ABSTRACT

EMERINE, SHERRIE ELLEN. The Biology and Control of Porcelain Berry (*Ampelopsis brevipedunculata*). (Under the direction of Robert J. Richardson).

Porcelain berry [*Ampelopsis brevipedunculata* (Maximowicz) Trautvetter] is a woody perennial climbing vine in Vitaceae. Porcelain berry is of concern in the eastern United States because of its rapid growth, vining habit, and high rate of seed germination. Greenhouse and field studies were conducted to determine efficacy of aminocyclopyrachlor, aminopyralid, glyphosate, imazapyr, and triclopyr for controlling porcelain berry. Greenhouse trials indicated that cut stem treatments with triclopyr provided 100% control and aminopyralid and glyphosate provided at least 93% control. Imazapyr and basal bark applications of triclopyr provided moderate control, and both cut stem and basal bark applications of aminocyclopyrachlor provided 44% control or less. Field trials indicated that all cut stem and basal bark triclopyr treatments and cut stem treatment with aminocyclopyrachlor provided at least 89% control of porcelain berry. Aminopyralid provided good control at 85%, while glyphosate and imazapyr provided 67% or less control.

Research trials were conducted to determine effects of interspecific competition between porcelain berry, Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch], and bushkiller [*Cayratia japonica* (Thunb.) Gagnep.]. Results indicated that bushkiller was the fastest growing and most robust species tested, with the greatest stem and root biomass. Bushkiller reduced stem length and mass of porcelain berry and Virginia creeper. Porcelain berry did not significantly impact Virginia creeper or bushkiller stem or root growth, and porcelain berry root weights were similar to those of Virginia creeper. Virginia creeper alone did not

negatively affect either other species. These results suggest that porcelain berry is less aggressive and vigorous than bushkiller and is competitively similar to Virginia creeper.

Porcelain berry seeds were subjected to several treatments to determine requirements for germination. Fresh seeds did not germinate when subjected to a range of temperatures. Seeds germinated at higher rates when exposed to alternating temperatures than to constant temperature. Seeds only germinated in dark conditions if exposed to alternating temperatures. Depth of germination tests indicated that porcelain berry did not germinate well at the surface or deeper than 6 cm, but germination of up to 80% occurred at depths between 1 and 4 cm. Perennation tests indicated that the ability of porcelain berry to resprout after being cut at ground level was poor in plants with fewer than 7 leaves, after which resprouting occurred in up to 50% of plants. However, some seedlings with only two true leaves were able to resprout.

Two climate models, CLIMEX and NAPPFAST, were used to determine the potential extent of invasion of porcelain berry and bushkiller in the United States. Both models indicated that porcelain berry could spread to some areas of the Midwest and the Pacific Northwest, as well as continue to spread in the southeast. CLIMEX predicted that some regions of the northern Midwest may be suitable for bushkiller, but the NAPPFAST model predicted that the upper Midwest is not suitable. Both models indicated that the climates of the Pacific Northwest and the Southeast are very favorable for invasion by bushkiller, but that most of the interior of the United States is not. The models predicted that both species have the potential to increase their extent of invasion in the United States.

The Biology and Control of Porcelain Berry (*Ampelopsis brevipedunculata*)

by
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BIOGRAPHY

Sherrie Emerine was born in Chattanooga, Tennessee to a military family and traveled extensively in her childhood. She pursued a degree in Fisheries and Wildlife at The University of Maryland and Virginia Polytechnic Institute and State University before settling down in a rewarding career as a veterinary technician. An avid hiker and backpacker, Sherrie began noticing the abundance of exotic invasive species in natural areas and determined to learn more about them. She finally earned a Bachelor of Science in Plant Biology at North Carolina State University with a minor in Geology, and continued on to pursue a Master of Science in the Weed Science program under the advisement of Dr. Rob Richardson. Sherrie's activities include gardening, hiking, kayaking, and spending time with her many pets.

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Chapter 1

An Introduction to the Biology and Control of Porcelain Berry [*Ampelopsis brevipedunculata* (Maximowicz) Trautvetter]

Abstract

Porcelain berry [*Ampelopsis brevipedunculata* (Maximowicz) Trautvetter] is a woody perennial climbing vine native to eastern Asia. Porcelain berry was introduced into the United States for ornamental purposes and has become a weed of riparian areas, early successional fields, pastures, and waste places. Its seeds are spread by animals and water and exhibit a high rate of germination. Porcelain berry can grow as much as 15 to 20 feet in a growing season. It is able to shade out other species and can damage trees and other supporting vegetation due to its large biomass. Porcelain berry may negatively impact communities in which it displaces native species. Control recommendations for porcelain berry include hand pulling of young stems in conjunction with foliar, cut stem, or basal bark applications of triclopyr. Glyphosate applied as a foliar spot treatment also has been recommended.

