AN ASSESSMENT OF THE INVASIVE POISON HEMLOCK AND ITS INSECT ASSOCIATES IN KENTUCKY

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AN ASSESSMENT OF THE INVASIVE POISON HEMLOCK AND ITS INSECT ASSOCIATES IN KENTUCKY

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture at the University of Kentucky

By
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Lexington, Kentucky

Director: Dr. James D. Harwood, Professor of Entomology
Lexington, Kentucky
2013

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AN ASSESSMENT OF THE INVASIVE POISON HEMLOCK AND ITS INSECT ASSOCIATES IN KENTUCKY

Poison hemlock, *Conium maculatum* (Apiaceae), is an invasive plant in North America with a unique toxic chemistry. Previous research on this plant has focused on identifying herbivores as potential biological control agents or describing the toxic plant alkaloids. However, none have examined the role of higher trophic levels in the food web surrounding poison hemlock. Generalist predators and food web interactions are an important component of studies investigating invasion effects, as plant or animal introductions can alter ecosystem functioning. In this study, predators in poison hemlock were sampled at the foliar and epigeal levels, resulting in 956 Carabidae and 321 Coccinellidae being collected. Predator connectedness to plant resources was quantified using molecular gut-content and chemical analyses. Foliar *Harmonia axyridis* (Coccinellidae) contained aphid DNA and plant chemicals, while *Harpalus pensylvanicus* (Carabidae) only contained alkaloids, suggesting that the ground predators were obtaining plant chemicals via alternative prey. Feeding trials between *H. axyridis* and their potentially toxic prey, *Hyadaphis foeniculi* (Aphididae), revealed that the exotic predator shows faster development when consuming aphids from poison hemlock compared to alternative diets. This study reveals that three Eurasian species may be facilitating one another, illustrating the importance of continued examination of invasive species interactions.

Keywords: Invasive species, Poison hemlock, Generalist predators, PCR, GC-MS

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March 29, 2013
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DEDICATIONS

I would like to dedicate this work to Dr. Edward Weiss and Dr. Michael Meyer of Christopher Newport University. Their instruction permanently changed my view of the world around me by introducing me to science in a way that I could appreciate and understand. Also, I dedicate this thesis to my family, for their unwavering love and support throughout my adventures.
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Chapter One: Introduction and Literature Review

1.1 General Introduction

Globalization has led to a world where borders no longer prevent the spread of alien species into new regions. While biological invasion is not a new phenomenon, the frequency at which it now occurs is following a steady upward trend linked to increased commerce and trade (Wells et al. 1986; di Castri 1989; Hulme 2009). Many invasions are accidental; however, some are the result of deliberate introductions. Ornamental plants such as the exotic saltcedar (*Tamarisk* spp. L. (Tamaricaceae)) and the tree of heaven (*Ailanthus altissima* (Mill.) Swingle (Simaroubaceae)) are now considered invasive (Ding et al. 2006; Swearingen and Pannill 2009; Zavaleta 2000). Saltcedar invasion costs the United States $121 to 291 million annually (Zavaleta 2000) and the tree of heaven is able to inhibit the growth of nearby native plants through the production of allelochemicals (Swearingen and Pannill 2009). Furthermore, the introduction of biological control agents has provided another source of invasive species, such as the multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). This generalist predator was originally introduced to the United States to control pest aphid populations, but has since become a nuisance in urban settings and outcompetes or consumes native lady beetle species (Ware and Majerus 2008; Brown et al. 2011). While the growing population of *H. axyridis* helps to suppress certain agricultural pests, and the sale of ornamental plants is beneficial for the landscaping industry, introduced organisms have the potential to strongly impact native communities (reviewed in Ehrenfeld 2010). Introductions have resulted in invasive species that negatively affect biodiversity (Dukes and Mooney 1999; Manchester and Bullock 2000), ecosystem functioning, and even atmospheric conditions (Dukes and Mooney 1999; Tilman 1999; reviewed in Hooper et al. 2005). These negative impacts ultimately lead to changed communities that require examination and re-characterization.

The Eurasian plant *Conium maculatum* L. (Apiales: Apiaceae), also called poison hemlock, has successfully invaded many regions around the globe, including North and South America (Parsons 1976; Holm et al. 1979, 1997). Similar to the saltcedar and the
tree of heaven, poison hemlock was once considered ornamental (Pokorny and Sheley 2000) but has since become problematic due to its invasive and toxic properties. The source of poison hemlock’s toxicity comes from its piperidine alkaloids, conine, γ-coniceine, conhydrinone, and conmaculatin, all of which have been characterized in previous studies as highly toxic to vertebrates and potentially insecticidal (Sperry et al. 1964; Berenbaum 1981; Widner 1984; Panter et al. 1988, 1989; Vetter 2004; Castells et al. 2005; Castells and Berenbaum 2006, 2008b; Radulovic et al. 2012). Community studies in poison hemlock have described a depauperate insect fauna surrounding the plant (Goeden and Ricker 1982) and arthropod assemblages have typically been classified to taxonomic Order as opposed to species-level identification (Fork 2010). However much remains unknown about the community interactions occurring around the plant and its alkaloids. The movement of plant chemicals, predator-prey relationships, and higher taxonomic resolution are all necessary for an accurate description of poison hemlock and its impact on native communities.

In the current study, feeding activities of generalist predators foraging in poison hemlock were revealed using a combination of molecular and chemical gut-content analysis techniques. DNA-based post-mortem gut-content analysis has the power to accurately detect which prey species generalist predators are consuming under field conditions through the use of species-specific primers (Harwood et al. 2007; reviewed in Harwood and Greenstone 2008; Harwood et al. 2009b; reviewed in Weber and Lundgren 2009; King et al. 2010; Eitzinger et al. 2013). Additionally, gas chromatography – mass spectrometry, a chemical analysis technique used for identifying compounds in complex mixtures (Hites et al. 1968; Blechschmidt et al. 1984; Stermitz et al. 1994; Castells et al. 2005; Boumba et al. 2006; Konoz et al. 2013) was applied to generalist predator gut content to quantify how the unique alkaloids produced by poison hemlock move through the food web. Foliar and ground level (or epigeal) sampling followed by family-level identification provided further insight into the current interactions occurring between poison hemlock and the arthropods in central Kentucky. As a final measure of the implications of poison hemlock invasion, feeding assays revealed the developmental benefits for some predators foraging in this community.
1.2 Invasive Species

In his seminal book, *The Ecology of Invasions by Animals and Plants*, author Charles Elton (1958) warned fellow ecologists that we are all witnessing history with the alarmingly high rate of ‘ecological explosions’ taking place. He used this term to characterize the “enormous increase in numbers of some kind of living organism […] bursting out from control of forces that were previously held in restraint by other forces.” Elton’s prescient book raised the red flag for biological invasions and the potential negative impacts that may follow, such as a loss of biodiversity and the initiation of harmful interactions. As predicted, global trade and deliberate species introductions have revealed a parallel increase in biological invasions and endangered or extinct organisms (van Wilgen et al. 1996; Rhymer and Simberloff 1996; Mooney & Cleland 2001). The process begins with the often anthropogenic dispersal of an organism, followed by its establishment and spread. Once an exotic species spreads out of control, native communities can be negatively impacted as energy flow and niches are shifted from their original state. When the consequences of these introductions outweigh the positive attributes of the organism, the species is termed as invasive (Mack et al. 2000; Sakai et al. 2001).

Notably, only a fraction of exotic species survive long enough to establish and proliferate, and even fewer become invasive (Williamson and Fitter 1996). It is also common for an organism to be established in a new region for an extended period of time prior to becoming invasive. This period, known as lag time, will sometimes end when environmental conditions shift in favor of the intruder (Sakai et al. 2001). What often tips the scale to this end is a phenomenon known as propagule pressure, or repeated introductions of the organism. The sum of several small introductions amounts to one larger introduction, thus resulting in successful establishment and spread (Lockwood et al. 2005). Free from the natural checks and balances provided in its native range, prolific species have the ability to change communities at genetic, landscape, and environmental levels (Mooney and Cleland 2001).

Human-induced species dispersal and climate change will continue to impact the global landscape and put natural communities at greater risk for invasion. Novel
interactions and altered ecosystems will be the ultimate result of this, making it imperative for the scientific community to continue describing the modes by which invaders are altering ecosystem functioning. For example, plant chemistry is a known driver of plant-insect interactions and has the capacity to exert significant pressure on the behavior of native species (Harvey and Fortuna 2012). Some introduced plants will act as a resource for other introduced arthropods, thereby facilitating them in their success (Crosby 1986; Simberloff and Von Holle 1999). Poison hemlock and its unique chemistry may provide similar examples of novel interactions occurring in the field, making it a candidate for further investigation.

1.2.1 Invasive Plants

Approximately 84% of invasive macroorganisms in the Unites States are plants (Pimentel et al. 2005). This has been attributed to the use of seeds for reproduction and dispersal (Higgins et al. 1996, 1999; Cain et al. 2000). Nutrients and physical protection extend the duration and viability of seeds prior to germination, as opposed to invasive animals that typically need to reproduce soon after introduction in order to become established. The seed’s extended lag phase could also translate into the introduction of more propagules over time (Rouget and Richardson 2003). New invaders will sometimes reduce their seed size to produce more seeds for greater dispersal range (Rejmanek & Richards 1996; Lockwood et al. 2005) and may also grow taller in areas of introduction (Crawley 1987). These trends follow the enemy-release hypothesis (Keane and Crawley 2002), stating that introduced organisms are released from their natural enemies and can reallocate their energy to other processes such as reproduction, thereby making them more competitive than their native counterparts.

Another contributing factor to successful plant invasion is their novel chemistry in areas of introduction. Allelopathic chemicals, or those that inhibit the growth of neighboring plants, provide outstanding benefits to introduced plants by killing off local vegetation (Bais et al. 2003). Plants may produce distasteful compounds as well, preventing them being consumed by local herbivores. These traits are best described by the novel weapons hypothesis (Callaway and Ridenhour 2004), or the phenomenon that
novel biochemical weapons are unusually powerful against native species. For example, poison hemlock displays a unique chemistry that has been described as insecticidal to indigenous herbivore species in the United States (Berenbaum 1981).

In addition to enemy release and the use of novel weapons, weeds require minimal resources for success in disturbed landscapes, such as those allocated for agriculture. Agricultural weeds, including poison hemlock, are costing the U.S. an estimated $27 billion annually (Pimentel et al. 2005). Furthermore, introduced plants may also hybridize with native species, creating super-weeds that have the ability to overtake large land areas, reduce genetic variability, and displace native species (Ellstrand and Schierenbeck 2000). Due to the economic and biological consequences of invasive weeds, such as loss of agricultural land, production of toxic chemicals, and displacement of native species, there is great need for continued study on how these traits affect invaded communities (Brown et al. 2002; Davis et al. 2006).

1.3 Poison hemlock

After its introduction to the United States from Eurasia in the 1800s as an ornamental plant, poison hemlock became less appreciated for its lacey umbels and more disliked by farmers for its noxious compounds and foul smell (Pokorny and Sheley 2000; Castells and Berenbaum 2006). Now the plant is considered invasive across the North American continent, Australasia and South America (Parsons 1976; Holm et al. 1979, 1997) and is listed as one of several noxious weeds that contributed to $2 billion dollars in annual U.S. rangeland impacts (Bovey 1987). These impacts include cattle abortions from poisoning and reduced grazing capacity (DiTomaso 2000). It comes as no surprise that insect herbivores have avoided the plant as reported in previous studies (Berenbaum 1981; Goeden and Ricker 1982). However, as poison hemlock becomes more naturalized in areas of introduction, generalist species that have adjusted to its defensive compounds may now be utilizing the plant. High impact invasive species must continually be reevaluated to assess changes in ecology of the plant and the new potential impacts on the community that may follow.
1.3.1 The Biology of Poison Hemlock

Poison hemlock grows biennially or as a monocarpic perennial dependent upon environmental conditions (Baskin & Baskin 1989; Pokorny & Sheley 2000). Although it can germinate during any season (Baskin and Baskin 1990), early spring is the most typical period for germination in Kentucky, revealing bright green pinnately compound leaves (Fig. 1.1). Notable physical features of the plant include a smooth, hollow stem supported by a large, white tap root resembling a carrot, and its distinct purple spotting along the stems set it apart from other plants. At the end of its life cycle, poison hemlock experiences significant growth and will reach two or more meters in height within a few months (Fig.1.2). This period, known as ‘bolting’, is followed by flowering which typically occurs in June. When in flower, the compound umbellate heads will open up to reveal an inflorescence consisting of many small white flowers (Fig. 1.3). After bolting and inflorescence exposure, the flowers will reduce to seeds that drop from the plant in late July or August (Fig. 1.4) (Baskin & Baskin 1989; C.Allen personal observation).

Figure 1.1 Poison hemlock germinating (September 28, 2011) (left) along an alfalfa field at Spindletop Research Farm, Fayette County, Kentucky (USA) (GPS coordinates 38°07N, 84°30W) and (right) in greenhouse waste (March 15, 2010), Fayette County, Kentucky (USA).
Figure 1.2 Poison hemlock invasion (May 9, 2012) at Spindletop Research Farm, Fayette County, Kentucky (USA) (GPS coordinates 38°07N, 84°30W).

Figure 1.3 Inflorescence (left) and *Chaulognathus marginatus* F. (Coleoptera: Cantharidae) in flowering umbellate heads (right) of poison hemlock (June 6, 2011) on Spindletop Research Farm, Fayette County, Kentucky (USA) (GPS coordinates 38°07N, 84°30W).
In its native range, it is restricted to wet soils running alongside riverbanks and streambeds (Pursh 1979; Pokorny and Sheley 2000); in regions of introduction, however, it will grow almost anywhere, preferring abandoned agricultural fields, unmown field margins, forest edges, roadsides, and floodplains (Pursh 1979; Goeden and Ricker 1982). The plant may also serve as a reservoir for a variety of plant viruses including the carrot thin leaf virus and celery mosaic virus (Sutabutra and Campbell 1971; Gracia and Feldman 1977; Howell and Mink 1977). As seeds are able to germinate during every season of the year and mature readily under poor soil conditions, poison hemlock is a prominent candidate for invading new regions.

1.3.2 The Chemistry of Poison Hemlock

Aside from its ability to invade non-indigenous landscapes, poison hemlock is toxic when consumed. Possibly the most well known fact about the plant is its legendary use in the trial against the Greek philosopher, Socrates, who was found guilty for corrupting the minds of youth and “not believing in the gods of the state”. He was sentenced to death by drinking a poison hemlock mixture (Hardin and Arena 1974). In the modern world, poison hemlock contributes to $100 million in annual livestock losses
from consumption of these plants (Vetter 2004). This toxicity is attributable to a series of piperidine alkaloids that are distributed throughout all aerial parts of the plant (Panter & Keeler 1989; Castells et al. 2005). It produces many secondary compounds, of which coniine and its related alkaloids, $\gamma$-coniceine, conhydrinone and conmaculatin are most abundant (Fig. 1.5) (Fairbairn 1971; Castells et al. 2005; Radulovic et al. 2012). Of all aerial parts, the reproductive tissues and seeds exhibit the highest alkaloid concentrations relative to the plant’s vegetative parts (Cromwell 1956; Khodzhimatov and Bobokhodzhaeva 1976). Although the plant exhibits an extensive chemistry, including the production of furanocoumarins (Berenbaum 1981), and steroids (Radulovic and Dordevic 2011) it is these few alkaloid compounds that are the source of the plant’s neurotoxicity, fatal to small children and poisonous to cattle and swine (Sperry et al. 1964; Widner 1984; Panter et al. 1988, 1989; Vetter 2004). Some have speculated if the unique plant chemistry has insecticidal properties (Berenbaum 1981). The broad toxicity and lack of understanding of the trophic consequences these compounds may have in invaded communities makes it necessary to characterize the mechanisms involved in alkaloid transfer through the food chain.

![Image of piperidine alkaloids](image)

Figure 1.5 Active piperidine alkaloids of *Conium maculatum* L. (Apiales: Apiaceae): (from left) coniine, $\gamma$-coniceine, conhydrinone and conmaculatin.

