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Assessment of Insects, Primarily Impacts of Biological Control Organisms and Their Parasitoids, Associated with Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) in Eastern Tennessee

Amy Lynn Kovach
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Amy Lynn Kovach entitled "Assessment of Insects, Primarily Impacts of Biological Control Organisms and Their Parasitoids, Associated with Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) in Eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Paris L. Lambdin, B. Eugene Wofford

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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Graduate Studies

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A Thesis
Presented for the
Master of Science
Degree
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Amy Lynn Kovach
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Dedication

This thesis is dedicated to all of my family and friends, who have encouraged and supported me. In particular, I dedicate this thesis to my father who instilled in me a life long respect for education as the key to success, and who is proudly looking down on my accomplishment.

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I am appreciative for all of those who assisted this research project. In particular, I acknowledge and thank Jerome Grant, my advisor, for his encouragement, professionalism, support, and guidance for my success throughout the duration of my studies. The availability of help from Paris Lambdin and Eugene Wofford, my committee members, is greatly appreciated. I thank Gregory Wiggins for his assistance with locating and sampling research plots, processing specimens, preparing presentations, and creating an enjoyable work environment.

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I extend my thanks to the following taxonomists: Jim Story of the Western Agriculture Research Center, Montana State University, for his confirmation of *Urophora quadrifasciata* (Meigen), Alfred Wheeler of Clemson University, for his confirmation of *Megalanotus sabulicola* (Thompson), E. Eric Grissell and Michael Gates both of the USDA-ARS-Systematic Entomology Laboratory and Roger Burks of University of California-Riverside, for their identification of Chalcidoidea specimens.

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Abstract

Spotted knapweed [*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek] (formerly *C. maculosa* Lam. and *C. biebersteinii* DC.) (Asteracea) (referred to here as *C. stoebe* L. *sensu lato*) a non-indigenous, invasive weed, has been the focus of a biological control program using a complex of insects for more than 30 years in North America. Spotted knapweed is a prolific seed producer and produces two phytotoxic chemicals (catechin and cnicin), both enhancing the invasiveness of the weed. In Tennessee, information about this common weed of roadsides and its associated insects is not well known.

This research consists of five components: (1) Determine family composition and seasonality of insects associated with spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek, in eastern Tennessee. (2) Determine the distribution of *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) on spotted knapweed in eastern Tennessee. (3) Assess the impact of *U. quadrifasciata* on the production of seeds by spotted knapweed in eastern Tennessee. (4) Determine the distribution of *Megalanotus sabulicola* (Thompson) (Hemiptera: Lygaeidae) with spotted knapweed in eastern Tennessee. (5) Identify and determine the effects of hymenopteran parasitoids on *U. quadrifasciata*. The hypothesis of this research is that biological control organisms will be present on spotted knapweed in eastern Tennessee, reducing the ability of the weed populations to spread.

Insects (n = 3,122) representing 108 families in 15 orders were collected from spotted knapweed using sweep-net, direct, and beat-sheet sampling. Hymenopteran pollinators (11 families) were prevalent in sweep-net and direct sampling. These hymenopterans (Anthophoridae, Apidae, Halictidae, Megachilidae, Sphecidae, and Vespidae) found throughout the summer months contribute to the previous limited knowledge of only honeybees acting as pollinators of spotted knapweed, enabling the plant to produce offspring.

The most numerous insect recovered from sweep-net, direct, and beat-sheet sampling was the biological control organism *U. quadrifasciata* (n = 605; 19.4% of all insects collected). *U. quadrifasciata* was intentionally released in Beltsville, Maryland, in 1983 and has since dispersed southward. This gall-forming tephritid was released as a component of a complex of 13 biological control organisms where each species targets a specific part of spotted knapweed. The gall that the larva of *U. quadrifasciata* forms in the capitulum replaces the available space for seed development and appropriates nutrients from other plant locations. In eastern Tennessee, *U. quadrifasciata* was found to reduce seed production by 24% in infested capitula. The low density in the capitula of *U. quadrifasciata* (mean of 0.47 ± 0.02 S. E. individuals per capitulum) was offset by the prolific seed production of spotted knapweed in infested (6.01 ± 0.19 S. E. seeds per capitulum) and non-infested (7.94 ± 0.17

S. E. seeds per capitulum) capitulum to effectively reduce the population. One or more *U. quadrifasciata* immature was present in 78.4% of all dissected capitula of spotted knapweed. Immature *U. quadrifasciata* were collected from May 2003 through January 2004; adults were collected from May through August 2003.

Three solitary parasitoids (n = 412) of immature *U. quadrifasciata* were reared from field-collected capitula. These included *Pteromalus cardui* (Erdös) (Pteromalidae), *Brasema* sp. (Eupelmidae), and *Eurytoma* sp. (Eurytomidae). *P. cardui* was the most numerous (n = 346) and reduced the *U. quadrifasciata* population by 33.5%. In combination, all three parasitoid species have the potential to dramatically reduce field populations of *U. quadrifasciata*.

Another potential biological control organism, *M. sabulicola* (Thompson), was found in only two locations in small numbers (n = 10). *M. sabulicola*, a naturalized forager, is present throughout the eastern United States. It feeds on dispersed seeds of *Centaurea* spp. and therefore, has the potential to reduce the establishment of new spotted knapweed plants by consuming the dispersed seeds prior to germination. *M. sabulicola* had a density too low to be considered successful; however, future research into its rearing and effectiveness should be investigated.

This research is the first official confirmed collection of *U. quadrifasciata*, *M. sabulicola*, and the three parasitoids; *P. cardui, Brasema* sp., and *Eurytoma* sp., from Tennessee and the southern United States. The data on these aforementioned insects contribute to the distribution and impact of insects in the spotted knapweed community.

This research contributes to the known range of spotted knapweed, the known associations of the insect community with the host plant, along with adding to the known distribution of both previously released biological control insects and the distribution of potential naturalized biological control insects. Research into spotted knapweed and its associated insects in eastern Tennessee should be continued since this paper provides a solid foundation from which to base future studies.

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I. Literature Review

i. Invasive Flora

The early arriving European immigrants into North America in the 17th century brought plants to cultivate for food, forage, seasonings, and medicine in the unknown land (Mack 2003). Some of these non-native plants escaped confinement and naturalized (i.e., they became permanent members of the local flora) (Mack 1996) without interfering with the ecosystem. Others became abundant and aggressive, soon damaging the native species and their environment, as was the case in 1758 with the first described invasive flora species yellow toadflax (*Linaria vulgaris* P. Mill) (Mack 2003). Since the first recorded instance of an invasive plant in the United States, non-native floras have continued to be introduced at an accelerated rate both deliberately, as erosion controls or as ornamentals, and accidentally as contaminants from increased global trade.

Populations of non-native plant species (also referred to as non-indigenous species, alien species, naturalized species, or as exotic species, but with slightly different connotations) are undesirable for both ecological and economical reasons (Randall 1996). Today, non-native species in general are considered to be the third most important threat to biodiversity, preceded by habitat destruction and direct exploitation (Randall 1996). The invasion of non-indigenous plant species is considered to be one of the primary threats to the integrity and function of ecosystems (Randall 1996; Blossey 1999) costing the United States more than $34 billion in control costs for exotic aquatic, pasture, crop, and turf weeds (Pimentel et al. 2000).

Non-native weed species have attributes favorable to recently disturbed sites that enable them to out-compete or exclude native species for water and nutrients enabling them to develop faster and grow larger in new habitats. These attributes include the lack of their natural phytophagous enemies, shorter developmental times, greater seed or vegetative structure production, longer seed dormancy, phytotoxins, and higher photosynthetic rates (Malecki et al. 1993; Mack 1996; Thomas and Willis 1998; Westbrooks 1998; Pimentel et al. 2000). Plant invaders alter ecosystem processes, such as decomposition, hydrology, nutrient cycling, gene dispersal through hybridization, and natural disturbance regimes like fire occurrence (Randall 1996; Vitousek et al. 1996). Therefore, non-native plants can ultimately produce monospecific stands, dramatically reducing the species number and species diversity of an area.

In 1999, the National Invasive Species Council, an intergovernmental agency, was enacted by Presidential Executive Order 13112 to prevent the introduction of invasive species (alien species whose introduction does or is likely to cause economical or environmental harm or harm to human health), to

provide for their control, and to minimize the economical, ecological, and human health impacts that invasive species cause (Clinton 1999). In combination with older laws, such as the Federal Noxious Weed Act (Anonymous 1975), Executive Order 13112 has provided the legislative foundation and financial support for the prevention, control, and management of non-indigenous weeds detected on federal lands, through commerce, by state agencies, as a seed contaminant, and in waterways. Further enhancing the control effort, an Invasive Weed Awareness Coalition was established soon after the enactment of Executive Order 13112 to provide aid to ranchers who had been spending $5 billion each year to combat the invasion and spread of newly introduced non-native plants, such as knapweed (*Centaurea* species) (Babbitt 1999). Consequently, *Centaurea* species have been categorized among the most economically destructive exotic invaders in North America (Bais et al. 2003), and one rangeland weed, spotted knapweed [*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek syn. *C. maculosa* auct. *Amer*. (Asteracea) formerly *C. maculosa* Lam. and *C. biebersteinii* DC.] has been a prime target for control. Throughout this thesis, spotted knapweed will be referred to as *C. stoebe* L. *sensu lato* (*s. l.*) because of the changes with its nomenclature.

ii. Spotted Knapweed

Background. Spotted knapweed (*C. stoebe* L. *s. l.*) (Fig. 1) was first introduced into North America in the 1890s as a contaminant in alfalfa (*Medicago sativa* L.) seed from Asia Minor, probably Turkmenistan, or within hybrid alfalfa seed from Germany (Maddox 1979). In 1893, spotted knapweed was officially documented in Victoria, British Columbia, Canada, and Washington, United States, where it was limited to the San Juan Islands until 1920 (Roche et al. 1986; Sheley et al. 1998). However, Müller-Schärer and Schroeder (1993) described the first record of spotted knapweed in the United States to have been from Montana in 1935. Xeric grassland steppes from western Asia to western Europe are the native habitat range of spotted knapweed and its many subspecies (Müller-Schärer 1991; Sheley et al. 1998).

After its initial introduction into the western United States, spotted knapweed quickly spread to many ruderal habitats and disturbed sites, such as road and railroad right-of-ways, waste places, and overgrazed rangeland (Watson and Renney 1974), and became an economically important weed (Müller et al. 1989) within these areas. The elimination of superior forage species by spotted knapweed resulted in the main economic loss ($12/hectare in 1979 dollars) (Harris and Cranston 1979). By 1994, spotted knapweed had reduced the carrying capacity of rangelands by 90% (USDA APHIS 1997). By 2001, spotted knapweed had invaded more than 1,600,000 hectares of rangeland (Todd 2001) in the western United States and had consistently been

Figure 1. Spotted Knapweed (*Centaurea stoebe* L. *s. l.*).

described as one of the most serious exotic weeds of that region, and southwestern Canada (Rice et al. 1997).

Since 1894, the eastern United States also has been faced with populations of spotted knapweed (A. Swanson, personal communication) that arose from subsequent introductions within discarded wool-waste (Fletcher 1913) and not from population spread. To date, spotted knapweed had been documented in 45 of the 50 states (not reported in Alaska, Georgia, Mississippi, Oklahoma, and Texas) (USDA 2004) with 39% of the populations found on land classified as disturbed and 47% of them on pasture or timbered rangeland (Mauer et al. 2001).

Characteristics That Make Spotted Knapweed Undesirable. As most wildland weeds are invasive and non-indigenous (Randall 1996), so too is spotted knapweed. This short-lived perennial, preadapted to disturbance (Roche et al. 1986), can live for at least nine years (Boggs and Story 1987) and is a prolific seed producer. If there is an 80% survival of all seeds produced by all capitula (flower heads), the annual reproductive capability for spotted knapweed is 800 viable seeds per plant (Watson and Renney 1974). But, because the density of spotted knapweed plants varies from a single plant to more than 400 plants/m^2, a range of 800 to 320,000 seeds/m^2 is more likely to be produced (Watson and Renney 1974) for each square meter of infested land. Not only is production of viable seeds high, but seeds can maintain 50% viability even after banked in the soil for five years (Davis et al. 1993).

Besides having a high reproductive potential, spotted knapweed also has two allelopathic properties that benefit its survival and colonization of new habitats. Spotted knapweed displaces native plant species by exuding the phytotoxin (-)-catechin from its roots, inhibiting growth and germination of neighboring native plants by triggering an internal reaction by producing oxidants and consequently cell death within one hour of contact (Bais et al. 2003; Moellenberg 2003). The allelopathic compound, cnicin, a sesquiterpene lactone, that inhibits the consumption by wildlife because of its bitter taste, also has been isolated from leaves and shoots of spotted knapweed (Landau et al. 1994). Related to manipulation of other vegetation under the soil, Callaway and others (1999b) have found that soil microbes from the home range of spotted knapweed have stronger inhibitory effects on its growth than microbes from its native range, forming a positive feedback for the invasiveness of one of the world's worst weeds.

Spotted knapweed has a long stout taproot that can penetrate 0.5 m into the soil and thus is well adapted to extremely well-drained, gravelly soils where standing water does not accumulate (Davis et al. 1993; Sheley et al. 1998). The rosette stage can withstand drought conditions because the stored carbohydrates supply it with nutrients (Watson and Renney 1974). Disturbed

habitats, such as rangelands and roadsides, are typical xeric habitat types favorable for the growth of the taproot of spotted knapweed.

More than 500 individual plant species have been designated as a "noxious weed" because they have been officially targeted for regulation by the Federal Noxious Weed Act of 1975 due to their aggressiveness, rapid spreading capability, and direct harm or injury to agriculture or public health (Lorenz and Dewey 1988). An international database for both the United States and Canada lists *Centaurea* spp. (including *C. maculosa* Lam., *C. diffusa* Lam., and *C. solstitialis* L.) as the most commonly regulated weed group (Skinner et al. 2000). Because age class hierarchy of spotted knapweed allows it to occupy most available niches and to form dense monotypic stands (Sheley and Jacobs 1997), it has been placed on noxious weed lists for 15 states (Westbrooks 1998), of the 39 states, including Tennessee, that have such laws. The accepted classification for spotted knapweed for each of the 15 states where it is described as a noxious weed is shown in Table 1 (Bowen et al. 2002; USDA 2004).

Spotted knapweed often forms high stem densities on natural vegetation sites causing reduced vigor of native plant populations (88% reduction in rough fescue, *Festuca scabrella* Torr. ex. Hook.), decreased plant diversity, reduced livestock forage production, degraded wildlife habitat (98% reduction of elk, *Cervus elaphus* L.), and increased erosion (56% increase in water runoff) (Lacey et al. 1995; Rice et al. 1997; Sheley et al. 1998). Regressions of diversity indices on rangelands showed that the indigenous perennial grass cover, species richness, species diversity, and biomass of Idaho fescue (*F. idahoensis* Elmer) were inversely related to the cover of spotted knapweed (Kedzie-Webb et al. 2001). Anthropogenic disturbances, such as overgrazing by domestic livestock and mechanical soil disturbance, accelerate the invasion of spotted knapweed in native vegetation (Rice et al. 1997). Therefore, the success of the survival of spotted knapweed to continue as an invasive weed depends upon its ability to produce a large number of seeds, maintain a seed reservoir in the soil, produce allelopathic chemicals, and withstand severe moisture stress (Upadhyaya 1985).

Taxonomy of Spotted Knapweed. Spotted knapweed, which gets its common name from the black spotted tips of the bracts of the capitula, has been in the center of much taxonomic confusion within the genus *Centaurea*. Spotted knapweed belongs in the family Asteraceae (Compositae), subfamily Tubuliforae, and tribe Cynareae (Cronquist 1980). Cronquist (1980) designated only one species in the genus *Centaurea,* American knapweed (*C. americana* Nutt.), as native to North America, while all other present *Centaurea* species have been introduced from Europe, the Mediterranean and Eurasia. But Müller-Schärer and Schroeder (1993) state there are no native *Centaurea* spp. in North America and the native *C. americana* should be treated as *Plectocephalus americanus* L.

Table 1. Accepted Classification for Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) as Described on 15 State Noxious Weed Lists.

State	Noxious Weed List Classification[a]
Arizona	Prohibited and Noxious Weed
California	Noxious Weed A List
Colorado	Noxious Weed
Idaho	Noxious Weed
Montana	Category 1 Noxious Weed
Nebraska	Noxious Weed
Nevada	Noxious Weed
New Mexico	Class A Noxious Weed
North Dakota	Noxious Weed
Oregon	B Designated Weed, Quarantine Weed
South Dakota	Regulated Non-Native Plant Species
Tennessee	Rank 2 - Significant Threat
Utah	Noxious Weed
Washington	Class B Noxious Weed, Noxious Weed Seed and Plant Quarantine
Wyoming	Noxious Weed

[a]Classification is based on individual criteria set by each state.

As evident from the above example, over the past 100 years, there has been no consensus with nomenclature, number of taxa, or species rank in the *Centaurea* genus that consists of about 500 species (Müller et al. 1989). Because spotted knapweed belongs in a large and difficult taxonomic group (Heywood 1975; Johnson 1975), many revisions since its initial identification as *C. maculosa* Lam. have occurred. The origin of the problem began when no type specimen for *C. maculosa* had been designated because no authentic material from Linnaeus existed in the herbaria, and other botanists, like Lamarck, that have cited him do not allow an unequivocal identification of their specimens (Ochsmann 2001).

Various different morphological and regional habitat concepts have been used by different authors enhancing the great confusion in the nomenclature (Ochsmann 2001). For instance, Moore (1972) divides *C. maculosa* Lam. into three subspecies [*C. m.* ssp. *micranthos* (Gmel.) Gugler, *C. m.* ssp. *maculosa* Lam., and *C. m.* ssp. *rhenana* (Bor.) Gugler]. Heywood (1975) also lists *C. maculosa* Lam. as having three subspecies, but with three different subspecies than Moore [*C. m.* ssp. *chaubardii* (Reichenb.), *C. m.* ssp. *albida* (Lecoq & Lamotte), and *C. m.* ssp. *subalbida* (Jordan)].

Stemming from the confusion over spotted knapweed, Dostal (1976) completed the first recent revision of *Centaurea* based on morphological data. Müller and co-authors (1989) investigating root herbivores as possible biological control agents and their synchronicity on similar appearing morphotypes of spotted knapweed also completed a revision of *Centaurea*. In the revision (Müller et al. 1989), *C. maculosa* ssp. *rhenena* (Boreau) Gugler is a synonym for *C. rhenana* Boreau, whereas *C. maculosa* ssp. *micranthos* Gmel. is a synonym for *C. micranthos* Gmel. ex. Hayek in addition to a synonym for *C. biebersteinii* ssp. *biebersteinii* DC. Consequently, *C. maculosa* Lam. was briefly referred to as *C. biebersteinii* DC. Adding to the confusion, Heywood (1975) lists *C. biebersteinii* DC. and its four subspecies [*C. b.* ssp. *australis* (Pančić), *C. b.* ssp. *rhodopaea* (Hayek & H. Wagner), *C. b.* ssp. *radoslavoffii* (Urum.), and *C. b.* ssp. *cylindrocephala* (Bornmüller)] as a separate species from *C. maculosa*.

The latest revision of *C. maculosa* occurred in 2001. Using morphological data (width of capitula, number and color of flowers, cilia on the phyllaries, pappus length, ecology, and number of stems) and molecular characteristics (chromosome count and DNA analysis) based upon 200 regional populations of spotted knapweed from more than 1,000 herbarium specimens in Europe and 3,500 specimens from North America, Ochsmann (2001) reclassified *C. maculosa* Lam. and *C. rhenana* Boreau as synonyms of *C. stoebe* L. ssp. *stoebe*. *C. stoebe* L. ssp. *stoebe* are only found in Europe. Plants referred to as *C. maculosa* in North America are actually the non-indigenous, invasive *C. stoebe* L. ssp. *micranthos* (Gugler) Hayek with synonyms of *C. biebersteinii* DC and *C. micranthos* Gmel. and a basionym of *C. maculosa* Lam. ssp. *micranthos* (Gugler)

Hayek. Surprisingly, Moore (1972) also recognized other authors using the name *C. stoebe* L., but they were disregarded in favor of those that used for *C. maculosa*.

The United States Department of Agriculture-Agriculture Research Service-Germoplasm Resources Information Network (USDA-ARS-GRIN) and other branches of the USDA have adopted the following nomenclature for the short-lived perennial tetraploid, spotted knapweed, in North America as *Centaurea maculosa* L. auct. *Amer*. [synonym *C. stoebe* L. ssp. *micranthos* (Gugler) Hayek] and those not found in North America as *C. maculosa* Lam. (synonym *C. stoebe* L. ssp. *stoebe*), and *C. maculosa* Lam. ssp. *micranthos* (Gugler) [synonym *C. stoebe* L. ssp. *micranthos* (Gugler) Hayek] (M. Skinner, personal communication). Only the subspecies present in North America are invasive.

