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The biology of Rosa multiflora (Rosaceae) and two of its biotic mortality factors

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The biology of *Rosa multiflora* (Rosaceae) and two of its biotic mortality factors

by

Laura Christine Jesse

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-majors: Entomology; Ecology and Evolutionary Biology

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CHAPTER 1: GENERAL INTRODUCTION

Dissertation Organization

The goal of this research was to examine the population biology of *Rosa multiflora* Thunb. (Rosaceae) and two of its biotic mortality factors. This dissertation is comprised of a literature review, four individual papers, and a general summary. Chapter 2 is a study to quantify the modes of spread of *R. multiflora* within a field. Allozyme markers were used to determine if large and small patches of *R. multiflora* at two locations in central Iowa consisted primarily of clones or genetically distinct plants germinated from seeds. In Chapter 3, to determine the insect pollinators visiting *R. multiflora* flowers in Iowa, I identified insects collected on yellow sticky traps placed on plants during the period of blooming. I also observed insects visiting flowers to determine their identity and their visitation rates. In Chapter 4, I examined the effects of habitat and disease symptoms on the abundance of *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae), the presumed vector of rose rosette disease, and other arthropods infesting *R. multiflora* leaves. Over a two year period, I collected *P. fructiphilus* and other arthropods on branch tips from healthy *R. multiflora* growing in the sun and shade and from plants with symptoms of rose rosette disease. In Chapter 5, I quantified the presence and distribution of larval *Megastigmus aculeatus* var. *nigroflavus* Hoffmeyer (Hymenoptera: Torymidae), a wasp whose larvae feed in the developing *R. multiflora* seeds in eastern, northeastern and southern Iowa. The effect of *M. aculeatus* infestation on the numbers of viable and aborted *R. multiflora* seeds within a rosehip was examined at 3 selected sites. The formats for Chapters 2, 3, 4, and 5 follow the guidelines for each journal.
Literature Review

*Rosa multiflora* Biology

Characteristics

*Rosa multiflora* Thunb. is native to Japan, Korea, and parts of China (Steavenson 1946), where it is not considered a weed (Hindal & Wong 1988). *Rosa multiflora* is a vigorous shrub with long arching branches that can grow to a height and width of 2.5 meters (Steavenson 1946, USDA 1971). The plant gets its name from the numerous white flowers it produces, with up to 200 flowers on a single panicle (loosely branched cluster of flowers) (Steavenson 1946). Each flower produces a single hip (fruit) containing the achenes (seeds); the fleshy hips are red and remain on the plant throughout the winter (USDA 1971). It is estimated that a medium sized plant can produce 200,000 seeds each year (J. Amrine, West Virginia Univ., pers. communication). This estimate was based upon multiplying the average number of seeds per hip (7), by the average number of hips on panicles (63), by the average number of panicles per cane (20) by the number of canes per plant (25) (Amrine, pers. communication).

Hardiness and Distribution in North America

*Rosa multiflora* was first introduced into the United States as an ornamental plant in the early 1800's (Rehder 1936). The first published record of *R. multiflora* in the U.S.A. is in the 1811 catalogue of the Elgin Botanic Gardens in New York (Rehder 1936). *Rosa multiflora* is classified by the United States National Arboretum as winter hardy through USDA hardiness Zone 5 (USDA, 2001a), which includes southern Iowa. However, the
National Arboretum criteria are designed for horticulturalists and are based upon whether an individual plant species performance in an ornamental garden situation is considered satisfactory or unsatisfactory (USDA, 2001b). A plant species may grow and survive winters north of the zones for optimal horticultural production. To determine frost resistance of several rose species, Ma & Chen (1991), exposed new shoots to −15°C for 24h, then cooled them at a rate of 2°C/1.5h to −25°C, −35°C or −45°C. Plants remained at their respective temperatures for 20h and then were warmed to 13°C for 5h. Freezing injury was determined by tissue electric resistance and by the percentage of plants that continued to grow (Ma & Chen 1991). The *R. multiflora* cultivar Albo-Plena showed 100% survival at −15°C, 93% survival at −25°C, and 53% survival at both −35°C and −45°C (Ma & Chen 1991). Based upon these results, an estimated 93% of *R. multiflora* would survive winters in Zone 5 in the U.S. (southern half of Iowa) which has a minimum temperature range of −23.3°C to −28.9°C. The Albo-Plena cultivar of *R. multiflora* would likely have a 53% survival rate through Zone 2 (central regions of Canada) where minimum winter temperatures range from −40°C to −45.6°C.

Currently, *Rosa multiflora* is distributed into southern Canada and throughout the eastern United States, and in Oregon and Washington (USDA 1971, USDA-NRCS Plants Profile). *Rosa multiflora* is considered a serious weed in the central U.S. and has been designated a noxious weed in Iowa, Illinois, Indiana, Kansas, Maryland, Ohio, Pennsylvania, Virginia and West Virginia (Amrine & Stasny 1993).
Reproduction

*Rosa multiflora* requires cross-pollination to produce seed. Self-compatibility in roses appears to be related to the ploidy level; self-compatible species typically have a higher ploidy level (Ueda & Akimoto 2001). Wild roses, e.g. *R. multiflora*, are diploid and have relatively low levels of self-compatibility. No fruit set was observed in *R. multiflora* var. *adenochaeta* that was self-pollinated (Ueda and Akimoto 2001). Fruit set in self-pollinated *R. multiflora* was documented in several clones of *R. multiflora* at different locations in Denmark, but none of the self-pollinated seeds germinated (Stougaard 1983).

Insect pollinators, including the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), bumble bee, *Bombus sp.* (Hymenoptera: Apidae), and syrphid flies (Diptera: Syrphidae) have been observed pollinating *R. multiflora* flowers in Europe and Asia. For example, *Bombus sp.*, *A. mellifera* and the syrphid fly, *Syrphus ribesii* L., visited *R. multiflora* flowers in the Netherlands (Stougaard 1983). Presumably these insects are involved in the natural pollination of *R. multiflora* in Europe. In Korea, which is within the home range of *R. multiflora*, the honey bee, *A. mellifera*, was the major pollinator of *R. multiflora* (Lee et al. 1995).

In addition to reproduction by seeds, *R. multiflora* reproduces by vegetative growth of the roots or arching branches that reach the ground (layering) (Klimstra 1956, USDA 1971). The classification of *R. multiflora* as a noxious weed is based on its invasiveness, however, the relative contribution of each mode of reproduction to the spread of *R. multiflora* is unknown. An understanding of the reproductive biology of *R. multiflora* in different environments will provide information on plant dispersal and provide a better fundamental basis for management of *R. multiflora*. 
Seed Dispersal

*Rosa multiflora* seeds are contained within a red fleshy hip, and are primarily bird-dispersed (Klimstra 1956, Scott 1965, Schery 1977, Willson 1986). Birds consume the entire rosehip, but the achenes inside are not digested by most song birds (Amrine 2002). Attributes of the bird species dispersing the seeds will influence the type of habitat in which the seed is deposited, the distance the seed is moved from the parent plant, and the density of seeds deposited in a given habitat (Chavez-Ramirez & Slack 1994). The length of time the seed is in the bird digestive tract and the post-foraging behavior will determine where and when seeds are deposited.

*Rosa multiflora* bushes are a common winter habitat for a variety of birds (Steavenson 1946, Schmid 1958, Morgan & Gates 1982), which was one of the primary reasons it was initially planted. In central New Jersey, *R. multiflora* seeds were found in the excrement of gray catbirds, hermit thrushes, American robin, and cedar waxwings captured in mist-nets from September to November (Suthers et al. 2000). Mockingbirds, *Mimus polyglottos* L., have been observed feeding on *R. multiflora* hips in New Jersey during February and March; 31.5% of the food items consumed by one individual mockingbird were *R. multiflora* hips (Stiles 1982).

Plants from bird-disseminated seeds often grow under fencerows and under isolated trees in fields (McAtee 1947). It has been observed that relatively few bird dispersed seeds are found in areas without some sort of perch site (Ferguson & Drake 1999). To determine the effect of vegetation height on bird-dispersed seeds, McDonnell (1986) collected
excrement in traps placed under artificial saplings, and found that 16.4% of seeds were from *R. multiflora*.

**Invasiveness of Rosa multiflora**

*Rosa multiflora* was planted as a living fence in pastures, as a means to control soil erosion, and for wildlife cover in the 1940's and 50's, primarily in midwestern and northeastern states (Klimstra 1956). During these two decades *R. multiflora* was considered an ideal plant for many habitat restoration needs, including livestock fences, reduction of soil erosion, and provision of wildlife cover for game birds (Burgess 1948, Edminster 1938, Steavenson et al. 1943, Steavenson 1946). Fields with hedges for fences are preferred for wildlife habitat because 60% more pheasants occur in fields with hedges (Steavenson 1946). Compared to other trees and shrubs, *R. multiflora* is used by a large number of bird species for nesting (Steavenson 1946). Rosehips also provide food for birds in the winter (Steavenson 1946). However, use of *R. multiflora* by wildlife was refuted a decade later by Klimstra (1956), when he reported that *R. multiflora* plantings were not preferred habitat for pheasants when native vegetation was available. Because insufficient research had been done prior to the promotion of *R. multiflora*, Klimstra (1956) concluded that real benefits of *R. multiflora* plantings for wildlife habitat and farm fences were not documented.

When *R. multiflora* was promoted for wildlife cover it was stated that it would not spread or become a nuisance (Steavenson 1946). However, it was subsequently observed that spread occurred due to seed dispersal and vegetative growth (Klimstra 1956). *Rosa multiflora* currently infests approx. 45 million acres (18.2 million hectares) in the eastern United States, reducing the value of land for grazing or recreational purposes because dense
patches of the plant are impassable and are not fed upon by most livestock (Underwood et al. 1996).

**Control Methods**

Because of the large seed bank, any control of *R. multiflora* must include long-term management plans to destroy new seedlings (Underwood et al. 1996). Currently mechanical and herbicidal control methods are the most commonly used methods to suppress *R. multiflora* (Underwood et al. 1996). However, infestations are common on steep slopes, along fence rows and in wooded areas that cannot be reached with spray equipment (Underwood et al. 1996). Use of herbicides is also expensive; a 10-year *R. multiflora* eradication project in West Virginia involving herbicide use on 36,500 hectares of infested land was estimated to cost more than $40 million (Amrine 1996).

Mechanical control methods such as pulling or removing individual plants are only effective when all roots are removed (Underwood et al. 1996). Mowing repeatedly will eventually kill most plants, however, a single late season mowing, which is often used in pastures, does not kill *R. multiflora* plants (Underwood et al. 1996).

Grazing sheep or goats on pastures can provide effective control of *R. multiflora* by defoliating plants (Bryan & Mills 1988, Underwood et al. 1996). Goats reduced brush cover in a pasture from 15% to 45% in a single summer (Underwood et al. 1996). However, effective use of grazing to control *R. multiflora* requires owning or obtaining sheep or goats, good fences, rotational grazing, acquiring enough animals for early summer defoliation, and adequate grass cover to minimize erosion as *R. multiflora* cover is reduced (Underwood et al. 1996).
Rose Rosette Disease

A rose with symptoms of rose rosette disease (RRD) was first observed in an unidentified rose species in Manitoba, Canada in 1940 and 1941 (Conners 1941, Conners 1942). RRD was reported in the U.S. in 1941 from an ornamental rose, *Rosa rubriflora*, in Wyoming; an unidentified native rose species was also noted to have symptoms of RRD in the same area in 1942 (Thomas & Scott 1953). Based on the disease symptoms and the ability to infect healthy plants by grafting, the causal agent of RRD was presumed to be a virus (Thomas & Scott 1953).

RRD is believed to be spread by an eriophyoid mite, *Phyllocoptes fructiphilus* (Allington et al. 1968, Amrine et al. 1988), however, Doudrick et al. (1986) could not demonstrate transmission of RRD to *R. multiflora* by *P. fructiphilus* in greenhouse trials. Doudrick et al. (1986) states that the inability to establish colonies of *P. fructiphilus* on *R. multiflora* is the reason they failed to observe mite transmission of RRD. On the basis of the studies conducted by Allington et al. (1968) and Amrine et al. (1988) it has been generally accepted that *P. fructiphilus* is the only vector of RRD.

*Phyllocoptes fructiphilus* has a typical 4 stage eriophyid mite life cycle: egg, protonymph, deutonymph and adult (Kassar & Amrine 1990). It is believed that *R. multiflora* is the primary overwintering host for *P. fructiphilus*, because mites were found overwintering on *R. multiflora* branches in Indiana that were collected in December (Kassar & Amrine 1990). In a 4-year study conducted in Indiana, Amrine (1996) observed higher mite populations on *R. multiflora* with RRD compared to populations on non-symptomatic *R. multiflora* (Amrine 1996).
The causative agent of RRD has not been isolated and identified (Epstein & Hill 1995, Epstein et al. 1997, Epstein & Hill 1999). Many attempts have been made to isolate the causal agent, but none have been successful (Rohozinski et al. 2001). Hill & Epstein (1990) found double stranded RNA associated with diseased tissue, indicating the causal agent could be a cryptic virus. Rohozinski et al. (2001) successfully transmitted a virus-like agent from *R. multiflora* to a *Nicotiana* sp., but observed no virus-like particles.

Arthropod transmission studies of RRD have only been conducted with *P. fructiphilus* and *Tetranychus urticae* Koch (Acari: Tetranychidae), the twospotted spider mite, primarily because *T. urticae* commonly infests greenhouse grown *R. multiflora*. *Tetranychus urticae* was not observed to transmit RRD (Amrine et al. 1988). My current study (Chapter 4) documented that other known vectors of plant diseases commonly occur on *R. multiflora*. Aphids, thrips, and some mites are known vectors of plant viruses (Maramorosch 1963), but it is unknown if they are involved in transmission of RRD.

RRD infects ornamental and native wild roses (Allington et al. 1968, Thomas & Scott 1953), therefore, rose growers are concerned about the effects of increasing RRD to suppress *R. multiflora* (Harwood 1994). *Rosa multiflora* is highly susceptible to RRD, thus this disease is a promising biological control agent. The causal agent of RRD needs to be identified so the risk posed to non-target roses can be quantified before RRD could be safely augmented.

*Megastigmus aculeatus* Hoffmeyer (Hymenoptera: Torymidae)

The rose seed chalcid, *Megastigmus aculeatus var nigroflavus* Hoffmeyer (Hymenoptera: Torymidae) may have the potential to reduce seed output of *R. multiflora*
(Mays and Kok, 1988; Nalepa, 1989). *Megastigmus aculeatus* is native to Asia and is believed to have been accidentally introduced into the U.S. in shipments of *R. multiflora* seeds (Weiss, 1917). Although *M. aculeatus* larvae feed on seeds of other *Rosa* spp. (Balduf, 1959), current data indicate that *M. aculeatus* var. *nigroflavous* infests only *R. multiflora* seeds (Amrine, 2002).

Female *M. aculeatus* lay their eggs directly in developing *R. multiflora* achenes (seeds within the rosehip) in May and June, soon after the petals fall from flowers (Milliron 1949, Balduf 1959). *Megastigmus aculeatus* overwinters as a larva within the achene and rosehip. In early spring the wasp pupates and emerges from the achene as an adult in May or June (Nalepa & Piper, 1994). *Megastigmus aculeatus* reprodes parthenogenically and has a strong female sex bias (Balduf, 1959). This seed predator may reduce the spread of *R. multiflora* by seed dispersal if it destroys a high percentage of seeds (Amrine & Stansy, 1993). Infestation of *R. multiflora* hips collected from 64 sites in Virginia averaged 25% with a range of 2-59% (Mays & Kok, 1988). In North Carolina, Nalepa (1989) observed an average of 42% of rose hips and 17% of seeds infested with *M. aculeatus*.

Birds commonly feed upon *R. multiflora* rosehips; *M. aculeatus* larvae within seeds are not harmed by passing through the digestive tract of birds with no gizzards (Nalepa & Piper 1994). The primary bird feeders on *R. multiflora* hips are robins, cedar waxwings, mockingbirds, cardinals and other songbirds (Klimstra 1956, Nalepa & Piper 1994). Birds are believed to be the primary means of dispersal for *M. aculeatus*, since it is presumed that the small size of the adult wasps limits long distance dispersal (Balduf 1959, Nalepa & Piper 1994). However, experiments have not quantified the dispersal of adult wasps. Because birds also spread viable seeds of *R. multiflora*, the dispersal of the wasp parallels the spread
of the host, making this a dynamic interacting system involving *R. multiflora*, a seed feeding wasp, and birds that eat and spread the seeds and wasps (Nalepa & Piper 1994).