### 1.4 Plant-Insect Interactions

Plants and insects have been forming coevolved relationships for as long as the two have existed together. These interactions have been extensively described (Verschaffelt 1910; Dethier 1941; Fraenkel 1959; Ehrlich and Raven 1964); however, current investigations continue to uncover evidence for the basis on which plant-insect interactions were once forged (Gardner and Agrawal 2002; reviewed in Gatehouse 2002; Desurmont and Weston 2011). Despite the ongoing debate as to whether it is the insects
or the plants that drive this coevolution, most scientists will agree that the two groups are interdependent (Crawley 1989; Fine et al. 2004). Herbivorous insects rely on plants as food material, often utilizing host-recognition cues from their plant-food of choice. In response to damaging herbivory, plants may develop methods to deter herbivores, whether it is through the production of toxic compounds or the growth of unpalatable structures. Likewise, the insects may further respond to these defenses by evolving such that they may overcome novel plant defenses. Herein lays the basis for the coevolutionary arms-race that occurs between plants and insects (Ehrlich and Raven 1964). The categories that will be further discussed in detail are host-plant recognition and selection, detoxification, sequestration, and tri-trophic interactions. What these all have in common is the use of plant secondary chemicals as a form of recognition and association between the two groups of organisms, creating complex interactions that often result in life history effects (Feeny 1976; Berenbaum 1981). Poison hemlock, with its array of alkaloid compounds, is no exception, and should be studied further to understand how the plant chemistry translates through the food web into insect consumers.

1.4.1 Host-Plant Selection

Host-plant selection is a critical process for all specialized plant-feeding insects. Finding the right host optimizes oviposition and therefore increases the survival of offspring (Myers 1985; Honda 1995; Jallow et al. 1999; Witzgall et al. 2005) and may provide refuge from predators for multiple instars. For example, leaf-rolling behaviors can protect Lepitoptera larvae from birds (Murakami 1999) and gall-forming insects such as *Pemphigus betae* Doane (Hemiptera: Aphididae: Eriosomatinae) (Whithman 1978) and *Euura lasiolepis* Smith (Hymenoptera: Tenthredinidae) (Craig et al. 1989) will oviposit into the most vigorous plant hosts to benefit their offspring. Mate-finding strategies are also sometimes dependent on host-plant location, as in the case of the male cabbage looper moth, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Males are not only attracted to female sex pheromones but are also drawn to the unique blend of plant volatiles produced by their shared host-plant (Landolt and Heath 1990). From these examples and many others not described here, volatile compounds and plant chemistry
are powerful indicators to insects that give information about the food quality, fitness, and potential for finding a mate.

This method of location used by insects may be highly detrimental to crops, as many insects have acquired pest status through a chemically-mediated attraction to plants of economic importance (Matsuda 1988). For example, the crucifer flea beetle, *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae), is drawn to the glucosinolates produced by cruciferous plants and this association will attract the beetle to high-value crops such as broccoli and collards that exhibit similar plant chemistry (Burgess 1977; Nielsen 1988; Henderson et al. 2004). Many lepidopteran pests, such as the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), are influenced heavily by plant chemistry. Larvae of this pest will feed upon solanaceous plants such as potatoes or tomatoes, thus allowing for major defoliation of these crops (del Campo et al. 2001). Herbivores will also shift from one host plant to another based on similar plant chemistry (Janz et al. 2001). It is therefore possible that native species feeding upon alkaloid-producing plants may shift to poison hemlock, illustrating once more the importance of this study and the movement of invasive plant chemicals through the food web.

**1.4.2 Sequestration of Plant Secondary Compounds**

Plant compounds, such as the alkaloids produced by poison hemlock, may be harmful to herbivores. Physiological adaptations allow some herbivores to overcome the harmful effects of defensive compounds via sequestration. By sequestering plant chemicals away from vital body parts, insects can exploit plant defenses as their own for deterring predators and parasitoids. For example, several genera from the beetle family Chrysomelidae will sequester unpalatable host-plant compounds into their elytra or gut compartments, discouraging predators from consuming them (Denno et al. 1990; Ehmke et al. 1991; Dobler et al. 1996; Tallamy et al. 2005; Burse et al. 2009). The monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), feeds on milkweed and sequesters cardenolides, making them unpalatable to predators (Brower and Moffitt 1974). Some Lycaenidae and other Nymphalidae (Lepidoptera) are also capable of
exploiting plant secondary compounds for their own defense against generalist predators and parasitoids (Fiedler 1996; Smilanich et al. 2009). Certain species of Romaleinae (Orthoptera: Romaleidae) will sequester alkaloids by feeding on chemical-producing plants. The sequestered alkaloids will doubly benefit the grasshoppers by first protecting them from predators and then aiding in the synthesis of aggregation pheromones (Whitman 1988; Bernays and Chapman 2000).

With so many mechanisms of host-plant exploitation by herbivores, plants have developed their own arsenal of weapons. The release of unique volatile compounds will attract the predators and parasitoids of the herbivores feeding upon the plants that are being consumed and exploited (Turlings et al. 1990; Gols et al. 1999; De Moraes et al. 2001). Similar to the plants listed in these examples, poison hemlock is typically distasteful to most herbivores. Insects that adapt to utilize it may eventually evolve the capacity to exploit its toxic alkaloids. Plant chemicals are a key component in the coevolutionary arms-race, and poison hemlock may eventually show evidence for similar interactions in regions of invasion.

1.4.3 Detoxification and Excretion

Not all insects are able to use plant secondary compounds to their advantage. In many cases, herbivores must adapt to their host plants by developing physiological strategies to detoxify their food and excrete harmful chemicals for minimizing internal damage (Bernays 1981; Chapman 1988; Bernays and Chapman 2000). Enzymatic detoxification and metabolic conversion of toxic plant compounds is a well-studied phenomenon in insects (Krieger et al. 1971; Brattsen et al. 1977; Ivie et al. 1983; Brattsen 1988). In many systems, it is important for the herbivore to detect unpalatable or toxic material to trigger a reduced feeding rate for proper detoxification of the food. This compensatory feeding accommodates enzymatic breakdown of consumed toxins (Zangerl and Berenbaum 1993). However, the slow growth / higher mortality hypothesis (Clancy and Price 1987) explains why this could prove fatal for developing herbivores. The hypothesis states that, while slower feeding may be physiologically beneficial, a reduced feeding rate that leaves insects immature for longer periods could also leave them more
vulnerable to predation or parasitism (Moran and Hamilton 1980; Grossmueller and Lederhouse 1985; Clancy and Price 1987; Benrey and Denno 1997). For example, the leaf beetle *Galerucella lineola* F. (Coleoptera: Chrysomelidae) experiences slower development in concert with higher predation rates when feeding on a low quality willow host while those developing on high quality food experience faster development and less predation (Haggstrom and Larsson 1995).

Although this process does not follow the same mechanisms as chemical sequestration, plant secondary chemicals are still present in the digestive tract of the herbivore for a period of time. The presence of these chemicals may therefore affect predators and parasitoids of the herbivores. For example, the parasitic wasp *Hyposoter exiguae* (Viereck) (Hymenoptera: Ichneumonidae) experiences negative effects, such as reduced adult size, from ingested alkaloids within the gut of its caterpillar host, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Campbell and Duffey 1979). Likewise, Vanhaelen et al. (2002) found that development and survival rates decreased when the aphidohagous hoverfly, *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae), was consuming aphids reared on their glucosinate-producing host-plants. The hoverfly also exhibited a marked decrease in fecundity after consuming their toxic prey. Similarly, generalist ladybird beetles (Coleoptera: Coccinellidae) may show compromised fitness, such as decreased adult size or slower development rates, by consuming aphids that are feeding upon toxic host plants (Francis et al. 2000). In the poison hemlock system, English-Loeb et al. (1993) found that the tachinid parasitoid (*Thelairia bryantii* Curran, Diptera: Tachinidae) of the generalist herbivore caterpillar (*Platyprepia virginalis* (Bvd.) (Lepidoptera: Arctiidae) had a lower rate of emergence when their host was feeding on poison hemlock as opposed to bush lupine. The ubiquity of these multi-trophic chemical interactions make it necessary to continue studying plant secondary chemistry to understand the consequences of such relationships in nature.
1.5 Associates of Poison Hemlock

The invasive status of poison hemlock has made it the subject of several studies for the identification of a biological control agent (Berenbaum and Harrison 1994; Castells and Berenbaum 2006, 2008a,b). However, the plant was a documented invader many years prior to these attempts for control and was described as “an alien weed attacked by few insects” (Goeden and Ricker 1982). This observation comes as no surprise considering the insecticidal properties exhibited by poison hemlock’s suite of chemicals. Plant toxicity dictates the necessity of a well-adapted herbivore to overcome plant chemical defenses. As a result, only two consistent insect associates in the U.S. have the ability to overcome poison hemlock’s toxins: an aphid and a moth.

Colonies of the honeysuckle or parsnip aphid, *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae), are typically found in the flowering umbels of poison hemlock from late May through June (C. Allen, personal observation). Similarly, the defoliating hemlock moth, *Agonopterix alstroemeriana* Clerck (Lepidoptera: Oecophoridae), also inhabits the plant in the springtime as a leaf-rolling caterpillar (Berenbaum and Harrison 1994, Castells et al. 2005, Castells and Berenbaum 2006, 2008a,b). Both herbivores are native to their host plant’s indigenous region of Eurasia, and while the presence of each has been recorded in the United States, only *H. foeniculi* has been observed in high numbers in Kentucky during this study (C. Allen, personal observation). For this reason, only *H. foeniculi* will be highlighted further as the predominant herbivore within this system.

It is true that poison hemlock harbors very few insects. However, Goeden and Ricker (1982) did not examine the possibility of *H. foeniculi* serving as an attractant for foraging predators. *Hyadaphis foeniculi* are adapted to consuming this unpalatable plant resource, and are thus able to reach high population densities. It is therefore unsurprising that predatory species, namely Coccinellidae (Coleoptera), would take advantage of these aphids when they are readily available. Other generalist predators, such as Carabidae (Coleoptera), are found at the ground level of poison hemlock stands, and may be capitalizing on plant resources and insect associates via epigeal foraging. The current study therefore investigates the role of poison hemlock as a food resource for generalist...
predators, and thus more thoroughly characterizes the food web connections in the system.

1.5.1 *Hyadaphis foeniculi*

*Hyadaphis foeniculi* is native to Eurasia and formed a relationship with poison hemlock upon its introduction into the United States in the early 1900s (Fig. 1.6) (Goeden and Ricker 1982; Voegtlin 1984). It appears pale green and will readily inhabit poison hemlock as a suitable summer host (Fig. 1.6 and 1.7) (Hedin and Phillips 1991). This cosmopolitan species has been implicated as a vector for *Carrot virus Y* (*Poyviridae: Potyvirus*) (Jones et al. 2006) and *Nucleorhabdovirus* spp., or the coriander feathery red-vein virus (*Rhabdoviridae*), a pathogen infecting coriander, celery, parsley, carrots, and others (Misari and Sylvester 1983). *Hyadaphis foeniculi* will also inhabit other apiaceous plants as well as invasive honeysuckle species (*Lonicera* spp., Dipsacales: Caprifoliaceae) (Mack 2003). While it currently remains a non-pest herbivore, having multiple invasive host plants has the potential to increase the aphid’s range, providing an opportunity for spread.

![Figure 1.6 Hyadaphis foeniculi (Passerini) (Hemiptera: Aphididae) feeding upon poison hemlock umbels (May 9, 2012) at Spindletop Research Farm, Fayette County, Kentucky (USA) (GPS coordinates 38°07N, 84°30W).](image)
Figure 1.7 *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae) on *Angelica archangelica* (Apiaceae), Montreal Botanical Garden, Montreal (Canada) (GPS coordinates 45°33’19.06N, 73°33’18.34W), Claude Pilon©, (Favret and Miller 2012).

Redescribed in 1984, *H. foeniculi* was originally identified as *Hyadaphis tataricae* (Ajzenberg), the monophagous honeysuckle aphid, but has since been separated into its own species. In contrast to *H. tataricae*, *H. foeniculi* is oligophagous and will inhabit alternate hosts, such as the aforementioned agricultural commodities, poison hemlock, and honeysuckle species. Although *H. foeniculi* will feed on honeysuckle, *H. tataricae* is the source of damage on the plant, such as the formation of ‘witch’s brooms’ that leave the vegetation shriveled and dry. Colonies of these two aphids may be found together on honeysuckle and are difficult to distinguish until they reach full maturity (Goeden and Ricker 1982; Voegtlin 1984).

Although aphids were once described as a defenseless group of soft-bodied organisms (Imms 1947), they have evolved some surprising defense mechanisms against predators, most notably their dropping behaviors. These behaviors are employed when an aphid is disturbed or attacked by a predator. Once threatened, the release of an alarm pheromone, such as (E)-β-farnesene, signals to other conspecific aphids that danger is near (Montgomery and Nault 1977; Dixon 1985). *Hyadaphis tataricae*, a close relative of *H. foeniculi*, is known to use this compound as a warning to other aphids on the same or nearby host plants (Hedin and Phillips 1991). Therefore, it is likely that when threatened, *H. foeniculi* also produces alarm pheromone and drops off its host plant. While aphids may escape immediate danger by dropping off the host plant, this behavior comes with a series of costs, such as desiccation, loss of food and refuge habitat, and vulnerability to
ground-dwelling predators (Loughridge and Luff 1983; Dill et al. 1990; Winder 1990; Losey and Denno 1998). Ground-dwelling predators foraging in poison hemlock could therefore be preying upon *H. foeniculi*, illustrating the importance of characterizing epigeal communities as well as foliar communities in areas of invasion.

1.5.2 *Coccinellidae*

*Coccinellidae* (Coleoptera), or lady bird beetles, are multivoltine predators known for their ability to control large aphid pest populations (reviewed in Hagen 1962; Obrycki and Kring 1998). For example, species such as *Coleomegilla maculata* (DeGeer), *Hippodamia convergens* (Guerin), and *Coccinella septempunctata* L. will reduce disease-vectoring or harmful aphid species in various crops such as sugar beet, grain sorghum, wheat, and potatoes (Kring et al. 1985; Landis and van der Werf 1997; Obrycki et al. 1998; Michels et al. 2001; Volkl et al. 2007). The last species, *C. septempunctata*, as well as *H. axyridis*, are two examples of lady beetle species introduced to the United States from Eurasia (Hodek and Honek 1996; Brown et al. 2011) that have been employed in biological control programs (Obrycki et al. 1998; Schmidt et al. 2008). A synchronous relationship between *Coccinellidae* and prey groups such as mealybugs or sedentary scale insects has been observed. This relationship is less pronounced with aphid prey but coccinellid abundance and fecundity are nevertheless significantly correlated with large aphid populations (reviewed in Hagen 1962). Although *Coccinellidae* show a great affinity for aphids and other prey taxa, including individuals within their own species, these generalists are not exclusively predatory and will forage for non-prey food such as pollen, extra-floral nectar, and plant material (reviewed in Hagen 1962; Hodek and Honek 1996; Moser et al. 2008). Poison hemlock has the potential to provide all of the aforementioned food resources for foraging *Coccinellidae* (Fig. 1.8), and should thus be studied for such interactions occurring between introduced organisms.
1.5.3 Carabidae

Carabidae (Coleoptera) follow a mono- or bivoltine life cycle (Matalin 2007), and several species play an important role in epigeal predation as certain Coccinellidae fill this niche at the foliar level. In fact, the presence of these two beetle groups may be complementary, as foraging by Coccinellidae facilitates increased dropping behavior of aphids, making them available to Carabidae. The synchrony between these two beetle groups can lead to increased suppression of aphid pests, such as the pea aphid (*Acyrthosiphon pisum* Harris, Hemiptera: Aphididae) (Losey and Denno 1998). Disease-vectoring Russian wheat aphids, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae) showed up to 69.5% falling rates in Colorado wheat (Kerzienik et al. 2010) revealing a great potential for aphid predation among Carabidae (Losey and Denno 1998). However, many carabids are not limited to aphids as prey and will consume a wide variety of species, such as earthworms, mollusks, and other arthropods (Sunderland 1975; Stork 1990; Symondson et al. 2000; Holland 2002; Symondson et al. 2002a; reviewed in
Symondson et al. 2002b). Furthermore, some Carabidae within the Harpalini and Zabrini tribes have a strong affinity for plant seeds and are considered important contributors to biological control of weeds (Zhavoronkova 1969; Brandmayr 1990; Jorgensen 1997; Honek et al. 2003; Lundgren et al. 2006; Lundgren and Lehman 2010). Despite the high toxicity of poison hemlock seeds (Cromwell 1956), some carabid species will consume them (C. Allen, personal observation), revealing another avenue of chemical transfer and resource utilization in this invasive community.

