. conicus* on buds and flowers of all eight thistle species recorded from Tennessee were conducted in 2008 and 2009 to test if *R. conicus* could utilize these species for reproduction and what impacts larval feeding of *R. conicus* may have on plant reproduction. While no impacts were observed on *C. altissimum*, *C. discolor*, or *C. muticum* (all native fall-blooming thistles, i.e., not phenologically synchronous), larvae of *R. conicus* completed development in heads of *C. carolinianum* and *C. horridulum* (native spring-blooming thistles). Reductions in seed numbers of both of these native species were observed in both years of the study, with significant reductions occurring during 2008. In these enclosure studies, *R. conicus* readily oviposited on both *C. carolinianum* and *C. horridulum* at levels similar to those observed on *Carduus nutans* in 2008, and both native thistles exhibited significantly greater egg loads than the two introduced *Cirsium* species (*C. arvense* or *C. vulgare*) during both years. Infested heads of *C. carolinianum* contained proportionately more *R. conicus* per cm of plant head width than any introduced thistle species in 2008, and both *C. carolinianum* and *C. horridulum* contained proportionately greater numbers of *R. conicus* per head than *C. arvense* or *C. vulgare* during both years of the study. This information, coupled with the lack of significant variation in body length between *R. conicus* reared on native thistles and its target host *Carduus nutans*, signifies that, while *R. conicus* maintains an apparent preference for *Carduus nutans* as a reproductive host plant in naturally-occurring populations in this region, this weevil has the ability to utilize the native thistles *Cirsium carolinianum* and *C. horridulum* as host species. The evidence of the potential for *R. conicus* to utilize these species is tempered by the fact that no non-target activity has been observed in naturally-occurring populations of either *C. carolinianum* or *C. horridulum*.

A study isolating adults of *T. horridus* on rosettes of all eight thistle species that occur in Tennessee was conducted in 2009 to evaluate the plant responses in height, seed production, bud and flower production, and the size of flowerheads of native and introduced thistles to feeding of *T. horridus*. While damage to meristems of rosettes exposed to *T. horridus* was documented at varying levels for all eight thistle species, exposure to *T. horridus* had no effect on the numbers of damaged meristems. Also, no impacts of *T. horridus* were observed on seed production, flowerhead width, or plant height for any thistle species. The number of heads per plant of *C. discolor* treated with *T. horridus* were lower than control plants, but this thistle was the only species to exhibit any detrimental response. Additionally, while plant death occurred at varying levels in plants of each species, *T. horridus* was not a significant contributing factor for any species. Although damage to meristems of rosettes was documented in this study, the degree to which *T. horridus* may utilize these native *Cirsium* species in naturally-occurring populations remains unclear. The levels of *T. horridus* to which plants were exposed during this study may have been too low to elicit responses in plant growth. In populations of *Carduus nutans* infested with *T. horridus*, many (ca. 40-50) larvae per rosette were necessary to impact plant growth or seed production. Low infestation levels observed in naturally-occurring populations of the five native *Cirsium* species in Tennessee may indicate that even if *T. horridus* is utilizing native *Cirsium* species, no impacts to plants are not at meaningful levels. *T. horridus* was first observed feeding on *C. discolor* in Virginia in the early-1980s, but no reductions in populations of *C. discolor* have been attributed to feeding of *T. horridus* since that time.

Based on the potential for *R. conicus* to negatively impact plant reproduction, and the occurrence of *T. horridus* on naturally-occurring populations of all five native thistles, monitoring of native thistles should be considered in land-management areas where conservation of native species is a priority. These monitoring efforts could provide early detection of non-target feeding by *R. conicus* if it should occur on these native species and improve baseline information on which to base appropriate management decisions.

The ability to predict where non-target activity may occur would be useful in establishing monitoring programs and potentially managing populations of native thistles infested with either weevil species. A study was initiated in 2005 to identify areas of potential non-target feeding by *R. conicus* and *T. horridus* by predicting habitats for their known introduced hosts (*Carduus nutans* and *Cirsium vulgare*) and two native thistle species (*C. carolinianum* and *C. discolor*) incorporating Mahalanobis distance (D^2) with spatial analysis. Predicting habitats of thistle species is important, in light of the fact that *R. conicus* has been shown to have the ability to impact seed production in some native species in Tennessee, and *T. horridus* has been observed occurring on all native *Cirsium* species in Tennessee in the field. The general thistle habitats within the study area were primarily represented in areas of relatively high solar insolation at low-middle elevations, with gentle slopes and slightly acidic soils containing ca. 25% clay, ca. 35% sand, and ca. 0.75% organic matter. For most thistle species, pH, solar insolation, elevation, and percent clay were the most influential attributes for habitats, and percent organic matter and slope had the weakest influence on thistle habitats. Poisson regression showed associations between D^2 values and plant counts at field-test sites for *Carduus nutans* and *Cirsium carolinianum*. Negative binomial regression detected no association between D^2

values and plant counts for *C. discolor* or *C. vulgare*. During this study the highest resolution spatial data available for the study area were used, but higher resolution spatial data (i.e., 10-m pixel size) may have enabled greater delineation and prediction of habitats. Studies using spatial data across a range of resolutions would be useful in determining the specific impacts of data resolution on habitat prediction. Chi-square analysis found associations between sites where *Carduus nutans* was found and the occurrence of both weevil species and between sites where *Cirsium vulgare* was found and the occurrence of *T. horridus*. No associations were documented between either weevil or either native thistle species. When the predicted habitats of *C. nutans* and *Cirsium carolinianum* were compared, the habitats of both species overlapped in ca. 12% of the total study area. Due to the associations between the introduced weevils and *Carduus nutans*, this area is where non-target may most likely occur.

