Biology and Control of Amur Honeysuckle

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DEDICATION

… To my late mother: Jenny Lynn Riley

Without your inspiration I never would have made it through my undergraduate years, let alone graduate school. I know you would be proud of the things I have been able to accomplish. You will continue to inspire me for the rest of my life.

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BIOLOGY AND MANAGEMENT OF AMUR HONEYSUCKLE (*Lonicera maackii*)

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ABSTRACT

Amur honeysuckle (*Lonicera maackii*) is an invasive weed species that is present in a majority of the United States. This weed has the ability to displace native plant species and develop monocultures in undisturbed areas. Little is known about the biology and control options for this plant. The objectives of this research were to: a) determine efficacy of various herbicides using postemergence and basal bark applications; b) determine the means of seed spread and time at which Amur honeysuckle seeds are viable; c) determine if germination of other species is effected by allelopathic or light variables. Research was conducted during 2010, 2011, 2012 and 2013 at multiple locations throughout central Missouri. Control of Amur honeysuckle was achieved with a foliar application of glyphosate (90 to 100%), aminocyclopyrachlor + metsulfuron (62 to 90%), and aminocyclopyrachlor + metsulfuron + imazapyr (96 to 100%). Greater than 83% viability was observed for Amur honeysuckle seeds harvested in October through November. Greater than 90% of berries were found to be predated from shrubs from September through March. Understory light intensity (µmol m^{-2} s^{-1}) was reduced by shrub cover in the spring (92%), summer (86%), and fall (75%). Lettuce germination was not reduced in shrub infested versus uninfested soils.

CHAPTER I

LITERATURE REVIEW

Research Justification

The spread of invasive and noxious plant species is a threat to native plant species in many geographical areas of the United States (Luken 1988; Myers 1983; Woods 1993). Invasive species are defined as an alien plant whose introduction does, or is likely to cause economic or environmental harm or harm to human health (USDA NAL 2013). A noxious species is any plant that has been designated by the U.S. government or any state government to require control due to its harmful impact to agricultural or native ecosystems as well as to livestock or the public health (Federal Noxious Weed Act 1974). The Federal Noxious Weed Act requires that any noxious weed on one's property be eradicated.

Currently, Amur honeysuckle (*Lonicera maackii*) is not classified as noxious in Missouri. However, in Connecticut, Massachusetts, and Vermont it is classified as banned, prohibited, and a noxious weed respectively (USDA NRCS 2013). These classifications are an evidence of the problematic effects of Amur honeysuckle.

Origin

The *Caprifoliaceae* (Honeysuckle) family contains 11 genera and 177 taxa (USDA NRCS 2013). Seven genera within the family are native to North America (Ferguson

1966). All honeysuckle and elderberry species are members of this family. The family is not economically important in the United States with the exception of the species in the *Lonicera*, *Weigela*, *Viburnum*, *Leycesteria*, *Abelia*, *Symphoricarpos*, and *Sambucus* genera that are cultivated as ornamentals (Ferguson 1966).

There are about 180 species of *Lonicera* with only about 20 native to North America. Honeysuckles that are native to the United States include grape honeysuckle (*Lonicera reticulate* Raf.), yellow honeysuckle (*Lonicera flava* Sims.), and limber honeysuckle (*Lonicera dioica* L.), which are not considered invasive (Missouri Vegetation Management Manual 1997). The *Lonicera* genus contains species with both vining and shrub growth habits. Common features of *Lonicera* species are entire, opposite, short petiolate leaves and long tubular flowers. These flowers give rise to few-seeded fleshy berries and ovate seeds (Ferguson 1966). *Lonicera* species have been used as ornamentals, in land reclamation, and for erosion control (Luken and Thieret 1996). Amur honeysuckle, like many other honeysuckle species, was imported for the fragrance of flowers, and was widely planted in urban ornamental settings. The flowers are known to emit this pleasing odor when in bloom. Land reclamation is the process of returning of land to a natural state after an industrial use such as mining. The Soil Conservation Service (now Natural Resources Conservation Service) utilized Amur honeysuckle as an erosion control measure throughout the U.S. for use in poorly structured soils (Luken and Thieret 1996). In Russia and Japan, blue honeysuckle *(Lonicera caerulea* L.) is harvested as an edible berry (Chaovanalikit et al. 2004). Oregon State University is currently conducting an experimental program to assess if blue honeysuckle cultivation is a viable option in the United States (Thompson and Chaovanalikit 2003).

Figure 1.1. Invasive Honeysuckle Pith.

Non-native bush honeysuckles; which originate from eastern Asia, include Amur honeysuckle (*Lonicera maackii*), Morrow's honeysuckle (*Lonicera morrowii*), Tatarian honeysuckle (*Lonicera tataria*), and Bell's honeysuckle (*Lonicera* X *bella*), a hybrid of Morrow's and Tatarian honeysuckles (Vermont 1998). All four of these honeysuckles are considered invasive species in the United States. There are several characteristics that distinguish invasive from native honeysuckle species (USDA NRCS 2013). Native honeysuckle species exhibit both vining and shrub growth habits. The exception to this is Japanese honeysuckle (*Lonicera japonica*) which predominantly grows with a vining growth habit. Stems of invasive honeysuckles have hollow piths between the nodes, whereas stems of native honeysuckles are solid (Figure 1.1 and Figure 1.2, Pringle 1973). Additionally, native bush honeysuckles have yellow flowers and the fruits are an elongated capsule, whereas invasive bush honeysuckles have white or pink flowers and red fruits (Sarver et al. 2008).

Figure 1.2. Native Honeysuckle Pith

All four bush honeysuckle species exhibit a shrub growth habit, very prolific seed production, and hollow stems. There are however, very distinctive characteristics to distinguish between these species. Morrow's honeysuckle is the shortest of the four species growing to a height of 2 m tall; plants have oval- to egg-shaped leaves that are pubescent on the adaxial surface. Flowers have long and pubescent stalks that are white in color, and turn yellow with age. Tatarian honeysuckle reaches a height up to 3 m and has oval- to egg-shaped leaves that are glabrous. Plants have long, pink, and glabrous flower stalks. Bell's honeysuckle phenotypic characteristics can vary greatly between those associated with Morrow's honeysuckle and Tatarian honeysuckle, and it is identified by a combination of traits that are inconsistent with either parent species. For example, a honeysuckle less than 3 m tall at maturity with white flowers and glabrous leaves would be considered Bell's honeysuckle. Amur honeysuckle cotyledons are ovate to oblong. Leaves are ovate, pubescent and opposite. For the first year of growth the plant is completely herbaceous (Bryson and DeFelice 2010). When mature, leaves are dark green and end in a sharp point at the tip, with hair along the veins on the underside of the leaf. A distinctive characteristic of Amur honeysuckle is the flower. The flowers are two-lipped, with five petals that comprise a tube that is approximately 1.5 to 2.5 cm long (Figure 1.3). The

Figure 1.3. Flowers of Amur honeysuckle (*L. maackii*).

flowers are paired at the nodes of mature shrubs (Bryson and DeFelice 2010).

Amur honeysuckle in particular has become increasingly troublesome since its introduction to the United States from Northeast Asia in the 1897 as an ornamental (Dirr 1983). From the 1960s to 1984 a program was sponsored by the Soil Conservation Service (now Natural Resource Conservation Service (NRCS)) for the improvement and development of cultivars of Amur honeysuckle for use in soil stabilization and reclamation programs (Luken and Thieret 1996). Since that time the invasion by Amur honeysuckle has been quick and widespread.

Amur honeysuckle is a problematic species because it exhibits characteristics that are common among successful non-native species: rapid growth rate; long range seed dispersal, in this case by birds; and phenotypic and habitat plasticity in response to light environment (Edgin 2007; Luken et.al. 1995). The fast growing nature of Amur honeysuckle contributes to its invasiveness; the maximum biomass produced is 1,350 g m^{-2} y^{-1} which is similar to the production of an entire woodland community (Whittaker 1975). Deering and Vankat (1999) found 3 years after establishment the average shrub height was 1 m and stem count was 4.3 per shrub, which is more growth than many forest species. Plants are very prolific and spread is facilitated by birds (Bartuszevige and Gorchov 2006; Bonner and Karrfalt 2008; Ingold and Craycraft 1983). Luken et al. (1997) reported that Amur honeysuckle is able to equal or exceed the branch growth and leaf mass of the native shrub spicebush (*Lindera benzoin*), a shade tolerant forest species, in low-light as well as high-light environments. Also, Powell et al. (2013) reported plant communities in Missouri invaded by Amur honeysuckle had reduced abundance of shade-intolerant native species compared to uninvaded communities.

Amur honeysuckle also has the ability to produce allelopathic chemicals (Dorning and Cipollini 2006) It has been reported that extracts from mature Amur honeysuckle leaves and fruits had allelopathic effects on both grasses and forbes (Dorning and Cipollini 2006; McEwan et al. 2010). Thirteen phenolic compounds have been characterized from leaf extracts; two of which have inhibitory effects on *Arabidopsis thaliana* germination (Cipollini et al. 2008).

Currently, Amur honeysuckle is widespread throughout the Eastern and Midwestern regions of the United States; from North Dakota to Texas and east to Massachusetts and Georgia (Luken and Thieret 1996; Rich 2000). This area includes 26 U.S. states, the District of Columbia, and the Canadian province of Ontario. In the U.S. the Soil Conservation Service's policy of recommendation of this species for erosion control and also the use of Amur honeysuckle as an ornamental likely contributed to the spread of this species (Luken and Thieret 1996). Amur honeysuckle is considered a noxious weed in Connecticut, Massachusetts, and Vermont (USDA NRCS 2013). In these areas it is required; by law; to be controlled. A survey conducted by the Northern Research Station – Forest Inventory and Analysis of USDA found that non-native bush honeysuckles were the second most frequent invasive plant across 1,264 0.4 ha test plots in 2005 and 2006 in Missouri (Moser et al. 2008).

Amur honeysuckle has the potential to overwhelm habitats into which it is introduced. Native species can be outcompeted by Amur honeysuckle in low and high light environments (Luken et al. 1997). Woods (1993) also found that evergreen and vining species are more tolerant of Tatarian honeysuckle, due to their use of year round light and higher canopy position, respectively, which suggests that light competition is of vital importance. In invaded areas, honeysuckle is the plant with the highest population in forest edges (Luken and Mattimiro 1991) which appears to be directly related to higher light environments (Luken and Goessling 1995).

The negative impact that bush honeysuckles as well as other invasive species have on native ecosystems has been extensively documented (Hartman and McCarthy 2004; Luken and Goessling 1995; Luken et. al. 1997; Schmidt and Whelan 1999). The most prominent factor is the lack of herbaceous diversity, which is a characteristic of a well-functioning ecosystem, that is displayed in forests and roadsides where bush honeysuckle species have invaded due to its high biomass production (Whittacker 1975). Hutchinson and Vankat (1997) found that in a southwestern Ohio forest the presence of Amur honeysuckle was negatively correlated with herb cover, tree seedling density, and species richness. Buddle et al. (2004) showed that the diversity of ground-dwelling spiders was reduced in infested hedgerows due to decreased ground cover. Due to increased transpiration Amur honeysuckle was found reduce natural stream flow 10 percent, which will shorten the life of ephemeral ponds and steams (Boyce et al. 2011). Additionally, Schmidt and Whelan (1999) found that the daily nest mortality rate for American robins (*Turdus migratorius*) was significantly higher in Amur honeysuckle than in native species due to lower nest height and the absence of thorns seen in native species. Though Amur honeysuckle berries provide a significant food source to avian species the poor quality of the berries as an energy source makes high frugivory a negative aspect (Ingold and Craycraft 1983).

Biology

Figure 1.4. Epigeal Emergence of Amur honeysuckle.

By understanding the biology of weeds it is possible to target weak points in growth and reproduction and thereby manage the problem more effectively. Amur honeysuckle seeds germinate from spring through summer in an epigeal fashion (Figure 1.4). This means that when emerging the seed comes above the ground with the cotyledons. According to Luken and Goessling (1995), the seeds of Amur honeysuckle are dispersed in a non-dormant state. However, Swingle (1939) found that 75 to 90 days of cold stratification were required for Amur honeysuckle germination. Little is known about the precise longevity of Amur honeysuckle seed. Luken and Mattimiro (1991) found that 80% of seeds sampled under existing *L. maackii* plants were viable. However, Hartman and McCarthy (2008) found as low as 6% viability in soil samples from long-invaded sites. This evidence suggests that there is community level variability exhibited by Amur honeysuckle. *L. maackii* would fall into the class of

Figure 1.5. Amur honeysuckle seedling.

seeds described by Canham and Marks (1985) that exhibits minimal delay between dispersal and germination and lack of a persistent seed bank.