1.6 Implications of Poison Hemlock Invasion

Disturbed agricultural landscapes are prone to exotic species invasion and cost the United States approximately $24 billion in crop losses, annually (Pimentel et al. 2005). As a contributor to this cost, poison hemlock readily invades commodities such as alfalfa, pasturelands, and hayfields (Montegut and Jauzein 1984; Jeffrey and Robinson 1990; DiTomaso 2000; Green 2011), making it particularly dangerous to foraging livestock. All vertebrates, namely horses, cattle, and swine, are susceptible to poison hemlock-induced toxicosis. Whether ingestion is accidental in the field or through the consumption of previously contaminated feed products, the alkaloids in poison hemlock have extremely harmful effects (Panter et al. 1988; Jeffrey and Robinson 1990). Symptoms of ingestion include hard breathing, muscle spasms, ataxia, arthrogypsis, still-born calves, and potential death (Panter et al. 1988; Vetter 2004). Poison hemlock contributes to the $100 million estimated cost of annual plant-poisoned livestock in the United States (Nielson and James 1985; James et al. 1992) and the most recent Agricultural Census of 2007 placed Kentucky as the second largest producer of horses and ponies (USDA census 2007). Furthermore, poison hemlock has been identified as a reservoir species for the bacterium causing Pierce’s disease in certain grape cultivars. Xylella fastidiosa Wells (Xanthomonadiales: Xanthomonadaceae) is abundant in the poison hemlock growing near streambeds that run adjacent to Napa Valley grape fields (Raju et al. 1980). The plant also acts as a host for several viruses, including the carrot thin leaf virus and celery mosaic virus, both transmissible between the infected weeds and healthy agricultural plants via phytophagous aphids in agricultural settings (Sutabutra and Campbell 1971;

In addition to the monetary impacts of poison hemlock, its unique chemistry and ability to colonize new regions may have ecosystem-level consequences. Much remains unknown about how this plant interacts with native communities, but plant secondary compounds are known drivers of plant-insect relationships (Feeny 1976; Berenbaum 1981) and the same may be true for this system. For example, some aphids have the ability to utilize plant-produced alkaloids as protection against generalist predators (Wink and Witte 1991). However, generalist predators and herbivores have evolved their own mechanisms for overcoming these defenses, thereby allowing them to utilize toxic resources (Ehrlich and Raven 1964; Brower and Moffitt 1974; English-Loeb et al. 1993; Burse et al. 2009). Now that poison hemlock has been a part of the North American landscape for more than a century, these potential relationships can be uncovered through the combined use of chemical and molecular techniques. The movement of piperidine alkaloids through the food web and the identification of interacting species within this system will provide a new perspective on poison hemlock, and moreover, a better understanding of how invasive plant species affect the communities they inhabit.
1.7 Research Objectives

The principal objectives of this research are as follows:

1. Characterize the predator-prey community in poison hemlock infested areas, with an emphasis on generalist Coleoptera.
2. Quantify food web linkages and the transfer of plant alkaloids to higher trophic levels in poison hemlock habitats.
3. Examine the quality of *Hyadaphis foeniculi* as prey for developing predators.
Chapter 2: Poison hemlock in central Kentucky—an alien weed, its resources, and the potential for interactions with higher trophic-level organisms

2.1 Summary

Past research has characterized poison hemlock as an invasive and highly toxic plant that hosts very few herbivores, both generalist and specialized. The investigation of organisms at higher trophic levels, such as generalist predators, has been neglected. However, generalist predators play an important role in the structure and functioning of ecosystems, and are also known to utilize alternative food resources for a balanced diet, such as seeds, pollen, and vegetative material. This group of organisms is therefore important to include when describing invasive systems such as poison hemlock that have the power to change the biodiversity and ecosystem functioning in regions of invasion. Despite a depauperate insect fauna, poison hemlock is an alternate host for *Hyadphis foeniculi* (Passerini) (Hemiptera: Aphididae), and is fed upon by generalist herbivores such as the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). By providing resources for herbivores, and therefore generalist predators, poison hemlock may create novel interactions in local settings.

To investigate the community and potential for interactions within this invasive plant, both foliar and epigeal predators were collected from poison hemlock in 2011, totaling in 956 carabid beetles and 321 lady beetles. It was determined that location and season were significant indicators of the Carabidae and Coccinellidae species constituents within and outside of poison hemlock stands. Densities of *H. foeniculi* were quantified in both 2011 and 2012, characterizing their dropping behaviors in poison hemlock and high numbers through June and July. Sticky traps showing dropping rates also showed the presence of several alternative prey species and poison hemlock seed falling patterns. Collection data revealed a strong synchrony between aphid populations and foraging coccinellids, as well as seed dropping rates and the presence of granivorous carabid species. After a century of naturalization in North America, poison hemlock is attracting introduced herbivores and predators indigenous to its native range, revealing the potential for interactions among non-native species in new regions.
2.2 Introduction

In their 1982 study, Goeden and Ricker characterized the herbivore community surrounding the invasive weed *Conium maculatum* L. (Apiales: Apiaceae). Commonly known as poison hemlock, it was introduced to North America from Eurasia in the 1800s as an ornamental (Pokorny and Sheley 2000). They reported that poison hemlock was “host to many fewer species of phytophagous insects […] than other alien herbaceous weeds surveyed in the past.” (Goeden and Ricker 1982). This lack of associates has been attributed to the plant’s unique chemistry partly comprised of toxic piperidine alkaloids (Fairbairn 1971; Castells et al. 2005; Radulovic et al. 2012), and only two herbivores were found consistently feeding on the plant. One of them, *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae), has been observed feeding on poison hemlock in central Kentucky. Similar to its host-plant, *H. foeniculi* originated in Eurasia; however, the two were introduced separately with the aphid arriving in the early 1900s (Goeden and Ricker 1982; Voegtlin 1984). Although poison hemlock’s toxicity makes it unpalatable to many insects, it provides several food items including a thriving aphid community, plant seeds, pollen, and vegetative material are all nutrient-rich resources found on the plant. Goeden and Ricker’s study on the herbivore community did not examine higher trophic levels and generalist predators utilizing these resources. Due to the central role predation may play in determining the structure and functioning of ecosystems (Holt and Polis 1997; Sih et al. 1998), it is important to include generalist predators when characterizing invasive communities (Hunt-Joshi et al. 2005).

Generalist predators, such as Carabidae, have the ability to rapidly colonize disturbed areas (Luff 1987), survive on limited food resources (Wiedenmann and O’Neil 1990), and become established on alternative prey (Settle et al. 1996). The ability to succeed on limited or varied nutrition is important for predators foraging in invaded areas, as the process of invasion can negatively impact resource availability in local communities (Maerz et al. 2005; Greene and Blossey 2012). Two groups of predators, ground-foraging Carabidae and foliar Coccinellidae (Coleoptera), are both considered generalists (Hodek and Honek 1996; Symondson et al. 2002a; Honek et al. 2003; Lundgren and Lehman 2010). Carabidae are a well-documented group of generalists that
consume a wide variety of prey, from earthworms, mollusks and arthropods, to non-prey resources such as plant seeds (Zhavoronkova 1969; Sunderland 1975; Brandmayr 1990; Stork 1990; Jorgensen 1997; Symondson et al. 2000; Holland 2002; Symondson et al. 2002a; reviewed in Symondson et al. 2002b; Honek et al. 2003; Lundgren and Lehman 2010). At the foliar level, Coccinellidae can obtain nutrients from aphids, thrips, arthropod eggs, pollen, extra-floral nectar, and even other Coccinellidae (reviewed in Hagen 1962; Hodek and Honek 1996; Cottrell and Yeargen 1998; Schade and Sengonca 1998; Mallampalli et al. 2005; Ware and Majerus 2008; Brown et al. 2011).

The current study characterizes arthropod assemblages in poison hemlock of central Kentucky with an emphasis on generalist predators. To test the hypothesis that predator abundance is correlated with high prey densities, Carabidae and Coccinellidae were sampled weekly during the spring and summer of 2011. Aphid counts indicated availability of *H. foeniculi* to foliar-foraging coccinellids over the course of the field season while epigeal sticky traps revealed prey resource availability for ground-foraging Carabidae. These data were also used to test the hypotheses that aphid falling rate is correlated with coccinellid presence and if fallen aphids provide a significant prey resource for epigeal predators. Goeden and Ricker’s study (1982) on the herbivore community was an initial step in understanding single trophic-level interactions surrounding poison hemlock. Now, the characterization of generalist predators and their relationship to prey availability throughout the season provides further insight to the multi-trophic interactions occurring around this toxic, invasive habitat.
2.3 Materials and Methods

2.3.1 Field Transect and Plot Design

The arthropod fauna, consisting of herbivores, generalist predators, and other species present in poison hemlock, were collected at the University of Kentucky Spindletop Research Station in Fayette County, Kentucky, USA (GPS coordinates 38°07N, 84°30W) during the summer of 2011. On the research station, dense areas of naturally occurring poison hemlock infestation, or areas containing a minimum five poison hemlock plants per 4m², were flagged and incorporated into a standardized transect and field plot design. Each transect consisted of five 2x2 m plots (designated to areas showing substantial poison hemlock growth) and separated by approximately 4.5 m, with each plot containing four trap sites set for arthropod and predator sampling (Fig. 2.1). This transect design was replicated four times at different locations throughout the station separated by a minimum of 30 m, totaling 20 plots and 80 trap sites in poison hemlock. A fifth control transect was created in alfalfa (Medicago sativa L., Fabales: Fabaceae) that contained no poison hemlock. In contrast to the four experimental transects in poison hemlock, the control transect did not require a plot designation, as alfalfa growth was uniform throughout the control sampling area. Therefore, 20 independent trap sites were designated, each separated by approximately 3 m. The control transect sampled for arthropods and predators in a poison hemlock-free zone.

Figure 2.1. Diagram of transects in poison hemlock separated by a minimum of 30 m, with black dots representing trap site (n = 4 / plot). Plots area = 4 m², distance between plots ~4.5 m.
2.3.2 Collection of Carabidae

Described previously in Section 2.3.1, all four transects in poison hemlock (Fig. 2.1) contained five 2 x 2 m plots, each plot containing four trapping sites for a total of 80 sites. Trapping sites included dry pitfall traps for sampling Carabidae, a highly effective method to estimate ground predator activity and use of predators in molecular gut-content analysis (described in Section 3.3.2) (after Harper et al. 2005). To collect carabid populations outside of poison hemlock, 20 additional dry pitfall traps were set in a linear transect in an alfalfa (control) transect. A pitfall trap consisted of two 18oz. plastic Solo™ cups (Solo Cup Company, Lake Forest, IL, USA), one inside the other, filling a hole previously dug in the ground. The inner sleeve cup was coated with ©BioQuip Insect-a-Slip Insect Barrier Fluon (BioQuip Products Inc., Rancho Dominguez, CA, USA), a polymer coating preventing predators and other arthropods’ escape from the traps. Before placing traps in the field, a round mesh divider (~7 cm diameter) of 0.3 cm wire hardware cloth was inserted into the middle of the fluon-coated sleeve cup. This allowed smaller insects (aphids and other prey) to pass to the bottom of the trap (after Harper et al. 2005), minimizing within-trap predation. A foam plate (22 cm diameter) suspended on two nails was used as a cover for each trap.

From April to September (2011), pitfall traps were opened for a 48h sampling period, with collections taking place at 7am, 1pm, and 7pm. The purpose of this regimen was to detect any trends of which species were most active throughout the diel cycle and to ensure that gut contents (molecular and chemical) did not decay over long periods of time in the trap. All Carabidae collected during the first 24h period were placed into individual pre-labeled 1.5mL microcentrifuge tubes containing ethanol and stored at -20°C for subsequent molecular analysis, while those collected during the second 24h period were placed into empty tubes and stored at -80°C for chemical analysis (described in Sections 3.3.2 and 3.3.3). During alternate weeks, this pattern was reversed and the chemical analysis specimens were caught on the first day of collection, leaving the second day for molecular analysis collection. For transport from the field to the laboratory, specimens were kept cool in a portable Engel MT15 freezer (Engel, Jupiter,
2.3.3 Collection of Coccinellidae

The previously described transect and plot design (Fig. 2.1, Section 2.3.1) was also employed for the standardized examination of foliar communities on poison hemlock. From April to September (2011), Coccinellidae located on poison hemlock within field plots were hand-collected twice during a 48h sampling period, with five minutes of hand sampling allotted per plot. To survey Coccinellidae outside of poison hemlock, sweep-net sampling was performed in the control transect located in alfalfa. Sampling was performed a minimum of 7 m away from trapping sites to prevent unwanted disturbance. A time-weighted collection method, such as the one used for Carabidae (Section 2.3.2), was unnecessary for Coccinellidae, as they are day-active hunters (Hagen 1962). However, assignment to laboratory analysis (molecular or chemical) followed the same pattern from Section 2.3.2. Individuals collected during the first 24h period were placed into a pre-labeled 1.5mL microcentrifuge tube containing ethanol and stored at -20°C for subsequent molecular analysis, while those collected during the second 24h period were placed into empty tubes and stored at -80°C for chemical analysis (described in Sections 3.3.2 and 3.3.3). Again, this sampling trend was switched during alternate weeks and the chemical analysis specimens were caught on the first day of collection, leaving the second day for molecular analysis collection. For transport from the field to the laboratory, specimens were kept cool in a portable Engel MT15 freezer (Engel, Jupiter, FL, USA). All Coccinellidae were included in population count data; techniques for molecular and chemical analysis are discussed in detail in Chapter 3.

2.3.4 Aphid Counts

In addition to assessing generalist predator groups in poison hemlock, the field transect and plot design described in Section 2.3.1 (Fig. 2.1) was utilized for the
estimation of *H. foeniculi* populations in poison hemlock throughout the spring and summer of 2011. Once a week from April to August (2011), five poison hemlock umbels were selected in each plot (n = 20 plots, 100 umbels per sampling day) and any aphids seen on the umbels were counted in the field using a GOGOTM hand tally counter (Zhejiang Newthinking Industrial Co., Yiwu City, Zhejiang Province, China). Umbel counts of *H. foeniculi* were compared to 2011 sticky trap counts (methods described in Section 2.3.5) to reveal any relationship between aphid abundance and aphid dropping patterns.

For validation of 2011 field count data and confirm whether in-field umbel counts over- or under-estimated total populations, field counts were paired with destructive laboratory counts. Five poison hemlock plants per sampling day (n = 6 days) were selected on the field station and the process described above was carried out once more: five umbels per plant were examined and aphids were counted in the field. Counted umbels were then clipped from the plant and placed in labeled plastic bags to be transported to the laboratory for destructive counting using a dissecting microscope. By destructively examining umbels collected from the field, true aphid count data was obtained, then compared to field count data. Field count underestimate values were calculated by dividing field count values by the destructive laboratory count values to determine the percent of aphids detected in the field.

### 2.3.5 Sticky Traps

The field transect and plot design from Section 2.3.1 incorporated four trapping sites per plot (Fig.2.1). Each trapping site included an epigeal sticky trap (based on Harwood et al. 2001, 2003; Peterson et al. 2010; Romero & Harwood 2010) placed below poison hemlock plants to intercept potential food resources falling from the plants onto the ground (n = 20 traps / transect / collection period). This provided a quantifiable measure of *H. foeniculi* falling behavior, poison hemlock seed-fall, and other potential prey that may become available to ground predators such as Carabidae.