Chromosome and Morphological Information. Life span confusion of spotted knapweed as a biennial, short-lived perennial, or perennial of fields, roadsides, and wasteplaces (Boggs and Story 1987) has contributed to the re-classification of this particular unfavorable plant. Morphological results have shown that spotted knapweed is a perennial forb (Davis et al. 1993; Ochsmann 2001) that adds one ring of secondary xylem to its roots annually (Boggs and Story 1987). Molecular analysis has shown that the North American species previously identified as both the haploid n=18 (Radford et al. 1968; Cronquist 1980) *C. maculosa* Lam. and the diploid 2n=18,36 (Gleason and Cronquist 1991) is actually the perennial, polycarpic tetraploid 2n=36 *C. stoebe* L. ssp. *micranthos* (Gugler) Hayek (Moore and Frankton 1953; Ochsmann 2001).

Other *Centaurea* species (*C. rhenana* and *C. stoebe* ssp. *stoebe*) that are morphologically similar to *C. stoebe* L. ssp. *micranthos*, but are not found in North America, are strictly biennial, monocarpic diploid 2n=18 and not invasive (Ochsmann 2001). When spotted knapweed, *C. maculosa* L. ssp. *micranthos* Gmel, was briefly reclassified as *C. biebersteinii* ssp. *biebersteinii* DC, it was described as perennial and tetraploid 2n=36 (Müller et al. 1989).

The genetic differences of the North American subspecies of spotted knapweed (*C. stoebe* ssp. *micranthos*) enable it to germinate and develop in native vegetation. Thus, it thrives as an invasive plant.

Botanical description. The botanical description of spotted knapweed is: stems erect or ascending, branched, pubescent, 30-100 cm high; leaves alternate, much divided (pinnatifid); flowering heads eradiate, corymbs or corymbose panicles, foliaceous, ovate and yellow-green to brown involucre bracts with black fringed tips; flowers tubular, purple, pink, rarely white; achenes brownish; pappus of simple bristles, 1-2 mm long, persistent (Watson and Renney 1974). Additional morphological information includes plant height of 15 to 60 cm, capitula 5 to 12 mm long, and lobed basal rosette leaves up to 20 cm long (Sheley et al. 1998; Wilson and Randall 2003).

Life Cycle. The life cycle of spotted knapweed is rather simple. Structurally, spotted knapweed can live for nine (Boggs and Story 1987) to 15 years (Harris 1991). In its native Eurasian habitat spotted knapweed is in the rosette stage (Fig. 2a) throughout the year, bolts (Fig. 2b) in May and June, flowers (Fig. 2c) in July and August, with mature dry seed heads (Fig. 2d) emerging in September and remaining on the plant until the spring of the following year (Müller et al. 1989). In North America, spotted knapweed follows a similar developmental timeline, flowering from June to October and forming mature seeds by mid-August (Watson and Renney 1974) with dried heads remaining on the plant for three years on the dead stalks (Davis et al. 1993).

Seed Production. Spotted knapweed produces a mean of 16.35 ± 4.44 S.E. capitula per plant on rangeland and an astounding 706.66 ± 64.81 capitula per plant in partially irrigated soil (Watson and Renney 1974). Individual capitula contain between 25 and 50 flowers (Sheley et al. 1998; Wilson and Randall 2003) that flower for 2 to 6 days (Mauer et al. 2001). Pollination typically occurs by insects, especially the honey bee (*Apis mellifera* L.) (Watson and Renney 1974), but can also occur through self-pollination (Lack 1976). Following successful pollination, each individual flower is capable of producing one achene (fruit or seed) (Fig. 3) with a mean of 26.64 ±2.88 S. E. and 35.75 ± 4.00 seeds per capitula on rangeland and in partially irrigated soils, respectively (Watson and Renney 1974; Strang et al. 1979). Harris (1990) found that 12.6 of those seeds produced, on average, are viable.

Seeds are 3 mm long, oval, brown to black, with pale longitudinal lines and pappus of simple bristles (Fig. 3) (Davis et al. 1993). Spotted knapweed only reproduces by seeds. The number of seeds required to start a new population of spotted knapweed has not been determined, but the number of seeds required to maintain a population of spotted knapweed has been determined. A population of spotted knapweed can be maintained via seed production if the plants can surpass the threshold of 1,500 seeds/m^2 (Roze 1974). Schirman (1981) estimated that only about 0.1% of survival of the seeds produced is required to maintain stands in highly disturbed areas. Consequently, populations of spotted knapweed are commonly maintained and expanded because more than 140,000 seeds/m^2 (Schirman 1981) are found in the sampled area, surpassing the threshold level nearly tenfold.

Dispersal. Seeds are dispersed when bracts of flower heads open when they are dehydrated, 2 to 3 weeks after maturity, and movement of the stem by wind or by passing animals flicks the seed a relatively short distance up to 1 m from the parent plant (Watson and Renney 1974). Seeds can be dispersed long

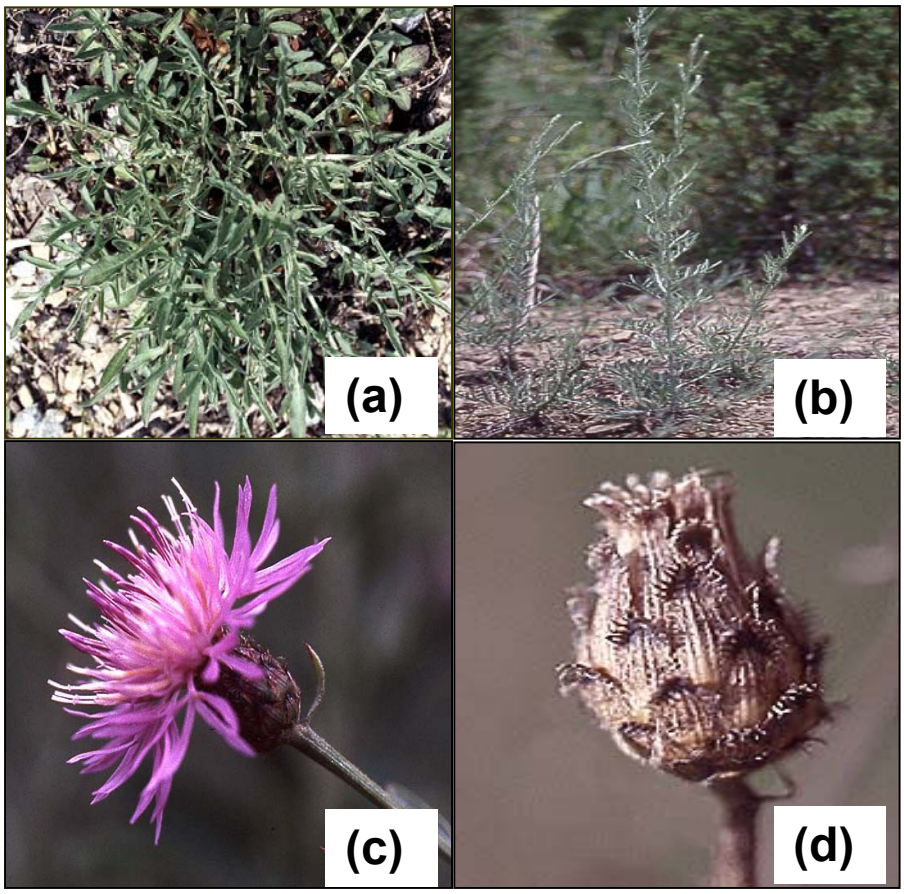

Figure 2. Developmental Stages of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*); (a) Rosette, (b) Bolting, (c) Flowering, and (d) Dry Capitula Stage.

Figure 3. Seeds (Achenes) of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*).

distances when attached to passing animals or mud stuck to vehicles and shoes and through bodies of water (Roche et al. 1986; Sheley et al. 1998). Seeds can be spread indirectly into uncolonized areas after passing through the digestive systems of deer mice [*Peromyscus maniculatus* (Wagner)] and great horned owls (*Bubo virginianus* Gmelin) (Pearson and Ortega 2001).

Germination. Because spotted knapweed is well adapted to extremely well-drained, gravelly soils where standing water does not accumulate, the availability of water for imbibition has a large effect on seed germination (Davis et al. 1993). Seeds germinate in the fall and early spring when water penetrates the hilum (Watson and Renney 1974). Optimum germination occurs at 19°C (range 7 to 34°C) within 5 cm below the soil surface (Watson and Renney 1974). Spotted knapweed produces three types of seeds with variable germination behavior: nondormant seeds (dark germinators), light-sensitive dormant seeds (germinate after exposure to red light) and light-insensitive dormant seeds (germinate without exposure to red light) (Nolan and Upadhyaya 1988); thus, the seeds can germinate in most environments.

Growth. Following germination the seeds develop into rosettes, the nutrient storehouses that remain above the soil throughout the year (Watson and Renney 1974). Within one year from germination, spotted knapweed will bolt a variable number of stems (Watson and Renney 1974) that will then begin a new generation through seed production and dispersal. It does not grow well in moist soils with vigorously growing grass (Harris and Cranston 1979). Spotted knapweed produces two allelopathic chemicals to suppress the growth of neighboring plants, cnicin in its leaves and shoots (Landau et al. 1994) and catechin in its roots (Landau et al. 1994; Bais et al. 2003; Moellenberg 2003).

iii. Biological Control of Weeds

Background. The first record of a biological control agent used against a weed was the accidental introduction in 1795, and then later cultivation and dispersal, of monacantha cochineal [*Dactylopius ceylonicus* (Green)] (Hemiptera-Homoptera: Dactylopiidae) in India for the control of Indian fig (*Opuntia vulgaris* Miller) (Crawley 1989; Goeden and Andres 1999). In Australia in the 1920s, one of the most well known historical textbook accounts in biological control of weeds occurred with the successful control of prickly pear cacti (*Opuntia inermis* deCandolle), by the prickly pear moth [*Cactoblastis cactorum* (Berg)] (Lepidoptera: Pyralidae) (Crawley 1989; Goeden and Andres 1999). Biological control of weeds in North America received prominent recognition in the 1950s with the successful introduction of two leaf beetles (Coleoptera: Chrysomelidae), the Klamath weed beetle [*Chrysolina hyperici* (Förster)] and the Klamathweed beetle [*C. quadrigemina* (Suffrian)], to control the rangeland weed St. John's

Wort, *Hypericum perfaoratum* L. (Malecki et al. 1993). Other recent successful programs of biological control of invasive, non-indigenous weeds, such as alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.] (Goeden and Andres 1999), and purple loostrife (*Lythrum salicaria* L.) (Blossey 1999), have demonstrated that with judicious research and testing procedures, long-lasting, cost-effective, environmentally sound, and effective biological control programs can be implemented for a variety of troublesome plant species (Malecki et al. 1993). To date, many other successful weed biological control programs have been documented in terrestrial and aquatic habitats.

The original implication of the term 'biological control' referred to the use of natural enemies to control insect pests (Smith 1919). A more recent definition of biological control has been broadened to include the study and utilization of parasites, predators, and pathogens to regulate populations of pests (both flora and fauna) (Harris 1991). Whereas, another recent definition applies just to the biological control of weeds from an economic perspective as the deliberate use of herbivorous organisms and pathogens to reduce the population density of a target species below its economic injury level (Müller-Schärer and Schroeder 1993).

Because naturalized weeds are often not native to the area where they are considered to be an unwanted plant (or plant out of place), and often have few host-specific natural enemies capable of controlling their populations, biological control agents that are used in their control are often from their geographic origin. Thus, biological control of weeds most often implements a "classical" approach, or the introduction of exotic agents from the native area of the weeds for permanent suppression of their populations (Müller-Schärer and Schroeder 1993). Classical biological control of weeds can also be stated as providing control on a continuing basis by maintaining populations of insects to keep the plant below its economic threshold level (Harris 1991) in selected locations. The biological control agents are expected to disperse in the environment, persist, reproduce and reduce the non-indigenous weed to a non-economic concern (Bellows and Headrick 1999).

The original method for selecting an agent for weed biological control was the researcher's intuition from limited study of what insect may be an effective weed pest, as is evident by the communication between Koebele and Perkins during their search for insects to control Lantana (*Lantana camara* L.) in Hawaii in 1902 (Harris 1973; Harris 1991). This method, although somewhat successful in limited instances, has led to a negative association with biological control (also referred to as biocontrol) in some ecological circles fostering debate over the ecological effects of classical biological control agents that has polarized biologists. One group views biological control as a reduction of chemical use, self-distribution, use and low economic cost; while another group views it as a threat to the structure and dynamics of complex biological communities from its non-target effects (Louda et al. 2003).

The case for biological control. The current standards for releasing biological control agents are much more stringent and ecologically safer than those practiced more than 100 years ago. Development of a biological control program begins with a systematic assessment of the weed problem: (1) assuring proper identification of the target weed, (2) charting the geographic range of the weed, (3) characterizing the habitats it infests, (4) ascertaining the losses caused by the weed, (5) determining the degree of control required, and (6) compiling a list of natural enemies already present or reported elsewhere (Goeden and Andres 1999). Once this information has been gathered, additional knowledge about the potential biological control agent is needed before it is considered for release in the United States today. This information includes the potentially released biological control agents to have the desirable attributes of ecological compatibility, temporal synchronization with target weed, density responsiveness, sufficient reproductive potential, searching capacity, dispersal capacity, host specificity and compatibility, food requirements and habitat assessment, minimal hyperparasitism, and culturability (Legner and Bellows 1999).

In the United States, scientists must demonstrate the environmental safety of plant-feeding arthropods as biological control agents of weeds. Studies with as many as 10 to 20 North American native plant species related to the target weed within its country of origin or at a domestic quarantine facility are conducted (Fisher and Andres 1999). All possible biological control insects for new introductions also must pass through United States Department of Agriculture (USDA)-Animal Plant Health Inspection Service (APHIS) Plant Protection Quarantine (PPQ) primary certified quarantine facilities (Fisher and Andres 1999) before research can be conducted on them. Procedures asking for scientific feedback and review, societal feedback, and permission have been implemented before any release of agents can occur (Bellows and Headrick 1999).

Advantages of the use of weed biological control agents include self-distribution, host-specificity with minimal ecological disruptions, non-impact on other community species, and relatively cost effectiveness (Goeden and Andres 1999). Some of the world's worst weeds, both terrestrial and aquatic, have been controlled biologically with herbivores, because it was the only option to treat the millions of hectares of native grasslands or waterways infested by the weeds (Bellows and Headrick 1999). Biological control also works well within an integrated pest management (IPM) program where other weed control methods, such as cultural and mechanical control, are part of the protocol.

The case against biological control. Even with its numerous successes, the use of biological control agents for non-indigenous weed control has generated several concerns because insufficient consideration is paid to

potential risks (Thomas and Willis 1998). One main concern is the unpredictability and irreversibility that suggests biological control must be viewed as risky and that specific projects should not be viewed as innocuous until substantial effort has been extended to support this view (Simberloff and Stiling 1996a). Likewise, the breadth of diet, potential host range, and ecological effects of the agent need to be investigated and then carefully weighed against the environmental costs and alternative management options (Louda et al. 1997) before the biological control agent is to be introduced. A concern in more recent years has occurred after a release of biological control agents with minimal monitoring of non-target species, particularly in sites and habitats far from the point of release (Simberloff and Stiling 1996b) where ecological harm to native species can occur without knowledge. Another concern is that for biological control to be successful, populations of the target weed must also persist at tolerable levels as a host for the biological control agent (Bellows and Headrick 1999); therefore, complete eradication cannot be expected and potential for future spread through seed or vegetative reproduction is always possible. Biological control programs should be closely monitored, since two-thirds of the agents released have not become numerous enough to inflict major damage to the targeted weed population (Harris 1991).

Additional concerns to the relevance of data obtained for weeds targeted for biological control also exist. Crawley (1989) states that: (1) weeds are generally alien plants growing in plant communities that are often quite different from those in which they evolved, so comparison data need to be taken from their introduced area along with the country of origin for a valid analysis, (2) the insects have been freed from their native natural enemies prior to release, so they may not show typical behavior as in their native habitat, and (3) the range of genetic variability in both plant and insect populations may be lower than in native communities as a result of the small size of the initial introductions, affecting both the characteristics of the plant and herbivory of the insects.

Disadvantages of biological control include: (1) an introduced agent cannot be recalled or limited to certain areas of the target plant range, (2) host-specificity tests for all possible hosts may never occur or take too long to complete, (3) it is a relatively slow process because it can take years for agents to become established or at high enough densities to reduce weed populations, (4) and it has only a 45% success rate (Bellows and Headrick 1999). Most of the failures of biological weed control occur for two reasons: (1) weed control is possible, but relatively unpredictable because of several insect species involved and (2) the weed species is difficult to control for various reasons (Crawley 1989). Both of these descriptions could apply to some non-indigenous weed species.

Success. Ultimately, success in biological control of weeds is measured in terms of the degree to which the density of the target weed is reduced below

its pre-release equilibrium (Müller-Schärer and Schroeder 1993) based upon the objectives of the project (Harris 1991). Phrased another way, success can be demonstrated by the reductions of populations of the targeted plant populations below their carrying capacity by the released phytophagous insects (Julien 1992). The first and most important step toward weed control success is the "establishment," defined as the survival of biological control agents for three or more years following an open release (Crawley 1989). Further successes in weed reduction can also be quantitatively determined such as "biological success," a measure of resource use by the agent in relation to the resource available, and "host impact," a measure of the decrease of reproduction or biomass of the weed at sites favorable to the agent (Harris 1991).

iv. Management Methods for Spotted Knapweed

Most efforts to manage spotted knapweed have focused on re-establishing valuable range, pasture, or cropland and were not from the perspective of restoring the native community (Mauer et al. 2001). Therefore, management of spotted knapweed is based on large scale and well established populations of the invasive, non-indigenous plant. As with most invasive species, prevention of spotted knapweed from spreading into or being introduced into a new area is the most cost effective and ecologically practical management strategy (Sheley et al. 1996; Sheley et al. 1998), although not always the one most funded. Even if more funds were available for the prevention of spotted knapweed, there are still more than one and one-half million hectares of spotted knapweed reproducing, therefore alternative methods need to be enacted to manage its encroachment onto valuable land. The following management methods, alone or in combination in an IPM program, have been used with varied success against spotted knapweed.

Grazing. Grazing is limited in its ability to reduce populations of spotted knapweed. Cattle (*Bos taurus* L.) prefer grasses over spotted knapweed because of the difficulty to reach the low-lying rosettes and because of its bitter taste, but low levels of grazing of spotted knapweed by sheep (*Ovis aries* L.) and goats (*Capra hircus* L.) have been observed with limited successful control (Sheley et al. 1998). Angora goats in a Montana National Wildlife Preserve also have been shown to reduce reproduction through long-term grazing (Sheley et al. 1998).

Fertilization. Fertilization typically improves the health, viability, and survival of plants. However, it should not be used as a management method of spotted knapweed. Nitrogen fertilizer enhances the ability of spotted knapweed to capture the nitrogen before desirable neighboring species can utilize it (Sheley and Jacobs 1997).

Cultivation/Tillage. Cultivation has had mixed results in reducing spotted knapweed. To evaluate the hypothesis that herbivory will be most destructive when competition is most pronounced, Müller-Schärer (1991) discovered that, when placed in pots with a common forage grass (*Festuca pratensis* Huds.), both growth and seed production by *C. maculosa* was reduced. Harris and Cranston (1979) found that spotted knapweed does not grow well under cultivation in irrigated alfalfa. Tillage of the soil to 20 cm increased the density of both spotted knapweed and its competitor, wheatgrass [*Elytriga intermedia* (Host) Nevski], but reduced the biomass of spotted knapweed one year after treatment (Velagala 1996).

Fire. The use of fire as a management tool for spotted knapweed has had conflicting results. High intensity annual burns have reduced low densities of populations of spotted knapweed by 5-90%, but single, low intensity burns also have disturbed the habitat promoting colonization of spotted knapweed (Morisawa 1999). Additional studies have shown that fire can cause different responses in spotted knapweed based upon infestation density and time of year and should not really be considered as an option (Sheley et al. 1998). Likewise, sturdy taproots and seeds banked in the soil can survive fire and return (Sheley and Roche 1982) reinforcing the use of fire for newly colonized areas.

Mowing. Mowing would be a useful management option in areas free of rocks and shrubs. When conducted 10 days after flower heads begin to open mowing dramatically reduced seed production to four per capitula (Mauer et al. 2001). Mowing twice a year, first when plants are beginning to bolt and second just prior to flower emergence, reduced plant density by 75% (Watson and Renney 1974). Mowing would be useful in reduction of spotted knapweed populations, but not in its eradication because of the potential growth from banked seeds.

Hand Removal. Hand removal, although time and labor intensive, may be possible to reduce small populations of spotted knapweed if done before seed dispersal. The goal of hand removal of the entire plant should focus on removing as much of the root as possible to prevent re-growth (Lacey et al. 1995). One study suggests the best time to utilize hand removal is in the summer when the soil is dry (Mauer et al. 2001), while another suggests when the soil is wet is better (Lacey et al. 1995). Hand clipping the capitula is under investigation to reduce small populations in Oregon with less threat to soil disturbance than hand pulling (Mauer et al. 2001).