Amur honeysuckle exhibits very distinctive characteristics when mature. It is generally thought to take 3 to 5 years to reach reproduction. Shrubs, which are defined as all stems that share a root stock, tend to arch over one another when mature (Bryson and DeFelice 2010). Mature Amur honeysuckles are up to 6 m tall and deciduous, or shed their leaves every fall. Shrubs exhibit a variety of growth habits depending on environment. Generally, shrubs are arranged with younger branches that grow in an arching manner over the older branches. Trisel (1997) reported that Amur honeysuckle initial leaf expansion in the spring is up to 6 weeks earlier than other species. Amur honeysuckle also retains its leaves longer, until the middle of December, than native species (Luken and Thieret 1996, McEwan et al. 2009; Shustack et al. 2009). The bark of Amur honeysuckle is tan to light brown and will often split or peel lengthwise when mature. Amur honeysuckle grows in dense thickets along forest edges and roadsides in Missouri. These shrubs can live as long as 25 years (Luken and Mattimiro 1991).

The prolific nature of Amur honeysuckle is a major problem for the control of this weed as it can easily replace itself each year. Deering and Vankat (1999) reported that only 5.7% of Amur honeysuckle shrubs were reproductive at 3 years of age, however more than 50% were reproductive at age 5 in an Ohio woodlot. They also reported that all shrubs over 2.5 m in height were reproductive, however shrubs less than 1 m in height were not. Additionally, age was not a significant factor in the ability of Amur honeysuckle to reproduce, whereas height was. This makes control of taller shrubs a priority over shorter scrubs.

Inflorescence timing varies from geographic location within the species; however it is generally in late spring or early summer (Bonner and Karrfalt 2008). The nectar and pollen of Amur honeysuckle is used by a wide variety of insect species (Goodell et al. 2010), therefore Amur honeysuckle is able to be pollinated readily. Goodell and Iler (2007) found that pollinator visit is required for seed production. These flowers give rise to fruits that are bright red berries.

A distinctive characteristic of Amur honeysuckle is its opposite bright red berries (Luken and Thieret 1996). The fruits and seeds are eaten and then dispersed by birds (Ingold and Craycraft 1983). Berries are 4 to 7mm in diameter, and paired in leaf axils (Bryson and DeFelice 2010). They contain from 1 to 10 seeds and individual branches may produce hundreds of berries (Goodell et al. 2010).

Management

The management of invasive species, such as Amur honeysuckle, is often difficult because plants are integrated into habitats with desirable, native species. However, failure to control Amur honeysuckle will lead to greater exclusion of native species. For Amur honeysuckle, control strategies must focus on elimination of established plants as well as prevention of berry production. Because Amur honeysuckle grows in non-disturbed areas and is not found in agronomic fields and pastures, reports of effective management techniques are limited. It is likely that Amur honeysuckle is not found in agronomic fields because it is a perennial that is unable to with stand soil disturbance. Control techniques include biological control (the use of other species for selective weed control), mowing/clipping (mechanical removal of above ground biomass), controlled burning (the use of fire for selective control) and herbicide application (Franz and Keiffer 2000; Fuchs and Geiger 2005; Hartman and McCarthy 2004; Love and Anderson 2009; Missouri Vegetation Management Manual 1997; Rathfon and Ruble 2007).

Biological control of honeysuckle species may be difficult. With the exception of the honeysuckle aphid (*Hyadaphis tataricae*), which reduces plant vigor (Hahn and Kyhl 1999), Amur honeysuckle has few natural enemies. However, the honeysuckle aphid is readily controlled by native ladybeetle (*Hippodamia convergens*), green lacewing (*Chrysoperla carnea*), and syrphid fly (*Syrphidae* family) larvae (Keith 2001). Additionally, the honeysuckle aphid is not selective for invasive honeysuckle species.

Mowing is a common practice used by the Missouri Department of Transportation (MODOT) for controlling growth of roadside vegetation. However, the typical mowing practice includes the first 5 meters adjacent to the road only. With most of the roadside right-of-way wider than this and Amur honeysuckle found next to shaded areas, few plants are typically mowed. After Amur honeysuckle is established, its woody growth habit renders mowing more difficult. Mowing has only controlled Amur honeysuckles marginally because of its readiness to resprout from crowns after cutting (Luken and Mattimiro 1991). Luken and Mattimiro (1991) also reported that shrubs would readily resprout for up to three years with repeated cutting; however, repeated clipping for 3 years would reduce the shrub density of forest shrubs by greater than 50%. With a reduction in shrub density seed production is also reduced.

Controlled burning is the use of fire, in a planned and safe manner, to control unwanted vegetation. For Amur honeysuckle, fire is fatal to seedlings but only causes minor injury to mature plants. Frequent controlled burning for 5 years can be effective, but seed germination can result in new established plants (Missouri Department of Conservation 2011; Smith 1997). In communities where controlled burning is appropriate, timing is optimal in early spring (Missouri Department of Conservation 2011) when desirable plants remain dormant. Controlled burns will be most effective when used in conjunction with other control methods such as herbicide applications and mowing.

Herbicides remain one of the most effective means for management of invasive weeds, including Amur honeysuckle. When dealing with a weed species that possesses a woody stem, various application timings and herbicide placements are available: basal bark; cut stump; stem injection; and foliar herbicides (Franz and Keiffer 2000; Hartman and McCarthy 2004; Love and Anderson 2009; Rathfon and Ruble 2007). The dense growth of Amur honeysuckle, and proximity to wooded areas, necessitates specialized equipment for postemergence applications. Herbicide treatments range from \$12.25 to \$329.03 hectare^{-1} depending on chemical choice and application method (Rathfon and Ruble 2007). These costs are lower than those of manual cutting and removal. Systemic herbicides are known to be effective for control of perennial weeds. The herbicide modes of action most effective for honeysuckle include plant growth regulators, inhibitors of acetolactate synthase (ALS), and the aromatic amino acid synthesis inhibitor glyphosate. Though inconsistent levels of control have been observed through the use of herbicides (Rathfon and Ruble 2007) the long growing season and woody growth habit of Amur honeysuckle provide many options for the use of multiple herbicide strategies.

Various herbicides have been employed as a foliar application in attempting to manage Amur honeysuckle. Three of the most commonly utilized herbicides are triclopyr, imazapyr, and glyphosate (Fuchs and Geiger 2005; Rathfon and Ruble 2007). Triclopyr is a plant growth regulating herbicide, and selectively controls a wide range of broadleaf weeds (Ross and Childs 1996). Triclopyr is effective on most brush species, including Amur honeysuckle (Missouri Vegetation Management Manual 1997). Imazapyr is an ALS inhibiting herbicide and provides Amur honeysuckle control when applied to 0.75-3 m tall shrubs (Rathfon and Ruble 2007). The residual activity of imazapyr may reduce the growth of other desirable species in treatment areas (Ross and Childs 1996). Glyphosate is a non-selective herbicide that is commonly used to control Amur honeysuckle (Conover and Geiger 1999). Rathfon and Ruble (2007) found that various mixtures of triclopyr, imazapyr, and glyphosate applied in the spring to shrubs from 0-3+ m in height resulted in greater than 65% control of Amur honeysuckle. If glyphosate is to be used in the fall, it should be applied before leaf color changes (Missouri Vegetation Management Manual 1997). Rathfon and Ruble (2007) also showed that, in an Indiana hardwood forest, foliar applications of glyphosate resulted in 40% greater shrub mortality than both basal bark and cut-stump treatments.

Another herbicide application method for control of unwanted woody plants is a basal bark application. Basal bark applications are made with specialized herbicide formulations that are mixed with carrier oil. The mixture is then applied to the bark of the treated plant from ground level to 40 cm up each stem. Basal bark applications are a common practice in the control of woody weeds because they are a more targeted application, though less area can be treated than with a foliar application. However, most basal bark applications have not provided an acceptable level of control (90%) of Amur honeysuckle in previous research (Rathfon and Ruble 2007).

Purpose of Study

The proliferation of Amur honeysuckle, through rapid spread of seed by birds and adaptability of rapidly growing plants to the habitat between forested areas and open grassways, has sparked interest in controlling infestations. Amur honeysuckle has many negative impacts; multi-stemmed shrubs, up to 6 meters in height, form dense thickets that crowd out native species. In natural areas infestations reduce the aesthetics and utility of parks and natural areas.

Little information is available on identifying the weak points in Amur honeysuckle biology to optimize control. Studies are necessary to determine effective management strategies. Determining the efficacy of herbicides on emerged Amur honeysuckle, including the residual control of seedlings, will enable researchers to identify proper chemical control methods. Further research is necessary to determine the timing of control techniques to preclude viable seed production and the timing of bird predation for seed spread.

This thesis research is divided into four parts:

A) Herbicide efficacy

Objective:

1) To determine efficacy of postemergence herbicides on previously mowed Amur honeysuckle as well as residual effects.

2) To determine efficacy of basal bark applied herbicides on Amur honeysuckle.

B) Seed Dispersal

Objective:

To determine the relative spread and timing of Amur honeysuckle seeds dispersed by avian species.

C) Seed Viability

Objective:

To determine the time at which Amur honeysuckle seeds are viable.

D) Inhibition of Germination

Objective:

To determine if seed germination is affected by allelopathic or light variables.

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CHAPTER II

RESPONSE OF AMUR HONEYSUCKLE (*LONICERA MAACKII*) TO HERBICIDES

S. A. Riley and R. J. Smeda*

Amur honeysuckle is an invasive shrub found throughout the Central and Northeastern U.S., with plants persisting in undisturbed areas along treelines. Despite widespread infestations, effective herbicide programs are poorly documented. Experiments were conducted to determine the efficacy of herbicides applied as foliar or basal bark treatments on Amur honeysuckle. Foliar trials were established at two sites in central Missouri in 2010 and 2011. Established Amur honeysuckle was mowed the fall prior to application of treatments on 1 m regrowth. Treatments included glyphosate, imazapyr, metsulfuron, sulfometuron, dicamba, fluroxypyr, triclopyr, picloram, aminocyclopyrachlor, and 2,4-D applied alone or in some combination. At 28 days after treatment (DAT), aminocyclopyrachlor + metsulfuron + imazapyr resulted in >90% visual control across all site years; control was variable for other treatments and ranged from 16 to 92%. By 60 DAT aminocyclopyrachlor + metsulfuron + imazapyr and aminocyclopyrachlor + metsulfuron resulted in >90% control for at least three of four

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site years. Greater than 95% Amur honeysuckle control was observed 120 DAT with aminocyclopyrachlor + metsulfuron + imazapyr, aminocyclopyrachlor + metsulfuron, and glyphosate. Control with other treatments was inconsistent and varied from 12 to 92%. Basal bark applications were made on mature honeysuckle at two locations near Columbia in fall 2011 and spring 2012. Treatments included triclopyr, triclopyr + fluroxypyr, and glyphosate as the undiluted, formulated herbicide, and imazapyr and aminocyclopyrachlor in basal blue oil. Efficacy of basal applications in the fall did not exceed 21% up to 6 months after treatment (MAT). For spring applications, >75% control was observed for aminocyclopyrachlor at 5 MAT, with all other treatments resulting in up to 46% control. Control of Amur honeysuckle is effective with treatments containing aminocyclopyrachlor or glyphosate as foliar applications. Spring basal applications were more effective than fall, but not effective alone for long-term control.

Nomenclature: Aminocyclopyrachlor; dicamba; fluroxypyr; glyphosate; imazapyr; metsulfuron; picloram; sulfometuron; triclopyr; 2,4-D; Amur honeysuckle, *Lonicera maackii* Rupr.

Key Words: aminocyclopyrachlor, basal bark, brush control.

Amur honeysuckle (*Lonicera maackii*) is a widespread, invasive shrub that was introduced to North America in the late 1800s (Dirr 1983). It is one of four species collectively known as bush honeysuckle (Amur, Morrow's, Tartarian, and Bell's) (Vermont 1998). Infested areas include treelines, fencelines, roadsides and other undisturbed areas. Amur honeysuckle is problematic because plants exhibit characteristics that are common among successful non-native species: rapid growth reaching up to 6 m tall; long range seed dispersal through birds; and phenotypic as well as habitat plasticity in response to light environment (Edgin 2007; Luken et.al. 1995). A survey conducted by the Northern Research Station – Forest Inventory and Analysis of USDA found that non-native bush honeysuckles were the second most frequent invasive plant across 1,264 0.4 ha test plots in 2005 and 2006 in Missouri (Moser et al. 2008).

Once established, Amur honeysuckle negatively impacts native plant species. In a forest habitat, Amur honeysuckle exhibited a higher relative growth rate, of >70 and 40% in full sun and 25% of full sunlight, respectively, compared to a desirable native species, spicebush (*Lindera benzoin*) (Luken et al. 1997). Within an Ohio forest, Hutchinson and Vankat (1997) found tree seedling density was <0.5 m^{-2} when Amur honeysuckle cover was ≥15%. Additionally, species richness was ≤8 in a 50 m transect when Amur honeysuckle cover exceeded 50% (Hutchinson and Vankat 1997). Amur honeysuckle presence in riparian habitats was found to reduce natural stream flow by 10%, due to increased transpiration, which will shorten the life of ephemeral ponds and steams (Boyce et al. 2012).