A sticky trap consisted of a platform (polystyrene petri dish, (A = 64 cm²), two 18 gauge galvanized wire stakes for securing the trap in the ground, and a pre-cut circular
3M™ write-on transparency film sheet (3M Company, St. Paul, MN, USA) (A = 64 cm²) coated with spray-on Tangletrap Insect Trap Coating Paste® (Tanglefoot Company, Grand Rapids, MI, USA). Holes were drilled at two opposing sides of the petri dish, allowing the wire stakes to be looped through and securely tied to the platform. To ensure that falling prey would land directly on the trap, the wire stakes were measured to stand a minimum of 3 cm above any ground-level vegetation within plots (Fig 2.2). On sampling days, each circular transparency film sheet was sprayed on both sides with the Tangletrap adhesive and left on a platform in situ for 24 h. The following day, traps were placed on pre-labeled foam-board and transported to the laboratory for identification of potential prey. To evaluate epigeal prey outside of poison hemlock, sticky traps were included in the alfalfa (control) trapping sites (Section 2.3.1) and the same methods were carried out in a linear fashion (n = 20). This process was repeated approximately once a week from May to August 2011 for a total of 100 traps collected on each of the eleven collection dates.

Figure 2.2 Model of a sticky trap used for quantifying epigeal prey resources, dropping behavior of *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae), and poison hemlock seed-fall (n = 80 in four poison hemlock transects, n = 20 in one control transect in alfalfa). Platform (A = 64 cm²) was a minimum of 3 cm above ground-level vegetation.
2.3.6 Statistical Analysis

Shannon-Wiener (H’) index of Diversity and Pielou’s Evenness (E’) index were calculated for 2011 field data for both Carabidae and Coccinellidae collected in poison hemlock. To detect significant differences in diversity and evenness index values throughout the different periods of collection, data were analysed using a one-way Analysis of Variance with a PROC GLM model (SAS® v. 9.3, SAS, Inc. 2012). Next, to detect differences in species abundance based on transect location and period of collection, a two-way ANOVA (PROC GLM model) was applied to the following two data sets: (a) count data of each species of Carabidae and Coccinellidae collected in poison hemlock and the control transect, and (b) field umbel counts and sticky trap counts of H. foeniculi. A post hoc Least Significant Difference test was then applied to Carabidae and Coccinellidae population data sets using PROC ANOVA (lsd tukey) for the detection of pair-wise differences between transects and collection periods. To show the relationship between field and laboratory validation counts for H. foeniculi on poison hemlock in 2012, a regression analysis was performed using the PROC REG function (SAS). Additional regression analyses were performed to show correlations between the number of foraging H. axyridis and its relationship to H. foeniculi population densities as well as H. foeniculi dropping rates (SAS).

2.4 Results

2.4.1 Carabidae Sampling

Pitfall traps captured eight different species of Carabidae (Coleoptera) in poison hemlock and seven different species in the alfalfa control (Table 2.1). The four most abundant in poison hemlock during 2011 were Chlaenius tricolor Dejean representing 37.6% of carabid beetles, followed by Cyclotrachelus sodalis (LeConte) at 18.3%, Harpalus pensylvanicus De Geer at 15.7%, and Poecilus lucublandus Say at 14.5% (Fig. 2.3). Chlaenius tricolor and P. lucublandus were dominant during the June collection period, while C. sodalis and H. pensylvanicus dominated the late summer and early fall period.
collections (Fig. 2.3). In the control transect, the most abundant group was *Scarites subterraneus* F., constituting 66.6% of carabids caught in a poison hemlock-free area, followed by *P. chalcites* (Say) at 25.7% (Fig. 2.4). Both *S. subterraneus* and *P. chalcites* populations peaked early in the season between May and June collection time periods (Fig. 2.4). A two-way ANOVA revealed that transect location and collection period had significant effects (*α* = 0.05) on the abundance of each species collected in poison hemlock and alfalfa, excluding *Agonum punctiforme* (Say), that showed no significant transect effect (Table 2.2). The post hoc Least Significant Difference test for the detection of pair-wise differences showed no detectable trend in differences between transects and collection periods for Carabidae.
Table 2.1 Total and mean numbers (+/- SE) of adult Carabidae captured per pitfall trap from transects located in poison hemlock and in alfalfa, 2011.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total count, Poison hemlock</th>
<th>Mean number / trap Poison hemlock</th>
<th>Total count, Alfalfa</th>
<th>Mean number / trap Alfalfa</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agonum punctiforme</em> (Say)</td>
<td>45</td>
<td>0.11 +/- 0.02</td>
<td>5</td>
<td>0.05 +/- 0.03</td>
<td>50</td>
</tr>
<tr>
<td><em>Chlaenius tricolor</em> Dejean</td>
<td>275</td>
<td>0.58 +/- 0.07</td>
<td>6</td>
<td>0.06 +/- 0.02</td>
<td>281</td>
</tr>
<tr>
<td><em>Cyclotrachelus sodalis</em> (LeConte)</td>
<td>134</td>
<td>0.33 +/- 0.04</td>
<td>3</td>
<td>0 +/- 0.02</td>
<td>137</td>
</tr>
<tr>
<td><em>Harpalus erythropus</em> Dejean</td>
<td>8</td>
<td>0.02 +/- 0.01</td>
<td>1</td>
<td>0.01 +/- 0.01</td>
<td>9</td>
</tr>
<tr>
<td><em>Harpalus pensylvanicus</em> De Geer</td>
<td>115</td>
<td>0.29 +/- 0.07</td>
<td>2</td>
<td>0.02 +/- 0.01</td>
<td>117</td>
</tr>
<tr>
<td><em>Poecilus chalcites</em> (Say)</td>
<td>1</td>
<td>0.003 +/- 0.003</td>
<td>58</td>
<td>0.57 +/- 0.1</td>
<td>59</td>
</tr>
<tr>
<td><em>Poecilus lucublandus</em> Say</td>
<td>106</td>
<td>0.26 +/- 0.04</td>
<td>0</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td><em>Scarites subterraneus</em> F.</td>
<td>47</td>
<td>0.12 +/- 0.02</td>
<td>150</td>
<td>1.5 +/- 0.25</td>
<td>197</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>731</strong></td>
<td></td>
<td><strong>225</strong></td>
<td></td>
<td><strong>956</strong></td>
</tr>
</tbody>
</table>
Table 2.2 Statistical results from two-way analysis of variance on log transformed data of species counts based on transect location and collection period of adult Carabidae captured in pitfall traps within poison hemlock, 2011.

<table>
<thead>
<tr>
<th>Species</th>
<th>F value, Transect</th>
<th>P, Transect (DF=4)</th>
<th>F value, Period</th>
<th>P, Period (DF= 4)</th>
<th>F value, Transect*Period</th>
<th>P, Transect*Period (DF= 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agonum punctiforme</em> (Say)</td>
<td>1.33</td>
<td>0.2567</td>
<td>17.55</td>
<td>&lt;0.0001</td>
<td>1.66</td>
<td>0.0502</td>
</tr>
<tr>
<td><em>Chlaenius tricolor</em> Dejean</td>
<td>33.10</td>
<td>&lt;0.0001</td>
<td>16.29</td>
<td>&lt;0.0001</td>
<td>5.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Cyclotrachelus sodalis</em> (LeConte)</td>
<td>21.65</td>
<td>&lt;0.0001</td>
<td>7.66</td>
<td>&lt;0.0001</td>
<td>2.18</td>
<td>0.0052</td>
</tr>
<tr>
<td><em>Harpalus erythropus</em> Dejean</td>
<td>2.43</td>
<td>0.047</td>
<td>2.43</td>
<td>0.047</td>
<td>4.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Harpalus pensylvanicus</em> De Geer</td>
<td>19.74</td>
<td>&lt;0.0001</td>
<td>9.36</td>
<td>&lt;0.0001</td>
<td>6.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Poecilus chalcites</em> (Say)</td>
<td>72.58</td>
<td>&lt;0.0001</td>
<td>18.84</td>
<td>&lt;0.0001</td>
<td>19.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Poecilus lucublandus</em> Say</td>
<td>31.35</td>
<td>&lt;0.0001</td>
<td>8.25</td>
<td>&lt;0.0001</td>
<td>2.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Scarites subterraneus</em> F.</td>
<td>53.32</td>
<td>&lt;0.0001</td>
<td>24.80</td>
<td>&lt;0.0001</td>
<td>10.31</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 2.3 Seasonal abundance of a) *Chlaenius tricolor* Dejean, b) *Cyclotrachelus sodalis* (LeConte), c) *Harpalus pensylvanicus* De Geer, and d) *Poecilus pensylvanicus* Say (Coleoptera: Carabidae) in poison hemlock in central Kentucky, 2011 (data points correspond to the mean number (±SE) of beetles captured per trap per collection period).

Figure 2.4 Seasonal abundance of a) *Scarites subterraneus* F. and b) *Poecilus chalcites* (Say) (Coleoptera: Carabidae) in alfalfa (control) in central Kentucky, 2011 (data points correspond to the mean number (±SE) of beetles captured per trap per collection period).
2.4.2 Coccinellidae Sampling

Twice weekly hand sampling in poison hemlock and alfalfa showed the presence of three lady beetle species (see Table 2.3), dominated by *H. axyrids* which represented 78% of all individuals collected. In alfalfa, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) was the dominant predator at 67% abundance. Both transect location and collection period had significant effects on the abundance of all lady beetle species (*α* = 0.05) (Table 2.4, Fig 2.5). As with Carabidae, the post hoc Least Significant Difference test for the detection of pair-wise differences showed no clear trends in differences between transects and collection periods for Coccinellidae.
Table 2.3 Total and mean numbers (+/- SE) of adult Coccinellidae captured per plot from transects located in poison hemlock and in alfalfa, 2011.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total count, Poison hemlock</th>
<th>Mean number / plot +/- SE</th>
<th>Total count, Alfalfa</th>
<th>Mean number / plot +/- SE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coccinella septempunctata</em> L.</td>
<td>42</td>
<td>0.42 +/- 0.2</td>
<td>10</td>
<td>0.4 +/- 0.12</td>
<td>52</td>
</tr>
<tr>
<td><em>Cycloneda munda</em> Say</td>
<td>24</td>
<td>0.24 +/- 0.07</td>
<td>1</td>
<td>0.04 +/- 0.04</td>
<td>25</td>
</tr>
<tr>
<td><em>Harmonia axyridis</em> Pallas</td>
<td>240</td>
<td>2.4 +/- 0.5</td>
<td>4</td>
<td>0.16 +/- 0.1</td>
<td>244</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>306</strong></td>
<td></td>
<td><strong>15</strong></td>
<td></td>
<td><strong>321</strong></td>
</tr>
</tbody>
</table>

Table 2.4 Statistical results from two-way analysis of variance on log transformed data of species counts based on transect location and collection period of adult Coccinellidae captured on poison hemlock plants, 2011.

<table>
<thead>
<tr>
<th>Species</th>
<th>F value, Transect (DF=4)</th>
<th>P, Transect</th>
<th>F value, Period (DF= 4)</th>
<th>P, Period</th>
<th>F value, Transect*Period (DF= 16)</th>
<th>P, Transect*Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coccinella septempunctata</em> L.</td>
<td>10.14</td>
<td>&lt; 0.0001</td>
<td>13.63</td>
<td>&lt; 0.0001</td>
<td>8.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><em>Cycloneda munda</em> Say</td>
<td>7.98</td>
<td>&lt; 0.0001</td>
<td>6.84</td>
<td>&lt; 0.0001</td>
<td>2.31</td>
<td>0.0061</td>
</tr>
<tr>
<td><em>Harmonia axyridis</em> Pallas</td>
<td>18.35</td>
<td>&lt; 0.0001</td>
<td>9.4</td>
<td>&lt; 0.0001</td>
<td>1.87</td>
<td>0.0319</td>
</tr>
</tbody>
</table>
Figure 2.5 Seasonal abundance of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in poison hemlock in central Kentucky, 2011 (data points correspond to the mean number (±SE) of beetles captured per trap per collection period).

Figure 2.6 Season and transect distributions of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) in alfalfa (control) in central Kentucky, 2011 (data points correspond to the mean number (±SE) of beetles captured per trap per collection period).
2.4.3 Food Availability in Poison Hemlock

Plant umbel counts showed that *H. foeniculi* populations were most abundant from June to early July in 2011, with an overall seasonal mean of four aphids per umbel. Both transect location and period had significant effects on aphid abundance \( F(27,672), P < 0.0001 \) (Fig. 2.7). *Hyadaphis foeniculi* numbers at the ground level (aphids caught on sticky traps) were most abundant at the end of June and showed an average dropping rate of 0.1 aphids per trap, per 24-hour collection period (Fig. 2.7). Dropping rates were also significantly affected by transect location and period \( F(44,855), P = 0.0001 \).

A significant positive correlation between average seasonal density of *H. foeniculi* and the number of *H. axyridis* foraging in poison hemlock was detected \( R^2 = 0.541, y = 0.5034x + 0.2081, F(1,18), P = 0.0002 \) (Fig. 2.8a). However, foraging *H. axyridis* in poison hemlock umbels did not significantly account for the proportion of aphids caught on epigeal sticky traps throughout the season \( R^2 = 0.1002, y = 0.0494x + 0.0261, F(1,18), P > 0.05 \) (Fig. 2.8b).

Epigeal sticky traps showed that *H. foeniculi* and poison hemlock seeds are available to ground predators. The seeds in particular showed a marked increase in epigeal abundance beginning in August. Although *H. foeniculi* exhibited dropping behaviors, their abundance at the ground level is lower than other arthropod species including Collembola, Thysanoptera, Cicadellidae (Hemiptera), and Mordellidae (Coleoptera) (Fig. 2.10).

2.4.4 Validation Counts of *Hyadaphis foeniculi*

Aphid count validations in 2012 showed a significant positive correlation between the number of aphids counted per umbel in the field and the number counted in the laboratory via destructive counting \( R^2 = 0.926, y = 1.4632x + 19.056 \) (Fig. 2.9). Field counts thus represented an average of 65% of total aphids present in umbels.
Figure 2.7 Seasonal counts of *Hyadaphis foeniculi* (Passerini) in poison hemlock umbels and seasonal capture frequency the aphids on epigeal sticky traps in poison hemlock in central Kentucky, 2011 (data points correspond to the mean number (±SE) of aphids counted per umbel and mean number (±SE) of aphids counted per 64cm² epigeal sticky trap).
Figure 2.8 Linear relationships between a) the log transformed number of *Hyadaphis foeniculi* (Passerini) in poison hemlock umbels and the log transformed number of foraging *Harmonia axyridis* Pallas in poison hemlock, 2011. $R^2 = 0.541$ ($y = 0.5034x + 0.2081$), and b) the log transformed number of foraging *H. axyridis* and the log transformed number of *H. foeniculi* intercepted by epigeal sticky traps, 2011. $R^2 = 0.1002$ ($y = 0.0494x + 0.0261$).
Figure 2.9 Linear relationship between destructive (laboratory) and in-field counts of *Hyadaphis foeniculi* (Passerini), 2012. $R^2 = 0.9266$ ($y = 1.4632x + 19.056$).