**Objectives**

*Rosa multiflora* biology (Chapters 2 and 3)

1) Determine the relative levels of asexual and sexual spread of selected *R. multiflora* in land managed as a pasture and park.

2) Determine the identity and abundance of insect pollinators of *R. multiflora* in selected sites in Iowa.

3) Determine what insect taxa associate with *R. multiflora* during bloom, and what insect taxa are observed visiting *R. multiflora* flowers.

4) Calculate the visitation rates of common insect pollinators (i.e. *Apis* spp. and *Bombus* spp.).

Rose rosette disease (Chapter 4)

5) Examine the effects of the *R. multiflora* habitat (open vs closed canopy) on *P. fructiphilus*.

6) Examine the effects of RRD symptoms on the abundance of *P. fructiphilus* on *R. multiflora* growing in open habitats.

7) Examine the abundance of selected phytophagous arthropods (aphids, thrips, and additional mite species) found in the growing tips of *R. multiflora* branches, the same
microhabitat used by *P. fructiphilus*, because these other arthropods are potential disease vectors.

*Megastigmus aculeatus* biology (Chapter 5)

8) Determine the presence and distribution of *M. aculeatus* in Iowa.

9) Determine the levels of *M. aculeatus* infestation over 2-3 years at 3 selected sites.

10) Quantify the relationship between viable seeds, aborted seeds, and *M. aculeatus* predation.

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CHAPTER 2: QUANTIFYING THE LEVELS OF SEXUAL REPRODUCTION AND CLONAL SPREAD IN THE INVASIVE PLANT, \textit{ROSA MULTIFLORA}

A paper to be submitted to Biological Invasions

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Abstract

\textit{Rosa multiflora} Thunb. (Rosaceae), an invasive plant in the eastern U.S., was planted in the 1940’s as a living fence, for wildlife cover and to prevent soil erosion. However, \textit{R. multiflora} rapidly spread from these original plantings via seeds (sexual reproduction) and clonally, invading pasture and wooded areas. The purpose of this study was to determine the relative levels of asexual and sexual spread of selected \textit{R. multiflora} in a pasture and park. Allozyme markers were used to determine if large (> 9 m cir.) and small (< 2 m cir.) patches of \textit{R. multiflora} consisted primarily of clones or sexually reproduced plants. Larger patches of \textit{R. multiflora} tended to be dominated by one genotype, but all of the patches (N=10) contained multiple unique genotypes. Six of 10 smaller patches of \textit{R. multiflora} consisted of

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a single genotype, but 3 patches had 2 genotypes and one had 3 unique genotypes. Our results indicate that at the scale of contiguous plants (patches) *R. multiflora* is spreading clonally and through sexual reproduction. Although there were multiple genotypes in the large patches of *R. multiflora*, overall genetic diversity of large patches was consistent with clonal reproduction.

**Introduction**

The negative effects of invasive species on native species, biodiversity and ecosystem functions have been widely recognized and documented (Myers and Bazely 2003). Nonetheless, there are many non-native species in the U.S. that are not disrupting natural systems, and it is estimated that only 0.1% of all introduced species will become serious invasive species (Williamson & Fritter 1996). Life history characteristics of invasive plants are of interest to researchers because they may shed light on why certain plants are invasive and allow us to predict invasive potential before a plant is introduced into a new continent (Sakai et al. 2001). In addition, understanding the life-history characteristics of an invasive plant will provide the basis for the most effective control measures.

*Rosa multiflora* was intentionally introduced to North America from eastern Asia in the 1940’s and 50’s to serve as a living fence in pastures, as a means to control soil erosion, and to provide food and cover for wildlife (Burgess 1948, Klimstra 1956). This multi-stemmed shrub is now invasive in eastern North America and can form impenetrable thickets that exclude native plant species and decrease pasture quality. *Rosa multiflora* is an insect-pollinated outcrossing species; seeds are dispersed primarily by birds and rodents (Klimstra 1956, Scott 1965). It also reproduces clonally via roots and by layering (arching branches
that reach the ground) (Burgess 1948, Klimstra 1956, USDA 1971). When *R. multiflora* was introduced to the U.S., it was presumed that seeds would germinate only under “ideal nursery conditions” and that propagation by layering would occur infrequently, when a branch was covered with soil (Burgess 1948). Consequently, invasive spread of this species was not predicted to be a problem (Steavenson 1946). Within 10 years it was apparent that *R. multiflora* was spreading from the original plantings and was considered to be a serious emerging problem as an invasive species (Klimstra 1956).

*Rosa multiflora* reproduces by seeds and spreads clonally, but the relative contribution of each mode of reproduction to its spread has not been examined. Management of *R. multiflora* requires a reduction in both the number of plants in the area, including the seed bank, and the potential spread of these plants. Two biological control agents of *R. multiflora* that have the potential to reduce its spread have been examined in the United States. One is rose rosette disease (RRD), whose causative agent has not been isolated and identified, which can kill *R. multiflora* plants within several years (Epstein & Hill 1997). The other, *Megastigmus aculeatus* var. *nigroflavus* Hoffmeyer (Hymenoptera: Torymidae), is a wasp whose larvae develop in the seeds of *R. multiflora* (Weiss 1917). If sexual reproduction does not contribute significantly to the spread of *R. multiflora*, the wasp would have little effect on biological suppression of *R. multiflora*.

Allozyme genetic markers have been used to determine the frequency and spatial distribution of individual plants produced sexually (via seed) or asexually (via clonal spread) (Hamrick et al. 1979). In our study we used allozyme methods to quantify the relative levels of *R. multiflora* spread by seed dispersal and clonal growth within two habitats: woodland parks and open pastures. In the park setting we expected a significant contribution to spread
would be by seeds, since trees and other plants serve as perching sites for birds, one of the primary dispersers of *R. multiflora* seeds. In pasture areas, in contrast, we expected layering to be a more important determinant of invasive spread because cattle often trample branches into the ground (Klimstra 1956), potentially promoting the production of clonal plants. As clonal patches become large, however, they increasingly form suitable perching places for birds so that greater evidence of sexual reproduction (recruitment by seed) may be expected for larger patches compared to smaller patches of plants in pastures. Consequently, in addition to comparing park and pasture effects, within each habitat we also examined patches of large (> 9 m cir.) and small size (< 2 m cir.) for the relative contributions of sexual and clonal reproduction to the spread of *R. multiflora*.

**Materials and Methods**

*Collection of material*

Samples of *R. multiflora* leaf tissue were taken from two sites in central Iowa (Boone Co. and Story Co.) that are heavily infested with *R. multiflora*. The Story Co. site (N42°11', W93°10') is a county owned park with a small pond, some wooded areas and open unmowed areas that are infested with *R. multiflora*. Four of the 5 *R. multiflora* patches sampled were growing, at least partially, under a tree. The Boone Co. site (N42°8', W93°55') is a privately owned cattle pasture with grazed grassy areas with scattered trees and an adjacent wooded area. *Rosa multiflora* grows as an understory plant in the wooded areas, under trees within the pasture and in open grassy areas. All of the *R. multiflora* patches sampled were growing, at least partially, under a tree. At each site, leaves were taken from 5 pairs of patches, each pair consisting of a large and a small patch of plants separated by less than 3 m. A small
patch was defined as what appeared to be a single multi-stemmed plant; a large patch appeared to consist of multiple plants and was over 9 m. in circumference (Table 1). Four samples were taken from each small patch (< 2 m. cir.) at the cardinal points and 8 samples were taken from each large patch, also at the cardinal points and at the primary intercardinal points. Pairs of patches were separated from other pairs by at least 5 meters.

Allozyme analysis

Fresh leaves were refrigerated before 1 to 2 leaflets from each leaf were ground in a mortar and pestle with extraction buffer. Total protein extracts were absorbed through Miracloth (Calbiochem, La Jolla, CA) into 3 x 7 mm filter paper wicks, which were stored at -70°C until analysis. After an initial screening of 3 extraction buffers, 6 buffer systems, and 18 enzyme systems, we identified 6 polymorphic allozyme loci exhibiting expected subunit structures and patterns of expression. Optimal banding resolution was obtained using the extraction buffer of Mitton et al. (1979). 10% starch gels and a 0.04 M morpholine-citrate gel-electrode buffer system adjusted to pH 6.1 (Murphy et al. 1996) were used to resolve aconitase (Acoh, EC 4.2.1.3), isocitrate dehydrogenase (Idh-1, Idh-2, EC 1.1.1.42), 6-phosphogluconate dehydrogenase (Pgd-1, Pgd-2, EC 1.1.1.44), and glucose-6-phosphate isomerage (Gpi, EC 5.3.1.9). Staining protocols for individual loci followed Soltis et al. (1983).

Data analysis

For each patch, we determined the number and frequency of multilocus allozyme genotypes. Three measures of genotypic diversity and evenness were used to evaluate these
data (Ellstrand and Roose 1987, Stoddart 1983). Genotypic diversity ($D$) was calculated as Simpson’s Index modified for a finite sample size (Pielou 1969):

$$D = 1 - \frac{\sum_{i=1}^{k} n_i(n_i - 1)}{N(N - 1)}$$

where $n_i$ is the number of ramets of genotype $i$, $N$ is the number of ramets sampled, and $k$ is the number of distinct multilocus genotypes detected in the population. Values of $D$ range from 0 to 1 and measure the probability that two ramets selected at random from a population of $N$ plants will have different multilocus genotypes. Genotypic evenness (Frager 1972, Ivey and Richards 2001, Novak and Mack 2000) was measured as:

$$E = \frac{D_{obs} - D_{min}}{D_{max} - D_{min}}$$

where $D_{obs} = D$ from above, $D_{min} = [(k - 1)(2N - k)]/[N(N - 1)]$, and $D_{max} = [(k - 1)N]/[k(N - 1)]$. Values of $E$ can range from 0, if the sample population is dominated by one genotype, to 1 if each genotype in the population is represented by the same number of ramets.

Finally we calculated the observed genotypic diversity ($G_o$) for each large patch of plants (Stoddart 1983):

$$G_o = \frac{1}{\sum p_i^2}$$

where $p_i$ is the frequency of genotype $i$ in a given population sample summed over all genotypes. $G_o$ can range from 1, if there is a single genotype, to $N$, if each genotype in the sample is unique. To test if our observed genotypic diversity differed significantly from expectations for a population reproducing only sexually we used simulations to construct a
distribution of expected genotypic diversity ($G_e$) for each patch under the null hypothesis of free sexual recombination. Following Ceplitis (2001), for a patch of sample size $N$, the null distribution of $G_e$ (and its mean) was determined by calculating $G_e$ for each of 999 simulated sets of $N$ multilocus genotypes constructed from study site allele frequencies assuming sexual reproduction. These simulated values of $G_e$ were ordered from lowest to highest, the rank ($r = 1$ to 1000) of the patch’s observed value ($G_0$) determined, and the value $r/1000$ used to determine the probability ($P$) of $G_0$ under the null hypothesis of pure sexual reproduction. To evaluate the statistical significance of each $P$-value, a sequential Bonferroni correction (Rice 1989) was used to adjust testwise error rates for multiple tests on patches of size $N$ within a site. In addition to individual patch level tests, a global test of $G_0$ for all patches of size $N$ within a site was obtained using Fisher’s combined probability test (Sokal and Rohlf 1995) in which $-2\sum \ln(P)$ is distributed as chi-square with $2k$ degrees of freedom ($k =$ the number of separate tests and probabilities). Further, for patches of size $N$, we tested for significant differences in the ratio $G_0/G_e$ between study sites (park vs. pasture) using Mann-Whitney U-tests.

**Results**

Large patches (>9 m cir.) of *R. multiflora* had 2-6 genotypes, with an average (SE) of 4.2(0.3) across both field sites in central Iowa (Table 1). Although the diversity of genotypes ($D$) in large patches ranged from 0.57-0.92, many patches were dominated by a single genotype ($E$ ranged from 0-0.83 with an average of 0.36).

The observed genotypic diversity ($G_0$) for large patches in Story Co. ranged from 2.29-5.33 (Table 1). After sequential Bonferroni adjustment, only one patch (Patch 3)
differed significantly from the genotypic diversity expected from complete sexual reproduction. The observed genotypic diversity for large patches in Boone Co. ranged from 2.00-2.91 with two of the patches (Patches 1 and 3) significantly different from the expected genotypic diversity after adjustment for multiple tests. We interpret these differences from expected as evidence of clonal spread within large patches of *R. multiflora*. While only a subset of large patches exhibited significant evidence of clonal spread, a global test of $G_o$ over patches was significant for both sites (Story Co.: $P < 0.05$; Boone Co.: $P < 0.001$) indicating that overall the observed genotypic diversity was consistent with clonal spread. The difference between the ratio $G_o/G_e$ for the large patches of *R. multiflora* growing in the two field sites was significant at $P = 0.056$ (Mann-Whitney two-tailed test), indicating that different habitat management (park vs. pasture) influenced *R. multiflora* spread. Large patches at the Boone Co. site (pasture) had a genotypic diversity more consistent with clonal spread compared to the Story Co. site (park).

In contrast to large patches, small patches of *R. multiflora* had 1-3 genotypes, with an average (SE) of 0.71(0.25) genotypes across both field sites (Table 2). The diversity of genotypes ($D$) in small patches ranged from 0.00-0.83; 60% of patches (6 of 10) consisted of a single genotype ($E$ ranged from 0-1.00 with an average of 0.1).

The observed genotypic diversity ($G_o$) for small patches of *R. multiflora* in Story Co. ranged from 1.0-2.67. After sequential Bonferroni adjustment, three patches (Patch 1, 4, and 5) differed significantly from the genotypic diversity expected under sexual reproduction. Small patches of *R. multiflora* growing at the Boone Co. site had observed genotypic diversities ranging from 1.0-1.6 (Table 2), with three patches (Patches 1, 2 and 4) differing significantly from expected values with sexual reproduction.
The observed genotypic diversity of the small patches of plants ranged from 1-2.67 at Story County; and 3 of the 5 patches differed significantly from expected diversity, indicating the patches consisted of clones from one individual. At Boone Co. the observed genotypic diversity ranged from 1-1.6 (Table 2) with all patches differing significantly from the expected diversity resulting from complete sexual reproduction. A global test of $G_0$ over patches was highly significant for both sites ($P < 0.001$), indicating that *R. multiflora* stems in small patches are clones.

Small patches of *R. multiflora* in the pasture site (Boone Co.) and in the park site (Story Co.) had unique genotypes compared to the nearby large patch in 3 of the 5 pairs of patches within each field site.

**Discussion**

Our results indicate that at the scale of contiguous plants (patches up to 32 meters in circumference) *R. multiflora* at two sites in Iowa is spreading clonally. Although there were multiple genotypes in the large patches of *R. multiflora*, overall genetic diversity of large patches differed from expected if the *R. multiflora* was only reproducing sexually at both field sites in Iowa.

Small patches of *R. multiflora* often (60%) consisted of a single genotype, indicating that they were established by seeds and then spread clonally. In addition, 6 of the 10 small patches had genotypes not detected in the nearby large patch, further indicating that the initial establishment of *R. multiflora* plants is often from seeds.

Eriksson (1989) examined demographic studies of 68 plant species that reproduce clonally to determine rates of seedling recruitment into their populations. Forty percent of
the clonal plants repeatedly included seedling recruitment into their populations. Greater seedling recruitment was observed in species growing in grasslands (verses woodlands), with above ground clonal growth, phalanx style genet structure (verses guerilla style), and short distance seed dispersal (Eriksson 1989).

The benefits of clonal growth include rapid increase in size, capturing of resources, reduced mortality of young ramets, and is less costly to plants than sexual reproduction (Silvertown & Doust 1993). However, one of the primary reasons plants seldom lose the ability to reproduce sexually is that the advantages of maintaining genetic diversity outweigh the cost of sex (Silverton & Doust 1993). In simulation models with the clonal plant *Ranunculus repens* L., Soane and Watkinson (1979) found that even occasional establishment of sexually produced seedlings maintained genetic variability within populations. *Mahonia aquifolium* (Pursh) Nutt., a clonal invasive shrub in Europe, is estimated to produce approximately 50% of new ramets from seedling recruitment (Auge & Brandl 1997). Ellstrand and Roose (1987) analyzed studies of genotypic diversity in clonal plants and found that most populations of clonal plants contained multiple unique clones. The multiclonal populations usually showed intermediate levels of diversity and evenness. Species that regularly produce sexual progeny had relatively higher levels of genetic diversity (0.29-1.0 clones / sample) (Ellstrand & Roose 1987). We observed a similar range (0.25-0.75) in the number of clones / sample size in the large patches of *R. multiflora* indicating that it also regularly reproduces sexually.