Herbicides. Herbicides have been effective for short-term reduction of small infested areas. Although spotted knapweed can be managed for two to three years using picloram (4-amino-3,5,6, trichloropicolinic acid) (0.28 kg/ha)

during treatment in the fall on the rosettes or in the spring on the buds, associated ecological problems (Sheley et al. 1998; Morisawa 1999; Mauer et al. 2001) from the instability of the chemicals (Maddox 1979) make it unable to be used on porous soil or near surface water (Morisawa 1999). Land that had been treated with picloram made the reseeding of grass problematic (Müller-Schärer and Schroeder 1993) because it remained active in the soil for up to four years after treatment (Harris and Cranston 1979). The use of picloram also is economically prohibitive over large areas of land with the low economic value of the land providing further reasons not to rely upon it (Maddox 1979). Plus, germination is not prevented from herbicide use, and the seeds that are already on the soil remain dormant longer than the phytotoxic residual period of picloram (Davis et al. 1993).

Two other herbicides, clopyralid and 2,4 D, have been used against spotted knapweed. Clopyralid (0.13-0.19 L/ha) has been successful when sprayed on the bolting or bud stage of spotted knapweed in addition to having less residual than picloram (Morisawa 1999). 2,4 D (0.18 kg/ha), once recommended to spray on rosettes of spotted knapweed, should no longer be used because it is not effective in stopping bolting (Morisawa 1999).

Biological Control. Biological control is an effective management tool against spotted knapweed. One recent study hypothesized that the increased competitive ability of non-indigenous plants to displace native plants may be a result of an evolutionary shift favoring the selection of genotypes with a larger biomass allocation, therefore promoting a successional use of phytophagous insects to reduce overall plant vigor (Blossey and Kamil 1996). While another study reported that although biomass differences are true in some cases, the absence of a general trend casts doubt on the biological control strategy of introducing sequences of phytophages, none of which independently delivers a fatal blow (Thebaud and Simberloff 2001). Nevertheless, implementing management of spotted knapweed using multiple trophic types of phytophagous insects has been a focus of rangeland management for spotted knapweed over the past 40 years.

Exploration of biological control agents for knapweeds began by Heinz Zwölfer at the Commonwealth Institute of Biological Control (CIBC) Laboratory in Switzerland on behalf of Agriculture Canada in the 1960s (Maddox 1979) for economic reasons. Implementing a biological control program cost the Canadian government more than $1.5 million, compared to the potential $50 million dollars they were facing in rangeland losses (Müller-Schärer and Schroeder 1993). From 1961-1964 surveys for herbivores of spotted knapweed (and diffuse knapweed) were conducted by the CIBC in France, Switzerland, Germany and Austria, later to be extended in 1965 to Slovakia, Hungary, Greece, Bulgaria, Romania, and Turkey (Müller et al. 1989; Müller-Schärer and Schroeder 1993). The first potential biological control agents selected for study and screening from 1967

until field study termination in 1971 were three seedhead-attacking species: banded knapweed gall fly (*Urophora affinis* Frauenfeld) (Diptera: Tephritidae), UV knapweed seedhead fly (*U. quadrifasciata* Meigen) (Diptera: Tephritidae), and knapweed seedhead moth (*Metzneria paucipunctella* Zeller) (Lepidoptera: Gelechiidae) (Müller-Schärer and Schroeder 1993). When it became apparent that seedhead-infesting insects had only a limited potential for reducing weed density, a detailed investigation of the complex of rosette and root-feeders was initiated in 1978 by the International Institute of Biological Control (IIBC) (Müller-Schärer and Schroeder 1993). The objective of the knapweed control program was to achieve <5% spotted knapweed cover on rangeland (Harris 1990).

USDA-APHIS perceived the need to respond and organize a regional effort of its own to an enormous weed problem in the United States (Lang et al. 2000). In 1987, USDA-APHIS-PPQ in cooperation and consultation with other groups (such as Agriculture Canada) began a biological control program using a three phase strategy to establish and redistribute biological control agents of both spotted and diffuse knapweed that consisted of the following (Lang et al. 2000). Phase I- to introduce approved biological control agents, often through quarantine facilities, for the establishment of field insectary sites (FIS) (weed infested location that will be managed to produce insects for eventual redistribution to other weed infested sites). Phase II- to serve to increase the number of FIS from agents reared in the original Phase I FIS and to involve state cooperators in the management and maintenance of the FIS. Phase III- to begin when the biological control agents at the FIS reach collectable population size and then the collection and the redistribution of beneficial agents become the responsibility of federal, state, county, and local cooperators in each state, instead of the federal government.

To date 13 insect species, all of which are synchronized to the life cycle of spotted knapweed, have been released mostly in the western regions, with some in the eastern regions of the United States, for the biological control of five additional knapweed species (*C. diffusa*, *C. virgata* Lam. ssp. *squarrosa* Gigl., *C. pratensis* Thuill., *C. nigra* L., and *C. jacea* L.) (Wilson and Randall 2003). Even though the agents were subjected to strict Canadian government scrutiny before introduction, all of the 13 insect species that have been released into the United States were tested for host specificity by the IIBC, European Station, Delmont, Switzerland or the USDA-ARS, European Biological Control Laboratory (Montpelier, France) before being approved for importation and release (Lang et al. 2000). Of those released, eight of the insect species feed within the capitula, while five feed on the roots (Story and Piper 2001). The idea for multiple organism release is explained by the cumulative stress model (Müller-Schärer and Schroeder 1993). The cumulative stress model indicates agents will work together to reduce viable seed production and stunt the overall growth and

strength of the plants (USDA APHIS 1997) together having a greater chance of contributing to the suppression of knapweed (Wilson and Randall 2003)

Of the eight insect species that are seedhead-feeders, four are flies (Diptera: Tephritidae): *U. affinis*; *U. quadrifasciata*; the green clearwing fly [*Terellia virens* (Loew)]; and the knapweed peacock fly (*Chaetorellia acrolophi* White and Marquardt); one is the moth (*M. paucipunctella*); and three are beetles (Coleoptera: Curculionidae): the lesser knapweed flower weevil (*Larinus minutus* Gyllenhal); the blunt knapweed flower weevil (*Larinus obtusus* Gyllenhal); and the broad-nosed knapweed seedhead weevil [*Bangasternus fausti* (Reitter)] (USDA APHIS 1997; Lang et al. 2000; Wilson and Randall 2003). All of the seedhead-feeding insects damage the plant when larvae consume immature seeds and other tissues in the capitulum. Feeding by the two *Urophora* spp. causes the plant to encase the larvae in tissue called a gall. In forming the gall, the insect serves as a metabolic sink draining valuable nutrients away from normal plant growth (Wilson and Randall 2003). Gall formers feed on actively dividing cells so they attack at the early stages of seedhead bud formation (Wilson and Randall 2003).

Seedhead feeders have specialized niches and are separated in time and space by the density of knapweed, larval feeding habitats, number of larvae in the head, and overwintering sites (Wilson and Randall 2003). Because spotted knapweed produces flowers throughout the spring and summer, a constant supply of capitula is available for consumption by the insects.

Of the five root feeders, three are moths: the sulfer knapweed root moth (*Agapeta zoegana* L.) (Lepidoptera: Cochylidae); the gray-winged knapweed root moth (*Pterolonche inspersa* Straudinger) (Lepidoptera: Pterolonchidae); and the brown-winged knapweed root moth (*Pelochrista medullana* Staudinger) (Lepidoptera:Tortricidae) and two are beetles: the knapweed root weevil [*Cyphocleonus achates* (Fahraeus)] (Coleoptera: Curculionidae) and the bronze knapweed root borer (*Sphenoptera jugoslavica* Obenberger) (Coleoptera: Buprestidae) (USDA APHIS 1997; Lang et al. 2000; Wilson and Randall 2003). All five root feeders damage the plant in the larval stage by feeding on the central vascular tissue or the cortex of the root below the epidermis (Wilson and Randall 2003). All of the larvae mine the roots depleting the carbohydrate reserves of the plant, while both beetle larvae and *P. inspersa* cause root galls forming a metabolic sink (Wilson and Randall 2003).

Both fungi and mites also have been introduced for the control of spotted knapweed. One fungus (*Sclerotinia sclerotiorum* Whetzel), a common soil inhabitant that is native to North American, can cause wilt and death to spotted knapweed under some conditions (Jacobs et al. 1996). However, both this soil fungus and another one, along with two mite species, are no longer reared for spotted knapweed because they did not negatively impact the plant (Wilson and Randall 2003).

Most recent effort associated with biological control organisms of spotted knapweed is not focused on exploring for new organisms because the pool of suitable biological control agents is practically exhausted (Müller-Schärer and Schroeder 1993). As of 1993, Agriculture Canada, British Columbia Ministry of Forests, British Columbia Ministry of Agriculture, Montana Department of Natural Resources, United States Department of Agriculture-Cooperative State Research Service (USDA-CSRS), USDA-ARS, USDA-APHIS, and various universities were involved with rearing and distributing biological control organisms (Müller-Schärer and Schroeder 1993). The biological control program was expected to become fully effective between 1989 and 1999 (Harris and Cranston 1979).

The magnitude and complexity of the spotted knapweed problem indicate that successful management requires the adoption of integrated strategies (Sheley et al. 1998). Integrated knapweed management of rangeland involves the use of several techniques in a well-planned, coordinated, and ecologically based strategy to maintain desired plant communities or shift plant communities to those that are desired (Sheley et al. 1996). Abella (2001) incorporated techniques that can be used in an integrated management plan based on percent cover of the spotted knapweed. However, most regulatory agencies have incorporated biological control insects into their regulatory objectives. For instance, the combined attack of *U. affinis* and *U. quadrifasciata* reduced seed production by as much as 80-95% (Story 1989). Likewise, picloram and picloram-2-4 D used in combination with *U. affinis* and *U. quadrifasciata* significantly reduced spotted knapweed in Idaho, without significantly increasing the mortality of the biological control agents (McCaffrey and Callihan 1988). Schematics have been designed for using herbicides, cultivation, burning, irrigation, and mowing to return a 98% spotted knapweed-infested community into a 10% spotted knapweed, 20% bluegrass (*Poa pratensis* L.), and 70% seeded species community (Sheley et al. 1996).

The sharing of information on the effectiveness of many of these strategies has occurred at regional and international knapweed symposia, along with the cooperation between federal and state agencies working with spotted knapweed. The use of biological control organisms has been one of the most commonly used tactics for management of spotted knapweed on rangelands. Further releases of biological control agents of spotted knapweed are now the responsibility of the states and the insectaries are under their management (Lang et al. 2000).

v. Selected Biological Control Insects for Spotted Knapweed

Biological control insects for spotted knapweed were selected using data from previous associations in eastern Tennessee. *U. quadrifasciata* was selected as an agent to investigate because it was found at numerous populations of spotted knapweed in preliminary investigations of agents. *Megalanotus*

sabulicola was selected as an agent because it was found in close association with the rosette cover of spotted knapweed in eastern Tennessee, and its potential as a naturalized seed feeder has not been investigated.

***Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae: Myopitinae).** The Tephritidae genus *Urophora* (100 spp.) includes eight species indigenous to the Nearctic Region and three Eurasian species [*U. quadrifasciata*, *U. affinis*, and *U. sirunaseva* (Hering)] that have been introduced into North America for the biological control of knapweeds (White and Koryneyev 1989).

Taxonomy. The genus *Urophora* was first identified as *Euribia* in a pamphlet by Meigen in 1800 (White and Koryneyev 1989). Later in 1830, it was transferred to *Urophora* by Robineau-Desvoidy, with acceptance as this official genus name in a 1963 ruling by the International Commission for Zoological Nomenclature (opinion 678) to suppress Meigen's work and to place all species described as *Euribia* in *Urophora* (White and Koryneyev 1989). White (1988) has completed the most recent revision of Palearctic and Nearctic species of *Urophora* adults associated with *Cardueae* species, particularly because of their use in biological control (White and Clement 1987). The shape of the female's aculeus, length of the wings of females, and sternites of the males are the morphological characteristics used in the latest revision (White and Koryneyev 1989).

Urophora quadrifasciata (Meigen) sensu lato (*s. l.*) (Fig. 4), first discovered in 1826, is native to western, central and southern Europe (White and Clement 1987). Recently, the Palearctic *U. quadrifasciata* has been identified as a species complex consisting of three forms that may be regarded as subspecies (*U. quadrifasciata.* ssp. *algerica*, *U. quadrifasciata.* ssp. *quadrifasciata*, and *U. quadrifasciata*. ssp. *sjumorum*) (White and Clement 1987; White and Koryneyev 1989). Each of the three morphologically distinct forms (based on aculeus shape) of *U. quadrifasciata* occurs within a section of the total distribution, and has a host list which does not overlap with the host lists of the other two subspecies (White and Koryneyev 1989). The subspecies of *U. quadrifasciata* used in North America (*U. quadrifasciata* ssp. *quadrifasciata)* as a biological control agent for *Centaurea* species came from the Ukraine (Harris and Shorthouse 1996).

The following taxonomic description of *U. quadrifasciata* ssp. *quadrifasciata* begins with the subfamily and ends with the subspecies (White and Clement 1987; White and Koryneyev 1989). Myopitinae subfamily diagnostic characters are a cell cup closed by a convex vein Cubital/Anal (Cu/A) vein 2, so that there is no cup extension; head with one pair of orbital setae; and dorsocentral setae present (White and Clement 1987). The *Urophora* Robineau-Desvoidy genus consists of an elongate proboscis with narrow refluxed labella; lower facial margin not protruding; and vein M ending at or close to wing tip

Figure 4. Female (left) and Male (right) of *Urophora quadrifasciata* (Meigen), the UV Knapweed Seed-head Fly.

(White and Clement 1987). *Urophora* associated with *Cardueae* species host plants have characteristics of the color predominantly black; scutellum yellow; legs and antennae mostly orange; labellum about 1.5 times as long as first flagellomere; palpi orange; wings banded; wing base yellow or hyaline; male distiphallus reduced to a narrow membraneous sack with no sclerotized areas; and female spermathecae not sclerotized (White and Clement 1987).

The *U. quadrifasciata* has the characteristics of wings with subbasal or discal crossbands fused from the Costa vein (C) to or almost to Radius (R) vein 4+5; femora black; discal and preapical crossbands not fused from midway between Medius (M) vein and Cu/A 1 to hind margin of wing; first flagellomere orange on inner surface; and aculeus truncate without subapical steps (White and Clement 1987).

The subspecies *Urophora quadrifasciata* ssp. *quadrifasciata* has the first flagellomere yellow to black; palpi yellow, darkening to orange apically; gena 0.25-0.30 times eye height; labellum 1.5 times length of first flagellomere; scutum with fine tomentum which does not obscure the underlying cuticle; basal scutellar seta on or near margin of central yellow and marginal black area; femora black; wing base yellow, subbasal crossband extending between veins C and A1 or between veins C and A2; subbasal and discal crossbands joined between veins C and R4+5; discal and preapical crossbands separate; hyaline area between preapical and discal crossbands usually about 0.75-1.5 times as broad along vein R4+5 as breadth of preapical crossband on vein R4+5; aculeus apex 1.3-2.1 mm; female wing length 2.2-3.0 mm; aculeus apex breadth 4-10 µm; and aculeus length/wing length = 0.55-0.75 (White and Koryneyev 1989).

Larval descriptions of *U. quadrifasciata* can be found in Hennig (1968) and in Ferrar (1987). Pupal characters of *U. quadrifasciata* are explained by White (1988).

Biology. *Urophora quadrifasciata* is bivoltine (having an obligatory second generation) (Myers and Harris 1980; Lang et al. 2000) with adult emergence synchronized with spotted knapweed phenology (Story et al. 1992). Synchronization within the period of host plant plasticity of cell development (Shorthouse 1986) is important for the successful development of gallformers. Emergence of the first generation has peaks in late June to early July and is closely synchronized with capitulum diameter (2-4 mm) (Story et al. 1992). Whereas, the emergence of the second generation peaks about 5 to 6 weeks after the first generation in mid August and is closely synchronized with the opening of early maturing seed heads for seed dispersal (Story et al. 1992; Harris and Shorthouse 1996). A third generation may occur in September at some locations (Story et al. 1992). Males generally emerged earlier than females, but the sex ratio for the population was almost 1:1 (Story et al. 1992; Mays and Kok 2003).

Oviposition. Females begin to oviposit on the second or third day after emergence, laying eggs singly among the stamens of the flower (Lang et al. 2000) in capitula that are 5 to 8 mm long (Berube and Myers 1983; Wilson and Randall 2003). Female flies are neither attracted to areas of high flower density, nor spread uniformly through the field, but rather independently choose oviposition sites as they encounter each flower bud (Myers and Harris 1980). If suitable stamens are available, females may lay several eggs in one capitulum before moving to another capitulum to oviposit (Myers and Harris 1980). Berube and Zacharuk (1984) have provided morphological evidence for the presence of pheromone glands in female *U. quadrifasciata* that are used in the marking of capitula after oviposition.

Eggs. The white, elongate shaped eggs of *U. quadrifasciata* are 0.6-0.8 mm long (White and Koryneyev 1989) and deposited singly among developing florets (Harris and Shorthouse 1996; Wilson and Randall 2003). The eggs hatch within 3 to 4 days (Lang et al. 2000).

Larvae. Larvae of *U. quadrifasciata* undergo three instars (White and Koryneyev 1989). The first-instar larva is creamy white, barrel-shaped, with a retracted head and elliptical dark brown anal plate; after hatching it chews down the floral tube into the ovary of a pollinated seed head (Harris and Shorthouse 1996; Lang et al. 2000; Wilson and Randall 2003) and begins to feed on the parenchymatous tissue (Shorthouse 1986).

A papery-thin, non-lignified gall from the tissue of the ovary wall (Story et al. 1987; Story et al. 1992; Nowierski et al. 2001) begins to form (one gall per larva) along the outer edge of the capitulum within eight days, causing the inner ovary cells to multiply and form nutritive tissue which reaches maximum size in 15 days (Harris and Shorthouse 1996; Lang et al. 2000). The larva consumes the ovule, cells of the inner layer of the ovary wall (the outer cells becoming the thin gall tissue), and adjacent receptacle tissue (especially if more than one larva is present in the capitulum) (Harris and Shorthouse 1996). By the end of the third instar (20 days after hatching) (Harris and Shorthouse 1996), the larva will have consumed nearly the entire gall, ultimately destroying the floret (Lang et al. 2000). Because larvae are generally immobile in their gall, their success depends upon where they have been distributed during oviposition.

Distribution of larvae and their galls within the capitula has been described as both clumped (Myers and Harris 1980) and uniformed (Nowierski et al. 1987) on spotted knapweed. A study with *U. quadrifasciata* on squarrose knapweed (*C. virgata* Lam. ssp. *squarrosa* Gigl.) suggests that a clumped distribution is favored by the quality of the capitulum and is negatively impacted by the density of other larvae/galls present (Reieder et al. 2001).

Pupae. Pupation of *U. quadrifasciata* occurs at 20-25 days after hatching, about the time achene development is complete (Harris and Shorthouse 1996). The second generation of larvae overwinter in the gall as prepupae and pupate in the spring (Story et al. 1992; Lang et al. 2000).

Adult. Adults of *U. quadrifasciata* are 4.5 mm long (Story 2002). Adults can be distinguished from other knapweed tephritids because they have a relatively dark body and dark bands in the classic shape of a "UV" pattern on their wings (Story 2002). The common name of the UV knapweed seed-head fly is derived from the wing pattern (Story 2002).

Behavior. Both adult male and female *U. quadrifasciata* exhibit 'rendezvous behavior' (link between their reproductive behavior and the attraction to their host plant) (Berube and Myers 1983). This rendezvous behavior consists of cruising (an apparent search behavior in which they walk up and down the stems and onto developing flower heads looking for a mate), scissoring (males move both wings simultaneously and repeatedly extended so the wing patter is displayed), and probing (females insert their ovipositors into capitula) (Berube and Myers 1983).

Mating occurs on the capitula with successful encounters lasting about 10 to 120 s exclusive of time *in copula* (Berube and Myers 1983). During normal mating, a receptive female will raise her oviscape after having been mounted by a male, the male then grabs the oviscape with his hind wings and guides the tip of his abdomen while attempting to insert his adeagus (Berube and Myers 1983). Resting *U. quadrifasciata* hold their wings in a partially extended position, perpendicular from their bodies (Berube and Myers 1983).

Use of *U. quadrifasciata* as a biological control organism. Within *Urophora*, *U. quadrifasciata* has the largest host list for the control of *Centaurea* species (White and Koryneyev 1989). It attacks spotted, diffuse, squarrose, meadow, black, and brown knapweeds (Wilson and Randall 2003). *U. quadrifasciata* was first introduced into North America at Ned's Creek, British Columbia, Canada, in 1971 as a biological control agent for the control of the two most invasive weeds at that time, spotted and diffuse knapweed (Harris 1991). At the time of the release in Canada, *U. quadrifasciata* was not approved for release in the United States because of taxonomic concerns, but nevertheless was first detected in Idaho in 1980 and Montana in 1981 immigrating a distance of 400 km from the Canadian release point (Gillespie 1983; Story 1985; Lang et al. 1997; Wilson and Randall 2003). After distribution and establishment in the western United States (Lang et al. 1997), *U. quadrifasciata* was intentionally released as larvae in bouquets of seed heads of spotted knapweed in New York and Maryland by USDA-ARS personnel in May 1983 (Hoebeke 1993). However, it was not approved for release as a biological control agent in the United States

until 9 August 1988 (Lang et al. 2001). By July 1990, *U. quadrifasciata* was first found to be established on *C. dubia* Stuter in New York (Hoebeke 1993), and is now widely distributed across the United States (Story 2002).