Figure 2.10 Epigeal prey caught on sticky traps in poison hemlock in central Kentucky, 2011 (data points correspond to the mean number ($\pm$SE) of aphids, seeds, or alternative prey counted per sticky trap per collection period).
2.4.5 Diversity and Evenness of Carabid and Coccinellid Populations in Poison Hemlock

Carabidae showed a seasonal average $H'$ value of 0.38 and $E'$ value of 0.19 (Fig. 2.10) while Coccinellidae $H'$ and $E'$ seasonal averages were both 0.06 (Fig. 2.11). There were no significant differences between collection periods for diversity ($F_{(4,15)}, P = 0.54$) or evenness ($F_{(4,15)}, P = 0.63$) for Carabidae. In contrast, Coccinellidae diversity and evenness values were significantly different across collection periods in 2011 ($F_{(4,15)}, P = 0.032$).
Figure 2.11 (a) Shannon-Weiner Diversity Index (H’) and (b) Pielou’s Evenness Index (E’) of for Carabidae captured in poison hemlock, 2011 (data points correspond to the mean H’ or E’ (±SE) per transect).
Figure 2.12 (a) Shannon-Weiner Diversity Index (H’) and (b) Pielou’s Evenness Index (E’) of for Coccinellidae captured in poison hemlock in central Kentucky, 2011 (data points correspond to the mean H’ or E’ (±SE) per transect).
2.5 Discussion

Sampling for generalist predators in poison hemlock during the spring and summer of 2011 revealed that both Carabidae and Coccinellidae are foraging in a toxic, invasive system. A total of 731 carabid beetles from eight different species were captured in poison hemlock, featuring four dominant species: *C. tricolor*, *C. sodalis*, *H. pensylvanicus*, and *P. lucublandus*. Together, these represented 86% of all carabids in poison hemlock (Table 2.1 and Fig. 2.3). The epigeal arthropod assemblage in the poison hemlock-free control greatly contrasted that of the invaded areas. A total of 225 carabid beetles from seven different species were accounted for; however, only two species, *S. subterraneus* and *P. chalcites*, were consistently captured. Together, these two groups represented 92% of all carabids captured in alfalfa (Table 2.1 and Fig. 2.4). Carabidae showed no significant variation in diversity or evenness in poison hemlock transects throughout collection periods (Fig. 2.11). Coccinellidae, however, were significantly more diverse and even during collection periods two and three which took place in June. This may be attributed to an overwhelming majority of coccinellids being collected during those times in contrast to the much more sparse periods of one, four, and five (Fig. 2.12). Three different species of Coccinellidae were captured, with 306 caught in poison hemlock and 15 sampled from the control. The invasive multi-colored Asian lady beetle (*Koch and Galvan 2008*), *H. axyridis*, dominated poison hemlock umbels at 78% capture frequency while *C. septempunctata*, represented 67% of the coccinellids found in the alfalfa control.

Poison hemlock provided a varied and abundant set of food resources with a potential to support predator diversity in this invasive system (*Wallin et al. 1992; Toft 1995; Evans et al. 1999; Messelink et al. 2010*). Sticky traps characterizing the epigeal prey community revealed that *H. foeniculi* and poison hemlock seeds were intercepted by the traps at an average rate of 0.1 aphids and 0.4 seeds per sticky trap per day (*A = 64 cm², 24h*) (Fig. 2.8b and 2.10). At the foliar level, field counts of aphids in 2011 showed an average of four aphids per umbel on poison hemlock. However, validation counts in 2012 revealed an underestimation of field counts, with an average of 65% of the actual aphid population being included in original field counts. As a result, the corrected value
of average number of aphids per umbel in poison hemlock during 2011 was approximately six aphids per umbel. To confirm that methods carried out in 2011 represented true aphid population trends in poison hemlock, a regression analysis of the 2012 field and validation count data was performed. Results supported the accuracy of the methods with a strong positive correlation between field and laboratory validation counts (Fig. 2.9).

Although it is possible to estimate an average number of aphids per umbel, aphid distributions in the field were more heterogeneous, with some umbels containing no aphids while others contained hundreds of individuals at a time. These larger populations represent a nutrient-rich habitat for foraging coccinellids, and may additionally translate to greater aphid dropping rates (Losey and Denno 1998), as was the case in this study. Greater numbers of *H. axyridis* had a positive correlation with *H. foeniculi* dropping behaviors in poison hemlock (Fig. 2.8b). With an average of six aphids per umbel and 0.1 aphids caught per sticky trap (A = 64 cm²), the aphid falling rate was approximately 1.7% of aphids. For an umbel containing 300 aphids, an estimated five dropping aphids may land within each 64 cm² area at the base of the plant. In this example, it is important to remember that sticky traps (64 cm²) only represented a small fraction of the total area onto which aphids may have fallen, so it is possible that hundreds of aphids reached the ground level around poison hemlock plants, and were therefore available to Carabidae. This supports the hypothesis that *H. foeniculi* may serve as a significant source of nutrients for ground predators. In addition, the spring of 2012 may have provided more aphid predation opportunities for ground predators as validation counts reached up to 450 aphids per umbel after mild winter conditions.

Sticky trap and aphid count data provided additional information on the temporal availability of different prey items and their synchrony with certain predator species. Interestingly, a distinct temporal linkage was detected between two predators and their respective food resources. The presence of the seed-eating carabid, *H. pensylvanicus*, was not recorded until poison hemlock seeds began to drop from the plants in late July and early August of 2011 (Fig. 2.3c and Fig. 2.10), implying that poison hemlock seeds may have act as an attractant or an arrestant to *H. pensylvanicus* (Sasakawa 2010; Diehl et al. 2012). Similarly, large aphid populations may have brought in foraging coccinellids, as
*H. axyridis* populations surged in concert with the increasing *H. foeniculi* numbers throughout the month of June 2011 (Figs. 2.5, 2.7, and 2.8a) (Muller and Godfray 1997; Leroy et al. 2012). Both predator species were principal in their respective foraging niches, suggesting the potential strength of these interactions around this Eurasian invader.

Previous studies in poison hemlock focusing on herbivore species have not reported the presence of generalist predators (Goeden and Ricker 1982; Berenbaum and Harrison 1994; Castells and Berenbaum 2006; Castells and Berenbaum 2008a,b), and to my knowledge, this is the first study to explore higher trophic levels in this system. Higher trophic levels and generalist predators have the ability to strongly influence community interactions (Holt and Polis 1997; Sih et al. 1998; Hunt-Joshi et al. 2005). In addition, invasive species like poison hemlock have the potential to reshape community interactions through the provision of novel resources such as aphids or other small arthropods, seeds, and pollen. The combination of novel resources and foraging generalist predators may be further affected by the Eurasian origin of poison hemlock, *H. axyridis*, and *H. foeniculi*. These three species have the potential to be pre-adapted to one another, allowing them to immediately begin interacting in a new region (Simberloff and Von Holle 1999; Ricciardi 2001). However, the current data cannot confirm or refute the occurrence of interactions between these nutrient sources and the generalist predators foraging in the system. In the following chapter, these relationships will be unlocked by utilizing both molecular and chemical techniques to track poison hemlock-derived resources through the food web, thereby revealing the significant role generalist predators play within this invasive system.
Chapter 3: The role of plant chemistry in complex food webs: using GCMS and PCR to reveal consumer-resource interactions in poison hemlock

3.1 Summary

Invasive species, such as poison hemlock (Conium maculatum L., Apiaceae), have the potential to change ecosystem structure and introduce novel interactions into native communities. To date, studies on poison hemlock, have focused on the identification of herbivore associates or the plant’s potent alkaloid chemistry. However, no study has investigated higher trophic levels and their association to poison hemlock resources. Higher trophic levels, specifically generalist predators, are crucial in defining the structure and functioning of ecosystems, as their predation on lower trophic levels has the capacity to exert considerable pressure on both prey and alternative food taxa alike. Recent studies on poison hemlock in Kentucky revealed the presence of several generalist predator species and their prospective food sources at the foliar and epigeal levels around the plant (C. Allen, Chapter 2); however, the relationship between the predators and their prey remains unknown.

In this study, molecular and chemical techniques were utilized for the determination of trophic linkages between predators foraging in poison hemlock and their food. Individual Coccinellidae and Carabidae were collected during the spring and summer of 2011 and divided into molecular and chemical analysis groups. Molecular gut-content analysis involved the screening of individuals for aphid DNA from Hyadaphis foeniculi (Passerini) (Hemiptera: Aphididae), a small herbivore that colonizes poison hemlock umbels in the spring months. Chemical analysis screened predators for the presence of poison hemlock-derived alkaloids, thereby detecting the consumption of non-prey food groups such as seeds. Harmonia axyridis Pallas (Coleoptera: Coccinellidae) was the dominant predator in poison hemlock, with 11% testing positive for DNA from the aphid H. foeniculi, and 28.5% testing positive for the poison hemlock alkaloid γ-Coniceine. No carabid beetles tested positive for aphid DNA, possibly due to the abundance of alternative prey at the epigeal level. However, 50% of Harpalus pensylvanicus De Geer (Coleoptera: Carabidae) screened for alkaloid were positive for γ-
Coniceine, revealing the potential for seed predation or tri-trophic transfer through non-aphid routes in poison hemlock stands. These results highlight the importance of utilizing multiple laboratory techniques for detecting multi-trophic interactions and the prospect that both native and exotic predator species are utilizing poison hemlock resources.

3.2 Introduction

“Every animal is closely linked with a number of other animals living round it, and these relations in an animal community are largely food relations.” Charles Elton’s Animal Ecology initiated the unraveling of food web ecology and emphasized the importance of connectedness between organisms in an ecosystem (Elton 1927). Elton’s use of functional groups eventually led to Raymond Lindeman’s “trophic-dynamic viewpoint” that defined the importance of “energy-availing relationships” between species. In essence, the survival of higher trophic level groups is dependent upon their efficiency in extracting energy from their lower trophic level food, and the further one moves up the food chain, the further the individual is from the most abundant energy source: the sun (Lindeman 1942). As a consequence, many predators occupying higher trophic levels rely on a generalized diet to ensure that they obtain the proper nutrition (Elton 1927; Lindeman 1942). Predators exert substantial pressure on their prey through these energy-obtaining interactions, often resulting in top-down effects that can reshape entire communities (reviewed in Schmitz et al. 2000; Terborgh et al. 2001; Costamagna and Landis 2011). For example, a study performed from 1993 to 2001 on a series of small islands off the coast of Venezuela showed that the absence of predators resulted in 10 to 100 times more herbivores leading to significantly reduced canopy vegetation (Terborgh et al. 2001).

In lieu of their significance in ecosystems, many ecological studies have focused on predator-prey relationships, as these interactions are driving forces in determining ecosystem functions (Holt and Polis 1997; Sih et al. 1998). To answer the question of “who-eats-whom”, some studies have relied on predator gut dissection and the subsequent identification of partially digested prey species while others have relied on direct field observation of predation events. Although these two methods provide some
information on predator-prey connections, both are subject to a wide range of errors. Dissection, for example, is not always an option, as 79% of terrestrial predatory arthropods consume their prey in liquid form (reviewed in Cohen 1995). There also remains the problem of taxonomic resolution when attempting to identify partially digested prey body parts, a particularly difficult process when studying smaller organisms (Chiverton and Sotherton 1991; Holopainen and Helenius 1992; Bo et al. 2011). Observational field studies, prone to disruption, may prevent the occurrence of authentic animal behaviors including prey capture and consumption (Elliott et al. 2000; Navntoft et al. 2009). Furthermore, obtaining a significant amount of data utilizing this method requires a large time commitment, where one must adhere to predator’s feeding habits that may be nocturnal or occurring during inclement weather (Elliott et al. 2000; Pfannenstiel 2008). The development of modern laboratory analysis techniques solves the aforementioned problems.

Molecular methods, including polymerase chain-reaction (PCR), have more recently gained popularity in field-based ecology studies (Sheppard and Harwood 2005; Harwood et al. 2007; reviewed in Harwood and Greenstone 2008; Traugott and Symondson 2008; Harwood et al. 2009b; reviewed in Weber and Lundgren 2009; King et al. 2010; Eskelson et al. 2011; Eitzinger et al. 2013). DNA-based post-mortem gut-content analysis allows predators to be captured under open-field (non-manipulated) conditions, and processed in the laboratory. The relative ease of development of primers that are subsequently used to amplify prey DNA leads to accurate visualization of predation events at the species level. Additionally, predators may be screened several times for a multitude of different prey species, giving an objective assessment of predator diet-breadth. In addition to the more recent development of DNA-based methods, the longstanding use of gas chromatography-mass spectrometry (GC-MS) has been recognized as a reliable method for identifying compounds in complex mixtures for several decades. GC-MS has been used in various fields such as food toxicology, forensic science, and organismal biology (Hites et al. 1968; Blechschmidt et al. 1984; Boumba et al. 2006; Konoz et al. 2013). The principle of this analytical technique relies on the coupling of gas chromatography (the separation and quantification of components in a mixture) and mass spectrometry (subsequent ionization and identification of each
A chromatogram from GC provides a series of peaks, the areas of which represent the concentrations of each constituent within the mixture. Mass spectra data provided by the MS process reveals the identity of each peak. Together, these data answer the question of “what” and “how much” of a target compound are present in a sample (Smith 1988; Millar and Haynes 1998).

The combination of DNA-based gut-content analysis and GC-MS alkaloid analysis provides two mechanisms for identifying predation in chemically unique systems. For example, Aebi et al. (2011) used both methods to illustrate their utility for environmental risk assessment studies prior to the release of biological control agents, such as the chemically defended multi-colored Asian lady beetle, *H. axyridis*. He argued that PCR alone may not be the ideal method for unraveling complex food webs, especially those involving intraguild predation (IGP) and cannibalism. *Harmonia axyridis* exhibits predation on other coccinellid species, but these predation events may be tracked using the species-specific chemistry of their lady beetle prey (Sloggett et al. 2009; Sloggett and Davis 2010; Hautier et al. 2011). This chemical tracking may also be performed on plant compounds, such as piperidine alkaloids (Castells et al. 2005; Castells and Berenbaum 2008a), and is therefore useful for illustrating how plant materials move through complex food webs. Including plant chemistry in food web studies provides further insight into community structure and interactions, especially when generalists are known consumers of non-prey food such plant seeds, pollen, extra-floral nectar, and vegetative material (reviewed in Hagen 1962; Zhavoronkova 1969; Brandmayr 1990; Hodek and Honek 1996; Jorgensen 1997; Honek et al. 2003; Harwood et al. 2007; Moser et al. 2008; Lundgren and Lehman 2010).

These complex interactions are significant in all ecosystems, but may prove especially compelling in the ever-changing environment brought on by globalization and the subsequent increase in biological invasions. Over the past century, invasive species have caused major losses in biodiversity (Hooper et al. 2005) and have brought on approximately $1.4 trillion in damages, worldwide (The Nature Conservancy 2012). While studies on invasive species continue to focus on prevention, mitigation and prediction of invasive species (DiTomaso 2000; Mack et al. 2000; Perrings 2005; Phillips and Murray 2012), there is a reality that many invasive species are past the point of fixing.
the problem. It is in our best interest in these situations to continue studying the species undergoing naturalization in new regions so that we may fully understand the implications of invasion and how exotic species forge new relationships with native and nonnative species alike. *Conium maculatum*, poison hemlock, is a good example of an invasive species that is becoming increasingly naturalized across North America. As the name suggests, the plant produces highly toxic piperidine alkaloids, but nevertheless provides a variety of food resources for generalist predators including an exotic aphid species (*H. foeniculi*), plant seeds, and other small arthropods (C. Allen, Chapter 2).

Its unique chemical makeup, invasive status, and ubiquity in the U.S. make poison hemlock a prime candidate for the combined use of molecular and chemical analysis techniques to unlock novel interactions occurring within this system. The objective of this study was to quantify food web linkages and the transfer of plant alkaloids to higher trophic levels in poison hemlock habitats. Both PCR and GC-MS were used to decipher the feeding activities of generalist predators foraging in poison hemlock-dominated areas. I hypothesized that epigeal-foraging Carabidae and foliar-foraging Coccinellidae would screen positive for *H. foeniculi* via DNA-gut content analysis. I next hypothesized that poison hemlock’s signature alkaloids would be detectable in the predator gut due to consumption of alkaloid-containing aphid prey or alkaloid-containing non-prey plant-derived resources. The results of these two complementary hypotheses would reveal whether or not certain predator species were receiving alkaloids through the consumption of live prey.

### 3.3 Materials and Methods

#### 3.3.1 *Hyadaphis foeniculi* Sequencing and Sequence Submission to GenBank

*Hyadaphis foeniculi* were collected from poison hemlock at the University of Kentucky Spindletop Research Farm in Fayette County, Kentucky, USA, (GPS coordinates 38°07N, 84°30W) and placed in individual microcentrifuge tubes containing 95% ethanol to be transported back to the laboratory in May, 2011. Specimens were stored in a freezer at -20°C prior to DNA extraction, and then identified by referencing
Voegtlin (1984) at the time of processing. Total DNA was then extracted from the crushed whole specimen using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, CA, USA) following the manufacturer’s animal tissue protocol. The resulting 200µl extractions were stored again at -20°C preceding polymerase chain reaction (PCR).