Control of Amur honeysuckle can be accomplished through the use of herbicides. However, proximity to desirable species complicates control measures adjacent to wooded areas. Also, shrub height can restrict adequate spray coverage with herbicides. Foliar herbicides recommended for control of Amur honeysuckle include glyphosate, triclopyr and imazapyr (Hartman and McCarthy 2004; Rathfon and Ruble 2007). Up to 95% control of Amur honeysuckle was reported with a spring application of glyphosate (Rathfon and Ruble 2007). Additionally, glyphosate exhibited 85% control of Japanese honeysuckle (*Lonicera japonica*) six months after treatment (MAT) (Regehr and Frey 1988). Up to 70% control of Amur honeysuckle was observed with triclopyr and triclopyr + imazapyr one year after treatment (YAT) (Rathfod and Ruble 2007). Aminocyclopyrachlor is a recently introduced growth regulator herbicide that appears to be effective on shrub and brush species (Anonymous 2011). However, the activity of this herbicide on Amur honeysuckle is unknown.

To avoid damage to desirable species, basal bark, cut stump, and stem injection applications of herbicides are used in forest habitats. Additionally, these applications are suitable for controlling brush species on uneven terrain. Applications are made typically in the fall and winter for easier access and reduced disturbance of native wildlife (Nelson et al. 2006). Hartman and McCarthy (2004) reported 99% mortality of Amur honeysuckle using a cut stump application and stem injection of glyphosate. Triclopyr and imazapyr (applied basally) are used to control many forest species (Nelson et al. 2006; Radosevich et al. 1980). However, triclopyr only exhibited up to 40% mortality of Amur honeysuckle (Rathfon and Ruble 2007). Basal bark applications have also been used to control shrub species such as lanata (*Lanata camara*) and hiptage (*Hiptage benghalensis*) (Dohn et al. 2013; Vitelli et al. 2009).

Rapid spread of Amur honeysuckle underscores the importance of control for sustaining native plant species. The objective of this research was to determine the efficacy of herbicides applied as foliar and basal bark treatments on Amur honeysuckle.

Materials and Methods

Foliar Applications. Field trials were established in fall of 2010 and 2011 at two sites in central Missouri. Amur honeysuckle was established along the edge of a woodland or roadside. In 2010, locations included Rothwell Park in Moberly (39.41°N, 92.45°W) and along a roadside (MO-740) in Columbia (38.94°N, 92.37°W). The locations in 2011 included Rothwell Park (distinct area from 2010 trial) (39.41°N, 92.46°W) as well as the Charles W. Green Conservation Area near Ashland (38.81°N, 92.25°W). Moberly and Ashland locations were undisturbed sites, whereas Columbia was disturbed due to road construction. Soil types for 2010 trials at Moberly and Columbia were a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) and a Weller silt loam (fine, smectitic, mesic Aquertic Chromic Hapludalfs) along an urban roadside area, respectively. Soil at Moberly had a pH of 4.8 and 1.3% organic matter content. Columbia soil had a pH of 7.3 and 7.4% organic matter content. In 2011, the soils at Moberly and Ashland were a Gorin silt loam (fine, smectitic, mesic Aquertic Chromic Hapludalfs) and a Keswick silt loam (fine, smectitic, mesic Aquertic Chromic Hapludalfs), respectively. Moberly soil had a pH of 4.5 and 2.0% organic matter content. Soil at Ashland had a pH of 5.0 and 2.2% organic matter content. Experimental areas were mowed during October 2010 and November 2011 for applications the following year (2011 and 2012) on 1 m regrowth. Plots were 2 by 7.6 m and treatments were made at a speed of 4.8 km hour^{-1} with a CO_2 pressurized backpack sprayer equipped with XR 8002 (TeeJet®: Spraying Systems Co., Wheaton, IL) flat fan nozzle tips calibrated to deliver 374 L ha^{-1} at 172 kPa. Treatments consisted of herbicides commonly used for brush control at rates labeled for use on *Lonicera* or a similar shrub species (Table 2.1). Applications in 2011 were made on June 27 and July 8 for Moberly and Columbia, respectively, and July 9 and 18 in 2012 for Moberly and Ashland, respectively. Average monthly temperature and total monthly precipitation following application of treatments through final visual ratings are shown in Table 2.2. Visual control ratings (0 = no control, 25 = meristem death, 50 = exstensive chlorosis, 75 = moderate necrosis, 100 = plant death) were recorded at 28, 60, 90, 120, and 270 days after treatment (DAT) for all site years.

Basal Bark Applications. Field trials were established in fall 2011 along a roadside (I-70 Drive SW) (38.96°N, 92.38°W) and at the Grindstone Nature Area (38.92°N, 92.31°W), both in Columbia MO. The spring 2012 trials were located along the Bear Creek Trail (38.98°N, 92.35°W), in Columbia, and the Charles W. Green Conservation Area (38.81°N, 92.25°W), near Ashland. All sites except I-70 Drive SW were undisturbed. Mature shrubs (berry development observed) adjacent to wooded areas, were selected randomly and ranged from 1.5 to 3 m in height. Selected shrubs consisted of 1 to 3 stems each, with diameters ranging from 2.5 to 7.5 cm at ground level; shrubs of similar stem diameter were grouped in the same replication. Herbicides were applied uniformly on the lower

45 cm of each shrub stem, with 9 mL stem^{-1} shrub^{-1} applied. Treatments included: triclopyr, triclopyr + fluroxypyr, glyphosate, imazapyr, aminocyclopyrachlor, and an untreated control. Imazapyr and aminocyclopyrachlor were dissolved in Tolex® basal blue oil (Exacto Inc., Sharon, WI) at 9.4 and 10% v/v, respectively, according to labels and recommendations (Table 2.3). All other treatments were applied as undiluted, formulated herbicides. Applications were made with distinct polyethylene spray bottles (Ace Hardware Corporation, Oak Brook, IL) for each herbicide. Applications were made on November 22 and 28, 2011 for the park and roadside location, respectively, and May 15 and 18, 2012 for the conservation area and Bear Creek Trail, respectively. Maximum air temperature and total precipitation the day of chemical application are listed in Table 2.4. Visual control of Amur honeysuckle was estimated using the scale described previously, monthly from 4 to 12 MAT and from 1 to 11 MAT for fall and spring applications, respectively.

Statistics. Foliar experiments were designed as a randomized complete block with five replications. Basal bark experiments were conducted as a randomized complete block with three replications. The ANOVA for foliar and basal bark control were conducted using the GLIMMIX procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). Due to a significant location by treatment interaction for foliar applications, all site years were analyzed separately. No location by treatment interaction was found for basal bark applications, therefore site years were combined. Means were separated using Fisher's Protected LSD at P=0.05.

Results and Discussion

Foliar Applications. Amur honeysuckle control was variable. Both excellent (>90%) and poor (<55%) control was observed at different rating dates and across site years with specific treatments (Table 2.5 and 2.6). By 28 DAT, >80% control was observed for only 3 of 9 treatments; aminocyclopyrachlor + metsulfuron + imazapyr was effective (92 to 94%) at all four site years. Picloram + fluroxypyr control was <30% across all site years. Control exhibited by all other treatments ranged between 30 and 85% depending on site year. These results were consistent with Enloe et al. (2013) who found that picloram + fluroxypyr only resulted in 34% control of another shrub species, Macartney rose (*Rosa bracteata*). The slow response of Amur honeysuckle likely reflected dilution of herbicide throughout the large root system. Luken et al. (1995) has shown that Amur honeysuckle possesses a large root system.

By 60 DAT, average control of Amur honeysuckle across site years improved by 11 to 70% (Table 2.5). Greatest increases in control over 28 DAT evaluations were observed for 2,4-D + dicamba + fluroxypyr (25%), triclopyr + fluroxypyr (19%), 2,4-D (17%) and triclopyr + imazapyr (11%). However, greater than 90% control was observed with aminocyclopyrachlor + metsulfuron + imazapyr for all site years, aminocyclopyrachlor + metsulfuron for three site years and glyphosate for two site years. Other treatments exhibiting >80% control for two site years included: 2,4-D, 2,4-D + dicamba + fluroxypyr, sulfometuron + metsulfuron, and triclopyr + fluroxypyr. Picloram + fluroxypyr was ineffective, with control ranging from 20 to 37%.

Herbicide efficacy improved for most treatments at 90 DAT (Table 2.6). At all site years, aminocyclopyrachlor + metsulfuron + imazapyr exhibited excellent control (98 to 100%). Control of Amur honeysuckle was 90 to 97% at 3 site years with aminocyclopyrachlor + metsulfuron. Greater than 80% control was recorded for 2 site years with triclopyr + fluroxypyr. Glyphosate control was variable (74 to 99%) across site years. Aminocyclopyrachlor is reportedly effective on woody species such as black locust (*Robinia pseudoacacia*), red oak (*Quercus rubra*), and tulip poplar (*Liriodendron tulipifera*), with >90% control up to 1 YAT (Johnson et al. 2010).

Little change in efficacy occurred from 90 to 120 DAT. However, optimum efficacy on Amur honeysuckle was observed at 120 DAT. Greater than 90% control was observed with aminocyclopyrachlor + metsulfuron + imazapyr for all site years and aminocyclopyrachlor + metsulfuron for three site years (Table 2.6). Greater than 80% control was observed for 8 of 9 treatments at various site years. Glyphosate, 2,4-D + dicamba + fluroxypyr, and triclopyr + imazapyr exhibited >80% control for 3 site years. At 2 site years, 88 and 83% control were observed with 2,4-D and triclopyr + fluroxypyr, respectively. Oneto et al. (2010) found that glyphosate, imazapyr, and triclopyr provided >90% control of the perennial shrub scotch broom (*Cytisus scoparius*) 1 YAT.

Amur honeysuckle survival was assessed by regrowth of plants the following growing season (270 DAT; Table 2.6). Glyphosate and aminocyclopyrachlor + metsulfuron + imazapyr resulted in almost complete mortality (90-100%) at all site years. Aminocyclopyrachlor + metsulfuron control was at least 90% at three site years. Greater than 80% control was observed with 2,4-D and 2,4-D + dicamba + fluroxypyr at two site years. For glyphosate applications in the spring, Rathfon and Ruble (2007) observed >90% control of Amur honeysuckle 1 YAT. However, triclopyr + imazapyr provided similar levels of control. Reduction in the control of Amur honeysuckle from 120 to 270 DAT was an indication of regrowth. Lack of complete control for many herbicides suggests sequential applications will be necessary.

Treatments exhibiting high levels of control were consistent across variable environmental conditions (Table 2.2). Air temperatures were similar across site years, but precipitation levels varied. From July through September 2011, Columbia received 21.5 cm more rainfall compared to Moberly. In 2012, March through April precipitation at Columbia was 24.7 cm greater than for Moberly.

Basal Bark Applications. Response of Amur honeysuckle was poor with fall applications (Table 2.7). For fall applications, herbicide efficacy ranged from 0 to 51% 4 MAT. Control was only 0 to 21% at 6 MAT, with few differences observed among treatments. Glyphosate resulted in the greatest control (51%), triclopyr and triclopyr + fluroxypyr exhibited the poorest response. Rathfon and Ruble (2007) also reported that basal bark herbicide applications on Amur honeysuckle exhibited only up to 30% mortality using triclopyr and imazapyr applied in winter. On a different species, Vitelli et al. (2009) found only 38% mortality of hiptage plants following winter basal bark applications of fluroxypyr or triclopyr + picloram.

Amur honeysuckle response to basal herbicide applications in spring was better than with fall applications (Table 2.8). Control ranged from 29 to 78% by 4 MAT. At 4 MAT, aminocyclopyrachlor provided significantly better control (78%) than all other treatments. Other treatments varied from 29 to 45% control. Efficacy at 5 MAT was similar to 4 MAT. A large reduction in efficacy was observed 6 MAT; treatments resulted in 5 to 46% control. Aminocyclopyrachlor only exhibited 38% control and triclopyr exhibited 5% control at 6 MAT. Dohn et al. (2013) reported only 40% mortality of lanata after a spring application of triclopyr + picloram. These data suggest that neither fall nor spring basal applications are effective for long–term control of Amur honeysuckle.

Amur honeysuckle was more susceptible to spring versus fall basal bark herbicide applications. Lanini and Radosevich (1982) found that manzanita shrub species (*Arctostaphylos patula* and *Arctostaphylos viscida*) were more susceptible to spring versus fall basal bark herbicide applications of triclopyr (41 and 25% mortality) and glyphosate (76 and 21% mortality). They attributed higher activity with periods of increased photosynthesis, which likely improved translocation of applied herbicides to the meristem. Meyer and Bovey (1986) reported honey mesquite (*Prosopis glandulosa*) was more susceptible to spring versus fall basal bark herbicide applications. Control of honey mesquite with clopyralid was reduced from 100 to 10% and 73 to 3% with picloram with May and September applications, respectively.