Metabolic sink. Insects belonging to the gall-inducing guild have evolved the ability to redirect the growth and differentiation of plant cells near the larval feeding sites into structures which provide shelter and a rich food supply (Shorthouse 1986). Not only do gall-makers "rob" the host plant of the consumed tissue, but they also cause the plant to alter tissue that would otherwise serve productive functions in plant growth and reproduction (Abrahamson and McCrea 1986). Galling in general can act as a nutrient sink, reducing other vegetative growth and, if galling occurs in reproductive tissues, reducing reproduction (Dennill 1988). Galls formed by the consumption of spotted knapweed tissue by *U. quadrifasciata* larva form a metabolic sink that can reduce spotted knapweed seed production by 1.9 seeds per gall per seed head (Harris 1980). Feeding by larvae of *U. quadfasciata* on the unmodified cells of the ovary wall within a gall cause the inhabited florets and adjacent florets to abort (Harris and Shorthouse 1996). Therefore, the presence of *U. quadrifasciata* reduces the number of seeds that can be produced and dispersed.

Even with its ability to reduce seed production, recent evidence suggests that *U. quadrifasciata* is insufficient by itself to reduce the density of spotted knapweed in North America (Harris 1980; Müller-Schärer and Schroeder 1993). Although *U. quadrifasciata* forms galls fairly early in development when galling has the greatest effect to be a metabolic sink (Harris 1980), it is a weak metabolic sink because galls are initiated after the vascular differentiation in the capitula (Harris and Shorthouse 1996). Therefore, the vascular system of the gall is rudimentary and many nutrients are not redirected to it and away from other developing parts of the plant. Likewise, the final instar larva of *U. quadrifasciata* contain on average 17.8 ± 0.6 kJ, which is less energy than is required to produce a single knapweed seed (Harris and Shorthouse 1996). Even though, *U. quadrifasciata* does destroy florets, it only affects seed production in attacked seed heads and does not deter the number of capitula produced as *U. affinis* does (Harris 1980; Harris and Shorthouse 1996).

Success of *U. quadrifasciata* as a biological control agent in the role of an effective gall former depends upon the agent's ability to damage its host as it relates to the power of the gall as a nutrient sink (Harris and Shorthouse 1996). The impact of the larvae of *U. quadrifasciata* on the relocation of nutrients of spotted knapweed is not very effective. Nevertheless, *U. quadrifasciata* was selected as a biological control organism because of its rapid dispersal ability (Harris 1980; Story et al. 1987) and ability to attack remote knapweed infestations (Myers and Harris 1980). It has value as a biological control agent because of its supplemental damage done to larger seed heads not targeted by

U. affinis, a related knapweed capitula feeder (Harris 1991; Harris and Shorthouse 1996).

Mortality. Mortality of *U. quadrifasciata* can occur from both biotic and abiotic factors. From stomach content analysis, *U. quadrifasciata* was found to be the primary food item in diets of deer mice for most of the year (247 larvae/mouse/day in winter months), negatively reducing *U. quadrifasciata* populations below a threshold necessary to effectively control spotted knapweed (Pearson et al. 2000).

Deer mice, along with white-tailed deer [*Odocoileus virginianus* (Zimmerman)] and black-capped chickadees (*Parus atricapillus* L.), were the primary predators of *U. quadrifasciata* larvae, and a native spider (*Dyctina major* Menge) preyed heavily on adult flies (Story et al. 1995). Yellow-pine chipmunks (*Tamias amoenus* J.A. Allen), pine siskins [*Carduelis pinus* (Wilson)], and house finches (*Carpodacus mexicanus* Muller) have been observed feeding on *U. quadrifasciata* (Pearson et al. 2000). *M. paucipunctella,* another introduced biological control against spotted knapweed, acts as an interference competitor as its larvae bore into the galls of *U. quadrifasciata* and kill the fly larvae (Story et al. 1991).

Parasitism by native species of *U. quadrifasciata* seems to be inconsequential and the result of accidental encounters (Harris and Shorthouse 1996). Because the introduced populations lack parasitoids, it is proposed *U. quadrifasciata* would reach higher densities in British Columbia than their densities in Europe (0.56 ± 0.29 S.E. larvae per head) where they are native and are impacted by parasitoids (Myers and Harris 1980).

Cold temperatures are the most important abiotic factors that affect the mortality of *U. quadrifasciata*. Periods (more than 5 days) of extremely cold temperatures (-28 to -30°C) is the most important mortality factor affecting overwintering survival in *U. quadrifasciata* (Nowierski et al. 1999).

Parasitoids of *U. quadrifasciata*.

Background. Parasitoids are organisms that live on (ectoparasitoids) or within (endoparasitoids) a host organism, eventually killing it. Parasitoids have a unique relationship with their host because unlike parasites which rely on the host for long-term survival, parasitoids rely on the host for only certain developmental stages. In the insect world, parasitoids are typically hymenopterans or dipterans.

An important protocol component of biological control programs involves the effort to introduce biological control insects without also introducing their own parasitoid natural enemies from their area of origin (Turner et al. 1990). However, after biological control organisms are released in the field, there is no way to prevent their exposure to indigenous parasitoids.

Because parasitoids are so morphologically diverse and structurally reduced, the life histories of only a small portion of the predators and parasitoids described in the taxonomic literature have ever been studied in detail. It is difficult to identify if a biological control organism would be within a suitable host range (set of species that can support all requirements of development) for all potential native parasitoids (Strand and Obrycki 1996). Host selection can vary under ecological circumstances (Strand and Obrycki 1996) creating opportunities for native parasitoids to attack introduced biological control agents if they are under stress or if they are the only potential hosts available. Bellows and Headrick (1999) stated that introduced herbivores often become attacked by existing parasitoids or predators. However, indigenous parasitoids often have little impact on insects introduced for biological control of weeds, only on occasion reducing their effectiveness (Goeden and Louda 1976).

Because of their high level of diversity, parasitoids are often categorized by the life stage of the host the female oviposits in and where her offspring develop (i. e., egg, egg-larval, larval-pupa parasitoid) (Strand and Obrycki 1996). Koinobionts, which have a narrower taxonomically defined host range, are parasitoids whose hosts continue to grow after parasitism, and idiobionts are parasitoids whose hosts do not develop further after parasitism (Strand and Obrycki 1996). Larval endoparasitoids are usually koinobionts, whereas egg and pupal endoparasitoids are usually idiobionts (Strand and Obrycki 1996). Many koinobionts oviposit in early instar larvae but do not kill the host until the late-larval, pre-pupal, or pupal stage (Bradford et al. 1997).

Importance of parasitoids on *U. quadrifasciata*. Two Eupelmidae species, *Hyssopus* nr. *novus* Girault and *Prototalia carlinarum* (Szelenyi and Erodös) (Hymenoptera), have been documented as parasitoids of *U. quadrifasciata* in Canada. Their collective impact was less than 10% parasitism (Harris and Shorthouse 1996) on *U. quadrifasciata*. In Michigan, the hymenopteran *Pteromalus purpuriventris* (Pteromalidae) has been the only parasitoid associated with *U. quadrifasciata* (Marshall and Storere 2003). No parasitoids of *U. quadrifasciata* have been recovered from specimens in Virginia, indicating that parasitism is not a limiting factor for *U. quadrifasciata* in that area (Mays and Kok 2003).

A few parasitoids have been found on *U. affinis*, a close relative of *U. quadrifasciata*, but with little adverse effects on this tephritid (Hoebeke and Wheeler 1996; Lang and Richard 1998). Lang and Richard (1998) conducted a late winter to early spring dissection of capitulum of spotted knapweed and found only 16 non-identified parasitoids (within 15 capitula), out of more than 7,500 examined capitula, associated with *U. affinis*, not *U. quadrifasciata*. One such Palearctic hymenopteran parasitoid that has been field collected and identified from *U. affinis* is *Pteromalus elevatus* (Walker) (Hoebeke and Wheeler 1996). Some parasitoids emerged from *U. affinis* that was brought into the

USDA Biological Control of Weeds Laboratory in Albany, California to be reared; however, these were killed and not identified (Maddox 1982).

The Nearctic Hymenoptera *P. coloradenis* (Ashmead) has been found on a tephritid (*Paracantha gentilis* Hering) introduced for biological control of thistles (*Cirsium californicum* Gray and *Cirsium proteanum* J. T. Howell) in California (Headrick and Goeden 1989), but not on *U. quadrifasciata*. All of these pteromalids that have been found associated with biological control organisms of spotted knapweed are solitary (one individual develops) parasitoids (Strand and Obrycki 1996).

Many examples of niche specialization (resource partitioning) are found among parasitoids (Strand and Obrycki 1996) facilitating their great diversity. Spatial patterns where the presence of the parasitoids is independent of the host density are most common (Redfern et al. 1992). Parasitoid-induced mortality actually appears higher on exotic species (Bradford et al. 1997) such as introduced biological control organisms. Consequently, organisms such as the exotic biological control agent *U. quadrifasciata* need to be examined for potential parasitoids that could reduce their numbers or effectiveness.

Megalanotus sabulicola.

Importance. *Megalanotus sabulicola* (Thompson) (Hemiptera: Lygaeidae: Rhyparochromine) (Fig. 5) is a Palearctic seed-feeder accidentally introduced into California through discarded ballast and into both Philadelphia and New York via plant material at ports of entry (Slater and Sweet 1958). In the United States *M. sabulicola* has been found in California, Connecticut, Delaware, Maryland, New Jersey, New York, Oregon, Pennsylvania, Rhode Island, Virginia, Washington, and West Virginia (Slater and Sweet 1958; Slater 1964; Wheeler Jr. 1989). Its native range is from Sweden and England south to the Mediterranean and into Asia Minor and east to Russia (Wheeler Jr. 1989).

In Europe, *M. sabulicola* was considered a sub-species of *M. chiragra* (F.), but has since been described as a distinct, sympatric species (Wheeler Jr. 1989). In the United States, *M. sabulicola* was first identified by Van Duzee in 1923 as *Rhyparochromus chiragra* var. *californicus*, but is now also known to be *M. sabulicola* (Slater and Sweet 1958).

In the mid-Atlantic region, *M. sabulicola* feeds mainly on fallen seeds of spotted knapweed (Wheeler Jr. 1989). *M. sabulicola* has also been observed carrying fallen seeds of spotted knapweed and bachelors' buttons (*Centaurea cyanus* L.) to a sheltered site under the leaves of these plants for later feeding (Sweet 1964). Although *M. sabulicola* feeds on fallen seeds and has been present in the United States for more than 60 years, it is not known to be a threat to native lygaeids because it competes poorly with native lygaeids since it is limited to feeding on the seeds of *Centaura* spp. (Sweet 1964; Wheeler Jr. 1989).

Figure 5. Adult *Megalanotus sabulicola* (Thompson).

Post dispersal seed-feeding insects (such as *M. sabulicola*) can reduce plant recruitment in years or in places where recruitment is seed limited (i.e., in deserts or semiarid open woodland). However, following dispersal of many seeds, seed-feeding insects typically have no effect on the numbers of plants that become established (Crawley 1989). *M. sabulicola* should be considered a beneficial immigrant because it destroys spotted knapweed seeds, but its effect as a post-dispersal seed-feeding insect on density of spotted knapweed would be negligible (Wheeler Jr. 1989).

Life Cycle. The bivoltine *M. sabulicola* overwinters as an adult and can be found in this stage throughout the year (Butler 1923; Wheeler Jr. 1989). Adult females of *M. sabulicola* attach eggs to litter, sand or plant fuzz (Sweet 1964). In England, the nymphs appear during the summer in June and July with the final molt in August (Butler 1923). A second generation was not described. In the United States, the first generation of nymphs of *M. sabulicola* began to appear in June, with adults occurring in July and the second generation of nymphs found in August with adults occurring in October (Wheeler Jr. 1989). A three-week difference in phenology throughout the United States may occur (Wheeler Jr. 1989).

In its native range of England, *M. sabulicola* was found under dead leaves and moss and at the roots of grasses living in open sites where the earth is warmer than the woodlands (Butler 1923). In the United States, *M. sabulicola* is typically associated with *Centaurea* species beneath dry litter, especially where the substrate was free of plants and somewhat gravelly and sandy (Slater and Sweet 1958). *M. sabulicola* is also associated with *Centaurea* spp. in shaly slopes along embankments, wet clayey soil, vacant lots, gravelly soil at the edge of parking lots and fine sooty and sandy ballast along railroads (Wheeler Jr. 1989).

Morphology. Adult and fifth instar *M. sabulicola* are hairy, medium-sized, brown lygaeids with a rostrum extending to the bases of mesocoxae; punctuate pronotum and scutellum; erect bristle-like setae on head, pronotum and scutellum; meso- and metafemora black apically, yellowish basally; tibiae yellowish, with stout dark spines; and antennal segment II and hind tibiae almost wholly yellow. For a more detailed morphological description of the first through fourth instars of *M. sabulicola*, see Slater and Sweet (1958) and Wheeler (1989).

vi. Research Objectives

Spotted knapweed is a prevalent non-indigenous plant in the western United States with millions of dollars spent over the past 30 years on its management. However, its potential impact along roadways and pastureland of the southeastern United States has not been investigated. The opportunity to

determine the community level interactions between spotted knapweed, the associated insects, and any previously released biological control organisms has been presented for eastern Tennessee.

The overall goal of this research was to contribute to the information known about the insect community associated with spotted knapweed, especially in the southeastern United States where little is known. In this study, the following objectives were investigated to reach this goal:

(1) Determine family composition and seasonality of insects associated with spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek, in eastern Tennessee.

(2) Determine the distribution of *Urophora quadrifasciata* (Meigen) on spotted knapweed in eastern Tennessee.

(3) Assess the impact of *U. quadrifasciata* on the production of seeds by spotted knapweed in eastern Tennessee.

(4) Determine the distribution of *Megalanotus sabulicola* (Thompson) with spotted knapweed in eastern Tennessee.

(5) Identify and determine the effects of hymenopteran parasitoids on *U. quadrifasciata*.

The hypothesis of this research is that biological control organisms will be present on spotted knapweed in eastern Tennessee, reducing the ability of the weed populations to spread.

II. Abundance and Diversity of Insects on Spotted Knapweed

i. Introduction

Spotted knapweed [*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek] (formerly *C. biebersteinii* DC. and *C. maculosa* Lam.) (referred to here as *C. stoebe* L. *sensu lato*] (Asteraceae), an invasive, non-indigenous perennial forb (Ochsmann 2001), has been described as one of the world's worst weeds (Rice et al. 1997; Callaway et al. 1999a). First introduced in the western United States in the 1890s as a contaminant in alfalfa seed (Maddox 1979), spotted knapweed is now prevalent on more than one and one half million hectares of land (Todd 2001). Characteristics of individual plants of spotted knapweed that reduce the vigor, species richness, and species diversity of both native and cultivated forage plants (Lacey et al. 1995; Rice et al. 1997; Sheley et al. 1998; Kedzie-Webb et al. 2001) include a reproductive capability of more than 800 viable seeds (Watson and Renney 1974), a drought tolerant rosette stage (Watson and Renney 1974), and the production of the allelopathic chemicals catechin (Bais et al. 2003; Moellenberg 2003) and cnicin (Landau et al. 1994).

Although primarily an economic pest in western xeric rangelands (Müller et al. 1989), spotted knapweed is also common in disturbed habitats such as roadsides, uncultivated fields, and waste areas of the northeastern and mid-Atlantic region of the United States (Hoebeke 1993; Mays and Kok 1996). Throughout these regions of the United States, the management of spotted knapweed has relied primarily on biological control using the combined impact from 13 introduced Palearctic phytophagous insect species that are synchronized to the life cycle of spotted knapweed and target different vegetative structures (capitulum, rosette, and root) (USDA APHIS 1997; Lang et al. 2000; Story and Piper 2001; Wilson and Randall 2003). However, when biological control was first implemented, populations of spotted knapweed in eastern states were not considered for release (Lang et al. 1997). The closest introductions of biological control insects of spotted knapweed to Tennessee have been for *Urophora affinis* Frauenfeld (Diptera: Tephritidae) and *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae) in southwestern Virginia (Mays and Kok 1996) and *U. quadrifasciata* (Meigen) in Beltsville, Maryland (Hoebeke 1993).

While the resource exploitation and rate of dispersal from individuals of the 13 biological weed control insect species are well documented (USDA APHIS 1997; Lang et al. 2000; Story and Piper 2001; Wilson and Randall 2003), little has been published about other insects (native or introduced) that are attracted to spotted knapweed and utilize it both directly as a source of carbohydrates (as with honey bees, *Apis mellifera* L., obtaining nectar and pollen) (Watson and

Renney 1974), and/or indirectly as resting places and as finding sources of prey for predatory insects.

Even though it commonly occurs along roadsides and along the edges of pastures (personal observation), spotted knapweed is not considered an economically important weed in the southeastern United States where little has been studied about its distribution. Even less management of spotted knapweed has occurred in this region because it is not economically affecting important pastures, such as the damage caused by the invasive musk thistle (*Carduus nutans* L.).

In Tennessee, spotted knapweed is listed on the Invasive Exotic Pest Plants List as a Rank 2 - Significant Threat (Bowen et al. 2002) with populations documented in 29 counties (Wofford 2002). Because spotted knapweed is not considered to spread as easily into native plant communities in the southeastern United States, the current isolated populations of spotted knapweed in eastern Tennessee will provide opportune sampling locations for the valuable assessment of insects associated with spotted knapweed.

The specific objective of this research was to contribute to the knowledge of the abundance, diversity, and seasonality of insects present in the spotted knapweed community. Emphasis was placed on sampling throughout the various developmental stages of spotted knapweed to obtain seasonality for family composition of selected insects, including biological control organisms that may have become established in eastern Tennessee.

ii. Materials and Methods

a. Selection of Insect Diversity Sites. The purpose of these methods was to assess insect richness and diversity, with special attention to biological control organisms on spotted knapweed. Representative stands of spotted knapweed in eastern Tennessee were located using University of Tennessee Herbarium records. These stands were canvassed to determine if they were suitable locations to sample for spotted knapweed. Criteria for suitable locations consisted of: (1) confirmation of presence of spotted knapweed from University of Tennessee Herbarium director, B. Eugene Wofford, (2) ease of access, (3) >75% ground cover by spotted knapweed from qualitative visual surveys, (4) within an hour and one half driving distance from the University of Tennessee, and (5) current land use (disturbed or natural habitat). These criteria enabled stands to be sampled in a timely manner for habitats dominated by spotted knapweed as typical of previous studies.

Preliminary Assessment. In December 2002 through April 2003, a preliminary assessment of the insects present at locations that met the criteria listed above was conducted. Preliminary assessment of spotted knapweed

consisted of direct 30-minute observations both on randomly chosen spotted knapweed plants and rosettes, along with the removal of 100 capitula (two per 50 plants) that were then set aside for examination of insect emergence. During the direct 30-minute preliminary field observations, two capitula each were hand removed from approximately 15 plants and dissected to examine for the presence of any insects. Also during the preliminary observations, rosettes of approximately 15 plants were lifted with the surrounding vegetation, and soil surface was examined for insect presence.

Based on the earlier preliminary findings, six locations of spotted knapweed infestations within four eastern Tennessee counties (Cocke, Grainger, Greene and Hamblen) were established as research sites (Fig. 6). Four subplots (12 m x 15 m) were delineated using orange flagging at each site in May 2003. Coordinate locations of each site were obtained from a Garmin® 12-channel Global Positioning System (GPS) unit while standing in the corner of one of the four subplots (Table 2).

Sampling of Insects. Insects associated with spotted knapweed at each of the six sites were sampled eight times between the hours of 1000 and 1400, approximately every 3 to 4 weeks from May 2003 through August 2003, once in October 2003, and once in January 2004 using a sweep net, beat sheet and direct observations. For each of the six sites, the order of the sampling technique varied from site to site and sampling date to sampling date. Sampling at each site took approximately 3 hours dependent upon weather conditions and insects that were present.

Sweep net. A sweep net (30 cm diameter x 31 cm x 61 cm net on a 1 m pole) was swung ten times in an 180° arc while walking in a random zig-zag pattern through each subplot. The contents from each subplot sample were emptied into their own labeled, re-sealable plastic bag (26.8 cm x 27.9 cm), placed in a cooler, and taken to the laboratory for processing, sorting, and identification to family level. This procedure was repeated for each of the four subplots at each site.