The findings of this project help clarify the interactions among *R. conicus* and *T. horridus* and thistle species in Tennessee. Comparisons of phenologies of the weevils and thistle species reveal that two native species, *C. carolinianum* and *C. horridulum*, are most at risk to non-target feeding by *R. conicus*, and that the rosette stage of all five native thistle species is vulnerable to feeding by *T. horridus*. Furthermore, adults of *T. horridus* were documented occurring at some level on all five native thistle species during a three-year study period. The occurrences of adult *T. horridus* in field populations, coupled with the damage exhibited to meristems of rosettes in caged plant studies, show that *T. horridus* has the ability to utilize native thistle species that occur in Tennessee. While no *R. conicus* were observed on any species of native thistle in naturally-occurring populations, in caged plant studies *R. conicus* did show the ability to utilize both *C. carolinianum* and *C. horridulum* as host species and negatively impact seed numbers. Monitoring of native thistle populations is crucial to determine the extent of future impacts of these weevils on native thistles. The spatial model generated during this study accurately predicted habitats of both *Carduus nutans* and *Cirsium carolinianum*. Because the occurrence of *Carduus nutans* predicts the occurrence of both weevil species, sites of *Cirsium carolinianum* could be established to monitor *R. conicus* and *T. horridus* activity in areas of overlapping habitats of *C. carolinianum* and *Carduus nutans*. The development of similar models for use with other *Cirsium* species could assist land managers in efforts to detect if non-target activity is occurring and aid future management decisions and practices. Only through continued efforts can the associations of these introduced weevils and native thistles be better understood.

This research provides a basis from which future studies can be modified or expanded. Modifications to studies conducted with *T. horridus* on native thistles could include the use of insecticides to prevent unwanted herbivory, the use of whole-plant cages to mitigate some cage effects, the propagation of plants in greenhouses or field plots to lessen potential site effects, and the application of larvae of *T. horridus* (instead of adults) to plants to lessen ambiguity of impact of larval feeding. Although there are difficulties associated with finding and removing larvae of *T. horridus* from plants infested in the field or maintaining colonies of *T. horridus* in the laboratory, the application of larvae to plants would enhance future studies of plant responses to larval feeding of *T. horridus*. Future studies of both introduced weevils and native thistles could investigate the chemical, visual, or other cues that may be used by each weevil species to detect suitable host species. If, for example, specific chemical cues could be identified, those chemicals may possibly be synthesized and applied to an area where rare, threatened, or endangered (RTE) thistles occur. The mass inundation of these semiochemicals could disrupt feeding and oviposition of the introduced weevils and possibly lessen the occurrence of non-target feeding.

Information gained during this research also may be used to enhance future biological control introductions. Although several genera of European thistles were tested for host suitability, no thistles native to North America (i.e., the area of release) were tested prior to release of either *R. conicus* or *T. horridus*. Current host testing protocols now include native species related to the introduced host species. As phenological synchrony is an important factor in non-target feeding, future non-target host testing should especially focus on native congeners of the target host that have phenologies synchronous with the proposed biological control agent. Additionally, spatial models that incorporate georeferenced plant population data from herbaria or natural resource agency databases could be used to evaluate the risk of non-target activity through modeling the habitats of target host species and possible native host species. The consideration of the phenologies of potential native host species, in conjunction with risk assessment using spatial analysis, should greatly improve the evaluation of non-target activity and reduce the likelihood of unintended host expansion observed with *R. conicus* and *T. horridus* on native thistles.

This research has demonstrated through a progression of studies that conditions are suitable for non-target feeding by introduced weevils to occur on native thistles in Tennessee. This potential non-target activity could have ecological impacts in the form of reduced seed numbers, thus potentially reducing population densities of some thistle species, which could result in changes in composition of local plant communities of which these thistles are a component. While no thistle species are listed as RTE in Tennessee, some thistle populations may occur in areas where conservation of native plants or restoration of native plant communities is a priority. The identification of the potential for ecological impacts in this study emphasizes the importance of monitoring populations of native thistle species in these areas of conservation/restoration to detect the presence and levels of activity of *R. conicus* and/or *T. horridus* and subsequently effectively manage their populations.

This research also may have ecological and economical benefits to areas outside of Tennessee and in other systems involving introduced insects and potential native host plants. The innovative approach to determining the current extent of non-target feeding, quantifying potential impacts based on field observations and studies, and estimating where impacts may occur using spatial analysis provides a basis for implementing similar programs in the future dealing with introduced insects and native plants.

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VITA

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