Control of established Amur honeysuckle is challenging. Although Amur honeysuckle responds to herbicides, the performance of foliar applications was superior to basal bark. Glyphosate and products containing aminocyclopyrachlor can effectively control the regrowth of mowed shrubs, but sequential applications on regrowth may be necessary. 2,4-D + dicamba + fluroxypyr also provides an option as it provided acceptable control for 3 site years. Applications must be targeted to avoid contact with sensitive, desirable species. Basal bark applications were relatively ineffective. Single basal bark applications of herbicides do not control Amur honeysuckle. Spring applications may be sufficient to preclude reproduction, but sequential applications are likely necessary. Continued observations over time will be necessary to determine if re-treatment is needed to control this slow responding shrub.

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Table 2.1. Herbicide treatments for postemergence control of Amur honeysuckle at two Missouri locations in 2011 and 2012. Herbicides were applied at 374 L ha^{-1} to 1 m shrub regrowth.

Treatment	Trade Name	Rate (g ae ha^{-1})	Surfactant
Glyphosate	Roundup WeatherMax	1577*	3.38 kg ha^{-1} AMS
2,4-D	2,4-D Amine	1317.6	0.25** NIS
2,4-D + dicamba + fluroxypyr	Escalade 2	785 + 98.1 + 98.1*	-
Triclopyr + imazapyr	Garlon 3A + Arsenal PowerLine	3 + 0.125**	1** MSO
Picloram + fluroxypyr	Surmount	375.3 + 302.8	0.5** NIS
Sulfometuron + metsulfuron	Oust Extra	69 + 18.4***	0.25** NIS
Triclopyr + fluroxypyr	PastureGard	578.2 + 192.7	1** NIS
Aminocyclopyrachlor + metsulfuron	Streamline	131.5 + 41.9***	0.5** MSO
Aminocyclopyrachlor + metsulfuron + imazapyr	Viewpoint	271.4 + 87 + 376.8***	0.5** MSO

*Formulated herbicide contains surfactant.
**Percent volume per volume of solution.
***g ai ha^{-1}; grams of active ingredient per hectare.

Table 2.2. Total monthly precipitation and average air temperature for foliar herbicide trial locations in 2011 and 2012 in Missouri. Weather data recorded for municipal airports near Moberly, Columbia, and Ashland.

	Moberly		Columbia/Ashland*	
	Precipitation (cm)	Temperature (C)	Precipitation (cm)	Temperature (C)
June, 2011	12.2	23.6	15.1	24.7
July	3.3	27.6	10.0	28.5
August	3.9	24.7	11.9	25.2
September	2.2	18	9.0	17.9
October	2.2	14.5	4.4	14.2
November	13.4	8.2	12.0	9
December	8.5	3.4	10.2	3.4
January, 2012	0.9	1.7	2.5	1.8
February	3.5	3.5	10.4	4.0
March	9.8	14.4	19.5	14.6
April	8.4	14.3	23.4	14.5
May	6.6	20.8	7.6	21.3
June	5.5	24.3	3.7	24.8
July	2.2	28.6	1.6	29.3
August	7.4	24.7	4.7	25.2
September	4.6	18.8	5.6	19.1
October	8.2	11.9	8.9	11.9
November	3.9	7.5	2.5	7.0
December	2.7	2.9	4.6	3.4
January, 2013	5.2	-0.2	6.1	0.4
February	3.9	0.2	9.1	0.9
March	11.0	2.3	8.0	3.4
April	18.2	10.7	23.4	11.5

*Columbia and Ashland were reported together because both trial locations were within 10 km of the Columbia Regional Airport.

Table 2.3. Basal Bark herbicide treatments on Amur honeysuckle at two locations in Missouri. Applications were made in November 2011 and May 2012. Herbicide solutions (9 ml stem^{-1}) were sprayed on lower 45 cm of each shrub. Treatments were either undiluted herbicides or mixed with basal blue oil.

Treatment	Concentration (% v/v*)	Carrier (%)
Triclopyr	100	-
Triclopyr + fluroxypyr	100	-
Glyphosate	100	-
Imazapyr	9.4	basal blue oil (90.6)
Aminocyclopyrachlor	10	basal blue oil (90)

*percent volume of herbicide per volume of solution.

Table 2.4. Maximum air temperature and total precipitation for basal bark herbicide trial locations in 2011 and 2012 in Missouri. Weather data from municipal airports within 10 km of experimental areas.

Location	Application Date	Total Precipitation (cm)	Maximum Air Temperature (C)
Grindstone Nature Area	November 22, 2011	0.3	8.3
Columbia Roadside	November 28, 2011	0	2.2
Green Conservation Area	May 15, 2012	0	35.0
Bear Creek Trail	May 18, 2012	0	29.4

Table 2.5. Visual control of Amur honeysuckle using foliar applied herbicides. Amur honeysuckle plants were treated at three locations in Missouri (Moberly, Columbia, Ashland) in 2011 and 2012. Visual control ratings from 28 to 60 days after treatment (DAT) were estimated using a scale of 0 (no effect) to 100 (complete plant death).

	Moberly 2011		Columbia 2011		Moberly 2012		Ashland 2012	
	Days after treatment (DAT)							
	28	60	28	60	28	60	28	60
Treatment	Visual Control (%)							
Glyphosate	92 a[a]	100 a	61 bc	63 bc	37 c	55 c	79 bc	55 c
2,4-D	59 c	74 b	43 d	59 bc	55 b	89 ab	52 d	89 ab
2, 4-D + dicamba + fluroxypyr	56 c	92 a	45 cd	60 bc	59 b	85 ab	30 e	85 ab
Triclopyr + imazapyr	63 c	87 ab	39 d	54 cd	85 a	82 b	67 c	82 b
Picloram + fluroxypyr	29 d	37 c	16 e	20 e	22 c	33 d	20 e	33 d
Sulfometuron + metsulfuron	67 bc	71 b	65 b	54 cd	80 a	80 b	87 ab	80 b
Triclopyr + fluroxypyr	53 c	83 ab	34 d	44 d	56 b	85 ab	26 e	85 ab
Aminocyclopyrachlor + metsulfuron	82 ab	93 a	73 b	72 b	78 a	90 ab	81 ab	90 ab
Aminocyclopyrachlor + metsulfuron + imazapyr	92 a	99 a	94 a	92 a	92 a	100 a	93 a	100 a

[a] Means within each column followed by the same letter are not significantly different using Fisher's Protected LSD at P=0.05.

Table 2.6. Visual control of Amur honeysuckle using foliar applied herbicides. Amur honeysuckle plants were treated at three locations in Missouri (Moberly, Columbia, Ashland) in 2011 and 2012. Visual control ratings from 90 to 270 days after treatment (DAT) were estimated using a scale of 0 (no effect) to 100 (complete plant death).

	Moberly 2011			Columbia 2011			Moberly 2012			Ashland 2012		
	Days after treatment (DAT)											
	90	120	270	90	120	270	90	120	270	90	120	270
Treatment	Visual Control (%)											
Glyphosate	99 a[a]	100 a	100 a	74 b	82 ab	99 a	78 ab	73 b	100 a	78 bc	95 ab	90 ab
2,4-D	68 bc	71 cde	62 cd	57 bcd	66 cd	58 bcd	91 ab	91 ab	90 ab	79 bc	86 bc	86 abc
2, 4-D + dicamba + fluroxypyr	82 abc	90 ab	77 abc	68 bc	70 bcd	65 b	73 b	87 ab	88 abc	74 bc	90 abc	90 ab
Triclopyr + imazapyr	84 ab	87 abc	65 bcd	63 bcd	59 cd	44 cde	92 ab	92 ab	90 ab	70 c	81 c	78 bc
Picloram + fluroxypyr	24 d	54 e	36 e	11 e	12 e	7 f	33 c	36 c	45 d	39 d	34 e	47 d
Sulfometuron + metsulfuron	62 c	68 de	48 de	50 d	57 d	35 e	77 b	74 b	74 c	86 abc	92 ab	82 bc
Triclopyr + fluroxypyr	66 bc	81 bcd	51 de	54 cd	56 d	40 de	90 ab	86 ab	80 bc	32 d	51 d	76 c
Aminocyclopyrachlor + metsulfuron	97 a	92 ab	90 ab	73 b	73 bc	62 bc	92 ab	97 a	90 ab	90 ab	92 abc	90 ab
Aminocyclopyrachlor + metsulfuron + imazapyr	99 a	97 ab	99 a	98 a	94 a	100 a	100 a	98 a	100 a	100 a	100 a	96 a

[a] Means within each column followed by the same letter are not significantly different using Fisher's Protected LSD at P=0.05.

Table 2.7. Visual control of Amur honeysuckle using basal bark applied herbicides in fall 2011. Plants were treated at two locations near Columbia, MO (roadside and Grindstone Nature Area). Visual control ratings from 4 to 6 months after treatment (MAT) were estimated using a scale of 0 (no effect) to 100 (complete plant death). Visual ratings are combined across locations for statistical analysis.

	Fall 2011			Fall 2012	
	(Months after treatment; MAT)				
	March 2012 (4)[a]	April 2012 (5)	May 2012 (6)	March 2013 (4)	April 2013 (5)
Treatment	Visual Control (%)				
Triclopyr	1 ab[b]	0 b	0 c	0 b	2 b
Triclopyr + fluroxypyr	2 ab	0 b	0 c	0 b	7 b
Glyphosate	3 ab	9 a	14 ab	51 a	37 a
Imazapyr	9 a	13 a	21 a	13 b	32 a
Aminocyclopyrachlor	0 b	0 b	5 bc	3 b	0 b

[a] Numbers in parenthesis represent months after treatment.

[b] Means within each column followed by the same letter are not significantly different using Fisher's Protected LSD at P=0.05.

Table 2.8. Visual control of Amur honeysuckle using basal bark applied herbicides in spring 2012. Plants were treated at two locations in Missouri (Columbia trail and Ashland conservation area). Visual control ratings from 4 to 6 months after treatment (MAT) were estimated using a scale of 0 (no effect) to 100 (complete plant death). Visual ratings are combined across locations for statistical analysis.

	Months after treatment (MAT)		
	4	5	6
Treatment	Visual Control (%)		
Triclopyr	31 b	35 b	5 b
Triclopyr + fluroxypyr	29 b	26 b	20 ab
Glyphosate	45 b	46 ab	46 a
Imazapyr	30 b	28 b	30 ab
Aminocyclopyrachlor	78 a	78 a	38 a

[a] Means within each column followed by the same letter are not significantly different using Fisher's Protected LSD at P=0.05.

CHAPTER III

INFLUENCE OF TIME ON SEED PREDATION, GERMINATION, AND VIABILITY OF AMUR HONEYSUCKLE (*LONICERA MAACKII*)

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Amur honeysuckle is a widespread, invasive shrub across the Central and Northeastern regions of the United States. Its attractive red berries are vectored by birds to virgin sites, contributing to the spread of infestations. However, little is known about the reproductive capacity of shrubs, the viability of seeds, and the timing of seed dispersal. Two studies were conducted to determine berry and seed production as well as characterize the timing of berry predation. In 2011 and 2012 studies at two locations focused on both germination and viability of seeds through berry maturation. Additional studies at two locations in 2011 and 2012 were used to assess mature shrubs for berry predation. Across four site years, seed production ranged, annually, from 2,844 to 7,161 seeds per shrub. Seed viability was first detected in September and reached an optimum of 90% by mid-November. Optimum viability corresponded with fruits reaching a full red color. From freshly harvested berries, germination of Amur honeysuckle was measured

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from seed of intact berries as well as seed extracted from berries. However, only 0.6% of seeds germinated within 8 weeks of berry maturation, indicating a lack of dormancy in some fruits. Overall, 97% of seeds did not germinate from intact berries or following extraction. Berries were harvested naturally by birds from mid-October to early December at a rate of 250 berries per week. Eighty-two percent of Amur honeysuckle fruits were predated from October through January, and >95% of all fruits were predated.

Nomenclature: Amur honeysuckle, *Lonicera maackii* Rupr.**;** tetrazolium

Key Words: berry predation, seed production, viability

The spread of invasive plant species is a threat to native species in many geographical areas of the United States (Luken 1988; Myers 1983; Woods 1993). Invasive species are the second largest threat, behind loss of habitat, to global biodiversity (Walker and Steffen 1997). Preventing introduction of unwanted species into novel areas is a key step to limiting the rate of spread.

Amur honeysuckle (*Lonicera maackii*) is an invasive shrub that has become widespread since being introduced to North America in the late 1800s (Dirr 1983). Currently, Amur honeysuckle is found throughout the Central and Northeastern regions of the United States; from North Dakota to Texas and east to Massachusetts and Georgia (Luken and Thieret 1996; Rich 2000). Amur honeysuckle is prevalent across Missouri, but has not yet been classified as noxious. A survey in 2005 and 2006, conducted by the Northern Research Station – Forest Inventory and Analysis of USDA, found that non-native bush honeysuckles were the second most frequent invasive plant across 1,264 0.4 ha test plots in Missouri (Moser et al. 2008).