Beat sheet. A beat sheet (75 cm x 75 cm) was placed below one spotted knapweed plant, and the plant was shaken for five seconds onto the sheet. All insects that fell onto the sheet were quickly aspirated or removed using forceps. Collected insects were placed in their own labeled, re-sealable plastic container (FISHERbrand® 17 x 100 mm vial 1.5 cm diameter x 1.5 cm x 9.5 cm) and transported to the laboratory within a cooler for processing, sorting, and identification to family level. This procedure was repeated three times at each of the four subplots at each site.

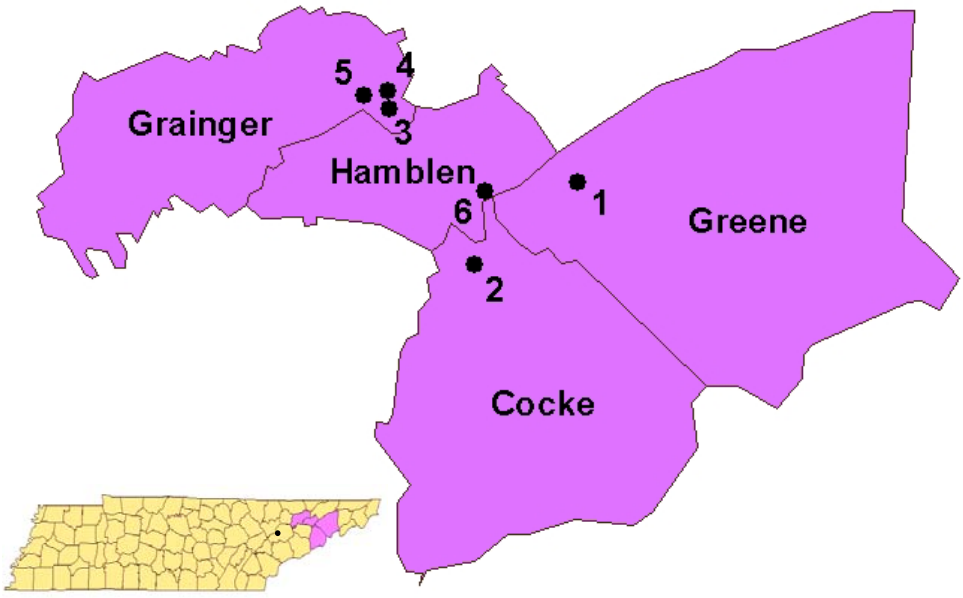

Figure 6. Location (GPS Coordinates are listed in Table 2) of the Six Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) Research Sites to Assess Insect Diversity in Eastern Tennessee. The Black Circle on the Inset Designates the Location of the University of Tennessee, Knoxville.

Table 2. Coordinate Locations[a] for the Six Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) Research Sites to Assess Insect Diversity in Eastern Tennessee.

Site Number	Latitude	Longitude
1	36° 13.408' N	083° 02.082' W
2	36° 06.533' N	083° 11.774' W
3	36° 19.727' N	083° 22.544' W
4	36° 21.621' N	083° 20.586' W
5	36° 20.981' N	083° 23.607' W
6	36° 11.558' N	083° 10.480' W

[a]Coordinate locations obtained with a Garmin® 12-channel Global Positioning System unit.

Direct Observations. On each sampling date, one subplot from each site was chosen randomly and between eight and twelve spotted knapweed plants were randomly examined for a total of 30 minutes. Any insect found under the rosette leaves or on the capitula or stem were noted and recorded. Some insects, such as the biological control organisms, pollinators, and those that were not previously collected, were removed using an aspirator or forceps. Collected specimens were placed in their own labeled, re-sealable plastic container (FISHERbrand® 17 x 100 mm vial 1.5 cm diameter x 1.5 cm x 9.5 cm) and transported to the laboratory within a cooler for identification to family level.

Search for *Megalanotus sabulicola* Thompson (Hemiptera: Lygaeidae). Five plants were randomly chosen in a subplot and examined specifically for the presence of *M. sabulicola*. *M. sabulicola*, an accidentally introduced seed-feeder of spotted knapweed seeds that are on the ground, was collected in eastern Tennessee during previous research (J. F. Grant, unpublished data). The direct search for *M. sabulicola* consisted of lifting the rosette leaves of one spotted knapweed at a time, removing any leaves, litter or rocks, and looking for nymphs or adults of *M. sabulicola*. This procedure was repeated for each of the four subplots at each site. All *M. sabulicola* present were collected using an aspirator or forceps, placed into labeled, re-sealable plastic containers (FISHERbrand® 17 x 100 mm vial 1.5 cm diameter x 1.5 cm x 9.5 cm), and transported to the laboratory within a cooler for processing, sorting, and confirmation of species.

b. Selection of Insect Distribution Sites. The purpose of these sampling methods was to provide a better overview of the distribution of insects, in particular the biological control organisms on spotted knapweed, throughout eastern Tennessee. The same criteria listed earlier were used to delineate sites using orange flagging for one-12 m x 15 m plot located in each of 11 counties (Anderson, Bradley, Campbell, Claiborne, Hawkins, Jefferson, Loudon, McMinn, Monroe, Sevier, and Sullivan) in eastern Tennessee (Fig. 7). Coordinate locations of each site were obtained from a Garmin® 12-channel GPS unit while standing in the corner of one of the four subplots (Table 3).

Sampling of Insects. Each plot was sampled once in the summer of 2003 (June or July) and once in the spring of 2004 (March or April) between the hours of 1000 and 1400. Following the methodology listed earlier, sampling consisted of a sweep net swung five times in an 180° arc while walking through the plot, a beat sheet placed beneath three randomly chosen spotted knapweed plants, and a 30-minute direct observation on randomly selected plants. A

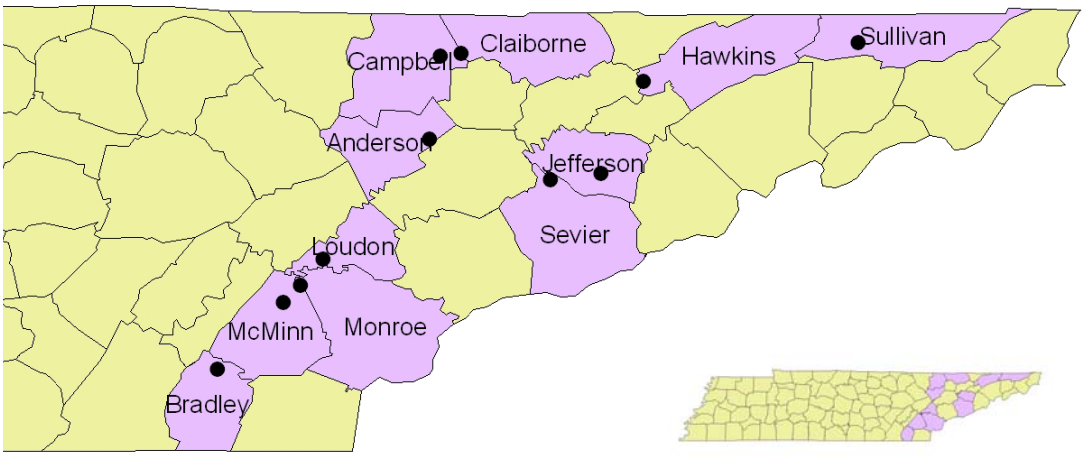

Figure 7. Location (GPS Coordinates are listed in Table 3) of the 11 Research Sites to Assess Distribution of Insects on Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) in Eastern Tennessee.

Table 3. Coordinate Locations[a] for the 11 Research Sites to Assess Distribution of Insects on Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) throughout Eastern Tennessee.

County	Latitude	Longitude
Anderson	36° 08.127' N	084° 08.127' W
Bradley	35° 17.392' N	084° 49.098' W
Campbell	36° 26.324' N	084° 00.120' W
Claiborne	36° 26.974' N	083° 55.536' W
Hawkins	36° 20.760' N	083° 15.443' W
Jefferson	36° 00.494' N	083° 24.586' W
Loudon	35° 41.610' N	084° 25.826' W
McMinn	35° 32.227' N	084° 34.671' W
Monroe	35° 36.024' N	084° 30.906' W
Sevier	35° 59.122' N	083° 35.854' W
Sullivan	36° 29.379' N	082°28.128' W

[a]Coordinate locations obtained with a Garmin® 12-channel Global Positioning System unit.

specific search for *M. sabulicola* under the rosettes of five randomly selected spotted knapweed plants along with collection of chosen insects was part of the 30-minute direct observation. All insects were collected using an aspirator or forceps, placed into labeled, re-sealable plastic containers within a cooler, and transported to the laboratory for processing, sorting, and identification to family level.

c. Identification to Family Level. All insect specimens collected from spotted knapweed were identified to family, where possible, using *An Introduction to the Study of Insects* (Borror et al. 1989). *U. quadrifasciata* and *M. sabulicola* were identified to species. Specimens that could not be conclusively identified were sent to expert taxonomists for identification to the most specific taxonomic level. Specimens were labeled and preserved according to standards for their family on either a pin or within a glass vial (9.5 ml) of 70% ethanol. Biological control specimens that had been identified and confirmed by experts were deposited in the University of Tennessee Insect Museum to be used as voucher specimens.

d. Data Analysis. All data (i. e., family, number of specimens collected, number of specimens per site, collection site, collection date, collection method, etc.) were entered into Excel® spreadsheets for analysis. Family richness, family evenness, Simpsons Index, and the Shannon-Weiner Index for families were determined using the Biodiversity Calculator (Maryland Sea Grant 2004). Total number of specimens per family, seasonality, and distribution of specimens were determined using Descriptives in the statistical program SPSS® 12.0 for Windows® (SPSS 2002).

iii. Results and Discussion

Sampling on spotted knapweed using beat sheets, direct collections, and sweep nets from April 2003 through January 2004 yielded 3,122 specimens of insects representing 108 families and 15 orders (Table 4). Of the 15 orders, Diptera (n = 1,038), Homoptera (n = 698), and Hymenoptera (n = 581) represented almost two-thirds (74.2%) of all specimens collected (Fig. 8). The numbers of species collected from each order were not unexpected for Diptera and Hymenoptera because these organisms are frequently found associated with roadside weeds. It was unexpected that the order Homoptera was so numerous, although most of the specimens were from the Cercopidae (n = 465) family.

Eight orders were represented by only one family. These orders and families were Collembolla: Entomobriidae, Mantodea: Mantidae, Neuroptera: Chrysopidae, Odonata: Coeniopterigidae, Plecoptera: Perlidae, Psocoptera:

Psocidae, Thysanoptera: Thripidae, and Trichoptera: Philopotamidae. Five of these families, Coeniopterigidae (n =1), Entomobriidae (n = 1), Mantidae (n =

Table 4. Families of Insects (n = 108) Collected from Stands of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) in Established Sampling Sites in Eastern Tennessee, 2003-2004.

Order	Family	Site[a]	Collection Method[b]	No. of Adult Specimens	No. of Immature Specimens	No. of Specimens[c]
Coleoptera	Bruchidae	4, Jefferson	S	3	0	3
	Cantharidae	4	D	2	0	2
	Carabidae	1, 3, 5, Hawkins, Monroe	D	7	0	7
	Cerambycidae	1, 2, 4, Sevier	B(3), D(1), S(1)	5	0	5
	Chrysomelidae	1, 2, 3, 4, 5, 6, Campbell, Claiborne, Hawkins, McMinn	B(4), D(20), S(11)	35	0	35
	Cleridae	1	S	2	0	2
	Coccinellidae	1, 2, 3, 4, 5, Sevier	D(4), S(7)	7	4	11
	Cucujidae	3	S	1	0	1
	Curculionidae	1, 2, 3, 4, 5, 6, Jefferson, Monroe, Sevier	B(7), D(12), S(45)	64	0	64
	Dermestidae	2	S	2	0	2
	Elateridae	3, 4	D(1), S(1)	2	0	2
	Lampyridae	3	S	1	0	1
	Languriidae	Loudon	B	1	0	1
	Meloidae	2	S	1	0	1
	Melyridae	1	B(1), D(1), S(1)	3	0	3
	Mordellidae	1, 2, 3, 4, 5, 6, Jefferson	D(7), S(13)	20	0	20
	Nitidulidae	1, 2, 3, 4, 5, 6, Campbell, Jefferson, McMinn	B(8), D(6), S(30)	44	0	44
	Scarabaeidae	3, 4, 5, Sullivan	D(5), S(2)	7	0	7
	Scolytidae	1	S	1	0	1
	Staphylinidae	6, Loudon	B	2	0	2
	Tenebrionidae	6	D(1), S(3)	4	0	4
Collembolla	Entomobriidae	2	D	1	0	1
Diptera	Anthomyiidae	2, 3, 4, 5, 6	D(2), S(6)	8	0	8
	Asilidae	1	S	1	0	1
	Bibionidae	1	S	1	0	1
	Bombylidae	2, 4	D	2	0	2
	Calliphoridae	3, Anderson, Bradley	B(1), D(1), S(1)	3	0	3
	Cecidomyiidae	1, 2, 6	D(2), S(18)	20	0	20
	Ceratopogonidae	2, 5, Jefferson	S	5	0	5

Table 4. Continued

Order	Family	Site[a]	Collection Method[b]	No. of Adult Specimens	No. of Immature Specimens	No. of Specimens[c]
	Chironomidae	1, 2, 3, 4, 6, Jefferson, Loudon	B(1), D(2), S(28)	31	0	31
	Chloropidae	2, 3, 4, 6	S	38	0	38
	Culicidae	3, Claiborne	B(2), D(17), S(6)	25	0	25
	Dolicopodidae	1, 2	S	4	0	4
	Drosophilidae	3, 4	S	2	0	2
	Lauxaniidae	1, 2, 5	B(1), S(5)	6	0	6
	Muscidae	1, 2, 3, 4, 5, 6, Anderson, Campbell, Claiborne, Hawkins, Monroe	D(21), S(26)	47	0	47
	Mycetophilidae	1	S	1	0	1
	Otitidae	3, Campbell	D	2	0	2
	Pipunculicidae	2	S	1	0	1
	Rhagionidae	1	B	1	0	1
	Sarcophagidae	2, 3, 4, 5, 6, Anderson	D(3), S(10)	13	0	13
	Sciaridae	1	S	1	0	1
	Sciomyzidae	1, 2, 3, 4, 6	S	8	0	8
	Sepsidae	1, 4	S	3	0	3
	Simuliidae	2, 4, 5	S	11	0	11
	Stratiomyiidae	4, Loudon, Monroe	D(2), S(2)	4	0	4
	Syrphidae	1, 2, 3, 4, 5, 6, Anderson, Campbell, Claiborne, Loudon, Monroe, Sevier, Sullivan	D(105), S(65)	170	0	170
	Tachinidae	1, 3, 4, 5, 6, Loudon	D(1), S(19)	20	0	20
	Tephritidae	1, 2, 3, 4, 5, 6, Campbell, Claiborne, Hawkins, Monroe, Sevier, Sullivan	B(39), D(340), S(227)	606	0	606
	Urophora quadrifasciata[d,e]	1, 2, 3, 4, 5, 6, Campbell, Claiborne, Hawkins, Monroe, Sevier, Sullivan	B(39), D(339), S(227)			
	Tipulidae	1, 2, Claiborne	D(2), S(2)	4	0	4
Hemiptera	Alydidiae	4, 5, Campbell	D(3), S(1)	4	0	4
	Anthocoridae	Bradley	B(1), S(1)	2	0	2
	Berytidae	3	D	1	0	1

Table 4. Continued

Order	Family	Site[a]	Collection Method[b]	No. of Adult Specimens	No. of Immature Specimens	No. of Specimens[c]
	Coreidae	2, 3, 4, 5	D(6), S(1)	7	0	7
	Cydnidae	4, Sevier	B(1), D(1), S(1)	3	0	3
	Flatidae	3	S	3	0	3
	Lygaeidae	1, 3, 5, 6, Campbell, Jefferson, Loudon, Monroe	B(16), D(18)	14	20	34
	Megalanotus sabulicola[d]	1, 6	B(1), D(9)			
	Miridae	1, 2, 3, 4, 5, 6, Anderson, Campbell, Claiborne, Monroe, Sullivan	B(18), D(18), S(52)	80	8	88
	Nabidae	Monroe	B	2		2
	Pentatomidae	1, 2, 3, 4, 5, 6, Bradley, Monroe, Sevier	B(12), D(7), S(18)	20	17	37
	Phymatidae	2, 5	D(1), S(1)	2		2
	Reduviidae	2, 3, 4, 5, 6, Jefferson	B(4), D(2), S(3)	5	4	9
	Rhopalidae	2, 3, 4, 5	B(1), S(5)	4	2	6
	Scutelleridae	6	S	1	1	2
	Tingidae	5, 6, Anderson, Bradley, Sevier, Sullivan	S	62	1	63
Homoptera	Acanalonidae	1, 3, 4, 5, 6	B(4), D(2), S(40)	45	1	46
	Aphidae	1, 2, 3, 4, 5, 6, Hawkins, McMinn, Sullivan	D(81), S(6)	81	6	87
	Cercopidae	1, 2, 3, 4, 5, 6, Anderson, Campbell, Claiborne, Hawkins, Loudon, McMinn, Sevier, Sullivan	B(7), D(154), S(304)	407	58	465
	Cicadellidae	1, 2, 3, 4, 5, 6, Bradley, Hawkins, Jefferson, McMinn, Monroe	B(6), D(2), S(47)	39	16	55
	Flatidae	3, 5	D(1), S(3)	4	0	4
	Membracidae	2, 3, 4, 5, 6, Anderson, Claiborne, Jefferson, Loudon, Sullivan	B(3), D(18), S(20)	41	0	41
Hymenoptera	Andrenidae	5	D	1	0	1
	Anthophoridae	1, 2, 4, 5	D(6), S(11)	17	0	17

Table 4. Continued

Order	Family	Site[a]	Collection Method[b]	No. of Adult Specimens	No. of Immature Specimens	No. of Specimens[c]
	Apidae	1, 2, 3, 4, 5, 6, Bradley, Loudon, Monroe, Sullivan	D(164), S(13)	177	0	177
	Braconidae	2, Campbell	D(20), S(2)	2	20	22
	Chalcidae	6	S	1	0	1
	Eumeniidae	4	S	1	0	1
	Eupelmidae	2	S	1	0	1
	Eurytomidae	2, 4	S	1	0	1
	Formicidae	1, 2, 3, 4, 5, 6, Claiborne, Loudon, McMinn, Sevier, Sullivan	B(14), D(128), S(38)	180	0	180
	Halictidae	1, 2, 3, 4, 5, 6, Bradley, McMinn, Monroe, Sullivan	D(72), S(20)	92	0	92
	Ichneumonidae	2, 4	B(1), D(2), S(4)	7	0	7
	Megachilidae	4, Sullivan	D(1), S(1)	2	0	2
	Mutilidae	2	D	1	0	1
	Pteromalidae	1, 2, 3, 4, 5, 6, Campbell, Claiborne	B(3), D(33), S(26)	62	0	62
	Pteromalus cardui	1, 2, 3, 4, 5, 6, Campbell, Claiborne	B(3), D(33), S(26)			
	Sphecidae	4	D	1	0	1
	Tiphiidae	1	S	1	0	1
	Vespidae	1, 2, 3, 4, 5	D	14	0	14
Lepidoptera	Arctiidae	1, 4	S		3	3
	Gelechidae	6	S	1	2	3
	Geometridae	1, 2, 3, 4, 5, Hawkins	S	1	6	7
	Hesperiidae	2	D(7), S(3)	10	0	10
	Lycaenidae	2, 3, 4, 6, Bradley	D(24), S(2)	26	0	26
	Noctuidae	1, 3, Anderson, Campbell	D(2), S(2)		4	4
	Papillionidae	Bradley	D	2	0	2
	Pieridae	2, Monroe	D	6	0	6
	Pyralidae	1, 2, 3, 5, 6	D(2), S(9)	11	0	11
	Satyridae	4	D	1	0	1
Mantodea	Mantidae	3, 6	D(1), S(1)	2	0	2
Neuroptera	Chrysopidae	2, 3, 4, 5, Sullivan	D	1	6	7
Odonata	Coeniopterigidae	3	D	1	0	1
Orthoptera	Acrididae	1, 2, 3, 4, 5, 6, Anderson, Campbell, Hawkins, McMinn, Monroe	B(1), D(61), S(58)	33	87	120

Table 4. Continued

Order	Family	Site[a]	Collection Method[b]	No. of Adult Specimens	No. of Immature Specimens	No. of Specimens[c]
	Gryllidae	1, 3, 4, 5, 6, Monroe	D(4), S(21)	14	11	25
	Tettigoniidae	1, 2, 3, 4, 5, 6, Jefferson, Loudon	B(1), D(9), S(46)	25	31	56
Plecoptera	Perlidae	1	S	1	0	1
Psocoptera	Psocidae	1, 2	B(11), S(18)	21	8	29
Thysanoptera	Thripidae	1, 2, 4, 5	S	4	3	7
Trichoptera	Philopotamidae	4	D	2	0	2
TOTAL	108			2,803	319	3,122

[a] Site: 1 (Disturbed Land), Greene County; 2 (Uncultivated Pasture), Cocke County; 3 (Natural Area), Grainger County; 4 (Roadside), Grainger County; 5 (Natural Area), Grainger County; and 6 (Roadside), Hamblen County; Anderson, Bradley, Campbell, Claiborne, Hawkins, Jefferson, Loudon, McMinn, Monroe, Sevier and Sullivan Counties (Single sites along the roadside).
[b] Collection Method: B = Beat sheet; D = Direct collection or Observation; S = Sweep net. Numbers in parentheses indicate the total number of specimens collected from each sampling method, if more than one method yielded specimens.
[c] Total number of specimens collected at all sites.
[d] Insects known to reduce the seed potential of spotted knapweed.
[e] Released biological control organism in Beltsville, Maryland.