*Rosa multiflora* seeds are contained within a red fleshy hip and are dispersed primarily by birds and rodents (Klimstra 1956, Scott 1965, Schery 1977, Willson 1986). *Rosa multiflora* bushes are a common winter habitat for a variety of birds (Steavenson 1946,
Schmid 1958, Morgan & Gates 1982), which was one of the primary reasons it was initially planted in the United States. In a 43 hectare area in central New Jersey, Suthers et al. (2000) observed *R. multiflora* seeds in the excrement of gray catbirds, hermit thrushes, American robin, and cedar waxwings captured in mist-nets from September to November. Stiles (1982) observed mockingbirds, *Mimus polyglottos* L., feeding on *R. multiflora* hips in New Jersey during February and March; 31.5% of the food items consumed by one individual mockingbird were *R. multiflora* hips.

Plants from bird-disseminated seeds often grow under fencerows and isolated trees in fields (McAtee 1947). Generally, relatively few bird dispersed seeds are found in areas without some sort of perch site (Ferguson & Drake 1999). In a study conducted in New Jersey to determine the effect of vegetation height on bird-dispersed seeds, McDonnell (1986) observed that 16.4% of seeds found in bird excrement collecting traps placed under artificial saplings were from *R. multiflora*. During the winter of 2003-2004, we collected an average of 125 seeds and 151 hips in four 1 m² boxes placed underneath four *R. multiflora* bushes in eastern Iowa. The whole hips may have been knocked off the bush by wind or foraging mammals. The individual seeds may have been in a hip consumed by a bird or chewed apart by a rodent or other mammal. Our findings demonstrate that *R. multiflora* seeds collect under established plants indicating that established plants serve as recruitment sites for *R. multiflora* seedlings.

Invasive plants that have been introduced multiple times into a new environment will presumably have higher genetic diversity (Pappert et al. 2000, Khudamrongswat et al. 2004). For example, kudzu, *Pueraria lobata* (Willd.) Ohwi, an invasive ornamental that was introduced multiple times into North America was found to have a Simpsons D average of
0.69 and E (Fragers) of 0.74 (Pappert et al. 2000) indicating a high level of diversity.

Compared to kudzu, *R. multiflora* had a slightly higher level of diversity, but lower evenness (average E=0.36). Giant reed, *Arundo donax* L., an aquatic invasive weed in the U.S., is presumed to only reproduce asexually. However, a moderate level of genetic diversity (average of 0.36 clones / plants sampled) was found in populations in California (Khudamrongksawat et al. 2004). Possible sources of diversity include somatic mutation, rare sexually produced individuals, heterogeneous environments, and multiple introductions of several genotypes (Khudamrongksawat et al. 2004). It is hypothesized that seedling recruitment is common in *M. aquifolium*, approximately 50% of new ramets are from seeds, because it was introduced as an ornamental, so plants may have been selected for high flower and seed production (Auge and Brandl 1997).

Our studies have documented that *R. multiflora* is spreading clonally, but like many clonal plants, has retained genetic diversity. The genotypic diversity of invasive weeds may affect the levels of control, e.g. high levels of genetic diversity may allow resistance to management practices, including biological control organisms (Khudamrongksawat et al. 2004). This genetic diversity may be a contributing factor in the difficulties managing *R. multiflora*. For example, *R. multiflora* in Iowa is very susceptible to rose rosette disease, however resistant individuals may be selected for, making this a less effective in the future. Seedling recruitment occurs in *R. multiflora* populations, so biological controls that reduce seed output may reduce spread. We observed a reduction in viable seeds per rosehip at *R. multiflora* sites when *M. aculeatus* larvae consume viable seeds (Chapter 5). It will likely be necessary to combine mechanical controls with any biological control for effective *R. multiflora* management. Dead *R. multiflora* plants are woody and will remain upright for
several years, serving as a focal point for bird perches and will aid in protecting new seedlings from grazing animals. Therefore, removing dead plants by mowing or burning should be considered as a component of *R. multiflora* management.

**Acknowledgements**

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Table 1: Genetic diversity in large patches of *R. multiflora* (*N* = 8 samples / patch) growing in two sites (Story Co. and Boone Co.) in central Iowa. *k* = the number of distinct multilocus genotypes per patch, *D* = genotypic diversity, *E* = evenness, *G₀* = observed genotypic diversity, *Gₑ* = the mean of simulated *Gₑ* values for a patch of size *N*. *P*-values in bold are significant after sequential Bonferroni adjustment.

<table>
<thead>
<tr>
<th>Site</th>
<th>Patch</th>
<th>Circ. (m)</th>
<th>k</th>
<th>k/N</th>
<th>D</th>
<th>E</th>
<th><em>G₀</em></th>
<th><em>Gₑ</em></th>
<th><em>P</em></th>
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<td>0.63</td>
<td>0.86</td>
<td>0.56</td>
<td>4.00</td>
<td>0.77</td>
<td>0.283</td>
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<tr>
<td>Story 2</td>
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<td>4</td>
<td>0.50</td>
<td>0.82</td>
<td>0.83</td>
<td>3.56</td>
<td>0.69</td>
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<tr>
<td>Story 3</td>
<td>18.3</td>
<td>4</td>
<td>0.50</td>
<td>0.64</td>
<td>0.00</td>
<td>2.29</td>
<td>0.44</td>
<td>0.024</td>
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<tr>
<td>Story 4</td>
<td>13.4</td>
<td>6</td>
<td>0.75</td>
<td>0.92</td>
<td>0.60</td>
<td>5.33</td>
<td>1.03</td>
<td>0.648</td>
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<tr>
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<td>5</td>
<td>0.63</td>
<td>0.78</td>
<td>0.00</td>
<td>3.20</td>
<td>0.62</td>
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<td>0.42</td>
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<td>2.91</td>
<td>0.53</td>
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<td>0.57</td>
<td>0.10</td>
<td>2.00</td>
<td>0.36</td>
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<td>0.043</td>
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<td>0.75</td>
<td>0.50</td>
<td>2.91</td>
<td>0.53</td>
<td>0.043</td>
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Table 2: Genetic diversity in small patches (<2 m cir.) of *R. multiflora* (4 samples / patch) growing in two sites (Story Co. and Boone Co.) in central Iowa. Parameters are as described in Table 1.

<table>
<thead>
<tr>
<th>Site</th>
<th>Patch</th>
<th>k</th>
<th>D</th>
<th>E</th>
<th><em>G₀</em></th>
<th><em>Gₑ</em></th>
<th><em>P</em></th>
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<td>0.00</td>
<td>1.00</td>
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<tr>
<td>Story 5</td>
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<td>0.007</td>
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<td>1.60</td>
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<td>1.00</td>
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<tr>
<td>Boone 5</td>
<td>2</td>
<td>0.50</td>
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CHAPTER 3: INSECT POLLINATORS OF THE INVASIVE PLANT, 

*Rosa multiflora* (Rosaceae)

Laura C. Jesse¹, John J. Obrycki², Kirk A. Moloney³

A paper to be submitted to Weed Biology and Management

Abstract

Invasive species often require mutualistic relationships to successfully invade new environments. Insect pollination is an example of a mutualism that is required for seed set in the invasive species, *Rosa multiflora* Thunb. (Rosaceae), an obligate outcrosser. To determine the insect pollinators visiting *R. multiflora* flowers in Iowa we collected insects on yellow sticky traps placed on plants during the period of blooming and visually observed insects visiting flowers. Common insect orders collected on sticky traps included Coleoptera, Diptera, Hemiptera, Hymenoptera, Mecoptera, and Thysanoptera. Many of the insects found on the sticky cards are pollinators or feed on pollen. However we did not collect Apidae (bumble bees and honey bees) on the sticky cards. We observed *Bombus* sp.

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and *Apis mellifera* foraging on flowers. Syrphidae were the most commonly observed taxa visiting flowers. Our results indicate that *R. multiflora* is utilizing common generalist insect pollinators in Iowa and pollination is not a limiting factor for this invasive species.

**Introduction**

Research on the ecology of invasive plants has traditionally focused on how competitive interactions affect the structure of a community and the ability of the invading plant species to establish (Callaway & Aschehoug 2000, Elton 1958, Levine & D’Antonio 1999, reviewed by Richardson et al. 2000). Invading plants with no close relatives within their new community are predicted to be more successful since they avoid direct competition for resources (Richardson et al 2000). Alternatively, invaders may also be more able to co-opt mutualistic relationships (e.g. pollination) of native species that are close relatives (Richardson et al. 2000)

When a species invades a new area it leaves behind associations it has evolved within its native range (Parker 1997). This may be beneficial to the invading plant when herbivores that negatively affect its population growth are left behind (Keane & Crawley 2002, Blossey & Notzold 1995). However, co-evolved mutualistic relationships are also severed, and this could potentially limit population growth of an invading species (Parker 1997). Establishing mutualistic interactions with organisms already established in the new ecosystem may greatly enhance the ability of the invader to establish and spread (Richardson et al. 2000, Simberloff and Von Holle 1999).

Pollination is one mutualistic relationship that has been studied for several invasive plant species (Barthell et al. 2001, Chittka & Schurkens 2001, Grabas & Laverty 1999,
Parker 1997, Brown & Mitchell 2001, Larson et al. 2002, Parker & Haubensak 2002, Waites & Agren 2004, Simpson et al. 2005, Morales & Aizen 2006). Many highly successful alien plants are self-fertilizing (e.g., garlic mustard (Alliaria petiolata), cheatgrass (Bromus tectorum)), but there are many invaders that are obligate out-crossers (e.g., purple loosestrife (Lythrum salicaria), spotted knapweed (Centaurea maculosa), multiflora rose (Rosa multiflora)). When a self-incompatible plant species that is insect pollinated is introduced in a new environment, its pollinators must be introduced with the plant, or the plant must attract insect species as pollinators to successfully reproduce (Parker 1997, Simberloff & Von Holle 1999). Pollinators may also be a factor in determining the rate of spread in invasive plants that do not spread clonally (Parker & Haubensak 2002).

*Rosa multiflora* Thunb. (Rosaceae) currently infests 45 million acres (18.2 million hectares) in the eastern half of the United States, reducing the value of land for grazing or recreational purposes because dense patches of the plant are impassable and are not utilized by most livestock (Underwood et al. 1996). *Rosa multiflora* has been declared a noxious weed in 9 states, including Iowa (Amrine & Stasny 1993). *Rosa multiflora* is a vigorous shrub with long arching branches that can grow to a height and width of 2.5 meters (Steavenson 1946). It produces numerous panicles, with up to 200 flowers on a single panicle (loosely branched cluster of flowers) (Steavenson 1946). Each flower produces a single hip (fruit); the fleshy hips are red and remain on the plant throughout the winter. A medium sized plant can produce 200,000 seeds per year (J. Amrine, West Virginia Univ., pers. communication). We are not aware of any published data on the pollinators of *R. multiflora* in North America, however honey bees, *Apis mellifera* (Hymenoptera: Apidae), bumble bees, *Bombus sp.* (Hymenoptera: Apidae), and syrphid flies (Diptera: Syrphidae)
have been observed visiting *R. multiflora* flowers on other continents. For example *Bombus* *sp.*, *A. mellifera* and the syrphid fly, *Syrphus ribesii*, visited *R. multiflora* flowers in the Netherlands (Stougaard 1983). Presumably these insects are involved in the natural pollination of *R. multiflora* in Europe. In Korea, part of the home range of *R. multiflora*, the honey bee, *A. mellifera*, was the major pollinator of *R. multiflora* (Lee et al. 1995).

The objectives of this study were to: 1) determine what insect taxa associate with *R. multiflora* during bloom, and what insect taxa are observed visiting *R. multiflora* flowers; and 2) calculate the visitation rates of common insect pollinators (i.e. *Apis* spp., and *Bombus* spp.).

**Materials and Methods**

**Sticky card samples**

In 2002, yellow sticky cards (28 x 22.8 cm) (Pherocon AM from Trece Incorporated) were used to sample insects occurring on *R. multiflora* plants during flowering (late-May to mid-June in Iowa). Sticky cards were set up at three sites in eastern Iowa (Johnson Co.) on May 25 and 26 and at two sites in central Iowa (Story Co.) on May 29, when *R. multiflora* had buds but was not yet flowering (Table 1 describes sites). At each site cards were placed on five *R. multiflora* plants growing approximately 4 m apart and were replaced weekly for three weeks, for a total of 15 sticky cards per site. Each card was folded open around the middle of a branch and the ends were secured to each other with staples or binder clips. The branches used all had flower buds and were about 1.5 m above the ground.

In 2003, sticky cards were placed on 3 plants at each of 4 sites (Table 1). As in 2002, the cards were replaced weekly for three weeks (a total of 36 sticky cards). Two sites were
located in Jackson Co. and 2 sites were located in Allamakee Co. in northeastern Iowa; all sites were pasture areas.

Each sticky card collected in 2002 and 2003 was divided into 63 (2.54 cm x 2.54 cm) squares. Ten squares were randomly selected on each card and the insects in those squares were identified to Order and, if possible, to Family. The number of insects on the 10 squares was used to estimate the number of insects on the entire card. Results are presented as the average number of insects per card for the entire sampling period.

Field observations

For three years, 2002-2004, direct observations were made of flowering *R. multiflora* to determine what insects were visiting the flowers. In 2002, observations and collections of pollinators were conducted in early June at two sites in Story Co. in central Iowa and 2 sites in Johnson Co. in eastern Iowa (Table 1). Four plants at each site were observed for 10 minutes each between 10:00 and 14:00 hours.

In 2003 and 2004, observations of pollinators were conducted at three sites infested with *R. multiflora* in central Iowa (Christiansen and Dakins Park in Story Co and a pasture in Boone Co. (Table 1)). We defined a pollinator as an insect that was on a *R. multiflora* flower and appeared to be actively consuming or collecting pollen. At each site, three *R. multiflora* growing in full sun were selected for observations. Two branches on each plant were observed for 10 minutes each. Observations were conducted between 10:00 and 15:00 hours.

In 2003 observations were conducted at Christiansen Park on June 4 and 10, Dakins Park on June 7, 12, and 16 and at the Boone Co. site on June 5, 10, and 13. In 2004 observations were conducted at Christiansen Park on May 28 and 31, Dakins Park on May
31, June 6 and 8, and at the Boone Co. site May 29, June 5 and 7. In 2003, when a honey bee (Apis mellifera) or bumble bee (Bombus sp.) was observed on a R. multiflora, the number of flowers visited on the plant was recorded to determine the visitation rate (number of flowers visited per minute).

Results

In 2002 and 2003 we collected Insecta, Acari, Arachnida, Opiliones on sticky cards on R. multiflora bushes during flowering. Insecta was the most common taxon, represented by 11 Orders and 48 Families in 2002 and 9 Orders and 33 Families in 2003 (Fig. 1). We report only groups that had at least three individuals captured during the entire sampling period (Fig. 1). We collected many species of Diptera, Coleoptera, Hymenoptera and other arthropod orders that could not be identified to family. We did not catch any Apidae on the sticky cards, possibly because they avoided the cards or were able to pull loose from the cards. Many of the insect families captured by the sticky cards are known to feed on pollen and serve as pollinators. There were fewer insect families collected on the sticky cards in 2003 (Fig. 1). This could be due to differences in sampling (fewer field sites in 2003) or to differences in the field sites; in 2002 we sampled R. multiflora growing in park areas and in 2003 we sampled R. multiflora growing in pastures.

Very few insect pollinators were observed visiting R. multiflora flowers in 2002. Insects were observed on flowers of 2 of 4 plants at Christiansen, 4 of 4 at Dakins, 1 of 4 at Kent, 3 of 4 at Frytown and 1 of 4 at Redbird. Apis mellifera and Bombus sp. were observed, but were not common. Sryphid flies were the most commonly observed pollinators. Insects
collected from flowers included Syrphidae, Formicidae, Chrysopidae, Cantheridae, Miridae, and Bombylidae.