Infestations of Amur honeysuckle negatively affect ecosystems. Relative growth rates of plants are >70 and 40% higher in full sunlight and 25% of full sunlight, respectively, compared to desirable native species such as spicebush (*Lindera benzoin*) (Luken et al. 1997). Relative growth rate is the average net increase in dry matter biomass accumulation per unit of plant dry biomass accumulation over time (Castro-Díez et al. 1998), and can signal the competitive ability of unrelated species. Within an Ohio forest, Hutchinson and Vankat (1997) found that when Amur honeysuckle cover was equal to or greater than 15%, tree seedling density was <0.5 m^{-2}. Additionally, species richness was ≤8 in a 50 m transect when Amur honeysuckle was >50% of the covered area (Hutchinson and Vankat 1997). Amur honeysuckle presence reduced natural stream flow by 10%, compared to uninvaded areas, which will shorten the flowing time of ephemeral ponds and streams (Boyce et al. 2011). McCusker et al. (2010) suggested that avian species are attracted to the denser understory of honeysuckle species for nesting, but Schmidt and Whelan (1999) estimated the daily nest mortality rate for American robins (*Turdus migratorius*) was significantly higher in Amur honeysuckle than in native species. This resulted from lower nest heights and the absence of thorns that deterred egg predators.

Aspects of seed biology for Amur honeysuckle are poorly documented. Development of red berries attracts birds and is likely a critical factor in vectoring seed spread. Levels of seed viability and germinability during berry maturation are factors to consider when developing weed management strategies. Luken and Mattimiro (1991) found that 80% of seeds sampled under established Amur honeysuckle were viable, but Hartman and McCarthy (2008) found as low as 6% viability in soil samples from sites invaded for longer than 12 years. Seed content among Amur honeysuckle berries is reported to range widely, from 1 to 10 seeds (Goodell et al. 2010). More understanding of seed production as well as the timing of seed viability and germination of Amur honeysuckle populations is necessary to slow unwanted seed spread.

Seed dispersal for plants with attractive fruit is difficult to prevent because it is largely facilitated by other species. Fruits are often consumed by birds and the seeds dispersed when birds defecate (Ingold and Craycraft 1983). Luken and Goessling (1995) found an increase in Amur honeysuckle seedling density from forest interiors (5 seedling m^{-2}) to forest edges (358 seedlings m^{-2}), suggesting seeds primarily deposited on forest edges. Seeds of Amur honeysuckle appear unaffected by passing through the digestive tract of birds, as Bartuszevige and Gorchov (2006) found seeds defecated by American robins were 86% viable, and were found primarily along the edges of woodlots and spurs (fencerow that leads out of a woodlot).

Development of management techniques for Amur honeysuckle must include the characterization of seed maturation and the timing of seed spread. The objectives of this research were to identify seed production of Amur honeysuckle as well as the timing of seed maturation and dispersal.

Materials and Methods

Seed Biology. Berry production and seed yield for Amur honeysuckle shrubs were assessed in October in 2011 and 2012 at two locations in Missouri. Locations included the Charles W. Green Conservation Area (Green Area), near Ashland (38.82°N, 92.26°W) and Grindstone Nature Area, in Columbia (38.92°N, 92.31°W). Locations were both undisturbed forested areas. For established, mature shrubs, berries were counted on October 4 and 18, 2011 and October 5, 2012. Fifty berries were harvested from nearby shrubs and seeds removed using forceps. The total number of seeds harvested from the berries were counted. The experiment was conducted as a randomized complete block with 4 replications; individual shrubs were considered replicates.

Seed maturation of Amur honeysuckle using germination was assessed from field collected berries harvested from August until November in 2011 and 2012 at two locations in central Missouri. Locations included the Green Area, near Ashland, and Proctor Park in Columbia (38.97°N, 92.34°W), in 2011; and the Green Area as well as Bear Creek Trail in Columbia (38.98°N, 92.34°W), in 2012. During this time period, berries matured from a green color to completely red. Samples of 100 berries were randomly harvested from five shrubs every 14 days from late August through mid-November. Under greenhouse conditions, 50 intact berries from each shrub were planted to a depth of 1 cm in 28 by 28 cm polypropylene containers filled with a 1:1 v/v mixture of Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs; pH of 5.9 and 1% organic matter) and commercial potting medium (Premier Tech, Rivière-du-Loup, Quebec, Canada). Containers were subjected to a 12 hour photoperiod with supplemental lighting for a total light output of 200 µmol photon m^{-2} s^{-1}. Air temperature was maintained at 15 to 30 C and containers were watered as needed. No fertilizer was used throughout the study. During January and February, containers were placed outside to vernalize seeds, then returned to the greenhouse in March. Average monthly temperature and total monthly precipitation during the vernalization period are listed in Table 3.1. The number of germinated seeds were recorded every 2 weeks until no germination was detected for four consecutive weeks. Germination was defined as the presence of fully developed cotyledons.

For the remaining 50 berries, seeds were extracted as described above. All seeds from 30 berries were planted in the greenhouse following the methodology described above for intact berries. Seeds extracted from the remaining 20 berries were subjected to a tetrazolium assay for assessment of viability (Miller 2010). The experimental design was a randomized complete block with 5 replications; individual shrubs were considered replicates.

A tetrazolium chloride assay was established to assess Amur honeysuckle seed viability, and followed a technique adopted from Miller (2010). Twenty Amur honeysuckle berries were dissected, and seeds extracted as described above. Seeds (15 to 60) were incubated in deionized water overnight at room temperature, then dissected the following morning. Dissection followed a two-step process (Figure 3.1). Initially, a scalpel separated the distal and basal ends; the distal end was used for treatment. Cut seeds were incubated in 1% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich Co., St. Louis, MO) in deionized water, and incubated overnight at 35 C. The following morning, a second cut using a scalpel exposed the cotyledons, allowing identification of embryo viability. Seeds with a fully stained embryo and cotyledons were considered viable; all other stain patterns were considered non-viable (Figure 3.2). The tetrazolium assay was designed as a randomized complete block with 5 replications; individual shrubs were considered replicates.

Seed Predation. Two sites in central Missouri with established stands of Amur honeysuckle were selected: the Green Area and the Grindstone Nature Area. Each location was used in both 2011 and 2012. Four shrubs were chosen at random at each location. The same randomly chosen shrubs were used in both 2011 and 2012. To facilitate the counting of abscised berries, Weed Block (black fabric used to prevent emergence of weeds) and Bird Block (1 cm mesh netting used to prevent bird predation) (Easy Gardener Products Inc., Waco, TX) were set up beneath each shrub. Fabric was placed on the ground to collect fallen berries and netting was suspended by wooden stakes 12 cm above the ground to prevent other animals from removing fallen berries. Initially, berries were counted on each shrub on September 19 and 30, 2011 for Ashland and Columbia, respectively and September 19, 2012 for both locations. Berries remaining on the shrub, as well as located on the Weed Block fabric were recorded every 15 days until March, when no visible berries remained on shrubs. The change in number of berries at each counting was considered predated. The experiment was designed as a randomized complete block with 4 replications; individual shrubs were considered replicates.

Statistics. The ANOVA for seed maturation and count date effects were conducted using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). The ANOVA for site year effects on seed production was conducted using the GLM procedure in SAS 9.2. The ANOVA for harvest date effects on seed viability was conducted using the GLIMMIX procedure with a logit link and binomial distribution in SAS 9.2. A transformation did not improve separation of treatment effects. Therefore, no transformation was carried out. Year, location, and replication were considered random effects while harvest date and counting date were considered fixed effects. Mean separation of data for all fixed variables was carried out using Fisher's Protected LSD at P=0.05.

Results and Discussion

Seed Biology. Greatest berry production of Amur honeysuckle was in mid-October and ranged from 1,554 to 4,173 berries per shrub (Table 3.2). Seeds per berry was significantly higher for 2011 (2.8 to 3.3), compared to 2012 (0.7 to 1.0). Seeds per berry could have been higher in 2011 due to more precipitation. Across all site years, berries contained an average of 2.1 seeds. Total seed production was not different for 2011 and 2012, and ranged from 2,844 to 7,161 seeds shrub per shrub. Another report (Anonymous 2013), indicated Amur honeysuckle berries contain from 2 to 6 seeds.

Viability of Amur honeysuckle seed was greater than 80% (Table 3.3). Emergence of Amur honeysuckle seedlings varied due to berry harvest date, but germination was significantly higher for mid-October (14.5%) and early-November (12.8%) compared to all other harvests (Table 3.3). Viability of Amur honeysuckle seed increased quickly during the fall (Table 3.3). No viability was measured in early September, but increased to 83% by mid-October. No statistical increase in viability occurred from mid-October to November. For all site years, berries were observed to become fully red by mid-October (data not shown). Goettemoeller and Ching (1999) found that sweetleaf (*Stevia rebaudiana*) seed germination (83.7%) was similar to viability assessment using a tetrazolium assay (76.7%). Results with Amur honeysuckle also agree with Luken and Mattimiro (1991), who found 80% viability of Amur honeysuckle seeds collected underneath both clipped and unclipped shrubs.

Germination of Amur honeysuckle seeds was assessed using intact berries as well as extracted seeds (Table 3.3 and Figure 3.3). Within 8 weeks of planting, seedlings from both intact berries and seed extracted from berries were observed, suggesting a lack of dormancy requirement for some portion of the population. Mean germination was lower for early (September), compared to late (November), harvest dates. For two of the last three harvest dates, germination of seedlings from intact berries was greater compared to emergence for extracted seed. Overall germination was higher for seed from intact berries (5.3%), compared to seed extracted from berries (2.9%), but this was statistically similar (Figure 3.3). This implies that separation of seeds from berry tissue was not required to trigger germination. Germination was low and similar for seeds extracted from berries across all harvest dates. Traveset and Verdú (2002) found that seeds of many species consumed by birds were 40% more likely to germinate than non-ingested seeds. This research did not attempt to simulate seed scarification during ingestion and digestion by birds.

Comparatively, overall germination of extracted seeds was low (<3%) compared to viability (>80%), suggesting other factors may play a role in triggering seed germination. The low viability of seeds following early and mid-September berry harvests (Table 3.3) likely explains the low seed germination of seed from berries (Figure 3.3). Mock privet (*Phillyrea latifolia*) also demonstrated low germination (39.4%) during the first year after harvest (Herrera et al. 1994). Low germination of mock privet was attributed to seed dormancy. Although, it has been shown that viability is associated with germination (Goettemoeller and Ching 1999); these data show that viability is not synonymous with germinability, and that other factors influencing germination are likely present.

Seed Predation. From a peak of 2,742 berries per shrub counted in early October, Amur honeysuckle berries on shrubs declined to 0 by the following March (Figure 3.4). From mid-October to late December, berry removal from shrubs was heavy, averaging 250 per week; fruit may have dropped from a failed attempt to consume them or simply matured and fell off shrubs. Of the berries removed, less than 7% were recovered beneath the shrubs. Over 70 berries were found beneath shrubs in early December, this is likely due to accumulation of abscised berries throughout the fall. Over all site years, >93% of Amur honeysuckle fruits were removed from shrubs and likely spread to adjacent areas. A large majority of fruits were likely predated. Nine bird species were found to consume Amur honeysuckle berries (Ingold and Craycraft 1983). On a similar species with production of purple fruit, Herrera et al. (1994) found less than 20% of mature (September) mock privet fruit remained on plants in February and March, with fruit removal attributed to birds. The importance of birds as a vector was shown by Sargent (1990), where 62% of southern arrowwood (*Viburnum dentatum*) fruit were removed from shrubs with high fruit producing neighbors compared to lower fruit producing neighbors.

Bird consumption of mature Amur honeysuckle berries in the fall appears to be a major natural contributor to the spread of infestations. Amur honeysuckle has the capacity to produce up to 2,700 berries annually with up to 3.3 seeds per berry (Table 3.2). Luken and Mattimiro (1991) indicated Amur honeysuckle shrubs can live greater than 20 years indicating a large capacity to spread infestations over an extended period of time. Berries mature to a bright red color by mid-October in mid-Missouri, which coincides with maximum seed viability (Table 3.3). Although most seeds are viable, less than 1% of seeds have the capacity to germinate in the fall. Birds consume berries quickly, with a measured rate of 250 berries per week from mid-October to December (Figure 3.4). A key component for managing the spread of Amur honeysuckle is to preclude seed production.

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Table 3.1. Mean monthly temperature and total monthly precipitation for Amur honeysuckle seed vernalization period in 2012 and 2013 in Missouri. Seeds were subjected to outdoor weather conditions in January and February each year. Weather data recorded for Sanborn Field (Missouri Agricultural Experiment Station; 38.94^{o}N, 92.32^{o}W) in Columbia, Missouri.

	Total Precipitation (cm)	Mean Temperature (C)
January, 2012	1.9	2.4
February, 2012	6.4	4.3
January, 2013	6.7	0.9
February, 2013	4.9	1.2

Table 3.2. Berry and seed production of Amur honeysuckle at two locations in Missouri in 2011 and 2012. Berries present on shrub were assessed at two locations [Grindstone Nature Area and Charles W. Green Conservation Area (Green Area)]. The same randomly selected shrubs were used at each location each year. Seed production per berry was assessed by dissection of 50 berries at each location each year. Total seed per shrub production is the product of total berry production and seed per berry production.