Biology and control

Ampelopsis brevipedunculata (Maximowicz) Trautvetter, commonly known as porcelain berry or Amur peppervine, is a woody perennial climbing vine in Class Magnoliopsida, Order Rhamnales, Family Vitaceae (ITIS 2010). Synonyms include *Ampelopsis heterophylla*

(Thunb.) Sieb et Zucc. var. *brevipedunculata* (Zheng et al. 2004), *Cissus brevipedunculata* Maxim., *C. humulifolia* var. *brevipedunculata* (Maxim.) Regel, *A. brevipedunculata* var. *maximowiczii* (Regel) Rehd., *Vitis heterophylla* Thunb., *A.heterophylla* (Thunb.) Sieb. & Zucc., non Bl., *A. regeliana* Carr., *A. heterophylla* var. *humulifolia* (Bunge) Merrill, *A. glandulosa* Lebas, and *A. brevipedunculata* forma *citrulloides* (Lebas) W. Lee (Ohwi 1984; USDA ARS 2011; Zhiduan and Wen 2007). Zhiduan and Wen (2007) list several other varieties as synonyms in China as well.

Porcelain berry superficially resembles the eastern North American native *Ampelopsis cordata* Michx. and several native *Vitis* species. Branches are terete and glabrous or with some pubescence, especially when young. Pith is white and is continuous through the nodes, which are thickened and jointed (Dirr 1998). Bark is thin, non-peeling, and with obvious lenticels (Brand 2001). The deciduous leaves are simple, unlobed or palmately lobed with three to five lobes, with a cordate leaf base, and are 4 to12 cm long and 4 to10 cm wide. Leaves may be glabrous or pubescent (Anonymous 1993). Leaf margins have obtuse or acute teeth (Brand 2001; Ohwi 1984). Non-adhesive tendrils form opposite most leaves, often bifurcating to form one tendril and one small inflorescence (S. Emerine personal observation). The inflorescence is a corymbose cyme (Zomlefer 1994). Flowers are 3 mm across, actinomorphic, hypogynous, and have five deciduous sepals and petals, which are not fused at the tips (Soejima and Wen 2006; Zomlefer 1994). Flowers are pollinated by ants, bees, wasps, and other anthophilous insects (S. Emerine personal observation). Fruit is a round berry, glabrous, 6 to 8 mm across, which varies from pale green when immature to amethyst to deep blue at maturity, with many stages of fruit on the same infructescence (Brand 2001, Dirr 1998, Ohwi 1984). Some berries in North Carolina populations also become greenish-white at maturity (S. Emerine personal observation). Berries usually contain two seeds, rarely one, three, or four (Gerrath and Posluszny 1989; Ohwi 1984). Zomlefer (1994) describes the seed as having a thin outer membrane and a hard inner layer, the sclerotesta, which forms grooves on the adaxial surface. The endosperm has three lobes. A ridge called the raphe extends between the grooves from the hilum to the seed apex, terminating on the abaxial surface in an elevated or depressed region called a chalazal knot.

A. brevipedunculata is native to eastern Asia. In Japan it is found in Hokkaido, Honshu, Shikoku, and Kyushu. In China its range includes the northeastern-most provinces of Heilongjiang, Jilin, and Liaoning. It is also known to occur in Korea and on the southern Kurile Islands (Anonymous 1993; Ohwi 1984; USDA ARS 2011). Two varieties are found in Taiwan. *A. brevipedunculata* var. *ciliata* (Nakai) Lu is found in southern China and northern Taiwan. *A. brevipedunculata* var. *hancei* (Planch.) Rehder is found throughout Taiwan as well as in southern China and the Philippines (Anonymous 1993).