In 2003, 99 individual pollinators were observed visiting *R. multiflora* flowers. Syrphidae were the most common pollinators, comprising 43% of the insects observed (Fig. 2). Other dipteran species were the second largest group (35%), which consisted primarily of calypterate muscoid flies. *Apis mellifera*, *Bombus* sp., and solitary bees made up 21% of observed pollinators.

In 2003 we counted the number of flowers visited by *A. mellifera* and *Bombus* species. Fourteen *A. mellifera* were observed at Christiansen Park (on June 4) and the Boone Co. site (June 5 and 13). The *A. mellifera* had a foraging rate of 9.7±0.6 flowers per minute. Five *Bombus* species were observed at Christiansen Park on June 4. *Bombus* species foraged at a rate of 15.6±3.4 flowers per minute.

In 2004, we observed 106 insects visiting *R. multiflora* flowers. Syrphids made up almost half of the insects observed (Figure 2). Honey bees and other Diptera (primarily calypterate muscoid flies) were the second most common pollinators. We observed 12 Coleoptera visiting flowers, most were Cantharidae and Staphylinidae.

**Discussion**

Our results indicate that similar pollinating insects visit *R. multiflora* in Iowa compared to other continents (Stougaard 1983, Lee et al. 1995). The two most common pollinators we observed visiting flowers were Syrphidae (hover flies) and *A. mellifera* (European honey bee), followed by other species of flies. *Bombus* sp. (bumble bees) and solitary bees, and Coleoptera were also observed visiting flowers. In the Netherlands,
Stougaard (1983) observed *Bombus* sp., *A. mellifera*, and Syrphidae and in Korea, *A. mellifera*, was the major pollinator of *R. multiflora*, and Diptera and Coleoptera were also observed visiting flowers (Lee et al. 1995).

In addition to the insects observed visiting flowers, a wide variety of insects were captured on sticky cards placed on flowering *R. multiflora* branches. Calypterateatae (Diptera), thrips (Thysanoptera), and Chalcidoidae (Hymenoptera), were common insects trapped on the sticky cards. These insects are known to feed on pollen and could serve as pollinators of *R. multiflora*. Some of the other insects found on the sticky cards, are probably feeding on *R. multiflora* leaves; Cicadellidae (Hemiptera), Aphididae (Hemiptera), Membracidae (Hemiptera), Fulgoridae (Hemiptera). Other insects captured on the sticky cards may have been attracted by the dead insects (Panorpidae), or are very common in the habitats where *R. multiflora* grows (Dolichopodidae, Cecidomyidae, Phoridae, Rhagionidae).

For some invasive plants, the rate of spread can be limited by lack of pollinator visits (Parker 1997, Larson et al. 2002, Parker & Haubensak 2002). For example, Scotch broom (*Cytisus scoparius*) and French broom (*Genista monspessulana*), and Japanese honeysuckle (*Lonicera japonica*) are three invasive plants that require insect pollinators and have been found to have low seed set due to a lack of insect pollinators (Parker 1997, Larson et al. 2002, Parker & Haubensak 2002). Although these plants are currently spreading it seems likely that lack of pollinators is reducing the rate of invasion in at least part of their range (Parker 1997, Parker & Haubensak 2002). Our study did not examine the effects of pollinators on output of viable seeds by *R. multiflora*. However, we have observed low numbers of viable seeds in dissected rosehips (Jesse, unpublished data). It is possible that an increase in insect visitation rates could increase seed output of *R. multiflora*. 
Our results are similar to previous studies examining pollinators of invasive weeds. For example, in Washington, the primary pollinators of the invasive shrub, *Cytisus scoparius* (Scotch broom), were *Bombus* sp. and *A. mellifera* (Parker 1997).

*Apis mellifera* appears to play an important role in pollinating invasive plants, although it was not the most common insect observed. *A. mellifera* comprised 14% of observed pollinators of *R. multiflora* in 2003 and 12% in 2004. In other studies *A. mellifera* was observed pollinating Scotch broom, *Cytisus scoparius*, and French broom, *Genista monspessulana* (Parker & Haubensak 2002) and was the most common pollinator of purple loosestrife, *Lythrum salicaria* (Grabes & Laverty 1999), and yellow star-thistle, *Centaurea solstitialis* (Barthell et al. 2001). It is not surprising that a generalist pollinator like *A. mellifera*, which has been introduced around the world for apiculture and pollination of fruit trees, is also serving as a pollinator for introduced plants.

Invasive plants with showy flowers or high nectar content may out-compete native plants for pollinators. Chittka & Schurkens (2001) found a reduction in pollinators and a corresponding reduction in seed set by the native *Stachys palustris* when grown with the invasive Indian balsam, *Impatiens glandulifera*. Purple loosestrife, reduced seed set in nearby native plants (*Eupatorium maculatum*, *E. perfoliatum*, *Impatiens capensis*) by pollen contamination or directly competing for pollinators (Grabas & Laverty 1999). Similarly, Brown et al. (2002) found reduced seed set in the native *L. alatum* when grown with *L. salicaria*. Future research is needed to determine if *R. multiflora* could be reducing pollination of native roses or other species blooming during the same time period.

In summary, it appears that there is a variety of insects in Iowa that serve as pollinators of *R. multiflora*. We observed common insect pollinators, such as syrphid flies,
A. mellifera, and Bombus sp. More research is needed to determine if low rates of pollination could be the cause of low numbers of viable seeds observed in dissected R. multiflora hips (Jesse, unpublished data).

Acknowledgements

We would like to thank the Story County Conservation Board for letting us conduct this survey in their parks. This research was partially funded by a grant from the College of Agriculture at Iowa State University. Journal Paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project Number 6628, supported by the Hatch Act and State of Iowa funds.

Literature Cited


Table 1: Description of the Iowa field sites used in this research that are infested with *R. multiflora*.

<table>
<thead>
<tr>
<th>Site</th>
<th>County</th>
<th>Owner</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dakins Lake</td>
<td>Story</td>
<td>CCB</td>
<td>Heavily infested park, plants in unmowed grassy areas and under trees adjacent to lake.</td>
</tr>
<tr>
<td>Christainsen Forest Preserve</td>
<td>Story</td>
<td>CCB</td>
<td>Moderate infestations in northern half of park in un-mowed areas. Forest under story with plants, esp. along the southern forest edge.</td>
</tr>
<tr>
<td>Redbird Farm</td>
<td>Johnson</td>
<td>Iowa DNR</td>
<td>Grassy slopes of heavily infested farm. Management with herbicides and mowing on parts of infestation.</td>
</tr>
<tr>
<td>Frytown</td>
<td>Johnson</td>
<td>CCB</td>
<td>Study site in infestation in an open sunny area that appears to have been planted with rows of shrubs.</td>
</tr>
<tr>
<td>Kent Park</td>
<td>Johnson</td>
<td>CCB</td>
<td>Study site east of Conservation Education Center in a ravine surrounded by trees, but mainly open and sunny.</td>
</tr>
</tbody>
</table>

CCB=County Conservation Board
Figure 1: The average number of insects per sticky card in a) 2002 (75 sticky cards) and b) 2003 (36 cards). The number of insects on the total card was estimated from a sub-sample of 10 of 63 squares. Solid bars indicate insect groups that are known to serve as pollinators or eat pollen.
Figure 2: The relative abundance of insect pollinators visiting *R. multiflora* at three sites in central Iowa over 8 sampling dates in late May or early June in a) 2003 and b) 2004.
CHAPTER 4: ABUNDANCE OF ARTHROPODS ON BRANCH TIPS OF THE
INVASIVE PLANT *ROSA MULTIFLORA* (ROSACEAE)

Laura C. Jesse\textsuperscript{4}, Kirk A. Moloney\textsuperscript{5} & John J. Obrycki\textsuperscript{6}

Weed Biology & Management (in review)

Abstract

*Rosa multiflora* Thunb. (Rosaceae) is an invasive species in the United States where it grows in pastures and wooded areas. A disease of unknown etiology, rose rosette disease (RRD), infects *R. multiflora* and other *Rosa* sp.. The goal of this research was to determine the effects of habitat and disease symptoms on the abundance of *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae), the presumed vector of RRD, and other arthropods on *R. multiflora*. We collected branch tips from healthy *R. multiflora* growing in the sun and shade, as well as RRD infected *R. multiflora* growing in the sun. Samples were collected June 2002 to April 2004 from three sites in Iowa, U.S.A. Samples were collected approximately every two weeks during the summer, monthly during the fall, and once during the early spring. Fall samples were only taken from RRD infected plants since they retain leaves throughout the winter. We found that *Phyllocoptes fructiphilus* was present on diseased and healthy *R.*

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multiflora growing in the sun and on healthy R. multiflora growing under trees (shaded), but the greatest numbers were observed on the diseased plants growing in the sun. Several other mite species, both predatory and phytophagous, Chaetosiphon sp. aphids, and thrips species Frankliniella exigua Hood and Neohydatothrips variabilis Beach occur in the same plant microhabitat as P. fructiphilus. Future research needs to isolate and identify the causal agent of RRD so it can be confirmed that, of the many arthropods feeding on R. multiflora, only P. fructiphilus vectors RRD.

Key words: Rosa multiflora, rose rosette disease, invasive species, biological control, non-target effects

Introduction

Rosa multiflora Thunb., (Rosaceae: Rosa) native to Japan, Korea, and parts of China (Steavenson 1946), was first introduced into the United States as an ornamental plant in the early 1800’s (Rehder 1936). In the 1940’s and 1950’s R. multiflora was planted as a living fence, for wildlife habitat, and as a means to control soil erosion in the eastern and midwestern U.S. (Klimstra 1956). Living fences are plants grown closely together for the purpose of containing livestock; R. multiflora and osage orange, Maclura pomifera (Raf.) Schneid. (Moraceae), were promoted as an affordable fencing alternative, and to make farms better habitat for game animals by providing wildlife cover (Edminster 1938, Lewis 1941, Steavenson et al. 1943). Initially, it was assumed that R. multiflora would not spread extensively or invade pastures and wooded habitats (Steavenson 1946), however, within a
decade *R. multiflora* was spreading due to seed dispersal and vegetative growth (Klimstra 1956).

*Rosa multiflora* currently infests approximately 45 million acres (18.2 million hectares) in the eastern United States, reducing the value of land for grazing and recreational purposes because dense patches are impassable and are not consumed by most livestock (except goats) (Underwood et al. 1996). *Rosa multiflora* has been declared a noxious weed in 9 states, including Iowa (Amrine & Stasny 1993). Infestations are most common in pastures and in nearby forested areas; similar to areas where it was intentionally planted (Edminster 1938, Steavenson et al. 1943, Steavenson 1946, Burgess 1948).

Because of a large seed bank, any reduction of *R. multiflora* must include long-term management plans to destroy new seedlings as they appear (Underwood et al. 1996). Currently mechanical and herbicidal control methods are most commonly used to suppress *R. multiflora* (Underwood et al. 1996). These methods are expensive, time consuming, often ineffective, and have negative environmental effects, including destruction of non-target vegetation (Underwood et al. 1996).

Biological control may offer a long-term, viable solution to *R. multiflora* management; one macro-organism and one disease have been identified as having potential to provide biological suppression of *R. multiflora*. The first is *Megastigmus aculeatus* (Hymenoptera; Torymidae), whose larvae feed in *R. multiflora* seeds. The second is rose rosette disease, a disease with unknown etiology and uncertain classification, which reduces plant vigor and eventually kills *R. multiflora*. Despite extensive study, the causative agent of rose rosette disease has not been identified (Epstein and Hill 1999).
Symptoms of rose rosette disease (RRD) (witches broom or mosaic discoloration) were first reported in an unidentified rose species in Manitoba, Canada in 1940 and 1941 (Conners 1941, Conners 1942). RRD was reported in the U.S. in 1941 from an ornamental rose, *Rosa rubriflora*, in Wyoming; an unidentified native rose species was also noted to have symptoms of RRD in the same area in 1942 (Thomas & Scott 1953). Based on the disease symptoms and the ability to infect healthy plants by grafting, the causal agent of RRD was believed to be a virus (Thomas & Scott 1953). Additionally, symptoms are observed when diseased plants are treated with the antibiotic tetracycline, which kills bacterial pathogens. Evidence also suggests that the agent is not a phytoplasma, because tests using polymerase chain reactions with primers known to detect phytoplasma failed to detect this type of pathogen (Epstein & Hill 1999). Thus, the causal agent, which is not identified, is presumed to be a virus.

RRD is presumed to be spread by an eriophyid mite, *Phyllocoptes fructiphilus* (Acari: Eriophyidae) (Allington et al. 1968, Amrine et al. 1988), however, Doudrick et al. (1986) could not demonstrate transmission of RRD to *R. multiflora* by *P. fructiphilus* in greenhouse trials. Doudrick et al. (1986) states that the inability to establish colonies of *P. fructiphilus* on *R. multiflora* is the reason they failed to observe mite transmission of RRD. On the basis of the studies conducted by Allington et al. (1968) and Amrine et al. (1988), it is generally accepted that *P. fructiphilus* is the only vector of RRD.

*Phyllocoptes fructiphilus* has a 4 stage eriophyid mite life cycle: egg, protonymph, deutonymph and adult (Kassar & Amrine 1990). *Phyllocoptes fructiphilus* were found on *R. multiflora* branches collected in December in Indiana (Kassar & Amrine 1990). In a 4-year
study conducted in Indiana, Amrine (1996) observed higher mite populations on *R. multiflora* with RRD compared to mite populations on healthy *R. multiflora* (Amrine 1996).

Previous researchers have noted an apparent lower incidence of RRD in multiflora rose plants growing in shaded areas under trees (Epstein & Hill 1995). It is unknown whether this is due to physiological differences in the plants (e.g., effects of lower light intensity on plant growth which may influence the disease) or if the mite prefers sunny locations (e.g., due to differences in mite dispersal behaviors). If differences in *P. fructiphilus* occurrence on *R. multiflora* in open canopy (sunny habitats) vs. plants growing in the understory (shaded habitats) are observed, this may indicate that RRD is not an effective mortality factor of plants in shady habitats, if RRD is only vectored by *P. fructiphilus*. Many *R. multiflora* infested pastures are adjacent to wooded areas that also contain multiflora rose. *Rosa multiflora* plants in these wooded areas may remain unaffected by RRD and serve as a seed source to re-infest pastures.

The objectives of this study were to: 1) document the effects of the *R. multiflora* habitat (open vs closed canopy) on *P. fructiphilus*, 2) examine the effects of RRD symptoms on the abundance of *P. fructiphilus* on *R. multiflora* growing in open habitats, 3) quantify the abundance of selected phytophagous arthropods (aphids, thrips, and additional mite species) found in the growing tips of *R. multiflora* branches, the same microhabitat used by *P. fructiphilus*, because these other arthropods are potential disease vectors.

**Materials & Methods**

Two sites were located in Story County Conservation Board Parks in central Iowa (Christiansen Forest Preserve (N41°54′, W93°34′) and Dakins Lake (N42°10′, W98°34′)),
and one site was located in an infested pasture in Boone County, IA (N42°06’, W93°56’). Samples were collected from June 2002 to April 2004 from the two sites in Story County (Dakins and Christiansen Parks) and from November 2002 to April 2004 at the Boone County site. Samples were collected approximately every two weeks during the summer, monthly during the fall, and once during the early spring. Fall samples were only taken from the RRD infected plants which, unlike the non-symptomatic plants, retain leaves throughout the winter.

On each sampling date 20 branch tips (approx 6 cm) were cut from each of 3 *R. multiflora* in open areas (no trees within 5 meters) and 3 plants growing as understory plants (shaded) with no visible symptoms of RRD, and from 3 *R. multiflora* rose also growing in open areas, with symptoms of RRD. No plants growing in the understory with symptoms of RRD were observed at these three sites. At the sites in Story Co., 42 samples were collected from *R. multiflora* in both sunny and shady sites, and 57 samples were taken from plants with RRD. At the Boone Co. site, *R. multiflora* growing in shade or sun were sampled 24 times and RRD plants were sampled 39 times.