Location	Total Berry (No.)	Seed per berry (No.)	Total seed per shrub (No.)
Grindstone, 2011	1,554 (366)[ab]	2.8 (0.1) a	4,477 (1,174)
Green Area, 2011	2,067 (633)	3.3 (0.1) a	7,161 (2,378)
Grindstone, 2012	3,172 (637)	1.0 (0.2) b	3,150 (1,034)
Green Area, 2012	4,173 (1,927)	0.7 (0.1) b	2,844 (1,031)

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[a] Number in parentheses indicates the standard error of the mean.

[b] Means within each column followed by the same letter or without letters are not significantly different using Fisher's Protected LSD at P=0.05.

Table 3.3. Mean viability and germination of Amur honeysuckle seeds harvested at various time points. Berries were harvested at two Missouri locations in 2011 (Charles W. Green Conservation Area and Proctor Park) and 2012 (Charles W. Green Conservation Area and Bear Creek Trail). Berries were harvested every 14 days from September through the first week of November. Early denotes first week of the month, mid denotes second and third weeks of the month, and late denotes fourth week of the month. Seed viability was assessed using a tetrazolium assay. Seed germination was assessed using greenhouse planting. Germination data were collected from November 2011 through May 2012, and November 2012 through May 2013. Data were combined across site years.

		Seed Germination (%)	
Berry harvest	Seed viability (%)	Intact berries	Extracted seeds
Early –September	0 d[a]	0 b	0 b
Mid-September	22 c	0.4 b	0.8 ab
Late-September	61 b	2.3 b	5.1 a
Mid-October	83 ab	14.6 a*	5.9 a*
Late-October	86 a	3.7 b	0.2 b
Early-November	90 a	12.4 a*	5.2 a*

[a] Means within each column followed by the same letter are not significantly different using Fisher’s Protected LSD at P=0.05.

*denote significant differences in germination of intact berries and extracted seeds within harvest dates.

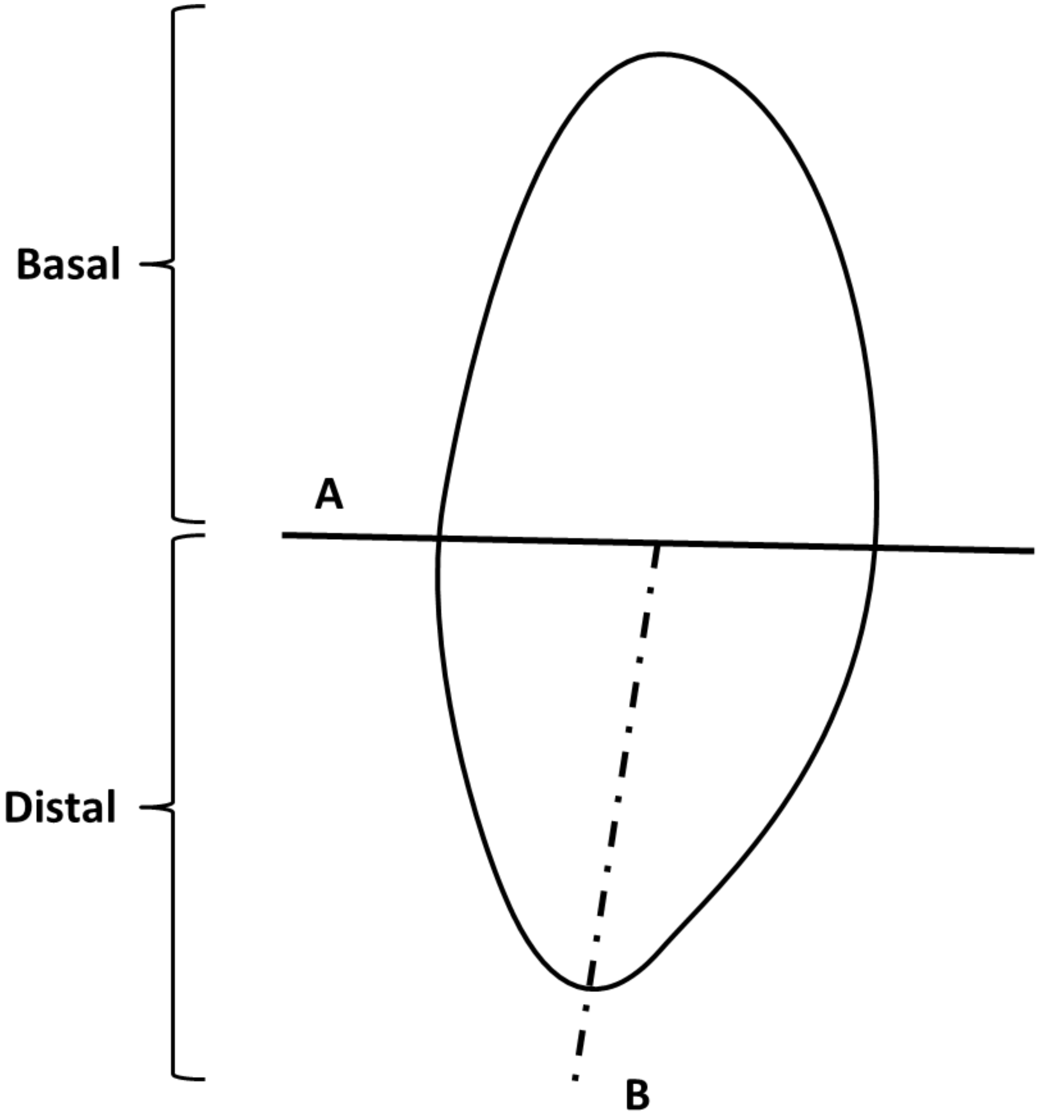

Figure 3.1. Methodology of Amur honeysuckle seed dissection during tetrazolium assay. Dissection followed a two-step process. Initially, seeds were cut laterally (A) and the distal end of the seed was retained for treatment. Seeds were then dissected longitudinally (B) to view embryo and cotyledons and assess viability.

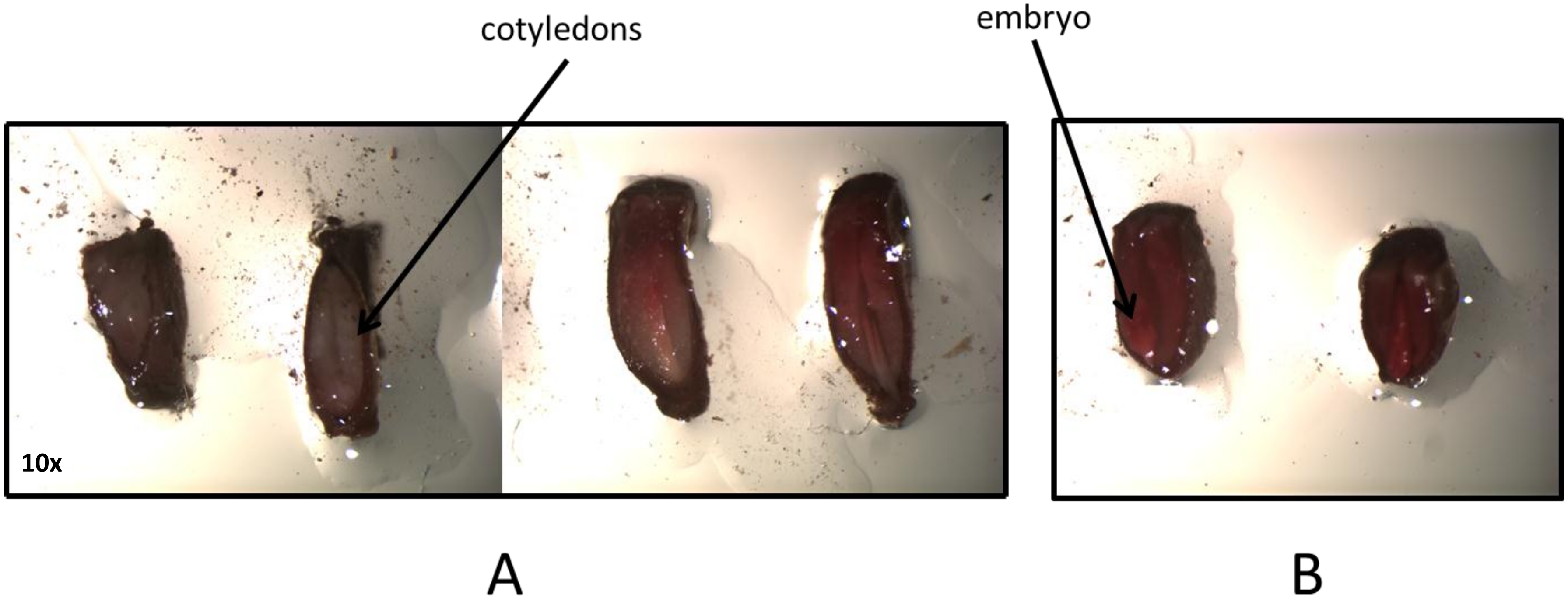

Figure 3.2. Representative seeds of Amur honeysuckle following treatment with tetrazolium chloride. Visual assessment of viability was taken after incubation. Seeds without any stained tissue (A; left) or with unstained embryos or cotyledons (A; right) were deemed non-viable. Seeds with stained embryo and cotyledons (B) were classified as viable.

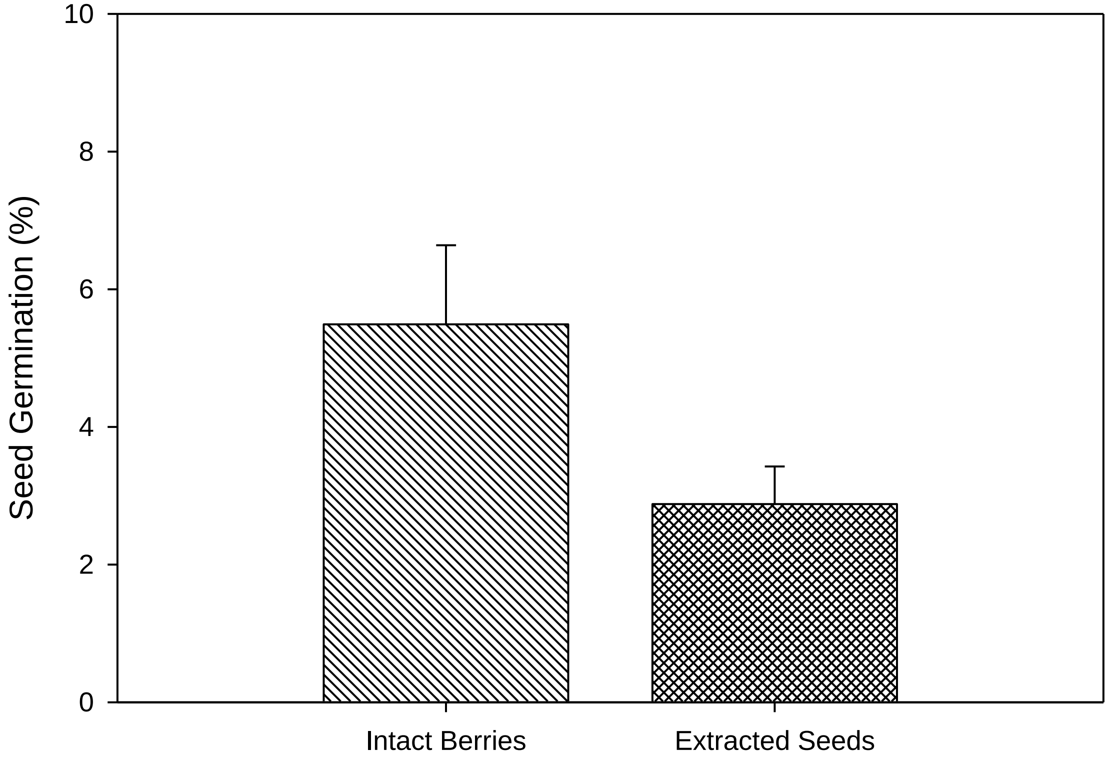

Figure 3.3. Mean germination of Amur honeysuckle seeds across all harvest dates for four site years in Missouri. Germination of intact Amur honeysuckle berries and extracted seeds was assessed. Germination data are cumulative. Berries were harvested from the Charles W. Green Conservation Area near Ashland, and Proctor Park in Columbia, in 2011, and the Charles W. Green Conservation Area and Bear Creek Trail in Columbia, in 2012. Harvest occurred every two weeks from September through November. Germination was defined as the presence of fully developed cotyledons. Germination data were collected from November 2011 through May 2012, and November 2012 through April 2013. Means without letters are not significantly different by Fisher's Protected LSD at p=0.05.