To obtain sequences, PCR was performed to amplify cytochrome c oxidase I (COI) of the previously extracted *H. foeniculi* DNA using the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR reactions (25µl) consisted of 1X Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each primer, 0.625U Takara *Ex Taq™* and template DNA from the previously extracted aphid DNA (1-5µl total DNA). Reactions were carried out in Bio-Rad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, USA) using optimal cycling protocols for Takara reagents as follows: 94°C for 1 min followed by 50 cycles of 94°C for 50s, 40°C for 45s, 72°C for 45s, and a final extension of 72°C for 5 min. Following the PCR, the success of the reaction was determined by electrophoresis of 10µl of PCR product in 2% SeaKem agarose (Lonza, Rockland, ME, USA) stained with 1X GelRed™ nucleic acid stain (Biotium, CA, USA).

Those that yielded positive PCR product for aphid DNA were sequenced by Advanced Genetic Technologies Center at the University of Kentucky. Forward and reverse sequences of the same individuals were assembled using Geneious (v. 5.4; Drummond et al. 2011). Further editing and aligning was performed using Bioedit Sequence Alignment Editor© (Carlsbad, CA, USA) and multiple sequence alignments were performed with MUSCLE (©European Bioinformatics Institute, 2011; available online at http://www.ebi.ac.uk/Tools/msa/muscle/). Final sequences were submitted to GenBank and the Barcode of Life Database (Ratnasingham and Hebert 2007). These submitted sequences (GenBank [Accession number JX239063 - JX239086]) were later used for the identification of *H. foeniculi* DNA from predator gut contents, described in Section 3.3.2.
3.3.2 DNA Decay Rate of *Hyadaphis foeniculi* in Predator Guts

*Hyadaphis foeniculi* used in feeding trials were obtained from poison hemlock plants located at the University of Kentucky Spindletop Research Station in Fayette County, Kentucky, USA (GPS coordinates 38°07'N, 84°30'W). Infested umbels were clipped, bagged, and transported back to the laboratory where they were frozen at -20°C. Two abundant predators, *Cyclotrachelus sodalis* (LeConte) (Coleoptera: Carabidae) and *H. axyridis*, were selected for DNA decay rate experiments. Thirty pitfall traps were set in poison hemlock-infested areas at the University of Kentucky Spindletop Research Station to collect *C. sodalis* during May and June of 2012. Upon collection, individuals were transported in a one gallon Rubbermaid® container (Rubbermaid®, Atlanta, GA, USA) back to the laboratory where they were separated into individual 4oz. plastic DART® containers (Dart Container Corporation, Mason, MI, USA). Until the commencement of feeding trials, individual *C. sodalis* were provided with a water-dampened cotton ball, an *ad libitum* diet of *Musca domestica* L. pupae (Diptera: Muscidae) (Oregon Feeders Co., Payette, ID, USA) and maintained at 22°C with a reverse light cycle of 14:10 (L:D) due to the nocturnal foraging habits of carabid beetles (Theile 1977, Kromp 1999). After a minimum acclimation period of one week, carabids selected for use in feeding trials were placed into new DART® containers for a starvation period of seven days during which only water was provided.

At the start of feeding trials, carabids were transported to a dark room and allowed to acclimate to red light for one hour prior to feeding. Next, all were presented with and observed to consume a single frozen *H. foeniculi*, followed by one chaser prey (*M. domestica*) to minimize the effects of starvation (Greenstone and Hunt 1993, Chen et al. 2000). The beetles were then separated into groups of ten for each decay rate time period being tested, starting with t = 0, followed by t = 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48h. After feeding and allowing the specified time to pass, beetles were placed in 1.5mL microcentrifuge tubes containing 100% EtOH and stored at -20°C prior to dissection and extraction (Section 3.3.2).

Adult *H. axyridis* used for feeding trials were collected during the spring of 2012 via hand collection directly from poison hemlock growing on the University of Kentucky
Spindletop Research Station. Similar to *C. sodalis*, individuals were stored in a one-gallon Rubbermaid® container and transported back to the laboratory to be separated into smaller individual DART® containers. All were maintained at 20°C with a 14:10 (L:D) cycle, a water-dampened cotton ball, and an *ad libitum* diet of live *Sitobion avenae* F. (Hemiptera: Aphididae) raised in greenhouse colonies (methods concerning greenhouse colony maintenance are provided in Chapter 4, Section 4.3.1). An acclimation period of 48h was followed by 24h of starvation with water, a sufficient period for Coccinellidae used in feeding experiments (Greenstone et al. 2007). Feeding trials were carried out as with *C. sodalis*, however, nocturnal conditions were unnecessary due to *H. axyridis* diurnal habits (Hagen 1962), and sugar water was provided instead of *M. domestica* following aphid consumption.

3.3.3 Predator Gut Content Analysis and the Detection of *Hyadaphis foeniculi*

Carabidae and Coccinellidae were collected as described in Sections 2.3.2 and 2.3.3 from April to September of 2011. To reiterate, all predators were procured from plots contained within transects at the University of Kentucky Spindletop Research Station in Fayette County, Kentucky, USA (GPS coordinates 38°07'N, 84°30'W) and stored in individual 1.5mL microcentrifuge tubes containing 95% ethanol. After being transported back to the laboratory, beetles were stored at -20°C before processing (identification and extraction) was carried out. Carabidae were identified referencing Lindroth (1961, 1963, 1966, 1968, 1969) and Gordon (1985) was used for identifying Coccinellidae. In adherence with the animal tissue protocols for QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, CA, USA), larger arthropod predators, such as Carabidae and Coccinellidae, required dissection of the alimentary canal prior to DNA extraction (after Symondson et al. 2000, Foltan et al. 2005). Next, DNA extraction was performed on the predatory alimentary canal as previously described in the above Section 3.3.1, after which total DNA was stored at -20°C preceding PCR analysis.

Gut content extractions were screened for the presence of aphid DNA using the general aphid primers, Aphid-413F and Aphid-565R (Chapman et al. 2010). PCR was carried out as above (Section 3.3.1) using Takara reagents and the following thermal
cycling conditions: 94°C for 1 min followed by 50 cycles of 94°C for 45s, 59°C for 45s, 72°C for 30s, and a final extension of 72°C for 5 min. Following PCR, the success of the reaction was determined by gel electrophoresis (Section 3.3.1). Predators screening positive for aphid DNA were sequenced, edited, and aligned as described in Section 3.3.1. BLASTN searches (Karlin & Altschul 1990, 1993) of GenBank and the Barcode of Life Database (Ratnasingham & Hebert 2007) were performed to determine whether the sequences significantly matched the previously submitted *H. foeniculi* sequences (Section 3.3.1). A significant match in GenBank and Barcode of Life Database (Ratnasingham & Hebert 2007) was considered to be ≥97% max identity (percent similarity between the query and subject sequences) or greater (after Hebert et al. 2002).

### 3.3.4 Seed-Feeding Trials (Carabidae)

During the summer of 2010, 20 pitfall traps were set in poison hemlock areas at the University of Kentucky Spindletop Research Station for the collection of Carabidae. Collection was carried out as described in the previous Section 3.3.3. During the same period, seed-fall for poison hemlock was imminent (Fig. 1.6), and umbels containing seeds were clipped, bagged, and transported back to the laboratory with collected carabid beetles. Individuals were separated into 4oz. plastic DART® containers (Dart Container Corporation, Mason, MI, USA) with a water-dampened cotton ball and maintained at 22°C with a light cycle of 14:10 (L:D). After an acclimation period of 24h during which no food was provided, individuals were presented with three poison hemlock seeds. Twenty-four hours later, remaining seeds in each container were counted and the condition of the carabid was recorded.

### 3.3.5 Predator Gut Content Analysis and the Detection of Piperidine Alkaloids

Predators were captured during the spring and summer of 2011 as described in Sections 2.3.2 and 2.3.3. Individuals captured for the purpose of chemical analysis were placed in empty individual 1.5mL microcentrifuge tubes and stored at -80°C in a SO-
LOW® freezer (So-Low Inc., Cincinnati, OH, USA). Prior to chemical analysis, specimens were identified (see Section 3.3.3) and all legs, elytra, and wings were removed. To determine how predators may be obtaining poison hemlock alkaloids in the field, *H. foeniculi* and poison hemlock seeds were included in chemical analysis. Both aphids and seeds were collected during the summer of 2011 as described in Sections 3.3.3 (aphids) and 3.3.4 (seeds). In addition to alkaloid testing, the highly concentrated seeds (Cromwell 1956, Khodzhimatov and Bobokhodzhaeva 1976) were used as a positive alkaloid control during chemical analysis. Prior to analysis, seeds were stored in 4oz. plastic DART® containers (Dart Container Corporation, Mason MI, USA) at room temperature and aphids were stored at -80°C with beetle predators. The following alkaloid analysis methods, based on Castells et al. (2005), were utilized for seeds, aphids, and predatory beetles.

Individuals (or seeds) were placed in a 2.0mL microcentrifuge tube with 1.5mL of acidified methanol solution (70% MeOH, 30% 0.1N HCl) and manually crushed using a pestle. Predator-methanol solutions were then vortexed, centrifuged (13500 rpm), and agitated for 1h, after which vortex and centrifugation (13500 rpm) were applied again. Next, 1.0mL of the remaining predator-methanol solution was removed and placed in a new 1.5mL tube with a previously perforated lid. Extracts were then concentrated in a Savant SpeedVac® concentrator coupled to a refrigerated condensation trap (1000rpm) (Savant Instruments Incorporated, Holbrook, NY, USA) for approximately 1.5h or until the volume was reduced to ~200µl. For the removal of nonpolar compounds, 150µl of HPLC grade hexane was added, and the mixture was vortexed and centrifuged (13500 rpm). The nonpolar nature of hexane caused layering in the resulting solution, thus allowing for easy removal and disposal of the top, nonpolar hexane. An additional hexane wash was performed as previously described and any residual hexane was evaporated off in the centrifugal evaporator for approximately 1m (1000rpm). Next, to basify the solution, 200µl of 10M NaOH was added. The remaining solution was extracted in 200µl of hexane containing 1ng/µl nonadecane for the internal standard. After applying vortex and centrifugate once more (13500 rpm), the hexane with internal standard portion was removed and placed in a new 1.5mL tube with a perforated lid for concentration in the centrifugal evaporator. Final extracts were concentrated to ~5µl.
The concentrated hexane was analyzed via GC-MS for the detection of piperidine alkaloids using a Hewlett-Packard® (HP®) 6890 GC with a HP® 7683 autosampler coupled to a HP® 5973 MS (Hewlett-Packard Company, Palo Alto, CA, USA). The high resolution gas chromatograph was equipped with a DB-5 column (30 m x 0.25 mm i.d. x 0.25 µm f.t.) held at 50°C for four minutes following the injection and increasing to 320°C at 10°C/minute with a scan range of 35-550 m/z. Samples showing positive results for poison hemlock-derived alkaloids using the National Institute for Standards and Technology (NIST) standard reference database were confirmed by matching the predator gut mass spectrum to the positive control mass spectrum (poison hemlock seed). Matching ions in decending order were 97, 110, 70, 82, 124, and 54 m/z.

3.3.6 Statistical Analysis

To determine the dominant predator groups among Carabidae and Coccinellidae, a Fishers exact test (PROC FREQ, SAS Institute, 2002) was applied to predation frequency of *H. foeniculi* as well as the frequency of alkaloid consumption. More specifically, comparisons between different carabid species revealed the dominant epigeal predator and the same was repeated for foliar coccinellids. A one-way ANOVA using the least squares means PROC GLM model (SAS) was used to detect differences in alkaloid concentrations between predator species screening positive for poison hemlock-derived alkaloids. The same procedure was then used to detect any significant differences in seed consumption between the three carabid species used in seed feeding trials.

3.4 Results

3.4.1 DNA Decay Rate of Hyadaphis foeniculi in the Predator Gut

Both general aphid primers and general COI primers were used to screen *C. sodalis* and *H. axridis* from laboratory feeding trials for the presence of aphid DNA following the consumption of *H. foeniculi* target material (Section 3.3.3). PCR performed on both predators’ gut contents showed negative results for aphid DNA at all decay rate
time treatments while results using general COI primers were positive, confirming the success of the extraction process (t = 0, 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48h).

3.4.2 Predator Gut Content Analysis and the Detection of Hyadaphis foeniculi

Weekly pitfall and hand sampling provided a total of 617 predators for molecular gut content analysis. None of the Carabidae involved in this analysis contained aphid DNA (Table 3.1), while 58% of Coccinellidae showed positive results (Table 3.2). Representing 78% of all lady beetles collected for gut content analysis, *H. axyridis* dominated the foliar community. *Harmonia axyridis* that screened positive for aphid DNA were sequenced and 15% contained *H. foeniculi* DNA (≥97% max identity) (Table 3.2). For Coccinellidae, *H. axyridis* showed significant dominance of foliar level predation on poison hemlock (n = 97, P = 0.005, Fisher’s Exact test). Figure 3.1 shows the relationship between rising *H. foeniculi* populations in the spring of 2011 and the subsequent rise of *H. axyridis* positive results for *H. foeniculi* DNA.
Table 3.1 Species of Carabidae collected from poison hemlock, total screened for aphid DNA, the number and percent that screened positive, and the number and percent that screened positive for *Hyadaphis foeniculi* (≥97% max identity).

<table>
<thead>
<tr>
<th>Predator Species</th>
<th>Total Screened</th>
<th>Number testing positive for aphid DNA</th>
<th>Number of beetles testing positive for <em>Hyadaphis foeniculi</em> (≥97% max identity)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agonum punctiforme</em> (Say)</td>
<td>32</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Chlaenius tricolor</em> Dejean</td>
<td>164</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Cyclotrachelus sodalis</em> (LeConte)</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Harpalus erythropus</em> Dejean</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Harpalus pensylvanicus</em> De Geer</td>
<td>68</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Poecilus chalcites</em> (Say)</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Poecilus lucublandus</em> Say</td>
<td>49</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Scarites subterraneus</em> F.</td>
<td>97</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>513</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
</tbody>
</table>
Table 3.2 Species of Coccinellidae collected from poison hemlock, total screened for aphid DNA, the number and percent that screened positive, and the number and percent that screened positive for *Hyadaphis foeniculi* (≥97% max identity).

<table>
<thead>
<tr>
<th>Predator Species</th>
<th>Total Screened</th>
<th>Number testing positive for aphid DNA</th>
<th>Number of beetles testing positive for <em>Hyadaphis foeniculi</em> (≥97% max identity)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coccinella septempunctata</em> Linnaeus</td>
<td>16</td>
<td>3 (5%)</td>
<td>0</td>
</tr>
<tr>
<td><em>Cycloneda munda</em> Say</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Harmonia axyridis</em> Pallas</td>
<td>81</td>
<td>48 (59%)</td>
<td>12 (15%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>104</strong></td>
<td><strong>51</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>
Figure 3.1 Seasonal abundance of *Hyadaphis foeniculi* (Passerini) (data points correspond to the mean number (±SE) of aphids counted per umbel per collection period) in relation to seasonal percent of *Harmonia axyridis* Pallas screening positive for *H. foeniculi* DNA, in poison hemlock in central Kentucky, 2011.
3.4.3 Seed-Feeding Trials (Carabidae)

Two-hundred and sixty carabid beetles were captured for laboratory seed-feeding trials, 192 S. subterraneus, 36 Amara convexa LeConte (Coleoptera: Carabidae), and 32 P. lucublandus. Amara convexa consumed significantly more poison hemlock seeds than S. subterraneus and P. lucublandus (F(2,257), P = 0.0005) which showed no significant differences in seed consumption. The highest average consumption rate for seeds, exhibited by A. convexa, was 1.9 seeds every 24h. Scarites subterraneus consumed an average of 1.2 seeds a day, and P. lucublandus consumed 0.8 seeds a day (Figure 3.2).