A wash solution of tap water, 0.2% dish soap, and 2% sodium hypochlorite was used to remove arthropods from the foliage (de Lillo 2001). The 20 branch tips from a single *R. multiflora* were weighed and then placed together in a 400 or 1000 ml glass beaker. Leaves were removed and counted from each stem to expose any arthropods in the basal attachment sites of the leaves. The bags which held the branch tips were rinsed with the washing solution; the contents poured into the beaker and additional washing solution was added until the leaf tissue was fully covered. A magnetic stirring bar was placed in the beaker and the plant material and washing solution was stirred for 5-10 minutes at room temperature (de
Lillo 2001). The solution was filtered through 4 stainless steel sieves stacked by descending mesh size; 850 μm, 180 μm, 53 μm, and 25 μm. Plant material was rinsed in tap water in the 850 μm sieve to dislodge mites and other arthropods (de Lillo 2001). *Phyllocoptes fructiphilus* range in size from 168-204 μm long and 50-60 μm wide, and so the 180, 53 and 25 μm screens were examined for mites and other arthropods. A wash bottle filled with the wash solution was used to rinse the screen contents into an 8.5 cm diameter (56.7 cm²) petri dish. One to 2 drops of dish soap were added to the petri dish and mixed by carefully moving the petri dish back and forth (de Lillo 2001). The petri dish was allowed to sit for at least 1 minute so the mites settled to the bottom, then the mites were counted under a dissecting microscope. Sub-sampling of the 53 and 25 μm screen contents was used to estimate arthropod numbers. A sub-sample consisted of counting arthropods in 0.5x0.5 cm squares arranged in a figure “8” shape across the petri dish, 38 squares were examined for a total of 9.5 cm². Only *P. fructiphilus* mites were observed in the 25 μm petri dish; if there were fewer than 10 *P. fructiphilus* in the 53 μm dish, the 25 μm dish was not counted. Selected arthropods were sent to the United States Department of Agriculture Systematic Entomology Laboratory for identification.

The arthropod abundance data were summarized per weight (gram) of the 20 branch tips collected from each plant. Data were log transformed prior to statistical analysis, so median values are presented. Analysis of variance was used to analyze site, habitat, and site*habitat effects. Within an effect, least squares means were compared with the Tukey test (SAS 2004).
Results

Plants with symptoms of RRD had the most leaves (Fig 1), which was expected because one of the symptoms of RRD is increased leaf production. We observed aphids, thrips, eriophyid mites, and other mite species on healthy and diseased *R. multiflora* growing in the sun and on healthy *R. multiflora* growing in shaded habitats (Fig 2). The most commonly observed arthropods were eriophyid mites on RRD symptomatic plants.

The median number of eriophyid mites found on *R. multiflora* was different depending on the habitat the plant was growing in (sun or shade) and whether or not the plant was diseased (Fig 3) (p<0.006). The greatest number of eriophyid mites was found on plants showing symptoms of RRD (p<0.0001), and plants in the sun had more eriophyid mites than plants growing in the shade (p=0.006). At the three sites the numbers of mites tended to be highest during the summer months, although eriophyid mites were present on the RRD plants on all sampling dates. There were significant differences among the number of eriophyid mites at the three field sites (p<0.0001). The number of mites at Dakins and Christiansen was similar (p=0.34), but Boone was different from both Dakins and Christiansen (p<0.0001). This is likely due to the different sampling times; Boone was not sampled during the summer of 2002. Even though sampling times differed, numbers at the Boone site were generally lower. Most of the eriophyid mites were identified as *P. fructiphilus*, however two mites from Dakins were identified as *Phyllocopetes adalius* Keifer (Table 1). *Phyllocopetes adalius* is not a vector of RRD (Amrine et al 1994).

Non-eriophyid mites found in the *R. multiflora* samples included predatory mites fungivorous mites and phytophagous mites (Table 1). The non-eriophyid mite species were most abundant during the summer. More non-eriophyid mites were found on *R. multiflora*
growing in the shade (p<0.0001). In the sunny habitat similar numbers of mites were observed on RRD symptomatic plants and apparently healthy plants (p=0.7). The number of mites at the three field sites was also significantly different (p=0.0006), with Dakins having the most and Boone the fewest mites.

Thrips species observed on *R. multiflora* included *Frankliniella exigua* Hood, and *Neohydatothrips variabilis* Beach, (Table 1). The highest number of thrips was observed at Dakins Park (p<0.0001), Christiansen Park and the Boone site had similar numbers of thrips (p=0.7). Habitat (shady or sunny) of the *R. multiflora* and disease status did not have an effect on the numbers of thrips present (p=0.08).

Aphids identified from *R. multiflora* were all *Chaetosiphon* sp. (Table 1). Aphids were abundant during the summer at the three field sites. There was a significant difference in the numbers of aphids at the three field sites (p=0.01), with the highest numbers at Boone and the lowest numbers at Christiansen Park. There was also a significant difference in the numbers of aphids in each the habitat (p=0.048). *Rosa multiflora* with RRD had the most aphids whereas healthy *R. multiflora* (growing in sun or shade) had similar numbers of aphids.

**Discussion**

*Phyllocoptes fructiphilus* populations were highest on *R. multiflora* with RRD symptoms. These results are similar to those reported by Epstein and Hill (1999) who observed much higher numbers of mites on symptomatic *R. multiflora* compared to unsymptomatic plants sampled from May-September from 1990 to 1992 at two sites in Iowa.
Amrine (1996) also reported mite populations on symptomatic *R. multiflora* were 14 times higher than those on un-symptomatic plants.

The lowest numbers of *P. fructiphilus* were observed on the *R. multiflora* growing in the shade as understory plants. Epstein & Hill (1995) found lower numbers of RRD symptomatic *R. multiflora* in shaded areas and a slower increase of disease incidence over a 6 year period in shaded areas compared to plants growing in full sun. They hypothesized that this could be due to lower ambient air temperature affecting the mites, or some other unknown factor that reduced the mites' capacity to vector RRD. We confirmed that there are fewer *P. fructiphilus* on plants growing in full shade, but we believe this could be a result of the absence of RRD, not the cause. Plants showing symptoms associated with RRD often have greatly increased density of leaf growth, thus providing many more leaf axil microhabitats for *P. fructiphilus* mite populations. This interpretation is consistent with the observation that there are fewer *P. fructiphilus* on plants in the sun with no symptoms of RRD.

Use of *Phyllocoptes fructiphilus* as a disease vector

RRD has the potential to be an important tool in managing *R. multiflora*. Epstein and Hill (1997) demonstrated RRD will readily spread to healthy plants in plots where RRD is augmented. RRD reduces flowering and seed set of infected plants and results in the death of the plant within approximately 5 years (Epstein et al. 1993). As the presumed vector of RRD it is important to understand the occurrence of *P. fructiphilus* on *R. multiflora*. Augmentation of the disease can be successfully accomplished by grafting buds from diseased plants on to healthy plants (Epstein 1995); however this process is too labor
intensive to be used in heavily infested areas. As an alternative, land managers cut branches of diseased plants and place them on healthy plants with the expectation that \textit{P. fructiphilus} present on the branches will move onto the healthy plants. There is no evidence that the mites will move onto the healthy plants, but this study clearly shows that higher mite populations are consistently found on \textit{R. multiflora} with symptoms of RRD.

There are several major concerns in the manipulation of \textit{P. fructiphilus} and RRD for biological control of \textit{R. multiflora}. The first is that ornamental roses are susceptible to RRD, particularly hybrid tea roses (Epstein & Hill 1999). Although movement of the mite onto ornamental roses causing infection appears to be limited, the risk of RRD is a great concern to rose growers and the rose industry (Harwood 1994, Dettmann & Pagliai 1993). Unfortunately, because RRD naturally occurs in \textit{R. multiflora} populations there will always be a source of inoculum with or without augmentation. A better understanding of the temporal and spatial dynamics of mite populations throughout the year will help determine ways to limit the potential spread of RRD from multiflora rose to ornamental roses.

The second problem with augmentation of RRD is that despite many the causative agent of RRD has not been isolated and identified (Epstein & Hill 1999, Rohozinski et al. 2001, Di et al. 1990). Many attempts have been made to isolate the causal agent, but none have been successful (Rohozinski et al. 2001). Di et al. (1990) found double stranded RNA associated with diseased tissue indicating the causal agent could be a cryptic virus. Rohozinski et al. (2001) successfully transmitted a virus-like agent from \textit{R. multiflora} to \textit{Nicotiana} sp., but observed no virus like particles.

Arthropod transmission studies of RRD have only been conducted with \textit{P. fructiphilus} and \textit{Tetranychus urticae}, the twospotted spider mite, primarily because \textit{T. urticae}
commonly infests greenhouse grown *R. multiflora* used in experiments. *Tetranychus urticae* was not observed to transmit RRD (Amrine et al. 1988). Our current study documented that other known vectors of plant diseases commonly occur on *R. multiflora*. Aphids, thrips, and some mites are known vectors of plant viruses (Maramorosch 1963), but it is unknown if they are involved in transmission of RRD.

*Chaetosiphon* sp. aphids were common on the *R. multiflora* and this genus transmits plant viruses. For example, *Chaetosiphon fragaefoili* (Cockerell), the strawberry aphid, and *Chaetosiphon jacobi* both transmit strawberry viruses (Frazier 1968, Krczal 1979). One of the thrips species (*Frankliniella sp.*) observed on *R. multiflora* is from a genera known to transmit plant tospoviruses (Maramorosch 1963). If these arthropods transmit RRD, they could spread the disease among *R. multiflora* plants and also to non-target ornamental roses.

Future research needs to isolate and identify the causal agent of RRD so it can be confirmed that, of the many arthropods feeding on *R. multiflora*, only *P. fructiphilus* vectors RRD. Until this is done, it is impossible to confirm that large scale augmentation of RRD to control *R. multiflora* can be implemented without increasing non-target RRD infections.

Use of eriophyid mites in weed biological control

Phytophagous mites are good candidates for successful weed biological control programs because of their limited host range; it is estimated that 80% of eriophyids are monophagous (Briese & Cullen 2001). In addition, mites have a high rate of population increase and often good dispersal abilities. Eriophyid mites have been used in biological control programs against invasive weeds, including St. John’s wort, rush skeletonweed, and field bindweed (Breise & Cullen 2001). Many eriophyids cause galling in plants, altering
resource allocation and reducing plant vigor. Non-gall forming eriophyids also affect plant
growth. For example, *Aculus hyperici* feeding in the growing tips of St. John’s wort causes a
decrease in leaf size and shortening of internode distances (Breise & Cullen 2001).
Currently, *P. fructiphilus* is the only eriophyid mite that is being considered for use as a
disease vector in biological control (Breise & Cullen 2001).

Acknowledgements

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Home Economics Experiment Station, Ames, Iowa; Project Number 6628, supported by the
Hatch Act and State of Iowa funds.

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Epstein A.H. 1995. Biological control of multiflora rose with rose rosette disease. Iowa State University, University Extension PM-1615, Ames, IA


Table 1: Identification of selected arthropods on the branch tips of *Rosa multiflora*.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Number of Individuals</th>
</tr>
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<tbody>
<tr>
<td><strong>Arachnida</strong></td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
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<tr>
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</tr>
<tr>
<td><em>Phytoseius sp.</em></td>
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</tr>
<tr>
<td>Stigmaeidae</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td><em>Tarsenemus waitei</em> Banks</td>
<td>1</td>
</tr>
<tr>
<td>Tetranychidae</td>
<td></td>
</tr>
<tr>
<td><em>Panonychus ulmi</em> Koch</td>
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<tr>
<td><em>Tetranychus sp.</em></td>
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<tr>
<td>Tydeidae</td>
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<td><em>Pronematus sp.</em></td>
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<tr>
<td><em>Neohydatothrips variabilis</em> Beach</td>
<td>5</td>
</tr>
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</table>

1Identified by Ronald Ochoa, USDA-SEL.
2Identified by Gary L. Miller, USDA-SEL.
3Identified by Sueo Nakahara, USDA-SEL.
Fig 1: The average number of leaves (± SE) on 20 *Rosa multiflora* branch tips sampled over a two year period in three field sites.
Fig 2: The average number of arthropods per gram on branch tips from 3 healthy *Rosa multiflora* growing in the shade and in the sun, and from 3 *R. multiflora* exhibiting symptoms of RRD at three locations in central Iowa, a) Christiansen Park, Story Co. b) Dakins Park, Story Co. c) Boone Co.
Figure 3: Average number of eriophyid mites per gram over time on branch tips from 3 healthy Rosa multiflora growing in the shade and in the sun, and from 3 R. multiflora exhibiting symptoms of RRD at three locations in central Iowa, a) Christiansen Park, Story Co. b) Dakins Park, Story Co. c) Boone Co.
CHAPTER 5: DISTRIBUTION OF *MEGASTIGMUS ACULEATUS* (HYMENOPTERA: TORYMIDAE) AND LEVELS OF SEED PREDATION OF *ROSA MULTIFLORA* (ROSACEAE) IN IOWA.

Laura Jesse¹, Michael Collyer², Kirk Moloney², John Obrycki³

A paper to be submitted to Biological Control

Abstract

*Rosa multiflora* Thunb. (Rosaceae), an invasive plant that currently infests millions of hectares in the eastern half of the United States, was initially planted in the 1940’s as a ‘living fence’, cover for game animals, and for erosion control. The larvae of *Megastigmus aculeatus* var *nigroflavus* Hoffmeyer (Hymenoptera: Torymidae), feed on the developing *R. multiflora* seeds and may have the potential to reduce seed output of *R. multiflora*. We collected rosehips from 49 sites across eastern and southern Iowa to determine the presence and distribution of *M. aculeatus* in Iowa. *Megastigmus aculeatus* larvae were found in 266 of the 979 rosehips dissected (27%) and at 31 of the 49 sites sampled (63%). An average of 0.5 seeds per hip contained a *M. aculeatus* larva. One larva per hip was most common (52%)

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of hips with larvae), but a range of 1-7 larvae was observed in rosehips. Next we determined
the levels of *M. aculeatus* infestation over 2-3 years at 3 selected sites, and we found that
more seeds were aborted than were viable or containing wasp larvae. The numbers of viable
seeds, aborted seeds and seeds containing larvae varied across the sampling time period.
Finally, we quantified the relationship between viable seeds, aborted seeds, and *M. aculeatus*
predation. We observed a negative relationship between numbers of both aborted and viable
seed and the probability of a rosehip being attacked by *M. aculeatus*, indicating that *M.
aculeatus* females are not preferentially selecting rosehips with higher numbers of viable or
aborted seeds for oviposition. There is a significant reduction in both viable and aborted
seeds in hips attacked by *M. aculeatus*. Detailed knowledge of *R. multiflora* demography is
necessary to determine at what level seed predation will reduce the recruitment of new
individuals into the population.

Introduction

*Rosa multiflora* Thunb. (Rosaceae) is an invasive plant that currently infests 45
million acres (18.2 million hectares) in the eastern half of the United States. It reduces the
value of land for grazing or recreational purposes because dense patches are impassable and
are not fed upon by most livestock (Underwood et al., 1996). Infestations are most common
in pastures and nearby forested areas presumably because the plant was promoted in the
1940’s as a ‘living fence’, cover for game animals, and for erosion control (Burgess, 1948;
Edminister, 1938; Steavenson et al., 1943; Steavenson, 1946).

The rose seed chalcid, *Megastigmus aculeatus var nigroflavus* Hoffmeyer
(Hymenoptera: Torymidae) may have the potential to reduce seed output of *R. multiflora*
(Mays and Kok, 1988; Nalepa, 1989). Presumably this species was accidentally introduced into the U.S. in shipments of *R. multiflora* seeds (Weiss, 1917), and, like *R. multiflora*, is native to eastern Asia. Although *M. aculeatus* Swed feeds on the seeds of other *Rosa* spp. (Balduf, 1959), available data indicate that *M. aculeatus* var. *nigroflavous* infests only *R. multiflora* seeds (Amrine, 2002).

Female *M. aculeatus*, lay their eggs directly in developing *R. multiflora* achenes (seeds within the rosehip) in May and June, soon after the petals fall from flowers (Balduf 1959). *Megastigmus aculeatus* over-winters as a larva within the achene and rosehip. In early spring the wasp pupates and emerges from the achene as an adult in May or June (Nalepa & Piper, 1994). *Megastigmus aculeatus* reproduces parthenogenically and has a strong female sex bias (Balduf, 1959). This seed predator may reduce the spread of multiflora rose by seed dispersal if it destroys a high percentage of seeds (Amrine & Stansy, 1993). Percentage infestation of *R. multiflora* hips collected from 64 sites in Virginia averaged 25% with a range of 2-59% (Mays & Kok, 1988). In North Carolina, Nalepa (1989) observed an average of 42% of rose hips and 17% of seeds infested with *M. aculeatus*.