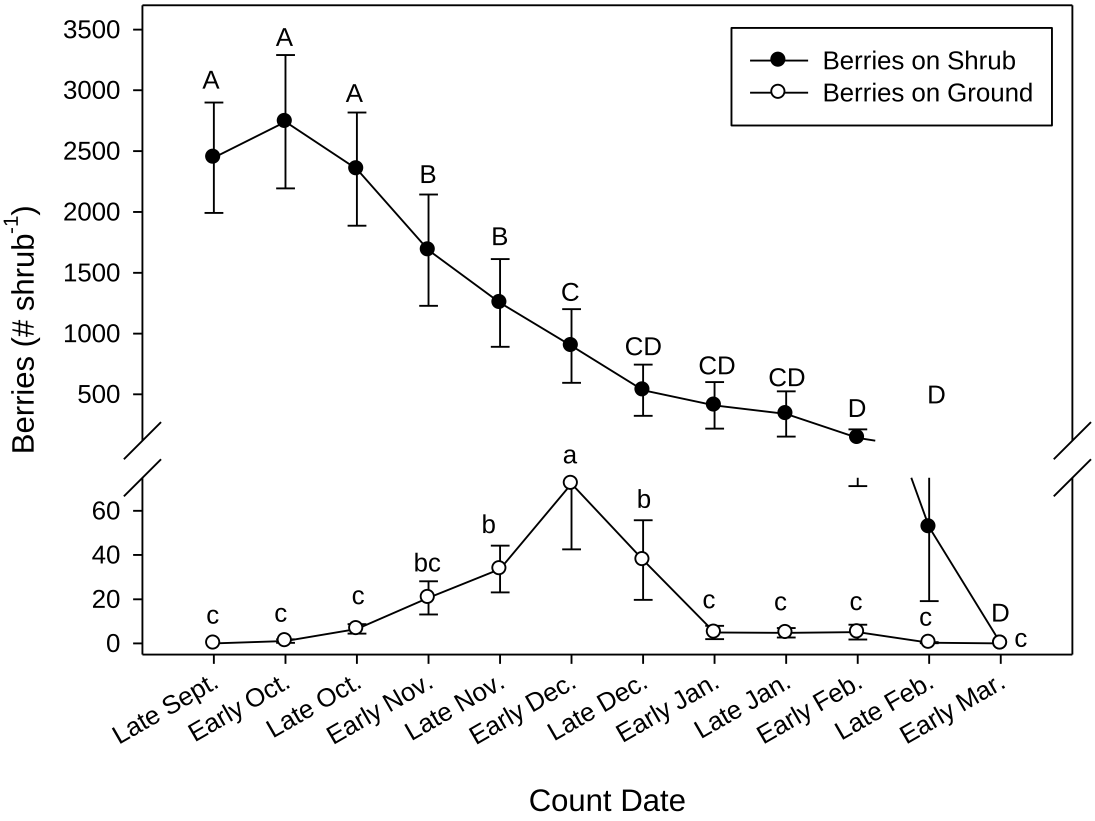

Figure 3.4. Change in number of Amur honeysuckle berries due to predation and berry abscission from September to March for 2011 to 2012 and 2012 to 2013. Counts were averaged over two locations, (the Charles W. Green Conservation Area and the Grindstone Nature Area) and recorded every 15 days. Average number of seeds per berry was 2.08 (±1.33). Vertical bars represent the standard error of the mean. Means followed by the same letter within each berry location are not significantly different by Fisher's Protected LSD at p=0.05. Capital letters denote mean separation of berries present on shrubs. Lowercase letters denote mean separation of berries present on the ground.

CHAPTER IV

FACTORS CONTRIBUTING TO THE SUCCESS OF AMUR HONEYSUCKLE (*LONICERA MAACKII*) INFESTATIONS IN MISSOURI

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Amur honeysuckle forms dense stands along forest edges, excluding native plants. Although widespread, few studies have identified factors contributing to the competitiveness of Amur honeysuckle among native species. Studies were conducted at two locations in Missouri from 2011 to 2013 to assess differences in light intensity beneath the canopy of Amur honeysuckle and to determine if Amur honeysuckle roots exhibited allelopathic activity. Along forest edges in the absence of Amur honeysuckle, photosynthetically active radiation (PAR) averaged 195.5 µmol m^{-2} s^{-1} during spring and summer. Comparatively, as much as 92.1 and 87% of PAR at ground level was reduced by the canopy of Amur honeysuckle from March through May and June through August, respectively. For fall (September through November), PAR beneath Amur honeysuckle was reduced 76.1% compared to cleared areas. PAR was not significantly reduced by Amur honeysuckle foliage during the winter (December through February). The

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longevity of reduced light penetration beneath Amur honeysuckle reflects the length of time plants retain leaves. Lettuce was planted into soils sampled beneath Amur honeysuckle shrubs and from open areas up to 10 m away. Averaged across samples for a given season (spring, summer, fall, winter), neither germination of lettuce, nor lettuce biomass accumulation was negatively affected by the presence of Amur honeysuckle roots. Competition for light appears to be a significant factor in the success of Amur honeysuckle, while allelopathic activity by roots is not.

Nomenclature: Amur honeysuckle, *Lonicera maackii* Rupr.; Lettuce, *Lactuca sativa* L.

Key Words: Allelopathy, light competition.

Amur honeysuckle (*Lonicera maackii*) is an invasive shrub that primarily occupies undisturbed areas along treelines, fencerows, and roadsides (Dirr 1983). The presence of Amur honeysuckle threatens the success of native species. Hutchinson and Vankat (1997) found within an Ohio forest, that tree seedling density was less than 0.5 m^{-2} when Amur honeysuckle cover was greater than or equal to 15%. Additionally, when Amur honeysuckle cover was greater than 50%, species diversity was less than 8 (Hutchinson and Vankat 1997). Mature plants may reach a height of 6 m, eliminating much of the open space at the edge of forest areas where populations of Amur honeysuckle are largest (Luken and Mattimiro 1991; Luken and Thieret 1996).

Negative impacts on native plants may be related to vegetative growth of Amur honeysuckle. Luken et al. (1997) found relative growth rates of Amur honeysuckle plants are >70 and 40% higher in full sun light and 25% of full sunlight, respectively, compared to desirable native species such as spicebush (*Lindera benzoin*). Plants exhibit a longer leaf retention time than native forest species. Trisel (1997) reported that Amur honeysuckle initiates leaf expansion in the spring, up to 6 weeks earlier than other species. Amur honeysuckle retains leaves as late as mid-December, which is longer than native species (Luken and Thieret 1996; McEwan et al. 2009; Shustack et al. 2009). Species with a long growing season can reduce the availability of resources to support native species. Luken and Mattimiro (1991) found Amur honeysuckle populations were highest along forest edges, which is directly related to higher light environments. Woods (1993) reported that the competitive ability of Tatarian honeysuckle (*Lonicera tatarica*) was suppressed due to the year-round canopy formation of evergreens and the ability of vining perennials such as blackberry (*Rubus* ssp.) species to grow over Tatarian honeysuckle.

Another factor that may increase the competitive ability of Amur honeysuckle is allelopathy. Amur honeysuckle produces allelopathic chemicals in leaves and fruits (Cipollini et al. 2008; Dorning and Cipollini 2006; McEwan et al. 2010). Several studies indicate extracts from mature Amur honeysuckle leaves suppressed germination and growth of grasses and forbes (Dorning and Cipollini 2006; McEwan et al. 2010). Dorning and Cipollini (2006) stated jewelweed (*Impatiens capensis*) germination was eliminated after treatment with Amur honeysuckle leaf extracts. Additionally, McEwan et al. (2010) found Amur honeysuckle leaf extracts delayed tall fescue (*Festuca arundinacea*) seed germination up to four days. Thirteen phenolic compounds were detected in honeysuckle leaves, two of which inhibited *Arabidopsis thaliana* germination up to 70% (Cipollini et al. 2008).

In many areas with established Amur honeysuckle in mid-Missouri, soil underneath shrubs is devoid of vegetation. Understanding factors that contribute to the competitive ability of Amur honeysuckle may indicate the proper design of effective management strategies to restore native species. The objectives of this research were to determine the amount of light penetration beneath Amur honeysuckle thickets, and to identify if soils containing Amur honeysuckle roots suppressed the germination and growth of an indicator species.

Materials and Methods

Light Inhibition. In the summer of 2011, two Missouri locations (the Charles W. Green Conservation Area, near Ashland (38.82°N, 92.26°W), and the Grindstone Nature Area, in Columbia (38.92°N, 92.31°W) were selected for establishing light inhibition experiments. The Ashland soil was a Keswick silt loam (fine, smectitic, mesic Aquertic Chromic Hapludalfs) with a pH of 6.0 and 2.8% organic matter. The Columbia soil was a Haymond silt loam (coarse-silty, mixed, superactive, mesic Dystric Fluventic Eutrudepts) with a pH of 6.2 and 2.9% organic matter. At each location, four mature shrubs of Amur honeysuckle within a continuous 30 m section of shrubs were selected, and all vegetation beneath the shrubs was removed. In four adjacent areas, Amur honeysuckle shrubs were cut at ground level with a chainsaw, and glyphosate at 860 g ae ha^{-1} was applied to remove existing vegetation. Thinning Amur honeysuckle resulted in four, 2 m areas of open space between shrubs. The intensity of photosynthetically active radiation (PAR) was recorded at ground level beneath shrubs and in open areas between shrubs. PAR was measured with a Li-Cor LI-250 light meter (Li-Cor Biosciences, Lincoln, NE) every 14 days, between 1:00 and 3:00 p.m. (solar noon), from September 2011 through March 2013. In full sunlight, away from all vegetation, the average PAR reading was 414 µmol m^{-2} s^{-1}. Data beneath shrubs were recorded regardless of cloud conditions. Data were averaged across recording dates for 3 months at a time, creating a seasonal mean. Seasonal means were used because it was of interest to determine seasonal fluctuations in PAR intensity. Seasons were defined as spring (March through May), summer (June through August), fall (September through November), and winter (December through

February). The experiment was designed as a randomized complete block with 4 replications; individual shrubs and individual cleared areas were considered replicates.

Allelopathy. At two locations in mid-Missouri, areas with mature Amur honeysuckle and adjacent areas lacking honeysuckle shrubs were identified. Locations included the Charles W. Green Conservation Area, near Ashland, and the Grindstone Nature Area, in Columbia. The Ashland soil was a Keswick silt loam, with a pH of 6.1 and 3.0% organic matter. The Columbia soil was a Haymond silt loam, with a pH of 6.4 and 2.8% organic matter. Soil samples (7.5 cm deep and a radius of 10 cm), beneath shrubs and in open areas (same environment but devoid of Amur honeysuckle), were taken monthly from October 2011 to November 2012. Once removed, soil samples were stored in a cooler at 2 to 7 C until assessment. Soils were crumbled by hand and placed in 28 by 28 cm polypropylene flats in the greenhouse, creating a 3 cm thick layer. Root fragments in soils were not removed during preparation. A mixture of 1:1 v/v commercial potting medium (Premier Tech, Rivière-du-Loup, Quebec, Canada) and sand was used as a control. Twenty-five seeds of lettuce (*Lactuca sativa* var. Iceberg) (American Meadows, Williston, VT) were planted into soil samples to a depth of 0.5 cm. Lettuce is often used as a test species because of it sensitivity to phytotoxic chemicals (Macías et al. 2000). Flats were subjected to natural light and supplemental lighting to provide a 12 hour photoperiod and minimum light intensity of 200 µmol photon m^{-2} s^{-1}. Greenhouse conditions included an air temperature of 15 to 30 C and flats were watered as needed. No fertilizer was used throughout the study. Lettuce emergence was recorded every 3 days until 15 days after planting (DAP). A laboratory test showed germination potential of lettuce seeds was 70%, and reached a maximum 10 days after initiation. Plants were considered emerged with fully developed cotyledons. At 15 DAP, emerged plants were harvested at ground level in each flat; tissue was dried for 3 days at 40 C and biomass recorded. Data were averaged across sampling dates to determine seasonal means. Seasons were defined as described above. The experimental design was a randomized complete block with 4 replications; individual shrubs and individual open areas were considered replicates.

Statistics. The ANOVA for light intensity, lettuce germination, and lettuce biomass data (dry weight) were carried out using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). For both experiments, fixed effects were shrub cover status and sampling date. Year and location were considered random effects. A transformation did not improve separation of treatment effects. Therefore, no transformation was carried out. Pooled mean contrasts were performed to test seasonal effects for the light inhibition experiment. Means were separated using Fisher's Protected LSD at P=0.05.