Porcelain berry was likely introduced into the United States in the 1870's for ornamental plantings (Huang and Sherald 2004, Mehrhoff et al. 2003). It is currently distributed from New Hampshire south to Georgia and west to Iowa (USDA NRCS 2011). The vine occurs in early successional fields, vacant lots, edges, pastures, railroad and utility rights-of-way, and along streams and rivers. It prefers moist soils and partial to full sun (Mehrhoff et al. 2003) and appears to be intolerant of deep shade (Robertson et al. 1994, Yost et al.1991). Porcelain berry fruits are said to be relished by birds and small mammals and seeds are dispersed in their droppings (Robertson et al. 1994; Young 2005). However, Witty et al. (2010) posit that porcelain berry deters mammalian herbivores by several methods discussed later. The berries float in water and may establish populations downstream from parent plants (Robertson et al. 1994). Seeds have a high rate of germination and once established, plants can grow as much as 15 to 20 feet in a growing season (Dirr 1998; Mehrhoff et al. 2003). Large vines will resprout from the vigorous taproot (Young 2005).

The vine is sold in the garden trade for its foliage and showy berries. There are two cultivars, 'Elegans', which has variegated foliage that may revert to the species type over time, and 'Citrulloides', which has deeply incised leaves with five lobes. Anecdotal reports indicate that the variegated cultivar may be less aggressive than the species in some areas (Brand 2001, Dirr 1998). One variety, *A. brevipedunculata* var. *maximowiczii*, has very deeply lobed sinuses and exhibits aggressive behavior (Dirr 1998; Mehrhoff et al. 2003).

Although porcelain berry is a host for several pests and pathogens, the species is generally hardy and is tolerant of disease and predation (Dirr 1998). Porcelain berry is an alternate host for *Xylella fastidiosa* Wells et al., which affects economically important crops such as peach, plum, and oak, and is the causal agent of Pierce's Disease in cultivated grape (Huang and Sherald 2004). Thiéry and Moreau (2005) found that the European grapevine moth, *Lobesia botrana* Den. and Schiff., a serious pest of wine grapes and subject to official control in the United States (NAPPO 2010), prefers and exhibits enhanced growth and reproductive rates on porcelain berry compared to cultivated grape. Japanese beetle, *Popillia japonica* Newman, consumes the leaves and may completely defoliate whole plants with little apparent harm (Brand 2001; Dirr 1998). Zheng et al. (2004) list an additional nine species of fungi and thirteen arthropod species as pests of porcelain berry.

Witty et al. (2010) report that, unlike most fruits, porcelain berry fruits contain layers of pigments which form colors and patterns more readily visible to birds than to mammals. Additionally, the plant and berries contain calcium oxalate raphides that deter predation by causing physical damage to soft tissues during mastication. Toxic oxalic acid is produced as a result of reactions between calcium oxalates and naturally occurring organic acids when animals chew the berries. Birds do not appear to be sensitive to oxalic acid (Witty et al. 2010).

Porcelain berry stems and roots have been used as anti-inflammatory, diuretic, and anti-hepatotoxic agents in Asian folk medicine (Yabe and Matsui 1997). Wu et al. (2004) found that plant extracts do demonstrate powerful antioxidant and anti-hepatotoxic activity, and Yabe and Matsui (2007) found that porcelain berry extracts inhibited damaging collagen formation and proliferative liver cells in rats. The plant contains tannins and novel oligostilbenes, called ampelopsins, which are assumed to deter predation. In addition, the berries contain multi-layer lignified cells that enclose cells containing tannins (Witty et al. 2010).

Young (2005) recommends hand pulling of vines before flowering for small infestations. Hand pulling combined with the herbides triclopyr or glyphosate has been shown to be effective. Foliar or cut stem applications of triclopyr amine or basal bark application of triclopyr ester with basal or other oil have been recommended (Smith 2008; Young 2005). Ding et al. (2006) propose that there are 22 natural enemies of *A. brevipedunculata* in its native habitat, but only four potential biocontrol agents suitable for study in the United States.

Justification

Pimentel et al. (2005) estimated that invasive exotic plants, animals, and pathogens cost the United States approximately $120 billion per year. For instance, treatment costs and productivity losses due to exotic agricultural weeds total about $27 billion, while totals for aquatic weeds are $100 million. Estimates for the economic and ecological costs of invasive plants to natural areas are more difficult to quantify. Invasive species can cause direct impacts to native species via predation, herbivory, parasitism, or competition, or indirect impacts such as habitat alteration or trophic changes (Sakai et al. 2001). Baker (1974) detailed several traits associated with invasive behavior in plants: sexual and asexual reproduction, rapid growth, phenotypic plasticity, high tolerance to environmental heterogeneity, prolific seed production, and self pollination. Later, Daeler (1998) added the traits aquatic or semi-aquatic habit, nitrogen-fixation, climbing habit, and abiotic pollination. Many of these traits are desired and selected for in ornamental and agricultural plants. In addition, repeated introductions have been shown to increase the likelihood of invasion success (Rejmánek 2000), and multiple introductions provide a source of propagules and genetic variation for incipient invasions (Sakai et al. 2001). Fridley (2008) states that eastern