The objectives of our research were to 1) determine the presence and distribution of *M. aculeatus* in Iowa, 2) determine the levels of *M. aculeatus* infestation over 2-3 years at 3 selected sites, and 3) quantify the relationship between viable seeds, aborted seeds, and *M. aculeatus* predation.
Materials & Methods

*Rosa multiflora* rosehips were collected from 49 locations (Fig 1) in 19 counties in southern, eastern, and central Iowa during the winter of 2001-2002 (Oct. 2001 - Jan 2002). Rosehips were collected from 1-5 plants at each site and brought to the laboratory. The seeds within 20 rosehips were dissected dry under a dissecting microscope using a razor blade. Seeds were categorized as viable if the embryo and endosperm were white and moist. Seeds were categorized as aborted if the seed was not fully formed or if the embryo and endosperm inside were brown and shriveled. Seeds were categorized as depredated if there was a full grown wasp larva within the seed coat.

In the winter of 2002-2003 we visited 11 sites with *R. multiflora* infestations in eastern, southern and central Iowa. At each site 10 rosehips were taken from each of 5 *R. multiflora*. In the winter of 2003-2004 we returned to 3 of the same sites and sampled 10 rosehips from the same 5 *R. multiflora*. In the winter of 2004-2005 we returned to two of the sites in northeastern Iowa and again sampled 10 rosehips from the same 5 plants. The seeds within the rosehips were dissected under a dissecting microscope with a razor blade. Seeds were categorized in the same manner as in 2001-2002. In addition, we used tetrazolium (1% 2,3,5 Triphenyltetrazolium chloride) as another test of seed viability (AOSA 1970). Seeds were not pre-treated with cold, and viability was not confirmed with germination tests. Seeds were hydrated in tap water overnight, then a razor blade was used to cut the seeds longitudinally through the embryo, and then the seeds were soaked in the tetrazolium solution overnight. Seeds were then dissected to examine the staining pattern and determine if the embryo was living (AOSA 1970).
**Statistical Analysis**

Relative portions of viable, aborted, and infected seeds, calculated from all hips collected, were determined for all site by year collections. From the collections made in 2003-2005, sites with prominent wasp predation were used to model the probability and rate of attack of rosehips as a function of viable and aborted seed numbers. The locations used in this analysis were 3 sites from Jackson Co. (eastern Iowa), 2 sites from Allamakee Co. (northeastern Iowa), and one site in Boone Co. (central Iowa), producing a data set of 599 rosehips. To determine if there was a year effect, a subset of these data (340 hips) were used that only included the 2 sites from Allamakee Co., because they were repeatedly sampled for three years.

To model variation in the probability and level of wasp infestation of seeds, we used a joint-response generalized linear model. Our response variables (collected for each hip) included a binary random variable for wasp infested (1 = wasp infested, 0 = non-infested) and a discrete random variable for larval count, assumed to follow a Poisson distribution. The independent variables included location as a fixed effect, hips nested within location as a random effect, and viable and aborted seed counts as covariates. For this subset of data, year was also included as a fixed effect, as well as the interaction of year and covariates. The generalized linear model uses differentiable monotonic link functions to allow a linear fit of the model based only on means and variances in the linearized form (McCullaugh and Nelder 2001), thus allowing non-normally distributed or non-continuous random variables to be modeled in a linear model framework. For the models we considered, bivariate responses were modeled with logit (binary infection probability) and log (larvae count) link functions. Model parameters were estimated with residual pseudo-likelihood with a subject-specific expansion method using the Glimmix procedure of SAS 9.1 (SAS Institute,
Tests of fixed effects were performed with a Satterthwaite approximation of the degrees of freedom to produce approximate F-values.

A contingency table test was used to determine if there was a significant difference in the relative portions of viable and aborted seeds in hips that were attacked by *M. aculeatus* compared to hips that were not (Sokal & Rohlf 1995). Data collected from winter 2002-2004 at the sites with higher levels of *M. aculeatus* attack (Jackson, Allamakee and Boone counties) were used because we had both attacked and unattacked rosehips occurring at the same site, allowing us to limit site differences affecting the production of viable seeds.

**Results**

In the winter of 2001-2002, 879 rosehips were collected and dissected, revealing an average of 8.6±0.1 seeds per rosehip. However, on average, there were only 1.5±0.1 viable seeds per rosehip; 35% of rosehips had no viable seeds (Fig 2). The rosehips dissected contained an average of 6.5±0.2 aborted seeds per rosehip (Fig 2). *Megastigmus aculeatus* larvae were found in 266 of the 979 rosehips dissected (27%) and at 31 of the 49 sites sampled (63%). An average of 0.5 seeds per hip contained a *M. aculeatus* larva. One larva per hip was most common (52% of hips with larvae), but a range of 1-7 larvae was observed in rosehips (Fig 2).

Many sites surveyed in the winter of 2001-2002 had very few *M. aculeatus* larvae, however 7 of the 19 counties samples averaged over one wasp larva per rosehip (Allamakee, Decatur, Jackson, Madison, Monroe, Story and Wapello Counties). Twelve counties had at least one sampling location where no *M. aculeatus* were found, three of these counties had another site with an average of over one wasp per rosehip.
For each field site sampled in 2001-2002 the numbers of aborted, viable and larval infested seeds were summed and then the proportion of each compared to the total number of seeds was graphed on a ternary plot (Fig 3). Many of the sites are clustered in the lower right of the graph because they had a high proportion of aborted seeds and low proportions of larval infested seeds or viable seeds. The two sites that are in the middle of the plot (one in Allamakee Co. and one in Jackson Co.) had higher levels of wasp predation than observed at other sites. No sites had more than 50% of their seeds viable or attacked by wasps, but all sites had more than 50% of their seeds aborted. Many sites had more than 70% of the seeds aborted (Fig 3).

During 2002, 2003, and 2004, 864 rosehips were collected at 11 sites. *Megastigmus aculeatus* larvae were present in 376 of the 864 (44%) rosehips dissected. We observed an average of $1.0 \pm 0.1$ wasp larvae per rosehip. This was higher than our observations from the winter 2001-2002 collections; likely due to smaller sample size and that we specifically revisited sites observed in 2001 to have *M. aculeatus* present. There was an average of $1.4 \pm 0.1$ viable seeds per rosehip and $5.7 \pm 0.1$ aborted seeds per rosehip.

At the three sites sampled over multiple years, more seeds were aborted than were viable or infested by wasp larvae. The numbers of viable seeds, aborted seeds and seeds containing larvae varied across the sampling time period (Fig 4). The percentage of viable seeds per rosehip at Allamakee (site 1) increased from 4% in 2003, 7% in 2004, to 12% of the seeds in 2005. Allamakee (site 1) had a relatively large percentage of seeds depredated by *M. aculeatus* per rosehip; in 2003, 21% of the seeds were infested, 30% were infested in 2004 and 24% in 2005. Most seeds in a rosehip were found to be aborted, 74% of the seeds, in 2003, 63% in 2004, and 64% in 2005. At site 2 in Allamakee Co. low percentages of
viable seeds per rosehip were observed; 4% in 2003, 7% in 2004, and 11% in 2005. *Megastigmus aculeatus* was also present at site 2, but the percentages of seeds per rosehip that were depredated were not as high as they were at site 1. In 2003, 21% of seeds per rosehip contained a *M. aculeatus* larva, 20% in 2004 and 24% in 2005. The greatest percentages of seeds per rosehip were found to be aborted; 75% in 2003, 74% in 2004, and 65% in 2005. At the site we sampled for two years in Jackson Co. we found higher percentages of viable seeds per rosehip than at the two sites in Allamakee Co. In 2003, 23% of seeds per rosehip were viable and in 2004, 41% were viable. Fewer seeds per rosehip were depredated by *M. aculeatus*; 2% in 2003 and 8% of seeds per rosehip in 2004. Similarly to the Allamakee Co. sites, the majority of seeds per rosehip were aborted at the Jackson Co. site. In 2003, 74% of seeds per rosehip were aborted and 50% were aborted in 2004.

The generalized linear model for variation in *M. aculeatus* attack rate (probability of a hip being infested and the number of larvae within a rosehip), conducted on Allamakee County (Goodness of Fit: generalized $\chi^2/df = 0.95$; $P = 0.80$) indicated that there were no significant year effects in the responses or significant interactions of year and viable or aborted seed number (Table 1).

Different years did not significantly affect the attack rates and larval counts, so we combined years and modeled the effects of location, viable seed number, and aborted seed number at sites where the wasp was present in higher numbers (at least 10 wasp larvae found in dissections of the 50-60 rosehips at Allamakee, Jackson and Boone Co.) (Table 2). This model was also not overdispersed (generalized $\chi^2/df = 1.01$) and indicated that location, viable seed number, and aborted seed number significantly affected seed infestation by *M.*
aculeatus (Table 2). The relationships between viable seed number and the probability of rosehip attack by *M. aculeatus* and the number of larvae within a rosehip were both negative (Table 3, Fig 5). This indicates that wasps are not preferentially attacking rosehips with larger numbers of viable seeds. The relationships between the number of aborted seeds and the probability of rosehip attack by *M. aculeatus* and the number of larvae within a rosehip were also negative (Table 3, Fig 5). This indicates that if a rosehip had greater numbers of aborted seeds there is a reduction in seeds available as food for a larva (initially viable seeds), therefore decreasing the probability of a hip having a *M. aculeatus* larva, as well as, the number of larvae that could survive in a rosehip (Table 3, Fig 5).

There was a significant reduction in the proportion of aborted and viable seeds in hips that were attacked by *M. aculeatus* compared to those that were not (Chi-square=66.5, p<0.0001). To determine if hips that were infested by *M. aculeatus* would have had a different number of viable seeds before infestation (based on the supposition that wasp larvae would survive only in seeds that were viable) we summed the number of viable seeds and larval infested seeds. We found that rosehips attacked by *M. aculeatus* would have had a greater number of potentially viable seeds if not attacked (Chi-square=90.9, p<0.0001). However, this relationship does not indicate if wasps preferentially attack rosehips with greater numbers of viable seeds, or simply have greater survival in hips with many viable seeds.

**Discussion**

*Megastigmus aculeatus* occurred in sites in eastern, central, and southern Iowa, with the largest populations occurring primarily along the northeastern edge of Iowa and central
Iowa. Many sites in the initial survey (winter 2001-2002) had very few *M. aculeatus* larvae, yet counties with higher levels of *M. aculeatus* larvae occurred throughout the sampling area. The patchy distribution of *M. aculeatus* could be due to environmental factors affecting the overall health of *R. multiflora*, or a lack of pollinators necessary to produce the viable seeds that are required for wasp larval development.

In 2001-2002 we found at least one *M. aculeatus* larva in 27% of the rosehips sampled. Surveys of *M. aculeatus* in *R. multiflora* rosehips have been conducted in several eastern states. Mays & Kok (1988) observed 25% of rosehips infested with *M. aculeatus* larvae in Virginia. Nalepa (1989) observed a higher percentage (42%) of hips infested in North Carolina.

All of the field sites sampled in 2001-2002 had high levels of aborted seeds (at least 50% of seeds were aborted). High levels of aborted seeds could be due to lack of pollinators, plant stresses or to enhance the success of other seeds (Mena-Ali & Rocha, 2005). Other studies have suggested that production of unfilled (full size seed without developing embryo inside) seeds may allow fertile seeds to escape predation by a seed predator that lays eggs indiscriminately (Traveset, 1993; Wright, 1994; Zangerl et al., 1991).

We observed a negative relationship between numbers of both aborted and viable seed and the probability of a rosehip being attacked by *M. aculeatus*. In addition, there was also a negative relationship between numbers of viable and aborted seeds and the number of *M. aculeatus* larvae within a rosehip. This indicates that *M. aculeatus* females are not preferentially selecting rosehips with higher numbers of viable or aborted seeds for oviposition. There is a significant reduction in both viable and aborted seeds in hips attacked by *M. aculeatus*. This was expected since the rosehip is a contained unit and any seeds that
are filled by *M. aculeatus* larvae will reduce the number of seeds that would otherwise have been viable.

Determining the effect an herbivorous insect has on the reproduction and spread of an invasive plant is difficult. Evaluations often focus on the establishment and spread of the control agent and not on the effects of the control agent on the weed (McFadyen, 1988; Blossey, 2004). Even when large impacts from an herbivorous insect species can be shown, it is necessary to demonstrate a reduction in the density or spread of the weed over time (Moran & Hoffman, 1989). In the case of *R. multiflora*, the seed predator was already present so we cannot conduct pre-release studies and exclusion experiments were not successful (Jesse, unpublished data).

Seed predators are often used in weed biological control projects, however, they may not be the best agent for controlling invasive plants because the spread of many plants is not limited by seed production (Myers & Bazely, 2003). Detailed knowledge of plant demography is necessary to determine at what level seed reduction will reduce the recruitment of new individuals into the population; in general, plant species are not seed limited. It has been estimated that for plants that are not seed limited, reductions of 95-99% of seeds will be necessary to reduce plant densities (Noble & Weiss, 1989; Hoffman, 1990; Myers & Risley; 2000, Myers & Bazely, 2003). In a study of four species of long-lived perennials, Andersen (1989) found that safe sites for seedlings were the most important determinant of population recruitment. Losses to seed predators of 95% of total seeds did not have an impact on population recruitment because of a rarity of safe sites and the buildup of a large seed bank (Andersen, 1989).
High losses of seeds to seed predators commonly occur for some plant species. Moran & Hoffman (1989) observed only 5 seed pods surviving on Sesbania punicea trees that had been attacked by the seed feeding weevil, Trichapion lativentre, for two years, compared to over 3,100 seed pods from trees without weevils. Annual seed production by Sida acuta was reduced from 8,000 seeds / m² to 731 seeds / m², by the seed feeding chrysomelid, Calligrapha pantherina (Lonsdale et al. 1995). The control of nodding thistle, Caradus nutans, in North America is attributed to the seed feeding weevil Rhinocyllus conicus (Myers & Risley, 2000; Myers & Bazely, 2003). Unfortunately this weevil also feeds on the seeds of rare, native thistle species (Louda et al. 1997).

Acknowledgements

We would like to thank Dennis Portz, Department of Horticulture, Iowa State University, for helping collect data and letting us conduct parts of this research on his family's property in Jackson Co. IA. This research was partially funded by a grant from the College of Agriculture at Iowa State University. Journal Paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project Number 6628, supported by the Hatch Act and State of Iowa funds.

Literature Cited


Table 1: Tests of fixed effects and covariates from the generalized linear model from the responses of probability of attack by *M. aculeatus* and the number of larvae in a rosehip at two sites in Allamakee Co. from 2003-2005.

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Table 2: Tests of fixed effects and covariates from the generalized linear model from the responses of probability of attack by *M. aculeatus* and the number of larvae in a rosehip combined over years for three locations with *M. aculeatus* present.

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<td>1182</td>
<td>7.51</td>
<td>0.0006</td>
</tr>
<tr>
<td>Aborted Seed Number</td>
<td>2</td>
<td>1182</td>
<td>81.18</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Table 3: Relationships between independent variables and responses for the generalized linear model described for Allamakee, Boone and Jackson counties.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Response Variable</th>
<th>Estimate</th>
<th>se</th>
<th>df</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable Seed Number</td>
<td>Infection Probability</td>
<td>-0.23</td>
<td>0.08</td>
<td>1182</td>
<td>-2.99</td>
<td>0.0029</td>
</tr>
<tr>
<td>Viable Seed Number</td>
<td>Larval Count</td>
<td>-0.08</td>
<td>0.03</td>
<td>1182</td>
<td>-2.63</td>
<td>0.0088</td>
</tr>
<tr>
<td>Aborted Seed Number</td>
<td>Infection Probability</td>
<td>-0.29</td>
<td>0.05</td>
<td>1182</td>
<td>-5.86</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Aborted Seed Number</td>
<td>Larval Count</td>
<td>-0.19</td>
<td>0.02</td>
<td>1182</td>
<td>-11.62</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Figure 1: Location of *R. multiflora* infestations where rosehips were sampled from Oct 2001-Jan 2002.
Figure 2: The frequency of a) viable seeds, b) aborted seeds, and c) seeds containing one or more *M. aculeatus* larvae in 879 *R. multiflora* hips collected from 49 sites in Iowa during the winter of 2001.
Figure 3: Ternary plot showing the location of each site based on compositional data summed for the field site of larval infested, viable, and aborted seeds, from winter 2001. The axes indicate the proportion of seeds in each of the three categories. Sites are numbered as follows: Allamakee County (1-3), Appanoose County (4-6), Cedar County (7), Clark County (8-10), Davis County (11-14), Decatur County (15-17), Jackson County (18-21), Johnson County (22-26), Lucas County (27-30), Madison County (31), Monroe County (32-34), Muscatine County (35), Polk County (36), Story County (37-38), Union County (39-40), Van Buren County (41-42), Wapello County (43-45), Warren County (46), and Wayne County (47-49).
Figure 4: The average number of viable, aborted and *M. aculeatus* infested seeds per rosehip in 2003 and 2004 in Jackson Co. (a) and 2003-2005 in two sites (b,c) in Allamakee Co. Iowa.
Figure 5: Surface plots for (A) probability of rosehip being depredated by a wasp larva, and (B) estimated larval count, as a function of viable and aborted seed number.
CHAPTER 6: GENERAL CONCLUSION

It seems unlikely that *Rosa multiflora* would be the serious weed it is today if humans had not intentionally spread and planted it throughout the eastern United States. So why was this plant promoted, when apparently its ability for invasive spread was not properly considered, and certainly underestimated? First of all I feel it is important to understand the historical context of the introduction of *R. multiflora*.