Results and Discussion

Light Inhibition. The canopy of Amur honeysuckle intercepted the majority of available light (Figure 4.1). Light availability at ground level was reduced beneath shrubs, compared to cleared areas, for the spring (92.5%), summer (86.7%), and fall (76.1%). This is evidence that Amur honeysuckle has a long growing season. Significant differences in light intensity between shrub and cleared areas existed for all seasons except winter. During winter, PAR levels were similar for areas with and without Amur honeysuckle; the 18% difference in the canopied area may be the result of branches. The presence of leaves of Amur honeysuckle over an extended part of the growing season (Luken and Thieret 1996; McEwan et al. 2009; Shustack et al. 2009; Trisel 1997) results in severe competition for light. Plant communities in Missouri infested with Amur honeysuckle had reduced abundance of shade-intolerant native species compared with uninvaded communities with the same species, suggesting interference mediated by reduced light availability from dense shading (Powell et al. 2013).

Removal of photosynthetically active radiation is a key factor in the competitiveness among plant species and is likely exacerbated in areas with species forming an understory beneath trees. In a forest habitat, Lei et al. (2006) reported that the presence of great laurel (*Rhododendron maximum*) reduced the amount of available light up to 88% compared to similar areas without this species. Uesaka and Tsuyuzaki (2004) found that the light intensity beneath both Salix willow (*Salix reinii*) and Japanese wintergreen (*Gaultheria miqueliana*), was 67% lower compared to bare ground. Light restrictions inhibited the germination of western pearly everlasting (*Anaphalis margaritacea*), a desirable native forb, up to 87% (Uesaka and Tsuyuzaki 2004). Therefore, the extensive period of reduction in light intensity found beneath Amur honeysuckle shrubs likely inhibits germination of desirable native shrubs and forbs.

Allelopathy. Lettuce emergence was not affected in soils containing Amur honeysuckle roots, compared to soils without roots, during any of the sample periods (Figure 4.2). Averaged over season, total lettuce germination was significantly greater for spring (57%) compared to winter (43%), fall (30%), and summer (15%) (data not shown).

Variation in lettuce germination is likely due to differences in greenhouse temperatures. Using a composite medium, averaged across all seasons, lettuce germination was 20% 3 DAP and increased to 33% by 15 DAP, but was not different than emergence in field collected soils (data not shown). McEwan et al. (2010) reported tall fescue germination was delayed four days after treatment with Amur honeysuckle leaf extracts. Additionally, jewelweed germination was suppressed completely with treatment of Amur honeysuckle leaf extracts; however, root extracts reduced germination by only 43% (Dorning and Cipollini 2006).

Similar to emergence data for lettuce, the soil environment and sampling time had no consistent effect on biomass accumulation of lettuce (Figure 4.3). Averaged over season, summer soil samples yielded higher lettuce biomass (0.23 g) than winter and spring samples (data not shown). Also, samples taken in the fall yielded higher lettuce biomass (0.17 g) than spring samples, averaged over season, likely due to greenhouse temperatures (data not shown). Lettuce biomass was significantly greater (240%) in soils containing Amur honeysuckle for fall samples compared to control soils (Figure 4.3).

The success of Amur honeysuckle in habitats along the edge of wooded areas is evident because of the observed monocultures. Exclusion of native species by Amur honeysuckle may result from competition for a needed growth factor or release of chemical suppressants. Our data suggests that the foliage of Amur honeysuckle restricts available PAR up to 92% over an extended part of the growing season. Release of allelopathic compounds from Amur honeysuckle roots was not apparent, suggesting allelopathy is not a contributing factor to Amur honeysuckle infestations. It is likely that encouraging the re-establishment of native species in areas infested with Amur honeysuckle begins with increasing levels of available light.

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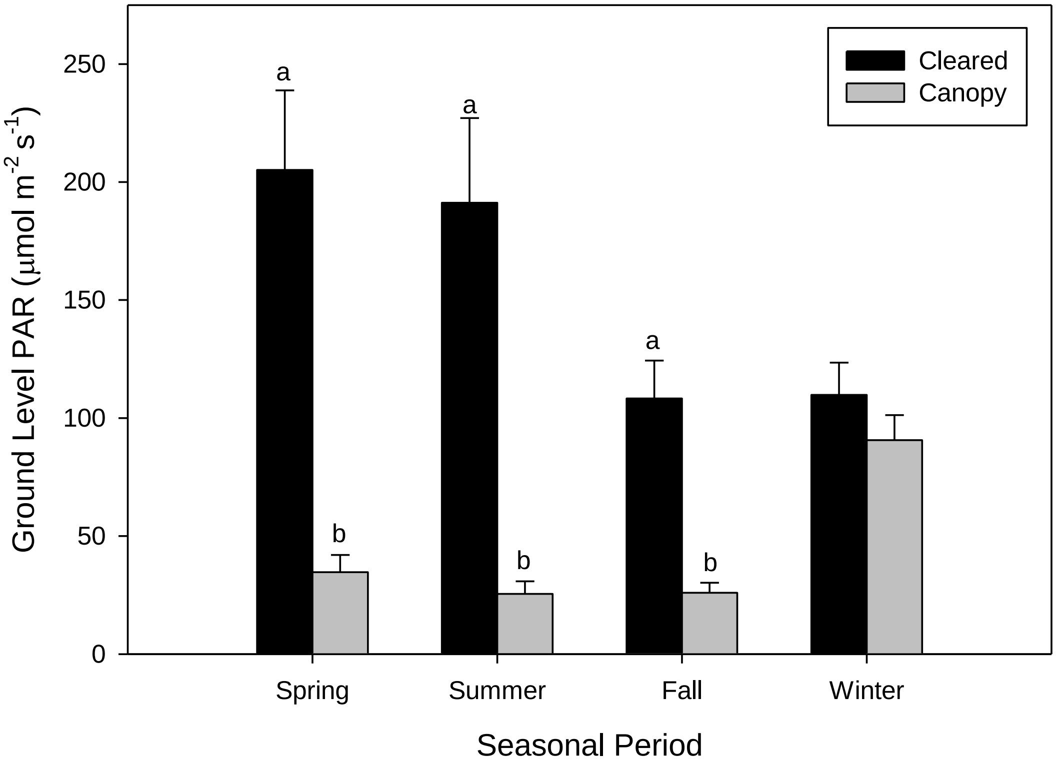

Figure 4.1. Mean photosynthetically active radiation (PAR) for seasonal periods from areas with Amur honeysuckle canopy and areas where Amur honeysuckle was removed. Seasonal periods included March through May (spring); June through August (summer); September through November (fall); and December through February (winter). Vertical bars indicate the standard error of the mean. Bars within a seasonal period with different latters are different as estimated by Fisher's Protected LSD at P=0.05, while bars within a seasonal period without letters are not significantly different.

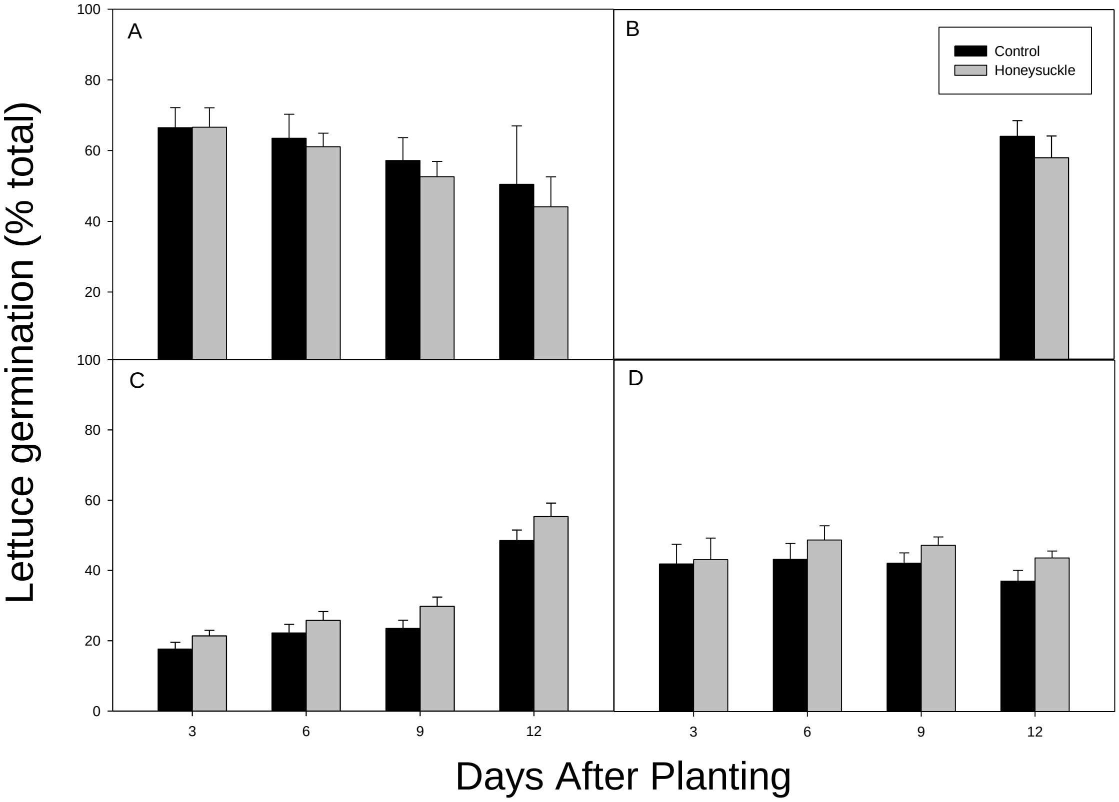

Figure 4.2. Emergence of lettuce planted into soils in the presence and absence of Amur honeysuckle roots. Soils were sampled monthly from October 2011 to November 2012. Germination results were averaged for spring (A), summer (B), fall (C), and winter (D) seasons. Seasonal periods included March through May (spring); June through August (summer); September through November (fall); and December through February (winter). Vertical bars indicate the standard error of the mean. For each seasonal period, means without letters within days after planting are not significantly different using Fisher's Protected LSD at p=0.05.

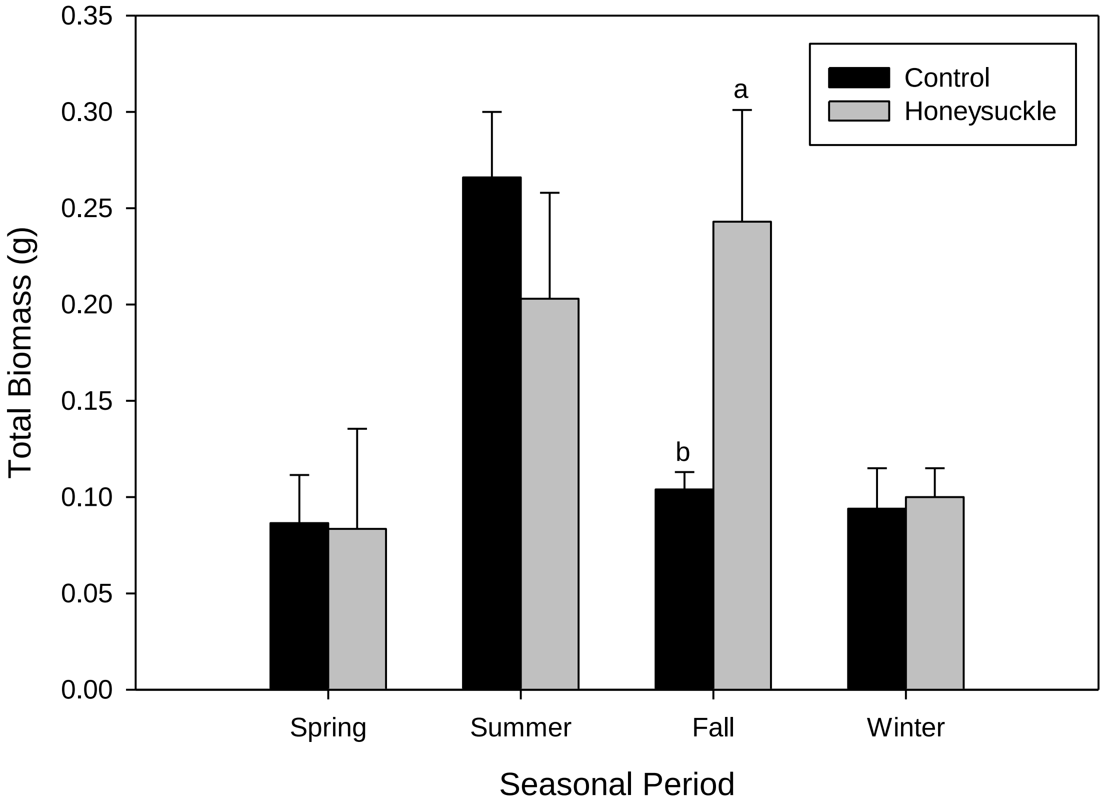

Figure 4.3. Dry weight biomass of lettuce (*Lactuca sativa* var. Iceberg) growing in soils containing or absent of Amur honeysuckle roots. Soils were sampled monthly from October 2011 to November 2012 and lettuce harvested 15 days after planting. Seasonal periods included March through May (spring); June through August (summer); September through November (fall); and December through February (winter). Vertical bars indicate the standard error of the mean. Bars within a seasonal period with different latters are different as estimated by Fisher's Protected LSD at P=0.05, while bars within a seasonal period without letters are not significantly different.