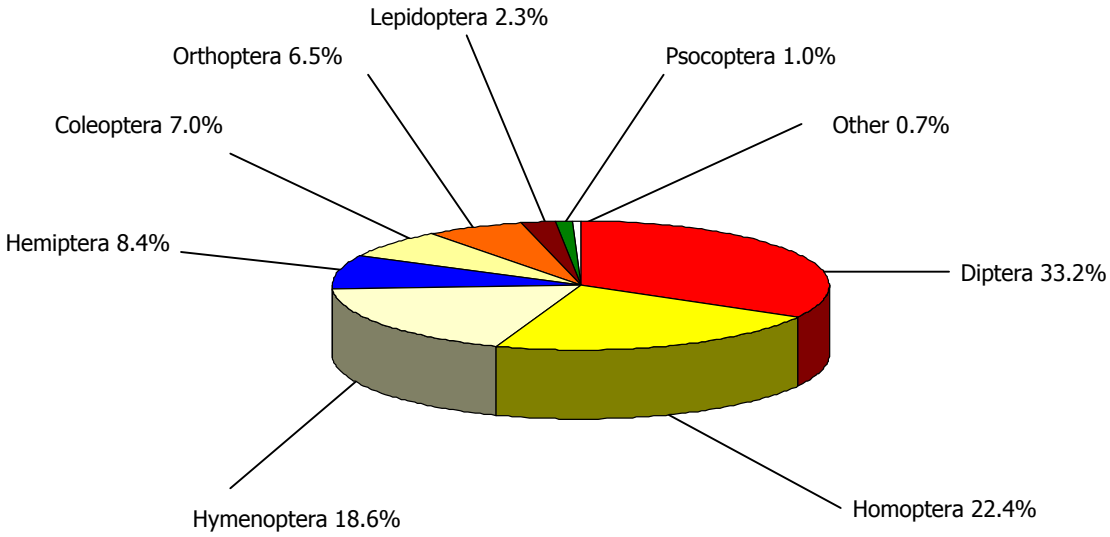

Figure 8. Proportion of Orders of Insects Collected (n = 15, 108 Families, and 3,122 Specimens) from Stands of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) in Eastern Tennessee, 2003-2004.

2), Perlidae (n = 1), and Philopotamidae (n = 2) had both a low incidence and low specimen total. The low incidence and low number of these singlet families provide evidence that they are probably incidentals, and were intercepted when they were using spotted knapweed as a resting location or searching for arthropods as prey. Chrysopidae (n = 7), Psocidae (n = 29), and Thripidae (n =7) were also singlet families, but they had a greater number of specimens collected from at least two locations. The collection of psocids and thrips from spotted knapweed can be associated with their phytophagous feeding. Chrysopidae was probably present on spotted knapweed as a predator of other insects. Other known predator families that were collected include: Coleoptera: Carabidae; Diptera: Asilidae; and Hemiptera: Anthocoridae, Nabidae, Phymatidae, Reduviidae. Likewise, these families were present on spotted knapweed not as a direct pest of the plant, but as opportunists for insect prey resources.

A total of 177 specimens from 33 families was collected using beat sheets, 1,449 specimens from 69 families using direct collection, and 1,488 specimens from 86 families using sweep nets. More specimens and more families were collected using sweep nets, than using either direct or beat sheets. These differences are primarily attributed to the behavior and feeding preference of the insects. For instance, sweep nets collect insect specimens from the flowers and stems, where most of the insects are located. Beat sheet collection, typically targets insects, such as weevils and true bugs, that fall to the ground when disturbed. Because most of the insects utilizing spotted knapweed are pollinators or flower feeders rather than foliage feeders, more were collected in the sweep nets. Direct collections allowed for specific insects to be targeted and removed from the plant. All three methods should be continued to be utilized for future collections because each method targets specific behavior of insects and specific areas of the plants leading to more complete assessment of insect diversity.

Family richness (S) (i. e., the number of families collected from one location) for the Insect Diversity Sites (Table 5) was highest at Site 4 (61 families) in Grainger County, followed closely by Site 2 (56 families) in Cocke County. Site 6 in Hamblen County had the least number of families collected (40). Family richness (Table 6) for the Insect Distribution Sites was highest (19 families) in Monroe County and lowest in McMinn County (8 families). The Monroe County site may have had the highest family richness among the Insect Distribution Sites because of its partially buffered roadside location in the median of the interstate providing a good resting or nutrient gathering spot for insects. The McMinn County site may have had the lowest family richness because it was adjacent to a heavily traveled highway and it was mowed frequently, thus preventing insects from easy access to the spotted knapweed.

Determining family richness does not take into account the proportion and distribution of each family; thus, Simpsons Index (D) [$D = \text{sum}(P_i^2)$] was

Table 5. Richness, Diversity, and Evenness for Families Collected from the Six Insect Diversity Sites in Eastern Tennessee, 2003-2004.

Site[a]	Family Richness[b] (S)	Simpsons Index[c] (D)	Shannon-Weiner[d] Index (H)	Family Evenness[e] (E)
1	50	0.300	2.193	0.561
2	56	0.074	3.122	0.776
3	53	0.052	3.307	0.833
4	61	0.050	3.427	0.834
5	46	0.080	3.006	0.785
6	40	0.122	2.615	0.709

[a]Locations of sites are shown in Figure 6.
[b]Family Richness is the total number of families collected from one location.l
[c]Simpsons Index (D) is calculated using the equation [$D = sum(P_i^2)$].
[d]Shannon-Weiner Index (H) is calculated using the equation [$H = -sum(P_i log[P_i])$].
[e]Family Evenness (E) is calculated using the equation [$E = H/log(S)$].

Table 6. Richness, Diversity, and Evenness for Families Collected from the 11 Insect Distribution Sites in Eastern Tennessee, 2003-2004.

Site	Family Richness[a] (S)	Simpsons Index[b] (D)	Shannon-Weiner[c] Index (H)	Family Evenness[d] (E)
Anderson	11	0.230	1.820	0.759
Bradley	10	0.190	1.898	0.825
Campbell	14	0.136	2.196	0.832
Claiborne	11	0.189	1.935	0.807
Hawkins	9	0.346	1.391	0.633
Jefferson	11	0.102	2.342	0.977
Loudon	13	0.167	2.166	0.845
McMinn	8	0.375	1.334	0.642
Monroe	19	0.107	2.637	0.896
Sevier	11	0.419	1.359	0.567
Sullivan	12	0.132	2.205	0.887

[a]Family Richness is the total number of families collected from one location.l
[b]Simpsons Index (D) is calculated using the equation [$D = sum(P_i^2)$].
[c]Shannon-Weiner Index (H) is calculated using the equation [$H = -sum(P_i log[P_i])$].
[d]Family Evenness (E) is calculated using the equation [$E = H/log(S)$].

calculated. Simpsons Index measures the probability that two randomly selected individuals will belong to same family (0 = infinite diversity, 1 represents no diversity) giving more weight to the more abundant family in the sample. Site 4 (0.050) in Grainger County was the most diverse of the Insect Diversity Sites (Table 5), followed closely by Site 3 (0.052) also found in Granger County. Site 1 (0.300) in Greene County was the least diverse. For the Insect Distribution Sites (Table 6), Jefferson County (0.102) was the most diverse and Sevier County (0.419) was the least diverse.

Another measurement of diversity, Shannon-Weiner Index (H) [$H = -\text{sum}(P_i \log[P_i])$], was calculated. This equation measures the disorder observed in a system and is more sensitive to the occurrence of a rare species, rather than the abundance. The diversity results of the Shannon-Weiner Index were consistent with those of the Simpsons Index for the Six Insect Diversity Sites (Table 5). Site 4 (3.427) in Grainger County and Site 1 (2.193) in Greene County were the most and least diverse locations, respectively. The Shannon-Weiner Index for the 11 Insect Distribution Sites calculated this site in Monroe County (2.637) and McMinn County (1.334) as the most and least diverse, respectively. Diversity values of these two counties can be explained by their family richness. Family richness has a direct relationship with the Shannon-Weiner Index. If the abundance of specimens are relatively similar, as did occur in the 11 Insect Distribution Sites, then diversity results will also be similar.

Family evenness (E) [$E = H/\log(S)$] is the measurement of how similar the abundance of different families are within a selected system. Family evenness ranges from 0 to 1 with 1 being the most even. Family evenness was calculated from the family richness and the Shannon-Weiner Index (H). When similar proportions of all families occur, family evenness is equal to one. Of the Six Insect Diversity Sites (Table 5), Sites 3 (0.834) and 4 (0.833) in Grainger County were the most even, while Site 1 (0.561) in Greene County was the least. All of these six sites showed moderate to high family evenness indicating a well distributed and similarly abundant number of families at each site. The Jefferson County (0.977) site was the most even and the site in Sevier County (0.567) was the least for the 11 Insect Distribution Sites (Table 6). The family evenness value for Sevier County can be explained by the relatively small numbers of specimens from ten families and large number of specimens from one family at the location. The overall high values (greater than 0.6) of family evenness numbers in the 11 Insect Distribution Sites can be attributed to the low number of families (range of 8 to 19) collected from each location along with the similar number of specimens collected from each family.

Cercopidae (Order: Homoptera) (n = 465), the second most commonly collected insect family, was the most distributed family occurring in eight of the 11 Insect Distribution Sites and all six of the Insect Diversity Sites. The well distributed nature of cercopids on spotted knapweed can be attributed to their early colonization of the newly-bolted plant in April and May compared to other

surrounding plants that have not yet begun to grow. Although cercopids were both numerous and well distributed on spotted knapweed, no typical damage such as yellowing or wilting of the plant was noticed indicating that the cercopids did not reduce its viability. Besides Cercopidae, 17 other families were collected from all six of the Insect Diversity Sites suggesting that spotted knapweed can maintain a large diversity of insect families throughout its growing season. Of the families collected from the 11 Insect Distribution Sites, 83.6% were from less than five of the sites, indicating that most insects were not well distributed on spotted knapweed in those locations.

The most commonly collected herbivore was the biological control organism, *U. quadrifasciata* (n = 605). The closest recorded intentional release of *U. quadrifasciata* to eastern Tennessee was in Beltsville, Maryland, in 1983. *U. quadrifasciata* was collected in large numbers from each of the six Insect Diversity Sites and six of the 11 Insect Distribution Sites (12 total sites) (Fig. 9). Of the six Insect Diversity Sites, Site 1 in Greene County had the most *U. quadrifasciata* collected (n = 317, 189 males and 128 females), while Site 3 in Grainger County had the least (n = 13, 5 males and 8 females). The greater number of *U. quadrifasciata* at Site 1 may be due to the integrity of the well established site which had numerous stems and flower heads. The spotted knapweed population also was isolated from other infestations; thus, it provided an accessible resource for the flies. Site 3 may have had the least number of *U. quadrifasciata* collected because it was mowed twice during the sampling season and was bordered on one side by a lake, preventing the movement of the flies from that direction. Of the 11 Insect Distribution Sites, the greatest number of *U. quadrifasciata* were collected in Claiborne County (n = 14, 10 males and 4 females), while the least number were found in Hawkins County (n = 1, 1 male). It is probable that Claiborne County had the most *U. quadrifasciata* because this county is close to southwestern Virginia, a possible dispersal pathway from its original release site in Beltsville, Maryland.

Direct collection yielded 37.5% (n = 227), and sweep net yielded 56.0% (n = 339) of the total *U. quadrifasciata* collected. Only 6.5% (n = 39) were collected using beat sheets. The greatest number of individuals of *U. quadrifasciata* collected using one sampling method at one location was 36 for the sweep net, 59 for direct, and 7 for beat sheet collection. The sex ratio collected for *U. quadrifasciata* was 345 males to 260 females (1.33:1), similar to the 1.11:1 ratio for *U. quadrifasciata* found in Virginia (Mays and Kok 2003). When compared to the total number of specimens collected from spotted knapweed, *U. quadrifasciata* comprised 19.4%.

These data represent the first confirmed record of *U. quadrifasciata* in Tennessee, expanding its already well established distribution (Story 2002) to include areas of the southeastern United States not traditionally examined for its presence. The rapid dispersal of more than 40 km/year for *U. quadrifasciata* (Story 1985) has been attributed to lack of capitula in the appropriate

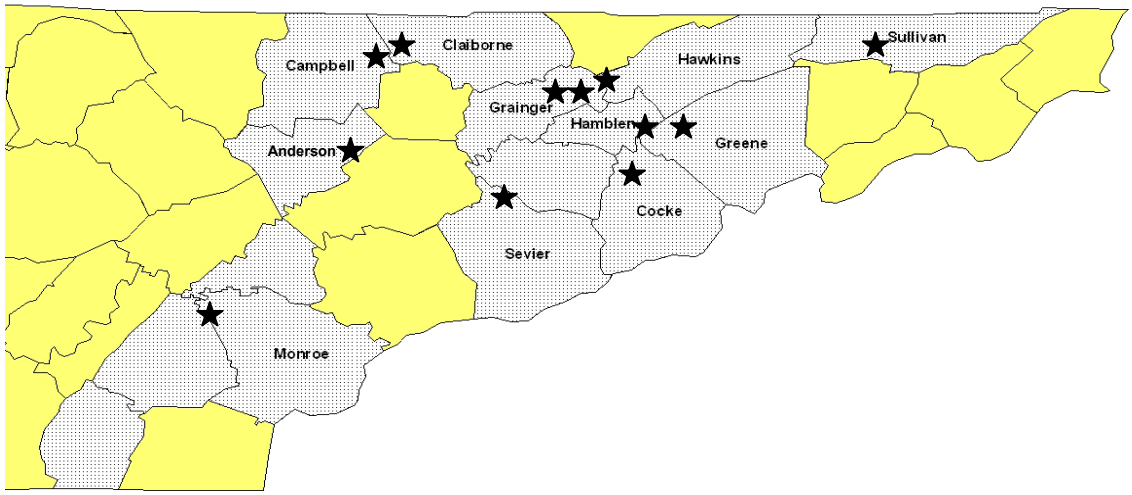

Figure 9. Distribution of *Urophora quadrifasciata* in Eastern Tennessee, 2003-2004. Shaded Counties Represent Those that Were Sampled. Stars Indicate Locations within the Counties from Where *U. quadrifasciata* was Recovered.

developmental stage for oviposition (Mays and Kok 2003). It is proposed that *U. quadrifasciata* will continue to expand southwestwardly across Tennessee and the southeastern United States where the appropriate developmental stages of spotted knapweed are available.

Urophora quadrifasciata was not collected from Anderson, Bradley, Jefferson, Loudon, or McMinn Counties. The fly may not have been recovered from these areas because of the lack of suitable capitula in which to oviposit, or because the populations of spotted knapweed were too isolated and with too little density as in Jefferson County. Continued monitoring of these counties where *U. quadrifasciata* is absent would add to the knowledge of its rate of spread. For instance, if collections in 2004 reveal the presence of *U. quadrifasciata* in a county where it was not found in 2003, distance from known points of establishment of *U. quadrifasciata* can be used to calculate its rate of spread. This calculation can then be used to predict where and when *U. quadrifasciata* could be found.

The earliest detection of adult *U. quadrifasciata* was on 22 May 2003 in Sevier County and the latest was on 22 August 2003 at Site 2 in Cocke County. The monthly seasonality of the sexes of *U. quadrifasciata* was consistent for more males than females collected, except in the month of August (Table 7). A direct seasonality occurrence with other insects besides Tephritidae was evident for most of the families corresponding to the opening of spotted knapweed flowers.

One particular species, *Pteromalus cardui* (Erdös) in the family Pteromalidae (n = 62) was collected using all three methods of collection (beat, n = 3; direct n = 33; sweep, n = 26) from all six Insect Diversity Sites and in two of the 11 Insect Distribution Sites (Campbell and Claiborne Counties). This hymenopteran parasitoid showed seasonality with the summer months from its collection from June through August. This summer activity of *P. cardui* is relevant to the development of the first generation of *U. quadrifasciata* because this hymenopteran has been described as a parasitoid of the biological control agent (Marshall and Storere 2003). Consequently, *P. cardui* should be studied throughout the year to determine if it significantly reduces the number of *U. quadrifasciata* during its summer adult stage.

Urophora affinis was not recovered from these collections, although it has been described as well established in southwestern and central Virginia (Mays and Kok 1996). The slow rate of spread (1.3 km/yr) and little pressure to spread since available capitula are abundant in Virginia (Mays and Kok 2003) may have contributed to *U. affinis* not yet establishing in eastern Tennessee. *M. paucipunctella* which has been recovered from spotted knapweed in southwestern Virginia in low numbers (Mays and Kok 2003) was not recovered from these samples. With more thorough sampling in the future, the two biological control agents that were intentionally released in Virginia in 1986 may be recovered from populations of spotted knapweed in upper eastern Tennessee.

Table 7. Seasonality of *Urophora quadrifasciata* in Eastern Tennessee.

	No. Collected[a]		
	Male *U. quadrifasciata*	Female *U. quadrifasciata*	Total
May	88	42	130
June	58	45	103
July	188	153	341
August	11	20	31
Total	345	260	605

[a]Number collected using sweep-net, beat-sheet, and direct sampling.

Only ten (eight adults and two nymphs) *M. sabulicola* were directly collected from two sampling locations, Site 1 in Greene County and Site 6 in Hamblen County (Table 4). Adults of *M. sabulicola* were collected from May through August with immatures collected during May, July, and August indicating an overlapping of generations. Although this naturalized seed-feeding bug has been described as a beneficial immigrant because it destroys spotted knapweed seeds that have already been dispersed (Wheeler Jr. 1989), the low numbers of specimens recovered suggest that it has a negligible effect on spotted knapweed establishment and survival in eastern Tennessee. The impact on the reduction of spotted knapweed seeds by laboratory-reared *M. sabulicola*, along with the ability of *M. sabulicola* to disperse and reproduce in natural settings, should be considered for future research.

Specific studies were not conducted on the occurrence of pollination from insects observed on, or collected from, open flowers of spotted knapweed. However the following families were most frequently collected from flowers: Coleoptera: Meloidae, Mordellidae, and Nitidulidae; Diptera: Muscidae and Syrphidae; Hemiptera: Coreidae, Cydnidae, and Rhopalidae; Hymenoptera: Andrenidae, Anthophoridae, Apidae, Formicidae, Halictidae, Megachilidae, Sphecidae, and Vespidae; Lepidoptera: Hesperiidae, Lycaenidae, Papillionidae, Pieridae, Pyralidae, and Satyridae; and Thysanoptera: Thripidae. All of the above mentioned familes of Lepidoptera were gathering nectar from the flowers, while the following families of Hymenoptera were observed collecting pollen from the flowers: Andrenidae, Anthophoridae, Apidae, Halictidae, and Megachilidae. The numerous families observed on flowers reveal that many more potential pollinators, other than Apidae (Watson and Renney 1974), should be investigated to determine their effectiveness as pollinators of spotted knapweed. Additional investigations of the biological associations between these organisms that may function in the fertilization of, and consequently development of seeds of, spotted knapweed would enhance the knowledge for the management of spotted knapweed.

Future investigations into the community level interactions between the collected insects and spotted knapweed should occur. Knowledge and insight into the relationship between spotted knapweed and the insects that utilize it for rest, pollen, nectar, food, and as an indirect source of arthropod prey would be beneficial to the management of spotted knapweed in eastern Tennessee.

iv. Summary

Within populations of spotted knapweed in 15 counties in eastern Tennessee, six Insect Diversity Sites and 11 Insect Distribution Sites were sampled for insects from April 2003 until October 2003. Sweep nets, beat sheets, and direct collection were used to collect insect specimens.

A total of 3,122 specimens representing 108 familes in 15 orders was collected. Diptera was the order with the most specimens collected (n = 1,038), as well as the order with the most families collected (n = 28). More specimens of Tephritidae were collected (n = 606) than any other family. Sweep-net collection gathered the most families (n = 86), followed by direct sampling (n = 69), and then beat-sheet sampling (n = 33). Some families, such as Cantharidae, were collected using one method, while others were collected using two or more methods.

Two insects that negatively impact the seeds of spotted knapweed were collected. One was the intentionally released biological control agent, *U. quadrifasciata,* that reduces seed development in the capitula. The other one was a naturalized species, *M. sabulicola,* that consumes already dispersed seeds. The greatest number of *U. quadrifasciata* were collected using direct collection (n = 339), followed by sweep-net sampling (n = 227). Adult *U. quadrifasciata* were present from May through August in eastern Tennessee and had a male to female ratio of 1.33:1. *U. quadrifasciata* is fairly well distributed in eastern Tennessee and it was collected from 12 of the 17 sites sampled. Only ten *M. sabulicola* were recovered from two sites, indicating that its ability to reduce spotted knapweed may be limited. However, since this seed feeder is already established, the quantitative impact of *M. sabulicola* on seed consumption in both laboratory and field conditions should be investigated further. This report is the first official documentation of both of these seed-reducing insects in Tennessee.