Asian species may be highly "preadapted" to the climate and forest associations of the eastern U.S. because native congeners exist for many of them. Invasive vines present particular challenges in their introduced ranges. Generally, woody vines allocate most of their available resources to stem and root elongation and canopy and reproductive tissue development. They often have greater photosynthetic biomass than other woody plants, as they have a high canopy- to- stem ratio. Additionally, they can colonize not only by seed in the understory before a disturbance, but also can quickly exploit new gaps as mature plants in tree-fall or logging events (Ewers et al. 1991). Woody vines may play an important role in community composition and structure as a result of their competitive abilities and effects on different tree species (Schnitzer and Bongers 2002). For example, Schnitzer et al. (2000) suggest that woody vines may delay succession by reducing competition from slow growing, shade-tolerant species by blanketing them.

Porcelain berry is of concern in the eastern United States because of its rapid growth, vining habit, and high rate of germination. Much like kudzu [*Pueraria montana* (Lour.) Merr.], it can damage trees and other supporting vegetation due to its large biomass, and it exhibits proliferative growth which can shade out other species (Young 2005). Birds and possibly other animals have spread the seeds into riparian and disturbed habitats, where the species may impact native plant and animal communities. Porcelain berry has physiological requirements similar to those of some of our native Vitaceae and may negatively impact communities in which it displaces native species. Perhaps because it is still sold in the horticulture trade, little information is available regarding its behavior as an invasive species or potential control options. It is a prohibited noxious weed in Massachusetts (USDA NRCS 2011).

The Vitaceae

The Vitaceae consists of approximately 14 genera and around 900 species, which are found mostly in the tropics of Asia, Africa, Australia, the Pacific Islands, and a few in temperate regions of Asia and eastern North America. The genera *Ampelopsis* and *Parthenocissus* exhibit disjunct distributions in eastern Asia and eastern North America (Ohwi 1984; Soejima and Wen 2006; Walters et al. 2006). The members are mostly woody or herbaceous climbers or small succulents. Leaves may be simple or compound, and vines generally have a repeating pattern of tendrils and inflorescences opposite leaves (Gerrath and Posluszny 1988; Wen 2006). The tendrils are thought to be modified shoots or inflorescences (Gerrath and Posluszny 1988; Soejima and Wen 2006). Posluszny and Gerrath (1986) propose that an "uncommitted primordium" appears identical whether it will mature into an inflorescence, a tendril, or an intermediate structure. Inflorescences are determinate and generally cymose- paniculate. Flowers are actinomorphic, perfect or imperfect, hypogynous, and minute. Sepals number 4 to 5 and are connate, and petals number 4 to 5 and are distinct or connate. There are two connate carpels and 4 to 5 distinct or connate stamens. A nectary disc is present. The ovary contains 2 locules with 1 to 2 ovules per locule. The fruit is a berry containing one to four seeds (Soejima and Wen 2006; Walters et al. 2006; Zomlefer 1994). Flowers are typically entomophilous with a few genera being anemophilous. Crosspollination is promoted by protandry, particularly in *Ampelopsis,* but self-pollination is common (Zomlefer 1994). Vitaceae often bear "pearl" glands, small spherical epidermal structures with a short stalk on their leaves. Ozawa and Yano (2009) purport that these pearl bodies may provide an alternative food source for predatory insects that may prey on plant pests. Vitaceae mesophyll contains mucilage cells and abundant calcium oxalate raphide crystals, which cause an acrid or stinging sensation when chewed. The fungitoxin stilbene resveratrol is present in wood and can be induced in leaves (Wen 2006). Important members of Vitaceae include *Vitis vinifera* L. (cultivated grape) and *Parthenocissus quinquefolia* (L.) Planch (Virginia creeper).