As early pioneers moved onto the prairies of the Midwestern U.S., wood for fencing was often scarce. Lewis (1941) states that in order to protect grain from roaming cattle it was up to the farmer to protect his fields; early cattlemen had fought hard to keep the public range open to free grazing. Further complicating the issue was that early settlers gained a monopoly on timber and would sell it to later immigrants at excessively high prices, so the search began for an alternative fence. Osage orange was the first “living fence” in the Midwest and was promoted in the mid-1800’s.

Barbed wire fencing was patented in 1875 and commercial production began. With this invention much less timber was needed for fencing and barbed wire fences became commonplace. However, this did not end the desire for living fences, because barbed wire fences were still expensive and in need of general maintenance and upkeep. A living fence was billed as lasting a lifetime, requiring no maintenance, and being a thing of beauty (Burgess 1948). Dambach (1942) states that fencerows of bluegrass sod or low shrubs will add to the attractiveness of farms, provide shelter for songbirds, and beneficial insects, such as lady beetles.
With this mindset, and the possibilities of metal shortages for barbed wire during World War II, the Soil Conservation Services in Missouri and Illinois set out to find a new living fence that would perform better than osage orange (Steavenson et al. 1943). Managing farms for wildlife, especially game animals, and controlling erosion were also important goals of the Soil Conservation Service during the 1940's and 1950's (Edminster 1938). This agency believed *R. multiflora* would be an ideal living fence because it successfully penned in most livestock, grew well in the Midwest, had few diseases, and provided food and shelter to wildlife (Steavenson et al. 1943).

It appears that enthusiasm for the positive aspects of *R. multiflora* may have prevented adequate consideration of its ability to spread. However, it only took a decade for the negative aspects of *R. multiflora* to be recognized and it was no longer planted. Unfortunately, *R. multiflora* was widely planted during the 1940's and 1950's and by the time planting ceased in the 1960's *R. multiflora* had become a noxious weed by spreading into pastures, parks and forests.

The overall objective of my research was to examine the population biology of *R. multiflora* and two of its biotic mortality factors. A better understanding of this plant and its mortality factors is needed to devise means for the long-term suppression of *R. multiflora*.

In Chapter 2, I explored the mechanisms by which *R. multiflora* is spreading. I found that larger patches of *R. multiflora* tended to be dominated by one genotype, but all ten large patches sampled contained more than one genotype. Smaller patches of *R. multiflora* tended to consist of a single genotype (6 of the 10 patches sampled), but 3 patches had 2 genotypes and one had 3 unique genotypes. It appears that like many clonally reproducing plants, *R.
multiflora is also routinely recruiting new members into its population by sexual reproduction.

My results in Chapter 2 showed that R. multiflora is reproducing sexually and since it is an insect pollinated obligate outcrosser, I investigated what insects were pollinating R. multiflora in Iowa. In Chapter 3, I collected insects on yellow sticky traps placed on plants during the period of blooming and visually observed insects visiting flowers to determine the insect pollinators visiting R. multiflora flowers. I observed several common generalist insect pollinators visiting R. multiflora flowers, including Apis mellifera, Bombus sp., and Syrphidae, and thus concluded that R. multiflora is not being limited by lack of pollinators. In the 16 hours of observations conducted over two years, I observed 205 insects visiting flowers. Further research will be needed to determine if an increase in visitation rates would result in higher seed set.

I next examined methods to manage infestations with biological control. Rosa multiflora is an interesting invasive plant because there has not been any classical biological control program for it, yet there is an introduced insect natural enemy and a native plant pathogen attacking it. Megastigmus aculeatus is from Asia and was accidentally introduced into North America with shipments of R. multiflora seeds. Rose rosette disease appears to be native to North America, as is its presumed vector, Phyllocopetes fructiphilus. Rose rosette disease also infects ornamental roses and attempts to augment the disease have caused concerns among rose growers. It appears that although the disease is native to the U.S. it was not a large problem before R. multiflora was planted. Rosa multiflora is highly susceptible to RRD and infestations are serving as a conduit for the spread of RRD and a constant reservoir of the disease.
A greater understanding of what RRD is and how it spreads is necessary before augmentation can be implemented, especially given the high non-target risks to other Rosa species. In Chapter 4, I focused on the effects of habitat and disease symptoms on populations of *P. fructiphilus* and other potential arthropod disease vectors on *R. multiflora*. *Phyllocopites fructiphilus* populations were highest on diseased *R. multiflora* in open sunny habitats and lowest on healthy *R. multiflora* growing in the shade. This may be due to disease symptoms (increased leaf growth) providing ideal micro-habitats for *P. fructiphilus*, since it prefers to live in leaf axils. I also observed other arthropods that are known to act as vectors of plant pathogens. For example, *Chaetosiphon* sp. aphids were common on *R. multiflora* and this genus of aphids transmits viruses in strawberries, another member of the Rosaceae. Until the causative agent of RRD has been isolated and identified it will not be possible to do the types of experiments necessary to prove that *P. fructiphilus* is the only vector of RRD, nor will it be reasonable to attempt to use it as a vector to augment RRD for *R. multiflora* control.

In Chapter 5, I examined a second biological control agent, *M. aculeatus*, to determine the presence and distribution of *M. aculeatus* in Iowa. I also determined if natural infestation rates were reducing the production of viable seeds by *R. multiflora*. I found *M. aculeatus* occurred throughout the areas I sampled in eastern, southeastern and central Iowa, although usually in low numbers. At three sites with higher numbers of *M. aculeatus* I found that there was a significant reduction in viable seeds. Further research is needed to determine if *M. aculeatus* can reduce *R. multiflora* seed production enough to reduce the spread of *R. multiflora*.
Biological controls of *R. multiflora* will need to be integrated with other control strategies to achieve satisfactory management. RRD may cause initial plant mortality, but from my observations, not all plants in an area will be infected and plants growing under tree canopies are often not infected. These plants will reproduce sexually and asexually and continue to spread. Plants that remain unaffected by RRD will need to be controlled with herbicide treatments, mechanical removal, or possibly prescribed burns. It is also important to remove dead *R. multiflora* by mowing or burning because they could serve as a focal point for bird perches and can protect new seedlings from grazing animals.

This work clearly demonstrated that management of *R. multiflora* with biological controls will require research to determine the cause of RRD and if *R. multiflora* is seed limited. At this time, I propose that herbicidal and mechanical management of *R. multiflora* are the most viable options available to manage infestations.

**References Cited**


Part 1: Rose rosette disease survey

Introduction

The primary basis for conflicts over the use of rose rosette disease for biological control of *R. multiflora* is centered on the possible effects of rose rosette disease on non-target plants, primarily domesticated roses. However, infestations of *R. multiflora* will have plants infected with RRD and be a constant source of inoculum to infect domesticated roses. Therefore both land managers and rose growers have a common goal -- reduction of *R. multiflora* densities.

While the occurrence of RRD is well documented in Iowa, the prevalence and cycles of the disease is poorly understood. It is impossible to access the risk to ornamental roses associated with augmentation of this disease without knowledge of its current distribution. In the summer of 2001 a survey of RRD in *R. multiflora* infestations was initiated in order to assess the occurrence of the disease in Iowa.

Materials & Methods

Transects were laid out by tying a string at about waist height between two fairly permanent landmarks, such as trees or fence posts at 7 sites over 2 years (Table 1). I sampled *R. multiflora* in one of two ways. For method one, I marked the locations of *R. multiflora* directly underneath the string and whether or not it had symptoms of RRD. Data are reported as the percent of area directly under string that had *R. multiflora* growing. In the second
method I counted the number of plants beneath the string. For each *R. multiflora* I record its height, width, and presence of symptoms of RRD, and flowering status.

**Results & Discussion**

Using method 1, I determined that 11-31% of the area under the string was covered by *R. multiflora*. At Johnson County none of the *R. multiflora* under the string showed symptoms of RRD, although I observed several plants in each site with symptoms of RRD. In Wayne and Decatur Counties (4 sites) 0-50% of the plants had symptoms of RRD (Table 1).

During the *Megastigmus aculeatus* survey conducted in the winter of 2001-2002 (Chapter 5), I also noted symptoms of RRD. I observed RRD symptoms in at least one site in 11 of the 14 counties sampled (Table 2).

These results show that RRD does occur naturally in *R. multiflora* infestations in eastern, northeastern, and southern Iowa.
Table 1: Summary of *Rosa multiflora* and RRD symptomatic plant densities at sites in southern, eastern and northeastern Iowa in summer of 2001 and 2002.

<table>
<thead>
<tr>
<th>Location</th>
<th>County</th>
<th>Year</th>
<th>Number of transects</th>
<th>Sample Method</th>
<th>Total meters</th>
<th>R. multiflora</th>
<th>RRD Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kent Park #1</td>
<td>Johnson</td>
<td>2001</td>
<td>6</td>
<td>1</td>
<td>210.6</td>
<td>11% of transect</td>
<td>0% of transect</td>
</tr>
<tr>
<td>Kent Park #2</td>
<td>Johnson</td>
<td>2001</td>
<td>5</td>
<td>1</td>
<td>111.0</td>
<td>31% of transect</td>
<td>0% of transect</td>
</tr>
<tr>
<td>Conway Rd</td>
<td>Allamakee</td>
<td>2001</td>
<td>4</td>
<td>1</td>
<td>140.7</td>
<td>20% of transect</td>
<td>5% of transect</td>
</tr>
<tr>
<td>Claude Neil</td>
<td>Wayne</td>
<td>2001</td>
<td>2</td>
<td>1</td>
<td>65.9</td>
<td>16% of transect</td>
<td>9% of transect</td>
</tr>
<tr>
<td>Claude Neil</td>
<td>Wayne</td>
<td>2002</td>
<td>2</td>
<td>1</td>
<td>62.6</td>
<td>17% of transect</td>
<td>4% of transect</td>
</tr>
<tr>
<td>Tim Runyon</td>
<td>Wayne</td>
<td>2001</td>
<td>2</td>
<td>2</td>
<td>200.0</td>
<td>16 plants</td>
<td>0 plants (0%)</td>
</tr>
<tr>
<td>Little River Lake</td>
<td>Decatur</td>
<td>2001</td>
<td>2</td>
<td>2</td>
<td>48.9</td>
<td>22 plants</td>
<td>11 plants (50%)</td>
</tr>
<tr>
<td>Little River Lake</td>
<td>Decatur</td>
<td>2002</td>
<td>2</td>
<td>1</td>
<td>47.4</td>
<td>11% of transect</td>
<td>2% of transect</td>
</tr>
<tr>
<td>John Rippey</td>
<td>Decatur</td>
<td>2001</td>
<td>3</td>
<td>2</td>
<td>73.7</td>
<td>11 plants</td>
<td>4 plants (38%)</td>
</tr>
<tr>
<td>John Rippey</td>
<td>Decatur</td>
<td>2002</td>
<td>2</td>
<td>1</td>
<td>43.7</td>
<td>11% of transect</td>
<td>0.7% of transect</td>
</tr>
</tbody>
</table>
Table 2: Counties where *R. multiflora* with symptoms of RRD were observed.

<table>
<thead>
<tr>
<th>County</th>
<th>Number of sites</th>
<th>RRD Symptomatic plants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allamakee</td>
<td>3</td>
<td>Y</td>
</tr>
<tr>
<td>Appanoose</td>
<td>3</td>
<td>Y</td>
</tr>
<tr>
<td>Cedar</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Davis</td>
<td>4</td>
<td>Y</td>
</tr>
<tr>
<td>Jackson</td>
<td>4</td>
<td>Y</td>
</tr>
<tr>
<td>Johnson</td>
<td>5</td>
<td>Y</td>
</tr>
<tr>
<td>Lucas</td>
<td>4</td>
<td>Y</td>
</tr>
<tr>
<td>Madison</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Monroe</td>
<td>3</td>
<td>Y</td>
</tr>
<tr>
<td>Polk</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Union</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Van Buren</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td>Wapello</td>
<td>3</td>
<td>Y</td>
</tr>
<tr>
<td>Warren</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Wayne</td>
<td>3</td>
<td>Y</td>
</tr>
</tbody>
</table>

* Observed *R. multiflora* with RRD symptoms in at least one of the sites in the county.
Part 2: Augmentation of RRD

Introduction

The augmentation of RRD for control of *R. multiflora* has been considered the most viable biological control method to suppress this invasive weed. However, grafting techniques that have been shown to work are a time consuming and often bloody management method. We attempted to augment RRD based on grower techniques that place *R. multiflora* stems showing symptoms of RRD on healthy plants (no symptoms observed). The progression of disease symptoms was monitored in *R. multiflora* with and without augmentative releases. The sites for these releases were selected to minimize possible non-target effects on domesticated roses. The three sites already had plants showing symptoms of RRD naturally occurring, so we did not feel that attempts to augment the disease within the site would be increasing the non-target risk.

Materials and Methods

Two field sites in Story Co, Iowa (Dakins and Christiansen Forest Preserve, both Story County Conservation Board Areas) and one site in Boone Co. with healthy and RRD symptomatic *R. multiflora* were selected. At each field site, 6 branch tips (approx 6 cm) were removed from a single branch on 3 RRD symptomatic plants. Branch tips were also removed from 6 branches on each of 3 non-symptomatic plants. The branch from which the tips were removed was marked. The branch tips were brought to the laboratory and the number of *Phyllocoptes fructiphilus*, the presumed mite vector of RRD, on all 3 tips were counted using methods outlined by deLillo (2001) (see Chapter 4). Mite population levels on
the RRD branches were categorized as high (>20 mites observed on the three branch tips) or low (<20 mites on the three branch tips). The remaining 3 branch tips from each plant were placed in a kill jar with ethyl acetate for at least 12 hrs to kill any arthropods.

Eighteen non-RRD symptomatic *R. multiflora* at each of three field sites were randomly assigned to 1 of 6 treatments: 1) RRD branch tips with high mite population, 2) RRD branch tip with low mite population, 3) RRD branch tip that had been in kill jar, 4) healthy branch tip with low mite population, 5) healthy branch tip that had been in kill jar, or 6) no branch tip. Three healthy *R. multiflora* were assigned to each treatment.

Branch tips used in treatments were collected from the marked branches at the field sites within 48 hrs of determining their mite populations. Branch tips were secured to the healthy *R. multiflora* branch tips with a twist-tie and left in place for 48 hours to allow tips to dry and mites to move onto plants. Treatments were applied at Dakins and Christiansen Parks on Sept. 10, 2002, treatments were applied at Boone on Sept. 13, 2002.

**Results**

Unfortunately, the treated *R. multiflora* at Dakins were destroyed by Boy Scouts doing brush removal in the summer of 2003. On Oct. 20, 2003 all 18 *R. multiflora* at Christiansen showed no symptoms of RRD. Results were more mixed at Boone Co. a year later (Oct. 21, 2003). Only one branch that a treatment had been applied to developed symptoms of RRD and the treatment was a RRD branch with a low mite population. Eight of the *R. multiflora* plants had developed symptoms of RRD, although on different branches than the treatments had been applied to. Two of them had been treated with healthy branches with low mite populations, and 2 had been treated with RRD branches with low mite
populations. One of the plants had been treated with a RRD branch with a high mite population. Two bushes that had been treated with branches that had been in a kill jar developed RRD (one of the treatment branches had been healthy and the other had RRD). Finally, 1 of the control bushes (no branch tip) developed RRD symptoms.