Thirty-four families of insects were identified on the flowers of spotted knapweed. Of these families, 11 have been described as pollinators and were characterized as gathering pollen and/or nectar from the flowers of spotted knapweed. Further studies of these pollinators need to be conducted to determine if they impact seed production.

The three collection methods provided valuable data for the abundance and the diversity of insects associated with spotted knapweed in eastern Tennessee. Some families of insects, such as Tephritidae, were abundant and well distributed on spotted knapweed throughout eastern Tennessee. Data also showed that some sites such as Insect Diversity Site 4 (n = 61) and Insect Distribution Campbell County Site (n = 19) were the most diverse in the number of families that were collected.

Data gained from this research provided new knowledge of both the invasive spotted knapweed and its associated insects. This knowledge is applicable to the management of spotted knapweed in eastern Tennessee.

III. Incidence, Distribution, and Impact of *Urophora quadrifasciata* on Spotted Knapweed

i. Introduction

Spotted knapweed [*Centaurea stoebe* L. *micranthos* Gugler (Hayek) formerly *C. biebersteinii* DC. and *C. maculosa* Lam.] (referred to here as *C. stoebe* L. *s. l.*) (Asteraceae) is a non-indigenous, invasive perennial (Watson and Renney 1974) that out-competes native plants and cultivated forages, ultimately replacing the community with a monoculture through its rapid development, prolific seed production (400-35,000 seeds/plant) (Watson and Renney 1974), and allelopathic chemicals (catechin and cnicin) (Landau et al. 1994; Moellenberg 2003). Since its first documentation in Washington in 1893 (Roche et al. 1986; Sheley et al. 1998), spotted knapweed has colonized more than 4 million hectares within the United States (Todd 2001) with most of that area occurring in the economically important western rangelands of the United States. Spotted knapweed has been present in the eastern United States since 1894 in Westford, Massachusetts (A. Swanson, personal communication). However, in the eastern United States, it has remained largely limited to roadsides and wastelands, and within some pastures (Hoebeke 1993; Mays and Kok 1996). The less invasive characteristics of spotted knapweed in the eastern United States are probably attributed to the less favorable and wetter habitat conditions. Consequently, a major effort to control the perennial weed using biological control with insects was primarily instituted in the western United States because spotted knapweed was not even recognized in the eastern United States (Lang et al. 1997) as a weed of concern at the time of initial release of the insects.

Populations of the Palearctic *Urophora quadrifasciata* (Diptera: Tephritidae), the UV knapweed seed-head fly, collected from the Ukraine (Harris and Shorthouse 1996), were first released in North America at Ned's Creek, British Columbia, Canada, in 1970 as a biological weed control for both spotted knapweed and diffuse knapweed (*C. diffusa* L.). Characteristics that make *U. quadrifasciata* a good biological control organism for spotted knapweed include: it is bivoltine (first generation in June or July and second generation in mid-August) (Myers and Harris 1980; Lang et al. 2000); it is well synchronized with knapweed development (Story et al. 1992); it forms a weak metabolic sink and reduces the number of seeds produced through the formation of a papery gall in a developing ovary of the capitulum by the larva (reduction of 1.9 seeds/gall/capitulum) (Harris 1980); and it disperses rapidly (40 km/year) (Story 1985; Lang et al. 1997). Sex ratio of male to female is close to 1:1 (Mays and Kok 2003).

Urophora quadrifasciata was not initially released in the United States, but dispersed from the Canadian populations, with first detection in Idaho in 1980 (Gillespie 1983) and in Montana in 1981 (Story 1985). Initial intentional releases of *U. quadrifasciata* in the United States occurred in New York and Maryland in 1983 by the USDA-ARS (Hoebeke 1993). However, *U. quadrifasciata* was not officially approved for release as a biological control agent in the United States until 1988 (Lang et al. 2001). Subsequent releases have occurred through both state and federal agencies throughout the United States (Wilson and Randall 2003). The range of *U. quadrifasciata* has continued to spread to include hundreds of counties across most of the United States where *Centaurea* spp. are present (Fig. 10) (Story et al. 1987; Story et al. 1992; Hoebeke 1993; Wheeler Jr. and Stoops 1996; Lang et al. 1997; Nowierski et al. 1999; Lang et al. 2001; Reieder et al. 2001; Mays and Kok 2003).

Mortality of *U. quadrifasciata* resulting from parasitism has been minimal (Myers and Harris 1980; Story et al. 1995; Mays and Kok 2003). Most incidence of death to *U. quadrifasciata* arose from consumption by deer mice [*Peromyscus maniculatus* (Wagner)] (Story et al. 1995; Pearson et al. 2000), black capped chickadees (*Parus atricapillus* L.) or white-tailed deer [*Odocoileus virginianus* (Zimmerman)] (Story et al. 1995).

In Tennessee, spotted knapweed is found in 29 counties (Wofford 2002), with most of the populations occurring in the eastern part of the state (Fig. 11). Spotted knapweed is listed on the Tennessee Exotic Pest Plants List as a Rank 2 - Significant Threat (Bowen et al. 2002) because it is not considered to spread as easily into native plant communities. It is typically present along disturbed habitats and the borders of interstates and the edges of roads, but is also occasionally found in some natural settings, such as pastures (personal observations). Consequently little, if any, chemical or mechanical control and no biological control have been used against spotted knapweed in eastern Tennessee. Because monitoring of the establishment and natural spread of individual and combinations of knapweed biological control agents needs to be continued (Lang et al. 2000), the specific objective of this research was to determine the incidence, distribution, impact, and parasitoids of *U. quadrifasciata* on populations of spotted knapweed in eastern Tennessee.

ii. Materials and Methods

a. Selection of Sites to Assess Incidence and Impact of *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae). To assess the incidence and impact of *U. quadrifasciata* on spotted knapweed in eastern Tennessee, the University of Tennessee Herbarium records were used to identify known locations of spotted knapweed. The locations were canvassed for appropriate permanent sampling locations of spotted knapweed. Criteria for suitable

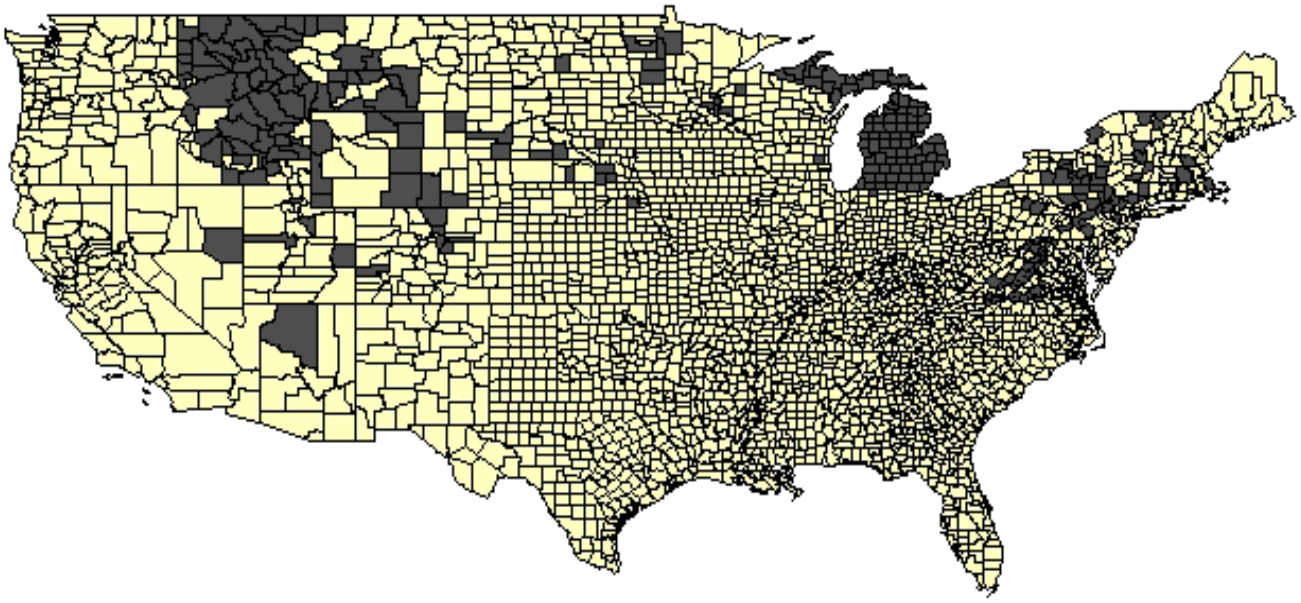

Figure 10. Distribution of *Urophora quadrifasciata* in the United States (Shaded Areas Indicate Establishment in Respective Counties) (From Story et al. 1987; Story et al. 1992; Hoebeke 1993; Wheeler Jr. and Stoops 1996; Lang et al. 1997; Nowierski et al. 1999; Lang et al. 2001; Reieder et al. 2001; Mays and Kok 2003) (2003).

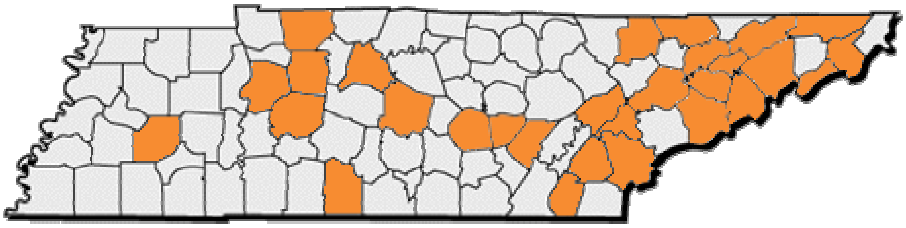

Figure 11. Distribution of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) within Counties of Tennessee, 2004.

locations consisted of: (1) confirmation of presence of spotted knapweed from University of Tennessee Herbarium director, B. Eugene Wofford, (2) ease of access, (3) >75% ground cover by spotted knapweed from qualitative visual surveys, (4) within an hour and one half driving distance from the University of Tennessee, and (5) current land use (disturbed or natural habitat). These criteria enabled stands to be sampled in a timely manner for habitats dominated by spotted knapweed as typical of previous studies.

Preliminary Assessment. In December 2002 through April 2003, a preliminary assessment of biological control organisms associated with spotted knapweed at locations that met the criteria listed above was conducted. Particular attention was made to the recovery of *U. quadrifasciata*, as confirmed by Dr. Jim Story, Western Agriculture Research Center, Montana State University. Preliminary assessment included the removal of 50 spotted knapweed capitula that were then taken to the laboratory in labeled, re-sealable plastic containers (FISHERbrand® 17 x 100 mm vials 1.5 cm diameter x 1.5 cm x 9.5 cm). Of these, 25 capitula were dissected and examined for insect presence, and 25 capitula were set aside and examined after 30 days for insect emergence. Laboratory conditions were maintained at a relative humidity of 60%, 10 L:14 D, and 23 ± 2 °C.

Based on the earlier findings, six locations of spotted knapweed infestations within four eastern Tennessee counties (Cocke, Grainger, Greene and Hamblen) were established as research sites (Fig. 6). Four subplots (12 m x 15 m) marked by orange flagging were established at each site in May 2003. Coordinate locations of each site were obtained from a Garmin® 12-channel Global Positioning System (GPS) unit while standing in the corner of one of the four subplots (Table 2).

Sampling for *Urophora quadrifasciata*. Sampling occurred eight times between the hours of 1000 and 1400 from May 2003 through January 2004. Capitula of spotted knapweed were removed at each of the six sites to assess the presence and impact of *U. quadrifasciata* on spotted knapweed. All six sites were sampled every three to four weeks from May 2003 to August 2003. Sampling also occurred once in October 2003 and once in January 2004. Sampling at each site took approximately one hour based upon weather conditions and developmental stage of spotted knapweed. To better assess the association and seasonal impact of *U. quadrifasciata* on spotted knapweed, the location of capitula collected from the plant were stratified vertically and classified as top, middle, or bottom (Fig. 12). Because of the development of spotted knapweed, the top stratification develops into flowers first, followed by the middle and then bottom early in the growing season. Later in the growing season, spotted knapweed may have similar stages of capitula in each of the three stratification sections.

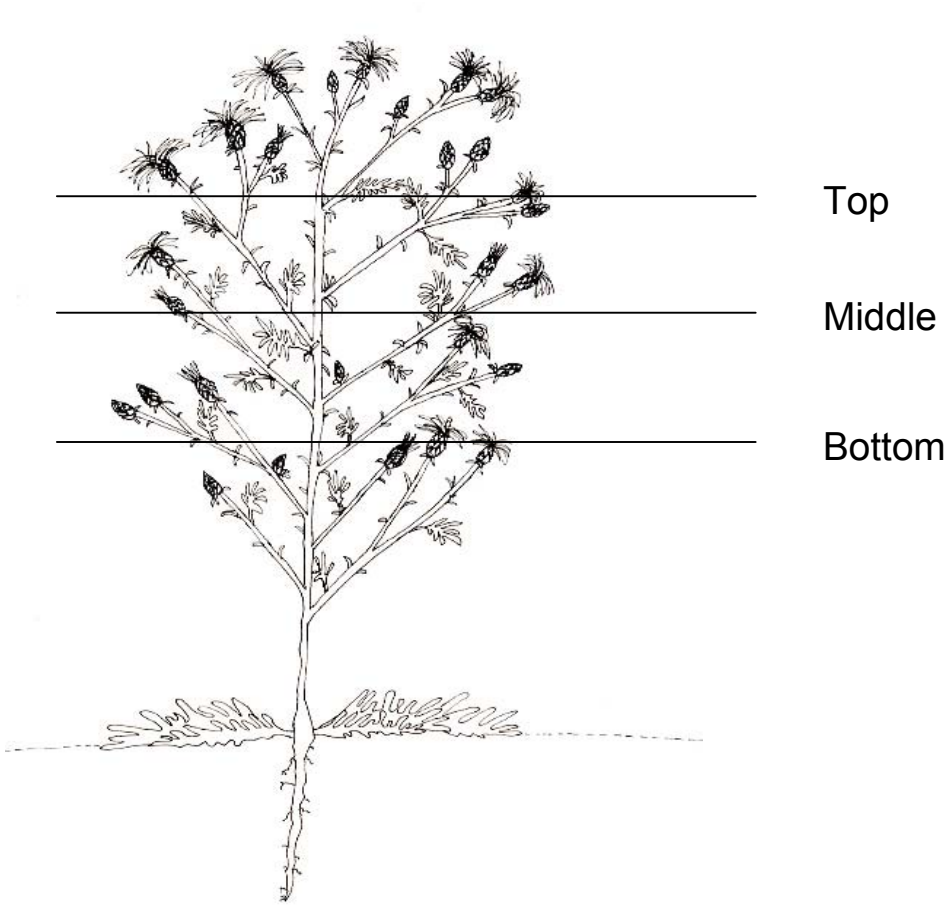

Figure 12. Vertical Stratification (Top, Middle, and Bottom) of Capitula of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*).

On each sampling date at the six sites, plants were chosen by walking in a random zig-zag pattern throughout each of the four subplots stopping every three to four steps. The plant directly in front of the sampler was chosen and four capitula per plant were removed by hand from a randomly selected stratification level (top, middle, or bottom) and placed in their own labeled, re-sealable plastic container (FISHERbrand® 17 x 100 mm vial 1.5 cm diameter x 1.5 cm x 9.5 cm). A total of 200 capitula from 50 plants was removed per site (or the most available capitula up to 200 if mowing had occurred). Because there were four subplots at each site, the 50 sampled plants were divided into 12 plants sampled from two subplots and 13 plants sampled from the other two subplots (200 capitula total = 4 capitula each x 50 plants = 2 subplots x 12 other plants x 4 capitula each + 2 subplots x 13 plants x 4 capitula each). All sites and numbers of samples were randomly assigned prior to each sampling date. All collected capitula were taken to the laboratory for processing, where two of the four capitula from each plant were removed from their containers and dissected. The other two capitula remained in the containers and were monitored for emergence while kept at a relative humidity of 60%, 10 L:14 D and 23 ± 2°C.

Collecting methods for total sample size (Nowierski et al. 1987; Lang et al. 1997; Mays and Kok 2003) and the number of capitula removed from each plant from prior studies were used for protocol (Nowierski et al. 1987) to minimize plant-to-plant differences. This protocol was utilized during collection of capitula at each of the sampled locations.

Dissection of Capitula. For every capitulum that was dissected, the length and width was first measured and recorded in millimeters using an electronic digital micrometer (Marathon Electronics 0-25 mm). Under a dissecting microscope (Zeiss Stemi SV6) the capitulum was pulled apart with forceps. The total number of achenes were counted and recorded; the achenes were then discarded. The total number of immature stadia of *U. quadrifasciata* (egg, larva, and pupa) were counted, recorded, and then preserved in a glass vial (9.5 ml) of 70% ethanol. The total number of other insects and their stadia present within the capitula were counted, recorded, and if a hymenopteran parasitoid was present, preserved in a glass vial (9.5 ml) of 70% ethanol for later identification to species or the lowest taxonomic level.

Emergence of *Urophora quadrifasciata*. Monitoring for the emergence of *U. quadrifasciata* and other insects from the capitula of spotted knapweed occurred once per week. Monitoring consisted of rotating each container with capitula, individually over a white letter-size sheet of paper (216 mm X 279 mm) for approximately 5 sec. while observing for insects in the container. Adult *U. quadrifasciata* could easily be seen and, if present, were removed and set aside for pin mounting and labeling. The site, date of collection, date of emergence, vial number, stratification level (top, middle or

bottom), and sex were recorded on a data sheet. If a hymenopteran parasitoid was present, it was removed and placed in a labeled glass vial (9.5 ml) of 70% ethanol for later identification to the most specific taxon possible; then associated characteristics (width, length, open or closed, and number of seeds) of the capitulum from which it emerged were recorded. For all parasitoids found, their site, date of collection, date of emergence, vial number, and stratification level (top, middle or bottom) were recorded on a data sheet. Once all of the containers from the site were examined, the process was repeated, so each container of two capitula was examined twice on the monitoring day to lessen the chance of human error not seeing an emerged insect.

b. Selection of Sites to Assess the Distribution of *Urophora quadrifasciata*. The purpose of these sampling methods was to provide a better overview of the distribution of *U. quadrifasciata* throughout eastern Tennessee. The same criteria listed earlier were used to delineate sites marked by orange flagging for one-12 m x 15 m plot located in each of 11 counties (Anderson, Bradley, Campbell, Claiborne, Jefferson, Loudon, McMinn, Monroe, Roane, Sevier, and Sullivan) in eastern Tennessee (Fig. 7). Coordinate locations of each site were obtained from a Garmin® 12-channel GPS unit while standing in the corner of one of the four subplots (Table 3).

Each plot was sampled once in the summer of 2003 (June or July) and once in the spring of 2004 (April or May). The same methodology was followed as mentioned earlier under sampling for *U. quadrifasciata*, except that 100 capitula (or most available capitula up to 100 if mowing had occurred), instead of 200 capitula, were removed from each county site per sampling time. Protocol for dissection and emergence as listed under sampling for *U. quadrifasciata* was the same.

c. Identification to Species or Lowest Taxonomic Level of Parasitoids. All hymenopteran parasitoid specimens collected from spotted knapweed capitula were identified to family, where possible, using *Hymenoptera of the World: An Identification Guide to Families* (Goulet and Huber 1993). These specimens were then sent to expert Chalcidoidea taxonomists (Eric Grissel and Michael W. Gates, both from the USDA-ARS Systematic Entomology Laboratory, and Roger Burks of the University of California, Riverside) for identification to species or the lowest taxonomic level able to be determined.

d. Data Analysis. All data were analyzed using the statistical program SPSS® 12.0 for Windows® (SPSS 2002). Pearson and Spearman's Two-Tailed Bivariate Correlations were conducted for the number of immature *U. quadrifasciata* and the number of spotted knapweed seeds present in a capitulum. A general linear ANOVA and pairwise comparison between sites was conducted for the number of immature *U. quadrifasciata* and number of seeds of spotted knapweed present in

each capitulum of each vertical stratification level of spotted knapweed. A general linear ANOVA was conducted for the number of parasitoids and the length and width of the spotted knapweed capitulum from which they emerged. A Pearson Correlation was conducted for the number of immature *U. quadrifasciata* and the number of parasitoids that emerged from each capitulum.

iii. Results and Discussion

Dissection. A total of 4,726 capitula of spotted knapweed was dissected for the six Incidence and Impact Sites. Dissected capitula revealed a total of 1,184 immature (333 larvae and 215 pupae) *U. quadrifasciata*. Empty puparia (n = 636) were also found indicating additional *U. quadrifasciata* had been present, but had successfully emerged before collection of the capitula. The number of immature *U. quadrifasciata* per capitulum ranged from 0 to 13.