![Figure 3.2 Frequency of poison hemlock seed consumption in the carabid species Amara convexa LeConte (n = 192), Poecilus lucublandus Say (n= 32) and Scarites subterraneus F. (n = 36) during laboratory feeding trials (data points correspond to the mean number (±SE) of seeds consumed every 24h period).]
3.4.4 Predator Gut Content Analysis and the Detection of Piperidine Alkaloids

Weekly pitfall trap and hand sampling yielded 422 Carabidae and 182 Coccinellidae for chemical analysis. Fifty three predators were successfully tested for the presence of poison hemlock-derived alkaloids (35 \textit{H. axyridis}, 12 \textit{C. tricolor}, and 6 \textit{H. pensylvanicus}). Similar to the results for molecular analysis (Section 3.4.1), \textit{H. axyridis} was the dominant foliar predator in the system, with 29\% screening positive for alkaloids (Table 3.3, Fig. 3.3a). At the epigeal level, \textit{H. pensylvanicus} was obtaining alkaloids, with 50\% screening positive (Table 3.3, Fig. 3.3b). The positive control for these samples, poison hemlock seeds, showed high concentrations of alkaloid at 1.297 milligrams per gram of sample (mg / g) and a GC retention time of 7.37 min.(Tables 3.5, Fig. 3.4a). GC retention time for alkaloids detected in predator guts was 7.41 min. (+/- 0.009) with a concentration of 285.77 ng / g (+/- 64.11) in predators (Table 3.4). A Fisher’s Exact test revealed that there was no significant difference between \textit{H. axyridis} and \textit{H. pensylvanicus} for alkaloid consumption frequency (n = 4, P = 0.036, Fisher’s Exact Test) and a one-way ANOVA showed no significant differences between \textit{H. axyridis} and \textit{H. pensylvanicus} for the amount of alkaloid consumed by each species (F(1, 11), P = 0.42). Additional chemical analysis on \textit{H. foeniculi} revealed that out of 25 aphids tested, only one contained detectable alkaloids (Table 3.5, Fig. 3.4b), with a GC retention time of 7.45 min. and a concentration of 0.025 mg / g.

Table 3.3 Species of Coleoptera collected from poison hemlock, total screened for poison hemlock-derived piperidine alkaloids, and the number and percent that screened positive for alkaloids via gas chromatography-mass spectrometry.

<table>
<thead>
<tr>
<th>Predator Species</th>
<th>Total Screened</th>
<th>Number (%) testing positive for piperidine alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Chlaenius tricolor} Dejean</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Harpalus pensylvanicus} De Geer</td>
<td>6</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>\textit{Harmonia axyridis} Pallas</td>
<td>35</td>
<td>10 (29%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>53</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>
Figure 3.3 Gas chromatograms of predatory Coleoptera showing positive results for poison hemlock-derived alkaloids in (a) *Harmonia axyridis* Pallas, Coccinellidae (720.8ng / g) and (b) *Harpalus pensylvanicus* De Geer, Carabidae (148.7ng / g).
Table 3.4 Species of Coleoptera collected from poison hemlock showing positive results for poison hemlock-derived piperidine alkaloids via GC-MS analysis, the amount of alkaloid per specimen, and alkaloid concentration in relation to the mass of the specimen.

<table>
<thead>
<tr>
<th>Predator Species</th>
<th>Alkaloid conc. in sample (ng)</th>
<th>Alkaloid conc. in specimen (ng / g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Harmonia axyridis</em> Pallas</td>
<td>24.78</td>
<td>720.80</td>
</tr>
<tr>
<td></td>
<td>9.40</td>
<td>273.34</td>
</tr>
<tr>
<td></td>
<td>29.31</td>
<td>852.45</td>
</tr>
<tr>
<td></td>
<td>5.31</td>
<td>154.55</td>
</tr>
<tr>
<td></td>
<td>8.28</td>
<td>240.91</td>
</tr>
<tr>
<td></td>
<td>7.65</td>
<td>222.58</td>
</tr>
<tr>
<td></td>
<td>4.84</td>
<td>140.72</td>
</tr>
<tr>
<td></td>
<td>8.62</td>
<td>250.64</td>
</tr>
<tr>
<td></td>
<td>8.27</td>
<td>240.51</td>
</tr>
<tr>
<td></td>
<td>2.05</td>
<td>59.73</td>
</tr>
<tr>
<td><em>Harpalus pensylvanicus</em> De Geer</td>
<td>18.77</td>
<td>148.70</td>
</tr>
<tr>
<td></td>
<td>24.82</td>
<td>196.71</td>
</tr>
<tr>
<td></td>
<td>26.93</td>
<td>213.40</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>13.77</strong></td>
<td><strong>285.77</strong></td>
</tr>
<tr>
<td></td>
<td>(±/− 2.67)</td>
<td>(±/− 64.11)</td>
</tr>
</tbody>
</table>

Table 3.5 Potential alkaloid sources collected from poison hemlock showing positive results for poison hemlock-derived piperidine alkaloids via GC-MS analysis, and individual specimen alkaloid retention time, peak area, concentration, and concentration within the specimen.

<table>
<thead>
<tr>
<th>Predator Species</th>
<th>Alkaloid conc. in sample (ng)</th>
<th>Alkaloid conc. in specimen (mg / g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyadaphis foeniculi</em> (Passerini)</td>
<td>7.18</td>
<td>0.025</td>
</tr>
<tr>
<td><em>Conium maculatum</em> L. (Poison hemlock), seed</td>
<td>3747.75</td>
<td>1.297</td>
</tr>
</tbody>
</table>
Figure 3.4 Gas chromatograms of the potential alkaloid sources for predatory Coleoptera foraging in poison hemlock, showing positive results for plant-derived alkaloids in (a) poison hemlock seeds (1296799 ng / g) and (b) *Hyadaphis foeniculi* (Passerini) (25124.20 ng / g).
3.5 Discussion

Previously described in Chapter Two, generalist predators foraging in poison hemlock showing a temporal linkage to food resources provided by the plant suggested that some species may be partaking in consumer-resource interactions. Using a combination of molecular and chemical analysis techniques, the current data confirms that certain generalist predator species, such as *H. axyridis* and *H. pensylvanicus*, are regularly consuming resources derived from invasive poison hemlock. These resources include, but are not limited to, *H. foeniculi* aphids living in plant umbels, poison hemlock seeds, pollen, and other small arthropods at the foliar and epigeal levels. Furthermore, the similar geographic origin of poison hemlock, *H. foeniculi*, and *H. axyridis* may indicate that these three species exhibit pre-existing interactions, a dynamic that has the potential to negatively impact native organisms (Simberloff and Von Holle 1999; Ricciardi and Maclsaac 2000; Agrawal et al. 2005; Engelkes and Mills 2013).

One crucial component of field-based molecular studies is laboratory feeding trials to measure prey DNA decay rate in the predator gut (Greenstone et al. 2007). Information from these experiments may be used to estimate the approximate timing of a predation event as well as a basis for comparison between predator species in a field study (Greenstone et al. 2007; Eskelson et al. 2011). Methods for these experiments (Section 3.3.2) were carried out for this purpose, and individuals from *H. axyridis* and *C. sodalis* were observed to feed on the target prey, *H. foeniculi*. Despite following these experimental protocols, subsequent PCR analysis of the predator gut content yielded no positive results. Several measures were taken to deduce the source of this problem. To ensure that DNA extractions were performed properly, individuals were screened again using general COI primers (LCO1490, HCO-700ME) (Folmer et al. 1994; Breton et al. 2006). Results were positive, confirming that the extraction process was carried out correctly. Next, to ensure that regurgitation was not the source of the negative results, feeding trials were repeated for $t = 0$ and $1$ h with *H. axyridis*. Instead of placing individuals directly into alcohol after feeding, all were frozen at -20°C and then stored in ethanol one hour later to prevent regurgitation of prey. Nevertheless, PCR performed on these individuals still showed negative results for aphid DNA.
After exploring these sources of error, there remained one likely explanation for the unusual results: genetic material of stock prey had significantly degraded prior to feeding trials. *Hyadaphis foeniculi* captured for these experiments were stored at -20°C in plastic bags prior to feeding trials. These bags of prey were thawed and refrozen several times over the course of the feeding trials, likely degrading the prey DNA in the process. For the optimization of these methods, future studies of prey DNA half-life should utilize live prey during feeding trials. If the use of live prey is not possible, field-captured prey should be divided into several smaller storage containers and frozen separately.

Separation into several containers will allow for the removal of only one batch of prey at a time so it can be used immediately in feeding trials and discarded after trial completion to avoid thawing and refreezing. Past research, however, has revealed that aphid DNA remains most detectable (>50%) within approximately three to five hours following consumption by certain carabid species. Although prey DNA decay rates are highly variable depending on both predator and prey species being analyzed, it is reasonable to deduce that aphid DNA is subject to rapid decay within the beetle gut (Foltan et al. 2005; Berg et al. 2008).

In contrast to laboratory prey DNA decay rate results, molecular gut content analysis performed on field-captured predators revealed that *H. axyridis* in poison hemlock were preying upon aphids at 59% frequency. Subsequent sequencing of these individuals revealed that 25% from the aphid-positive group contained *H. foeniculi* DNA (≥97% max identity, after Hebert et al. 2002, Section 3.3.3). It is possible that the other 75% showing positive results for aphid DNA but not for *H. foeniculi* were previously foraging on other aphid species in the area surrounding poison hemlock. However, the other 75% were showing significant results for *H. foeniculi*’s very close relative, *Hyadaphis tataricae* (Aizenberg) (Hemiptera: Aphididae). In fact, these two species were once considered the same until *H. foeniculi* was redescribed as a separate species in 1984 (Voegtlin 1984). Although *H. tataricae* is not a known herbivore of poison hemlock, its preferred host, honeysuckle vine, does occur in central Kentucky and in areas adjacent to collection sites for this study, providing the most likely explanation for these results. Nevertheless, 100% of *H. axyridis* containing aphid DNA in their gut was preying upon *Hyadaphis* spp. in or around an invasive plant.
Despite the presence of aphid dropping behaviors (Fig. 2.9, Chapter 2) molecular data for Carabidae showed no evidence for aphid predation at the ground level. The likely reason for this is the nocturnal foraging behavior of carabids (Theile 1977; Kromp 1999) which does not complement the diurnal patterns of aphids (Gomez et al. 2006; Narayasndas and Alyokhin 2006). In contrast to negative molecular results, chemical analysis later revealed that ground-foraging \textit{H. pensylvanicus} was obtaining poison hemlock alkaloids. It is likely that the source of these alkaloids are poison hemlock seeds, since conclusive laboratory feeding trials previously showed that carabid beetles readily consume the toxic seeds (Fig. 3.2). In addition, \textit{H. pensylvanicus} and other Harpalini are well-documented seed-eaters in scientific literature, and have even been used for the biological control of weeds (Zhavoronkova 1969; Brandmayr 1990; Honek et al. 2003; Lundgren et al. 2006; Lundgren and Lehman 2010). The native \textit{H. pensylvanicus}, in this case, was able to overcome poison hemlock’s toxic chemistry and utilize the invader as a source of nutrition.

Alkaloids also played a role at the foliar level, and the aphid predator \textit{H. axyridis} screened positive for the chemicals as well. The source of alkaloids detected in the gut of \textit{H. axyridis} is less clear than that of \textit{H. pensylvanicus}. A natural conclusion is that coccinellids were receiving alkaloids from their aphid prey. However, chemical analysis of \textit{H. foeniculi} revealed that out of 25 individuals tested, only one showed positive results. Some aphid species are known to avoid the uptake of deleterious plant chemicals by selectively probing their host-plants when feeding (Matile 1984; Dreyer et al. 1987), although this behavior was not confirmed during this study. With very few aphids showing positive alkaloid results and 29% of \textit{H. axyridis} screening positive for the plant chemicals, it is likely that these generalists were obtaining alkaloids from alternative food sources. Pollen and extrafloral nectar, both available in poison hemlock, are rich in nutrients and are known to be consumed by generalist predators and coccinellids (reviewed in Hagen 1962; Hodek and Honek 1996). Although it is not possible to confirm the precise source of these alkaloids, this is the first study in poison hemlock to conclusively demonstrate that the plant’s chemicals are moving through the food chain to higher trophic levels. Additionally, predator-prey relationships in this system were characterized for the first time using DNA-based molecular gut content analysis.
Ecosystem interactions around the globe continue to be altered by the increasing occurrence of biological invasions (Dukes and Mooney 1999; Tilman 1999; Manchester and Bullock 2000; reviewed in Hooper et al. 2005; reviewed in Ehrenfeld 2010). Among these interactions, predation can determine the structure and function of ecosystems (Holt and Polis 1997; Sih et al. 1998), and is therefore a critical component of any study characterizing invasive species and their impacts on local communities. Previous research on poison hemlock has focused primarily on plant-herbivore relationships (Goeden and Ricker 1982; Berenbaum and Harrison 1994; Castells and Berenbaum 2006; Castells and Berenbaum 2008a,b); however, our data show that generalist predators are consuming poison hemlock resources. This study is the first to elucidate the complex web of interactions occurring between lower and higher trophic-level organisms around poison hemlock. Results confirmed for the first time that *H. axyridis* is obtaining both *H. foenicui* DNA and poison hemlock-derived piperidine alkaloids at the foliar level. Additionally, the native generalist ground predator, *H. pensylvanicus*, exhibits the ability to consume and utilize toxic plant material as a food resource. These data provide an example of the complementary use of molecular and chemical analysis techniques to study unique food web systems using multiple approaches for the most accurate assessment of consumer-resource interactions.
Chapter 4: Aphid prey suitability for developing *Harmonia axyridis*

4.1 Summary

As invasive species become increasingly ubiquitous in ecosystems across the world, resulting novel interactions with local species are rearranging community structure and function. The consequences of shifting community structure and function have been characterized for several decades. Currently, however, high numbers of established invaders have led to the forging of new relationships among the exotics themselves. This phenomenon is gaining more attention and studies are beginning to unravel the impacts of invasive-invasive interactions. Multiple invasive species have the potential to facilitate one another’s success, creating a spiral of decline in local communities.

Poison hemlock, an invasive species with a unique alkaloid-based chemistry, was introduced to North America in the 1800s. Although studies in the past have reported a lack of interactions around this plant, this research in poison hemlock has revealed food-web linkages between higher trophic level predators and the resources provided by the plant. One predator in particular, the exotic *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), is a confirmed consumer of the exotic *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae) aphid. Colonies of these aphids grow to high densities in poison hemlock umbels during the spring in central Kentucky (Fig. 1.5, Section 2.4.3), and their exposure to toxic plant alkaloids has the potential to influence life history traits of their consumer. *Harmonia axyridis* will also lay their eggs on the plant’s leaves in close proximity to aphid colonies, likely to ensure their offspring will have access to an ample food supply upon hatching (Fig. 4.1). Success at early life stages can determine an individual’s fitness as an adult.

To assess the question of whether or not poison hemlock-derived alkaloids affect *H. axyridis* growth and development, beetle eggs were collected in the field and hatched under laboratory conditions. After hatching, immature *H. axyridis* were divided into four different diet treatments, two of which included *H. foeniculi*, another equivalent aphid diet with no *H. foeniculi*, and an aphid-free control diet. Results showed that *H. axyridis* developing on a diet containing the poison hemlock aphid grew significantly faster than
those not consuming the aphid. Therefore, three exotic species, poison hemlock, \textit{H. axyridis}, and \textit{H. foeniculi}, have formed a relationship in their region of introduction that may be facilitating the success of \textit{H. axyridis} in the context of the slow-growth-high-mortality hypothesis.

4.2 Introduction

Invasive species have become an integrated part of the global landscape and are important to continue studying throughout their naturalization, as novel interactions continue to develop and change over time. Changes that come with invasion are often detrimental to entire communities and can negatively impact biodiversity and ecosystem functioning (Dukes and Mooney 1999; Tilman 1999; Manchester and Bullock 2000; reviewed in Hooper et al. 2005). For example, the introduction and subsequent invasion of a single plant species may translate to the endangerment of a competing native plant. Released from natural enemies, the invader quickly out-competes the native plant that was once the primary food resource for an important herbivore in the community. Without their primary food resource, the herbivore rapidly declines and higher trophic level organisms, such as predators and parasitoids, are now without their food (Cronin and Haynes 2004; Fonseca 2009). Some organisms, however, have the advantage of a generalized diet and can quickly adapt to the ever-changing environment brought on by invasive species. Generalist predators in particular have the ability to rapidly colonize disturbed areas (Luff, 1987), survive on limited food resources (Wiedenmann and O’Neil 1990), and become established on alternative prey (Settle et al. 1996). These organisms may eventually go on to forge relationships with invasive species, thereby reshaping entire ecosystems (Bartomeus et al. 2008; Tallamy et al. 2010).