**Discussion**

We were unable to augment RRD at one field site and at the other field site the symptomatic *R. multiflora* appeared across all of the treatments. However, 6 of the 9 plants developing symptoms had been treated with branches with mites.

If I were to do this experiment again I would focus initially on the temporal aspects of disease transmission. It is possible that the vector competence of *P. fructiphilus* varies through the summer and the disease susceptibility of *R. multiflora* is also likely to vary over the summer. I would use only the high mite treatment and apply it to healthy *R. multiflora* before flowering, mid-summer, and late summer.
Part 3: Exclusion of *Megastigmus aculeatus* from *Rosa multiflora* hips to assess effect on viable seeds

**Introduction**

*Megastigmus aculeatus* is a potentially useful natural enemy of *R. multiflora* because the larvae feed on viable *R. multiflora* seeds. Each larva consumes a single seed within the rosehip, but other seeds within the hip can potentially be viable.

Experimental evidence is needed to assess if production of viable seeds is reduced by *M. aculeatus* infestations. In Chapter 5, I modeled data collected from sites with high numbers of *M. aculeatus* and found a significant reduction in both viable and aborted seeds in hips with *M. aculeatus*. However, exclusion experiments are preferred when possible, so the goal of this experiment was to exclude *M. aculeatus* from flowers on a branch of a *R. multiflora* and then compare the number of viable seeds produced with another branch of the plant on which *M. aculeatus* had access to the developing flowers.

**Material & Methods**

Four sites (two in Allamakee Co. and two in Jackson Co.) were selected with high populations of *M. aculeatus* (Chapter 5). Four *R. multiflora* were selected at each site. The flowers on 30 cm sections of 4 branches on each plant were hand pollinated. Two of the branches were enclosed within mesh bags to prevent access by *M. aculeatus* and two of the branches were enclosed within mesh bags that had access holes for *M. aculeatus*. Two additional branches had no treatment. The enclosures were left on the branches for at least two weeks after petal fall, the time period *M. aculeatus* females oviposit into rosehips. The
rosehips were collected in October and dissected to determine the number of viable seeds in
the enclosed and enclosed-with-openings sections of the branches.

Plants were bagged at the two Jackson Co. sites on June 11, 2003 and removed July
3, 2003. Plants were bagged at the two Allamakee Co. sites on June 18, 2003 and bags were
removed July 3, 2003. Hips were collected from Allamkee Co. on Oct 23, 2003. Several
plants had no hips or labels had been removed, so I only collected data from 2 plants at site 1
and one plant at site 2. I collected hips from the Jackson Co. site on Oct 25, 2003.

Results

I was not successful in preventing *M. aculeatus* from ovipositing in rosehips within
the bags. At the Allamakee Co. sites I had higher numbers of *M. aculeatus* in the bagged
treatments (Table 1). At the Jackson Co. sites I had similar numbers of *M. aculeatus* larvae
in all three treatments (Table 2). Across both fields sites the percentage of larvae were
distributed fairly evenly between bagged, cut bags and no treatment, with a slightly higher
percent in the bagged treatment (Table 3).

I do not think I accidentally enclosed wasp larvae within the bags, but it is possible
that female *M. aculeatus* were active before I put the bags in place. It is also possible that *M.
aculeatus* females were able to oviposit through the mesh. The ovipositors could definitely
fit through the mesh, I was hoping that the bags would make it difficult enough to reach the
hips that ovipositing females would move on to unbagged hips. The final possibility is that
the wasps oviposit over a long time period and I did not bag the hips long enough and they
oviposited after the bags were removed. I consider this the most likely possibility.
Conclusion

Exclusion experiments are good for assessing the effect of herbivores on a plant, however, it is often difficult to keep an insect from its food source. If I were to repeat this experiment I would leave the bags on the *R. multiflora* for a longer period of time. I think *M. aculeatus* adults emerge over a longer time period than the 2-3 weeks I left the bags on the branches. I hesitated to leave bags on longer because in the past I had found that all the flower panicles died when bagged for too long, but I think I should have left the bags on for about 6 weeks.
Table 1: The percent of viable seeds found in each treatment, percent of dead seeds found in each treatment, and the percent of *M. aculeatus* larvae found in each treatment at the two sites in Allamakee Co.

<table>
<thead>
<tr>
<th>County</th>
<th>Site</th>
<th>Plant</th>
<th>Bag</th>
<th>Cut bag</th>
<th>Nothing</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allamakee</td>
<td>1</td>
<td>Viable</td>
<td>4%</td>
<td>62%</td>
<td>35%</td>
<td>26</td>
</tr>
<tr>
<td>Allamakee</td>
<td>1</td>
<td>Dead</td>
<td>17</td>
<td>0</td>
<td>83</td>
<td>6</td>
</tr>
<tr>
<td>Allamakee</td>
<td>1</td>
<td>Larvae</td>
<td>3</td>
<td>52</td>
<td>45</td>
<td>64</td>
</tr>
<tr>
<td>Allamakee</td>
<td>1</td>
<td>Viable</td>
<td>84%</td>
<td>3%</td>
<td>14%</td>
<td>37</td>
</tr>
<tr>
<td>Allamakee</td>
<td>2</td>
<td>Dead</td>
<td>61</td>
<td>20</td>
<td>19</td>
<td>54</td>
</tr>
<tr>
<td>Allamakee</td>
<td>2</td>
<td>Larvae</td>
<td>75</td>
<td>11</td>
<td>15</td>
<td>123</td>
</tr>
<tr>
<td>Allamakee</td>
<td>2</td>
<td>Viable</td>
<td>76%</td>
<td>24%</td>
<td>0%</td>
<td>29</td>
</tr>
<tr>
<td>Allamakee</td>
<td>2</td>
<td>Dead</td>
<td>51</td>
<td>37</td>
<td>12</td>
<td>67</td>
</tr>
<tr>
<td>Allamakee</td>
<td>2</td>
<td>Larvae</td>
<td>57</td>
<td>35</td>
<td>8</td>
<td>93</td>
</tr>
</tbody>
</table>

Sum of Allamakee site 1 & 2

<table>
<thead>
<tr>
<th>County</th>
<th>Viable</th>
<th>Dead</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allamakee</td>
<td>59%</td>
<td>26%</td>
<td>15%</td>
</tr>
<tr>
<td>Allamakee</td>
<td>55</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>Allamakee</td>
<td>53</td>
<td>28</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 2: The percent of viable seeds found in each treatment, percent of dead seeds found in each treatment, and the percent of *M. aculeatus* larvae found in each treatment at the two sites in Jackson Co.

<table>
<thead>
<tr>
<th>County</th>
<th>Site</th>
<th>Plant</th>
<th>Bag</th>
<th>Cut bag</th>
<th>No Bag</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackson</td>
<td>1</td>
<td>Viable</td>
<td>30%</td>
<td>67%</td>
<td>3%</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Dead</td>
<td>15</td>
<td>56</td>
<td>29</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Larvae</td>
<td>56</td>
<td>44</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Jackson</td>
<td>1</td>
<td>Viable</td>
<td>29%</td>
<td>19%</td>
<td>52%</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dead</td>
<td>16</td>
<td>11</td>
<td>74</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Larvae</td>
<td>35</td>
<td>6</td>
<td>58</td>
<td>31</td>
</tr>
<tr>
<td>Jackson</td>
<td>1</td>
<td>Viable</td>
<td>40%</td>
<td>37%</td>
<td>23%</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Dead</td>
<td>31</td>
<td>55</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Larvae</td>
<td>30</td>
<td>53</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>Jackson</td>
<td>1</td>
<td>Viable</td>
<td>40%</td>
<td>27%</td>
<td>34%</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dead</td>
<td>18</td>
<td>58</td>
<td>25</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Larvae</td>
<td>38</td>
<td>33</td>
<td>29</td>
<td>200</td>
</tr>
<tr>
<td>Jackson</td>
<td>2</td>
<td>Viable</td>
<td>26%</td>
<td>17%</td>
<td>57%</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Dead</td>
<td>36</td>
<td>27</td>
<td>37</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Larvae</td>
<td>36</td>
<td>4</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td>Jackson</td>
<td>2</td>
<td>Viable</td>
<td>35%</td>
<td>56%</td>
<td>10%</td>
<td>524</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dead</td>
<td>38</td>
<td>44</td>
<td>18</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Larvae</td>
<td>67</td>
<td>0</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>Jackson</td>
<td>2</td>
<td>Viable</td>
<td>39%</td>
<td>0%</td>
<td>60%</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Dead</td>
<td>10</td>
<td>29</td>
<td>61</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Larvae</td>
<td>29</td>
<td>0</td>
<td>71</td>
<td>7</td>
</tr>
<tr>
<td>Jackson</td>
<td>2</td>
<td>Viable</td>
<td>11%</td>
<td>47%</td>
<td>42%</td>
<td>281</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Dead</td>
<td>11</td>
<td>56</td>
<td>32</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Larvae</td>
<td>11</td>
<td>50</td>
<td>39</td>
<td>154</td>
</tr>
</tbody>
</table>

**Sum of Jackson sites 1 & 2**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Viable</th>
<th>Dead</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31%</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>37%</td>
<td>46</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>32</td>
<td>36</td>
</tr>
</tbody>
</table>

**Table 3:** The percent of viable seeds found in each treatment, percent of dead seeds found in each treatment, and the percent of *M. aculeatus* larvae found in each treatment summed over all four field sites.

**Total Sum of Allamakee Co. and Jackson Co.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bag</th>
<th>Cut bag</th>
<th>No Bag</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable</td>
<td>32%</td>
<td>37%</td>
<td>32%</td>
<td>1849</td>
</tr>
<tr>
<td>Dead</td>
<td>27</td>
<td>44</td>
<td>29</td>
<td>802</td>
</tr>
<tr>
<td>Larvae</td>
<td>38</td>
<td>32</td>
<td>30</td>
<td>804</td>
</tr>
</tbody>
</table>
Part 4: Diapause in *Megastigmus aculeatus*

*Megastigmus aculeatus* is one of the natural enemies that may help manage *R. multiflora*. Female *M. aculeatus* deposit eggs, presumably within individual achenes, developing within a rosehip. In order to have food, the hatching *M. aculeatus* larva need to be in a fertilized achene that has a developing embryo. The synchronization of *M. aculeatus* adult emergence and oviposition and the development of *R. multiflora* seeds is necessary for the survival of *M. aculeatus*. Determining the environmental cues that *M. aculeatus* respond to after overwintering within rosehips will help us determine how they are synchronizing with a food source they evolved to utilize. The goal of this experiment was to determine the factors that maintain and terminate diapause in overwintering *M. aculeatus*.

Materials & Methods

Hips were collected monthly from Dec 2002-April 2003 from 3 sites in Jackson Co. Samples of ten hips were placed in glass 12 dram vials with a screw top and exposed to 12:12, L:D at 18°C, 22°C, or 26°C to assess response to temperature. Ten hips were also be placed outside in a shade box (white wooden box elevated off ground with ventilation slats). Temperatures in each cabinet and the outdoor shade box were measured with a data logger (HOBO® Pro Series). Samples of 10 hips were also placed in 10:14, 12:12, 14:10, or 16:8 L:D all at 22°C to measure the response of *M. aculeatus* to photoperiod. This method has been previously used to assess the response of diapausing *Chrysopa carnea, C. downesi* (Neuroptera: Chrysopidae) and *Perilitus coccinellae* (Hymenoptera: Braconidae) to daylength and temperature (Tauber & Tauber 1973, Obrycki & Tauber 1979, Tauber &
Tauber 1981). Ten hips from each treatment were also dissected to determine the number of live *M. aculeatus* larvae. Vials were checked at least twice a week for emergence of *M. aculeatus* adults. The number of days to adult emergence from the beginning of the treatments was calculated for each adult *M. aculeatus*.

**Results**

At the shortest photoperiod (10:14 L:D) wasps emerged only from rosehips collected in April (Table 1). The wasps from the earlier collection dates did not pupate or emerge as adults. More wasps emerged from hips exposed to longer daylengths, and the greatest number of wasps emerged from hips exposed to outdoor conditions (Table 1). More wasps emerged from hips exposed to 18 or 22°C compared to 26°C (Table 2).
Table 1: The average number of days between collection and emergence (number of adults) of *M. aculeatus* exposed to four light periods at 22°C and natural conditions in a shade box.

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Light period</th>
<th>10:14</th>
<th>12:12</th>
<th>14:10</th>
<th>16:8</th>
<th>Outside</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/1/2002</td>
<td></td>
<td>60 (1)</td>
<td>50 (1)</td>
<td>196 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/29/2002</td>
<td></td>
<td>45 (2)</td>
<td>43 (11)</td>
<td>170 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/7/2003</td>
<td></td>
<td>50 (2)</td>
<td>39 (1)</td>
<td>42 (2)</td>
<td>111 (6)</td>
<td></td>
</tr>
<tr>
<td>3/7/2003</td>
<td></td>
<td>46 (2)</td>
<td>39 (2)</td>
<td>98 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/15/2003</td>
<td></td>
<td>32 (12)</td>
<td>32 (11)</td>
<td>30 (11)</td>
<td>32 (9)</td>
<td>60 (5)</td>
</tr>
</tbody>
</table>

Table 2: The average number of days until emergence (number of adult wasps) of *M. aculeatus* exposed to differing temperatures at 12:12 L:D. Outside emergence times are same as Table 1.

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Temperature</th>
<th>18 deg. C</th>
<th>22 deg. C</th>
<th>26 deg C</th>
<th>Outside</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/1/2002</td>
<td></td>
<td>196 (14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/29/2002</td>
<td></td>
<td>54 (2)</td>
<td>170 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/7/2003</td>
<td></td>
<td>50 (2)</td>
<td></td>
<td>111 (6)</td>
<td></td>
</tr>
<tr>
<td>3/7/2003</td>
<td></td>
<td>58 (1)</td>
<td>46 (2)</td>
<td></td>
<td>98 (5)</td>
</tr>
<tr>
<td>4/15/2003</td>
<td></td>
<td>47 (8)</td>
<td>32 (11)</td>
<td>29 (3)</td>
<td>60 (5)</td>
</tr>
</tbody>
</table>
Part 5: *Megastigmus aculeatus* oviposition within rosehip

No information is available about the life history of *M. aculeatus* during the summer when the larvae are developing. It appears that females are able to oviposit eggs directly into the achenes developing within the rosehip, although this has not been confirmed. I hoped to find evidence of where, within the rosehip, *M. aculeatus* was depositing eggs.

Materials & Methods

Rosehips were collected from a *R. multiflora* infestation in Jackson Co. June 4, July 27, and Sept 18, 2004.

Whole hips and separated seeds were fixed in FAA (formalin-acetic acid-alcohol) for several days at 4°C. They were dehydrated in a graded ethanol series (24 hours each step), cleared with tertiary butyl alcohol, infiltrated and embedded using Paraplast paraffin (Fisher Scientific, Pittsburgh, PA). Sections of the seeds were made using an A/O 820 rotary microtome (Fisher Scientific, Pittsburgh, PA). Sections were cut at 10μm, collected onto slides, deparaffinized and stained with fast-green and saffranin, then dehydrated, cleared and coverslipped. Digital images were collected using a Zeiss Axiocam HRC on a Zeiss AxioPlan II (Carl Zeiss Inc, Thornwood, NY) compound microscope.

Results

We found one seed with damage consistent with an ovipositor cutting through the seed coat to insert an egg within the developing achene from the seeds collected June 4
(Figure 1). The processing was not successful on the hips and seeds collected in July and September. The older seed coats tended to shatter when sliced.
Figure 1: The tip of a *R. multiflora* seed showing what may be the damage caused by an ovipositing *M. aculeatus* female.
ACKNOWLEDGEMENTS

I would like to thank all the undergraduate students who helped me to complete this research, particularly Dennis Portz and Brad Tucker. The support of my fellow graduate students, Betsy Matos and Susan Chapman, made conducting this research possible and enjoyable. I would also like to thank my husband Mark for all of his help. I would particularly like to thank my parents, Robert and Dara, for their support and also for their willingness to help me string transects through a terrible, thorny weed and for driving with me to Allamakee County numerous times to collect rosehips.