Total number of seeds of spotted knapweed ranged from 0 to 42 per capitulum. Capitula with 1 to 13 immature *U. quadrifasciata* had a mean of 6.01 ± 0.19 S. E. seeds, compared to an average of 7.94 ± 0.17 seeds for those in non-infested capitula. If *U. quadrifasciata* are present, production of seeds is reduced by nearly two seeds per capitula (24.3% reduction in seed production).

A greater negative correlation existed between the number of immature *U. quadrifasciata* and the number of seeds present in closed capitula (n = 1,454), than in total (open and closed) capitula (n = 3,494). When compared using the Spearman's Two-Tailed Bivariate Correlation, total capitula showed a negative 0.067 correlation compared to a negative 0.239 correlation for just closed capitula. Both values were significant at $\alpha = 0.01$. The correlation between the number of immature *U. quadrifasciata* and the number of seeds in closed capitula is probably more accurate because it accounts for all immatures present, instead of some immatures that may have been dislodged from open capitula. However, both values show that as the number of *U. quadrifasciata* increases in the capitulum, the number of seeds decreases, which is consistent with previous data.

When capitula of spotted knapweed were stratified into top, middle, and bottom, the number of *U. quadrifasciata* along with the number of seeds found in the top stratification level was significantly less than those found in the middle or bottom strata (Table 8). This difference could be partially attributed to the development of spotted knapweed. The first capitula available for oviposition by *U. quadrifasciata* are typically in the top portion of the plant. However, as more overwintering *U. quadrifasciata* emerge later they have more ovipositional sites on the lower two strata of the plant as the capitula continue to develop throughout the plant. Timing of emergence of *U. quadrifasciata* could also positively influence utilization of capitula in the middle and bottom strata of

Table 8. Influence of Vertical Stratification of Capitula of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) (n = 4,725) on Seed Production and Incidence of *Urophora quadrifasciata* (n = 1,184).

Vertical Stratification	Mean No. (±S. E.) Spotted Knapweed Seeds Per Capitulum	Mean No. (±S. E.) Immature *U. quadrifasciata* Per Capitulum
Top	9.15 ± 0.21 a	0.36 ± 0.02 a
Middle	7.93 ± 0.25 b	0.51 ± 0.04 b
Bottom	5.89 ± 0.28 b	0.63 ± 0.06 b
Total Mean	7.39 ± 0.13	0.47 ± 0.02

*Numbers in each column followed by the same letters are not significantly different, where α = 0.05.

spotted knapweed, because once the flies have emerged the capitula in the top strata would have been too mature to support a developing larva.

Even if the correlation between *U. quadrifasciata* and seed production would have been higher, *U. quadrifasciata* are not effective energy sinks. Seed reduction occurs only in those capitula directly infested by the fly and does not affect the energy output of the whole plant (Harris 1980). Despite their importance in reducing knapweed seed production, the flies will not, by themselves, manage spotted knapweed (Schirman 1981). The mean infestation level of 0.47 ± 0.02 *U. quadrifasciata* per capitulum in eastern Tennessee (Table 6) is below the determined effective infestation level of 1.25 *U. quadrifasciata* per capitulum (Nowierski et al. 1987). This low infestation level reinforces that *U. quadrifasciata* on its own is currently not an effective control for spotted knapweed in eastern Tennessee. Perhaps in three to six years when *U. quadrifasciata* has been established in eastern Tennessee for a longer period of time it will show a greater impact on the seed production of spotted knapweed as evident from a reduction in stand size. The mean infestation level of *U. quadrifasciata* may also be low in eastern Tennessee because of parasitoids. These solitary parasitoids develop within the immature *U. quadrifasciata*, eventually killing it.

Confidence in the collection methods to obtain these data is high because the sampling size of 200 capitula (four subsamples of four capitula removed from 12 or 13 plants) removed from each of the six sites replicated six times was consistent with the optimal sampling size calculated using Taylor's Power Law Analysis for overall mean gall densities of *U. quadrifasciata* (21.86 capitula per subsample with 15 subsamples per site recommended when taken from five replications of 100 seed heads per site) (Nowierski et al. 1987). These collection methods were also supported by Mays (2003) and Nowierski (1987) who concluded that smaller sample sizes of 100 capitula are adequate and yield statistically similar results for determining if the density of *U. quadrifasciata* is at the effective infestation level of 1.25 *U. quadrifasciata* per capitulum as do sample sizes of 1,000 capitula.

Natural dispersal from the northern United States would seem responsible for the more southern distribution of populations of *U. quadrifasciata* (Wheeler Jr. and Stoops 1996). As of 1994, no *U. quadrifasciata* were established in Virginia, but they dispersed there from Maryland (Mays and Kok 1996). The distribution of *U. quadrifasciata* in eastern Tennessee (higher incidence in the northeastern counties and lower or none in the southeastern counties) can be compared to the initial distribution and establishment throughout Montana. Both distributions resulted in a linear decrease from the north to the south of the state from the origin of first establishment (Story et al. 1987). This north to south distribution in Tennessee provides a model of the dispersion of *U. quadrifasciata* among populations of spotted knapweed along Interstates 81 and 75 in Tennessee. The model can also be used to show the dispersion of *U.*

quadrifasciata from east to west along Interstate 40 in eastern Tennessee. Although *U. quadrifasciata* can be purchased from suppliers of biological control agents, its purchase and release in the eastern United States is highly unlikely because spotted knapweed is not considered an economical issue there (Wheeler Jr. and Stoops 1996).

Emergence. A total of 4,726 capitula was monitored for insect emergence. A total of 818 *U. quadrifasciata* (453 males and 365 females) (ratio 1.19:1) emerged from the capitula of spotted knapweed collected from the six Incidence and Impact Sites (Table 9). Site 5 in Grainger County had the greatest number of *U. quadrifasciata* emerge (n = 216), while Site 3, also in Grainger County, had the lowest number to emerge (n = 59). A total of 32 *U. quadrifasciata* (21 males and 11 females) emerged from the 11 Distribution Sites. It is probable that a greater mean (136.3 *U. quadrifasciata*/site) were collected from the six Incidence and Impact Sites compared to the mean of the 11 Distribution Sites (2.91 *U. quadrifasciata*/site) because they were sampled three times as often as the Distribution Sites and they comprised four times as much area.

Emergence of *U. quadrifasciata* from field-collected samples occurred from April 2003 through January 2004, but was probably increased by a few weeks due to the temperature conditions of the laboratory. Results suggest that *U. quadrifasciata* is well distributed and established in eastern Tennessee (see Chapter II). The data also reinforce the bivoltine life cycle of *U. quadrifasciata* because the tephritids emerged from capitula collected during various times of the year.

Four species of hymenoptera parasitoids (n = 367) were reared from *U. quadrifasciata* collected in the capitula of spotted knapweed from the six Incidence and Impact Sites (Table 10). Three of these parasitoid species [*Pteromalus cardui* (Erdös) (Pteromalidae), *Brasema* sp. (Eupelmidae), and *Eurytoma* sp. (Eurytomidae)] recovered from *U. quadrifasciata* are new records to the state of Tennessee. The parasitoids consisted of 337 *P. cardui* (Fig. 13), 25 *Brasema* sp., and 4 *Eurytoma* sp. One Dryinid species also was recovered, but it typically feeds on the eggs of froghoppers and spittlebugs (Homoptera: Cercopidae), and could not be directly linked to *U. quadrifasciata*. Its head and tibia morphology are unusual and is not a commonly recovered specimen.

Parasitoids reduced the number of *U. quadrifasciata* that emerged from the capitula by 33.5%. The number of parasitoids that emerged from the capitula ranged from 0 to 6 (mean of 0.44 ± 0.27 S. E.). More than one species of parasitoid emerged from one capitulum only 1.6% of the time (n = 1 for *P. cardui* with *Eurytoma* sp. and n = 6 for *P. cardui* with *Brasema* sp.), indicating that if more than one individual parasitoid utilized a capitulum, it was typically

Figure 13. Adult Female *Pteromalus cardui* (Erdös).

Table 9. Number of *Urophora quadrifasciata* (Meigen) that Emerged from Field Collected Capitula of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) at Six Incidence and Impact Sites, 2003-2004.

Site	Male *U. quadrifasciata*	Female *U. quadrifasciata*	Total *U. quadrifasciata* Per Site
1	96	86	182
2	65	53	118
3	33	26	59
4	75	80	155
5	129	87	216
6	55	33	88
Total	453	365	818

Table 10. Distribution of Four Species of Parasitoids that Emerged from Field Collected Capitula of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) from Each of the Six Incidence and Impact Sites in Eastern Tennessee, 2003-2004.

Site	*Pteromalus cardui*	*Brasema* sp.	*Eurytoma* sp.	Dryinidae sp.	Exit Holes[a]	Total Parasitoids
1	45	4	0	0	8	57
2	46	8	0	0	9	63
3	41	2	0	1	2	46
4	68	3	0	0	11	82
5	82	4	4	0	5	95
6	55	4	0	0	11	70
Total	337	25	4	1	46	413

[a]Exit holes were present in the capitula of spotted knapweed when they were field collected.

the same species. This species isolation could be an indication of the behavior of multiple parasitoids seeking out capitula with numerous *U. quadrifasciata*, or individual parasitoids seeking out capitula where other parasitoids of the same species have oviposited. *Pteromalus cardui* and *Brasema* sp. were present at all six sampling locations in Cocke, Grainger, Greene and Hamblen Counties, while *Eurytoma* sp. only emerged from Site 5 in Grainger County (Table 10). The Dryinid species only emerged from Site 3 in Grainger County (Table 10). Exit holes present in the capitula were equated to one parasitoid (the parasitoids have solitary development inside the *U. quadrifasciata*), although not assigned to a species. Exit holes were detected in capitula collected in April 2003, October 2003, and January 2004.

Parasitoids emerged from capitula collected in April 2003 through January 2004. Emergence of the parasitoids in the laboratory peaked in August 2003 (18.8%) and February 2004 (15.3%). However, emergence dates may be a few weeks earlier due to the higher temperature in the laboratory compared to the ambient field temperatures. Oviposition by the parasitoids based upon adult emergence was not significantly influenced by the width or length of the capitulum (Table 11). Because size does not seem to influence parasitoid oviposition of these three hymenoptera, chemical cues produced by *U. quadrifasciata* may be directing the parasitoids, as occurs with other parasitoid-prey relationships (Strand and Obrycki 1996).

A positive direct correlation was found between the number of immature *U. quadrifasciata* present per capitulum and both the number of total parasitoids (0.471) and the number of *P. cardui* (0.508) present in the same capitulum with a significance of $\alpha = 0.05$. These data show that as the number of *U. quadrifasciata* increase in the capitula, so does the number of all parasitoid species. *P. cardui* comprises 82% of all parasitoids, so when separated from the total parasitoids, it also has a positive correlation with an increase in *U. quadrifasciata*. This clustering behavior of the parasitoids would have a negative impact on their effectiveness at directing energy away from other regions of the plant. However, these parasitoids may target the *U. quadrifasciata* after it has already formed a gall, and finished its feeding, therefore not interfering with its productivity. The target stage and direct impact on *U. quadrifasciata* by the parasitoids need to be investigated.

Only nine parasitoids emerged from capitula collected from the 11 Insect Distribution Sites. These parasitoids were found in only two counties in eastern Tennessee, Campbell (n = 2) and Sullivan (n = 7). All of the nine parasitoids that emerged were *P. cardui*. *P. cardui*. may have been the only parasitoid to have emerged from capitula collected at the Insect Distribution Sites because of the density of infestation of *U. quadrifasciata*. It may not have been found at the other Insect Distribution Sites because density of *U. quadrifasciata* may have been too low and therefore of little value reproductively for the hymenoptera to oviposit there.

Table 11. Influence of the Width and Length of the Capitula of Spotted Knapweed (*Centaurea stoebe* L. *s. l.*) on Presence of Parasitic Wasps of *Urophora quadrifasciata*.

	Unstandardized Coefficients		Standardized Coefficients	t	Significance*
	B	Standard Error	Beta		
Length of Capitulum	-0.102	0.054	-0.112	-1.887	0.060
Width of Capitulum	0.043	0.038	0.066	1.110	0.268

*Numbers in the column are significantly different, where α= 0.1.

Experimental confirmation and quantification of the impact of predation on purposely colonized beneficial phytophagous insects is a badly neglected area of biological weed control research (Goeden and Louda 1976). The results presented in this research contribute to the knowledge of the impact of three parasitoid species, in particular *P. cardui* because of its abundance, on *U. quadrifasciata* found within the capitula of spotted knapweed.

According to Hoebeke and Wheeler (1996), even if *Pteromalus elevatus* (Walker), a parasitoid of *U. affinis*, were to become established, it would not significantly increase spotted knapweed populations by reducing the densities of the introduced seed feeders because by themselves they have not been successful in reducing the densities of target knapweeds (Harris and Cranston 1979). However, the impact of the three parasitoids collected from eastern Tennessee has not been studied on biological control organisms of isolated, less dense, patches of spotted knapweed, and may have the potential to significantly decrease the effectiveness of *U. quadrifasciata* and *U. affinis* when used in combination with other biological control organisms, and other IPM tactics.

iv. Summary

Urophora quadrifasciata was found to be well distributed throughout eastern Tennessee, spreading to new populations of spotted knapweed as the latter spread along roadways and disturbed areas. The current density of *U. quadrifasciata* was found to be ineffective to reduce spotted knapweed in eastern Tennessee, as was found in the western United States when not used in a complex of organisms (Harris 1980; Maddox 1982; Story 1989). The density of spotted knapweed is lower and more isolated in Tennessee than the western United States. Because *U. quadrifasciata* is already established in eastern Tennessee and two other biological control organisms (*U. affinis* and *M. paucipunctella*) are established in southwestern Virginia, future control by three organisms may be a management option. Therefore, if biological control insects are to be utilized as a management plan for spotted knapweed, a cumulative stress approach will be needed. Regardless, if a management plan is enacted for spotted knapweed, *U. quadrifasciata* will continue to spread into new areas.

Three species of parasitoids of *U. quadrifasciata* were collected in eastern Tennessee, showing that parasitism is a limiting factor for population increase of the biological control organism. These data oppose the regional findings of Mays and Kok (2003), where no parasitoids of *U. quadrifasciata* were recovered from any collected capitula in southwestern Virginia. These three parasitoids provide many new opportunities for research into their direct effect on *U. quadrifasciata*, their behavior, distribution, and subsequent impact on spotted knapweed.

Future successive collections of *U. quadrifasciata* should be investigated to monitor the continued expansion of its distribution. Future investigations to see if the wet summer of 2003 increased the 2004 spring population of *U. quadrifasciata* because of an increase in available capitula in 2004 as mentioned by Story and others (1992) should be observed.

The data collected from this research aid in the management of spotted knapweed in eastern Tennessee. Because *U. quadrifasciata* has been confirmed in eastern Tennessee other biological control organisms may be considered for introduction into this region to prevent the spread of spotted knapweed. The solitary parasitoids of *U. quadrifasciata* confirmed to be present in eastern Tennessee also provides support for future investigations into the role of parasitoids on biological control organisms.

IV. Conclusions

Spotted knapweed has been present in the eastern United States for more than 100 years (Connecticut, 1902; New Jersey, 1900; New York, 1914; Michigan, 1915; Massachusetts, 1894; Maryland, 1916) (A. Swanson, personal communication). It was identified in established populations in the eastern United States only one year after the first documented population in the western United States (Sheley et al. 1998). However, spotted knapweed in the eastern United States has not shown the same widespread monotypic colonization of pastures and rangelands as in the western United States, but has remained a plant of disturbed areas and roadsides.

Taxonomic and morphological confusion has also contributed to the management problem of spotted knapweed found in the United States. The accepted name of the non-indigenous, invasive plant found in the United States is *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek. A different subspecies, *C. stoebe* L. ssp. *stoebe* is found in the native habitat range from western Europe through eastern Asia. Both of these subspecies have previously been referred to and better known as *C. maculosa* Lam. and *C. biebersteinii* DC. Spotted knapweed has been referred to as *C. stoebe* L. *sensu lato* in this thesis because of the changes with the nomenclature.

Upon initial investigation to the concern of spotted knapweed as an economical problem in pastures in eastern Tennessee, 14 Middle and East Tennessee County Extension Agents were contacted. Only one of the County Agents was even aware of the plant, and all of the Agents responded that spotted knapweed has never been mentioned as a concern by their county residents, even though it has been present here for over 70 years (Wofford 2002). This lack of economical concern reinforces the atypical behavior of spotted knapweed in Tennessee to remain along roadsides and not form monotypic stands, as compared to the western United States. This difference in behavior leads to the possibility that climate or soil type may be influencing the plant, competition with other vegetation may be influencing it, or there may be a genetic component to its non-invasiveness.

The objectives of this research were to:

(1) Determine family composition and seasonality of insects associated with spotted knapweed in eastern Tennessee.

(2) Determine the distribution of *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) on spotted knapweed in eastern Tennessee.

(3) Assess the impact of *U. quadrifasciata* on the production of seeds by spotted knapweed in eastern Tennessee.

(4) Determine the distribution of *Megalanotus sabulicola* (Thompson) (Hemiptera: Lygaeidae) with spotted knapweed in eastern Tennessee.

(5) Identify and determine the effects of hymenopteran parasitoids on *U. quadrifasciata*.

The hypothesis of this research is that biological control organisms will be present on spotted knapweed in eastern Tennessee, reducing the ability of the weed populations to spread. Although biological control organisms were present, their success at managing spotted knapweed was not effective because of their low density offset by the prolific seed production of the weed.

Nearly all 108 families of insects (n = 107) were collected from spotted knapweed during the summer months of June through August when the flowers were in bloom. The only singlet found in May prior to full flower bloom was a Scolytid (Coleoptera). Seasonality of most native insects found to be associated with spotted knapweed is dependent upon the synchronicity of spotted knapweed development. Individual specimens of Cercopidae (Homoptera) were found in great numbers (n = 465) within spittle masses in early plant development and as adults in later plant development. Composition of insects associated with spotted knapweed also was impacted by the developmental stage of the plant. Sites with spotted knapweed plants in bloom had a higher diversity of insect families present than those that did not have plants in bloom.

Numerous insects important for the pollination of spotted knapweed were collected using beat sheet, sweep net, and direct collection. Most of these pollinators have not been described in previous literature as contributing to the successful seed production by spotted knapweed. The data on the pollinators have contributed to the knowledge of insects other than honeybees are assisting the plant to produce seeds.

Biological control insects have been used for the management of spotted knapweed for more than 30 years. *Urophora quadrifasciata* was the only previously released biological control insect recovered from spotted knapweed populations in eastern Tennessee. This report is the first official documentation of *U. quadrifasciata* in eastern Tennessee. *U. quadrifasciata* was well distributed throughout the populations of spotted knapweed. Its impact on reducing seed production by the formation of a gall in the capitula of spotted knapweed was statistically significant, but marginally successful compared to the number of seeds produced by a plant. Even though a 24% seed reduction occurred in the capitula when *U. quadrifasciata* was present, the low density of *U. quadrifasciata*, along with the number of seeds produced by spotted knapweed, contributes to the success of future plants. The reduction in seed production is consistent with Sheley et al. (1998) who reported that biological control insects by themselves were unsuccessful with the reduction of the density of spotted knapweed.

This report is the first official documentation of *M. sabulicola* from eastern Tennessee. *M. sabulicola,* a known seedfeeder on dispersed *Centaurea* spp. seeds, was collected from two research sites, but with numbers too low to be important as a biological control organism. Consequently, the distribution of *M. sabulicola* in eastern Tennessee is low. However, this naturalized species has

been recovered from locations of *Centaurea* sp. populations throughout the southeastern and Mid-Atlantic United States, providing potential sources of the organisms in the future.

Three species of hymenopteran parasitoids (n = 412) of *U. quadrifasciata* were also found well distributed throughout eastern Tennessee. Three of these species [*Pteromalus cardui* (Erdös) (Pteromalidae), *Eurytoma* sp. (Eurytomidae), and *Brasema* sp. (Eupelmidae)] were reared from *U. quadrifasciata* contained in the capitula of spotted knapweed. This report is the first documentation of these three species occurring concurrently on *U. quadrifasciata* and negatively impacting its success as a biological control organism. *P. cardui* was found to be the most numerous (n = 346), and also the most well distributed parasitoid in eastern Tennessee, recovered from six sampling sites. One additional parasitoid was from the family Dryinidae. The dryinid probably emerged from eggs of froghoppers in the family Cercopidae, not from *U. quadrifasciata*, so it is not considered to be a parasitoid of the biological control organism. The parasitoids reduced *U. quadrifasciata* that emerged from the capitula of spotted knapweed by 33.5%.

Future research in this area of the management of spotted knapweed using biological control organisms would include the continued monitoring for other agents in eastern Tennessee and establishing a centralized database to provide a complete listing of biological control agents present in regions of the country (to develop a unity between establishment in the eastern and western United States). Additional research on parasitoid emergence, behavior, oviposition, and development would add much to the limited information currently known about many Chalcidoidea. Emphasis should also be placed on preventing the spread of spotted knapweed, since prevention is the least expensive method of management. Finally, a paradigm shift will be needed to approach management of spotted knapweed from the perception of what is the desired plant community in the dynamic system.

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Vita

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