Being a generalist, however, may present several disadvantages. While monotypic species can rely on a single food source for optimal nutrition, generalists typically require a diverse diet for increased levels of fitness (Greenstone 1979; Toft and Wise 1999). Obtaining a diverse set of prey requires energy and time spent foraging, both of which may be costly to the organism (Sih 1980; Polis et al. 1989; Finke and Denno 2005). Prey quality is also less predictable for generalist predators, and the cost of foraging may not
be worth the reward (Snyder and Clevenger 2004). Utilizing a generalized diet also risks the consumption of harmful defense compounds that may have deleterious effects on the consumer (Feeny 1976; Berenbaum 1981; Haggstrom and Larsson 1995; Francis et al. 2000). This may be especially critical for developing consumers, as success at early life stages plays an important part in determining an individual’s success as an adult (Chen et al. 2012; Garcia et al. 2012). For example, the aphidophagous Adalia bipunctata L. (Coleoptera: Coccinellidae) shows slower development and reduced adult size after consuming aphids raised on brassica plants with high concentrations of allelochemicals (Francis et al. 2000). Nevertheless, generalist predators retain the ability to strongly influence ecosystem structure and functioning (Holt and Polis 1997; Sih et al. 1998), therefore making the study of prey quality in invaded systems a critical part of invasive species assessment.

The combined use of molecular and chemical analysis techniques in poison hemlock revealed that the generalist predator H. axyridis is linked to the toxic plant via consumption of H. foeniculi, an exotic aphid that feeds on poison hemlock during the spring (C. Allen, Chapters 2 and 3). Although H. axyridis and other coccinellids are considered predominantly aphidophagous, not all aphid species provide optimal nutritive quality for the predators consuming them. For example, two different phenotypes of H. axyridis performed differentially when consuming one of two different monotypic aphid diets consisting of Aphis fabae Scopoli or Myzus persicae (Sulzer) (Hemiptera: Aphididae) (Soares et al. 2005). Futhermore, the generalist nature of coccinellids suggests that they develop and reproduce more efficiently on a mixed diet in which they can obtain a variety of proteins, lipids, vitamins, and minerals for optimal success (reviewed in Hagen 1962; Hagen 1987; Wallin et al. 1992; Nielsen et al. 2000, Mayntz 2005). Therefore, I hypothesized that monotypic aphid diets would be less suitable for developing H. axyridis, resulting in slower development, lower average adult weight, and lower overall survivorship. It was additionally hypothesized that a mixed aphid diet would provide the optimal development and maturation in H. axyridis.

To make this assessment, newly hatched H. axyridis were raised on four different diet treatments: monotypic H. foeniculi, monotypic Sitobion avenae F. (Hemiptera: Aphididae), combined H. foeniculi and S. avenae, and sugar water as a control (Table
4.1). Results showed that instead of being inhibited by a potentially toxic diet, *H. axyridis* exhibited faster development when feeding on *H. foeniculi* in both mixed and monotypic diet treatments including *H. foeniculi*. We therefore conclude that *H. foeniculi* is superior to *S. avenae* as prey for developing *H. axyridis*, despite the possibility of exposure to plant toxins. This conclusion is supported by the slow-growth-high-mortality hypothesis (Feeny 1976; Clancy and Price 1987) stating that slower development of immature stages of insects makes them more vulnerable to predation or parasitism. This study provides another example of the importance in the continued study of invasive species in their regions of introductions, as novel relationships and unexpected outcomes may affect entire communities.

4.3 Materials and Methods

4.3.1 Greenhouse Colonies of *Sitobion avenae*

To maintain colonies of *S. avenae*, UC 603 barley (L. A. Hearne Co., King City, CA, USA) was planted in Pro-Mix® BX Mycorise® planting medium (Premier Tech Horticulture, Quebec, Canada) in 239 cm³ plastic pots. Barley was grown in the University of Kentucky’s greenhouse on a 14:10 (L:D) cycle, at 23°C:18°C (L:D). Plants were watered every 48 h and fertilized with Scotts Miracle-Gro® all purpose plant food (The Scotts Company, Maryville, OH, USA) following manufacturer instructions, once weekly prior to aphid inoculation. *Sitobion avenae* aphids were then collected from winter wheat growing at the University of Kentucky Spindletop Research Farm in Fayette County, Kentucky (GPS coordinates 38°07'N, 84°30'W), and transported back to the greenhouse where they were transferred onto full-grown barley. Inoculated barley was then transferred into 24x24x24cm BioQuip Observation BugDorms (Compton, CA, USA) where aphids and plants were maintained at the above conditions with an increased fertilizing regime (every 48 h with watering) to reduce plant stress during high levels of herbivory. As plants died off, they were periodically replaced with healthy barley to maintain the colonies. Aphids being used in feeding trials were dusted from plants into a
sorting tray, placed in plastic containers, and then refrigerated at 10°C for 48 to 72h prior to use.

4.3.2 Collection of Hyadaphis foeniculi

Due to the inconsistent perennial growth habit of poison hemlock, *H. foeniculi* could not be reared in the greenhouse. Instead, *H. foeniculi* were periodically collected fresh from the University of Kentucky Spindletop Research Farm (GPS coordinates 38°07N, 84°30W) every 48 to 72 hours. Collection was carried out shaking and dusting aphids from infested plant umbels over a large white sorting tray. Next, aphids were dusted into small plastic containers and taken back to the lab where they were stored in a refrigerator at 10°C for 48 to 72 hours or until they were used in feeding trials (Section 4.3.4).

4.3.3 Collection of Harmonia axyridis

To obtain *H. axyridis* eggs (Fig. 4.1), previously collected adult beetles from the University of Kentucky Spindletop Research Station were maintained at 20°C with a 14:10 (L:D) cycle. All were provided with a water-dampened cotton ball and an *ad libitum* diet of live *S.avenae* being raised in greenhouse colonies (Section 4.3.1). Adult females were checked every 24 h for egg batches, and those that laid eggs were removed from their original container and placed into a new one with a fresh food and water supply. Egg batches remained in their original container and checked every 24h until hatching. Once hatched, each *H. axyridis* larvae was placed into individual 4oz. plastic DART® containers (Dart Container Corporation, Mason, MI, USA) and provided a small cotton ball dampened with water. Their feeding treatments are described in Section 4.3.4.
4.3.4 Feeding Trials

In the same 24 h period, sixty newly hatched individuals were randomly separated into four groups of fifteen and each group represented a different feeding treatment. Treatments included field captured *H. foeniculi*, greenhouse reared *S. avenae*, a mixture of the two aphid species, and sugar water (Table 4.1). Individuals across all treatments were provided an *ad libitum* supply of their specified diet and progression of development was recorded every 48h until all individuals reached the pupal stage (Fig. 4.2). Individuals were then reared to adulthood and weighed.

Table 4.1 Diet treatments consumed by developing *Harmonia axyridis* Pallas in laboratory feeding trials, 2012.

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 15)</td>
<td><em>Hyadaphis foeniculi</em> (Passerini)</td>
</tr>
<tr>
<td>2 (n = 15)</td>
<td><em>Sitobion avenae</em> F.</td>
</tr>
<tr>
<td>3 (n = 15)</td>
<td>50 : 50 mixed <em>H. foeniculi : S. avenae</em></td>
</tr>
<tr>
<td>4 (n = 15)</td>
<td>sugar water (control)</td>
</tr>
</tbody>
</table>
4.3.5 **Statistical Analysis**

To detect differences in development between diet treatments, a one-way ANOVA using the least squares means PROC GLM model (SAS) was applied to both development time and adult weight data.

4.4 **Results**

Survivorship, time to pupae formation, and adult weight were measured in developing *H. axyridis* for four diet treatments (Table 4.1). No individuals in the carbohydrate-rich control diet group reached the pupal stage, with 100% mortality occurring within the first week of the experiment. Developing *H. axyridis* consuming only *S. avenae* had a 73% survival rate (Fig. 4.2) and took an average of 15.2 days to reach the pupal stage, significantly more time than the two diets including *H. foeniculi* ($F_{2,33} = 6.29$, $P = 0.005$) (Fig. 4.3). Diets with *H. foeniculi* showed similar results: the monotypic diet had the highest rate of survival at 93% while the mixed diet showed 80% surviving to adulthood (Fig. 4.2). There was no significant difference between the two *H. foeniculi* diets measuring time to pupae formation, and both showed an average of 13.7 days needed for development (Fig. 4.3). No significant differences were detected for average adult weight across all treatments ($F_{2,34} = 1.94$, $P = 0.16$) (Fig. 4.4).
Figure 4.2 Percent of *Harmonia axyridis* Pallas developing on different aphid diets successfully reaching adulthood.

Figure 4.3 Effect of different aphid diets on development time (days) of the predator *Harmonia axyridis* Pallas (data points correspond to the mean number (±SE) of days developing from hatch to pupae).
Diet Treatment

Figure 4.4 Effect of different aphid diets on adult weight of the predator *Harmonia axyridis* Pallas (data points correspond to the mean number (±SE) of days developing from hatch to pupae).

4.5 Discussion

Although aphids are generally recognized as an excellent source of nutrition for Coccinellidae, different species are not always equally suitable prey (Hodek and Honek 1996; zder and Saglam 2003). As a result, many generalist predators require a diverse diet to ensure optimal development and survival (Greenstone 1979; Toft and Wise 1999). In this study, it was hypothesized that *H. axyridis* consuming a mixed aphid diet would show superior survival, development, and adult mass in contrast to individuals consuming monotypic aphid diets. A portion of individuals across all aphid diet treatments reached adulthood in this experiment, while the carbohydrate-rich sugar water diet was not a sufficient control in this study and no individuals survived. Although the mixed diet performed well, it was statistically similar to the monotypic *H. foeniculi* diet, suggesting that *H. foeniculi* is more suitable for *H. axyridis* than *S. avenae*. More specifically, results showed significant evidence for faster development and a potential for increased survivorship in *H. axyridis* consuming *H. foeniculi* (Fig. 4.3). This suggests
a beneficial relationship between the predator and prey, according to the slow-growth-
higher mortality hypothesis, stating that prolonged development increases individuals’
vulnerability to predation and parasitism (Feeny 1976; Clancy and Price 1987).

Previous studies on exotic species’ interactions have revealed similar beneficial
relationships that eventually led to harmful mass invasions. For example, the continued
release of ballast water into the Great Lakes region from the Black, Caspian, and Azov
Seas during the mid- to late 1900s ignited a series of facilitating relationships among the
introduced species that subsequently eliminated overwhelming numbers of indigenous
species in the region (Ricciardi and MacIsaac 2000). This phenomenon is known as
‘invasional meltdown’ (Simberloff and Von Holle 1999; Ricciardi and MacIsaac 2000;
Agrawal et al. 2005; Engelkes and Mills 2013), and although the current study cannot
confirm that poison hemlock and *H. foeniculi* are the reason for *H. axyridis* success, these
three Eurasian species may be facilitating one another. More specifically, the plant is
providing a beneficial and abundant food resource for *H. axyridis*, and as the predator
preys upon colonies of *H. foeniculi*, they may diminish any damage incurred by
herbivory from the aphid colonies, thereby benefitting poison hemlock.

A suite of biotic and abiotic factors have the potential to play a strong role in food
web dynamics. For example, prey quality alone may be influenced by plant architecture,
climatic conditions, and competition. Therefore, each of these factors has the power to
determine a predator’s development, reproduction, and overall success (Grevstad and
Klepka 1992; Ferran and Dixon 1993; Nielsen et al. 2002; Soares et al. 2005). One
factor not often taken into account is the role of geographic origin in prey quality. In the
case of this study, compatible geographic origins between *H. foeniculi* and *H. axyridis*
may have led to a reunion among preadapted exotic species in a new region, North
America. Increased frequency of invasive species and geographic origin may reveal
unexpected consequences, affecting local food webs on large and small scales. This study
reveals the importance of the continued investigation of invasive species interactions and
the critical role that multiple invaders may play in one another’s success.
Chapter 5: Conclusions

This is the first study to investigate multi-trophic food web dynamics around the invasive poison hemlock plant in North America. Predator collection during the spring and summer of 2011 from four transects in poison hemlock and one control transect outside of poison hemlock resulted in eight different species of Carabidae and three different species of Coccinellidae. A total of 956 carabids were obtained, 76% of which were captured in poison hemlock, while coccinellids showed an overwhelming affinity for the toxic plant, with 95% of 321 total individuals being caught in invaded areas. Resources provided by the plant were assessed and quantified via foliar aphid counts and ground-level sticky traps, revealing that *H. foeniculi* aphids and floral resources were most abundant throughout the month of June (Fig. 2.7). Although aphids exhibited dropping behaviors, several other prey taxa, especially Collembola, were more abundant at the ground level throughout the season (Fig. 2.10). In early August, however, poison hemlock seeds became much more abundant on the ground, providing an additional food resource for epigeal predators. The abundance of two predator species, epigeal *H. pensylvanicus* and foliar *H. axyridis*, showed a distinct temporal synchrony with seed shed and high aphid populations, respectively. As a consequence, the predators showed positive results for aphid DNA, plant alkaloids, or both.

The complementary techniques, DNA-based molecular gut-content analysis and gas chromatography-mass spectrometry analysis, allowed the previously collected generalist predators to be analysed and conclusively linked to their prey. Screening for plant alkaloids revealed that both *H. axyridis* and *H. pensylvanicus* were consuming resources derived from poison hemlock. However, only the foliar predator, *H. axyridis*, was preying upon *H. foeniculi* in this system. Laboratory feeding trials later confirmed that *H. foeniculi* is a more suitable prey item for developing *H. axyridis* than the alternative aphid species, *S. avenae*. Immature lady beetles reached maturity at an accelerated rate compared to individuals reared on diets excluding *H. foeniculi* and significantly prolonged development occurred in predator larvae only consuming *S. avenae*. This emphasizes the potential role of geographic origin, as poison hemlock, *H. foeniculi*, and *H. axyridis* likely coevolved together in Eurasia, allowing them to
reassociate in regions of introduction. Although *H. pensylvanicus* is native to North America, the increasing naturalization of poison hemlock over the past century and the ubiquity of both beetle and plant may have provided the necessary means for these two species to form a novel association with one another. While the effects of poison hemlock seed consumption on *H. pensylvanicus* remains unknown, carabids are well-known consumers of apiaceous plants seeds (Thiele 1977), and seed consumption has been documented as beneficial to carabids’ life histories (Jørgensen and Toft 1997; Saskia and Jarosik 2001).

The multidimensional approach of this study provides a new perspective on poison hemlock invasion and the potential outcome of invasive species’ interactions in new regions. Introduced organisms are not only affecting native species; now, the prevalence of invaders has brought forth an entirely new set of consumer-resource interactions and invaders are beginning to connect with one another. While plant chemistry can be used as a novel weapon against native consumers, other introduced organisms may find a familiar resource based on a suite of unique compounds (Janz et al. 2001), inducing community-altering relationships between multiple invaders. However, two invasive organisms are not required to interact with one another to strongly affect the communities they invade; simply the sum of multiple invaders can harm local systems by making them more susceptible to future invasions (Howarth 1985; Simberloff and Von Holle 1999; Agrawal et al. 2005; Engelkes and Mills 2013).

Therefore, it is critical to continue studying organisms post-invasion, and to consider all trophic levels. Comparable to invasive species, generalist predators can exert significant pressure on their prey (reviewed in Hagen 1962; Zhavoronkova 1969; Brandmayr 1990; Hodek and Honek 1996, Jørgensen 1997; Honek et al. 2003, Harwood et al. 2007, Moser et al. 2008, Lundgren and Lehman 2010), thereby driving community interactions (Holt and Polis 1997; Sih et al. 1998). In the case of this study, poison hemlock was providing a substrate for *H. axyridis* to develop, feed, mate, and produce offspring, while *H. pensylvanicus* forged a completely novel relationship with poison hemlock, an example of an association that could alter invaded systems in the future.
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