The genus *Ampelopsis*

Ampelopsis contains approximately 30 species which are endemic to Asia and Central and North America (Zhiduan and Wen 2007). Most grow primarily in mountainous regions in temperate zones (Wen 2006). All members of the genus are woody vines with two- or three-branched tendrils. In some species tendrils can bear flowers, which may represent modified inflorescences (Zomlefer 1994). Zomlefer (1994) reports that the tendril may be a main axis that has been made auxiliary by growth of the opposite leaf axil. Pith is white and is continuous across the nodes (Young 2005). Leaves are simple, palmately, or pinnately compound. Inflorescence is a cyme and flowers are 5-merous with a well developed disc. The berry has 1 to 4 obovoid seeds (Zhiduan and Wen 2007). There are two native congeners in North America, *Ampelopsis cordata* Michx. and *A. arborea* (L.) Koehne (Ding et al. 2006), as well as *A. aconitifolia* Bunge, an introduced ornamental vine (USDA NRCS 2011).

Bushkiller (*Cayratia japonica*)

Cayratia japonica (Thunb.) Gagnep., known commonly as bushkiller, javan grape, or sorrel vine, is a perennial herbaceous vine in Vitaceae. It is native to and common in tropical and subtropical regions of southeast Asia, Australia, India, Malaysia, New Caledonia, and New Guinea (Hansen and Goertzen 2006; Hsu and Kuoh 1999; Ozawa and Yano 2009). Bushkiller was first documented in the United States in Louisiana in the 1970s, likely as an escape from cultivation (Hansen and Goertzen 2006). Its current distribution in the United States includes Alabama (Hansen and Goertzen 2006), Louisiana, Mississippi, North Carolina, and Texas (USDA NRCS 2011). Leaves are pedately compound with 5 to 7 leaflets. Leaves are alternate with tendrils opposite. The inflorescence is an axillary branching corymb. Flowers are hermaphroditic and 4-merous. Fruit is a purple globose berry with 2 to 4 seeds per fruit (Wen 2006). West (2009) reports that bushkiller does not produce viable seeds in its invaded habitat in North America, but spreads vegetatively by rhizomatous roots.

Virginia creeper (*Parthenocissus quinquefolia*)

Parthenocissus quinquefolia (L.) Planch, commonly called Virginia creeper or woodbine, is a North American deciduous woody vine in Vitaceae. It is distributed throughout the east from Canada to Florida, and west to Utah (USDA NRCS 2011). Virginia creeper is a component of early to late sere forests, open areas, and disturbed sites, and is often cultivated ornamentally (Bush 2002). It climbs using tendrils terminating with adhesive disks and can reach 60 feet in height (Bush 2002; Wen 2006). Virginia creeper is an important food and shelter source for wildlife. Birds and mammals eat the berries and deer browse foliage (Bush 2002). It prefers moist, well-drained soils but will grow in dry conditions (Bush 2002). *P. quinquefolia* is well adapted to tolerate low-light environments and is physiologically plastic, with a high photosynthetic rate at both low and high light levels (Carter and Teramura 1988). Leaves are palmately lobed with five serrated leaflets. The inflorescence is a corymb. Flowers are 5-merous and fruit is a small, glaucous, purple berry with 1 to 3 seeds. A portion of the primordium may form flowers, while the remaining tissue develops into a tendril (Wen 2006).

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Chapter 2

Efficacy of Selected Herbicides in Control of Porcelain Berry

Greenhouse and field studies were conducted in 2009 and 2010 to determine efficacy of aminocyclopyrachlor, aminopyralid, glyphosate, imazapyr, and triclopyr for controlling porcelain berry. In greenhouse trials, cut stem application of triclopyr provided 100% control and aminopyralid and glyphosate provided at least 93% control 8 weeks after treatment. Cut stem application of imazapyr provided good control at 83%, and basal bark treatments with triclopyr provided moderate control at 69%. Both cut stem and basal bark applications of aminocyclopyrachlor provided 44% control or less. In field trials, all triclopyr treatments and aminocyclopyrachlor cut stem treatment controlled porcelain berry at least 89%. Aminopyralid provided good control at 85%, while the remaining herbicides provided 67% or less control. Based upon these results, cut stem treatments with aminopyralid or triclopyr provided the best control under both field and greenhouse conditions.

Nomenclature: Aminocyclopyrachlor; aminopyralid; glyphosate; imazapyr; triclopyr; porcelain berry [*Ampelopsis brevipedunculata* (Maxim.) Trautv.].

Keywords: herbicide; invasive plant; invasive vine.

Porcelain berry [*Ampelopsis brevipedunculata* (Maximowicz) Trautvetter] is a perennial woody vine in Vitaceae. Native to eastern Asia, it was introduced into North America in the 1800s and has naturalized from New Hampshire south to Georgia and west to Iowa (USDA

NRCS 2011). Porcelain berry grows rapidly and blankets underlying vegetation, effectively shading out other species and weighing down supporting vegetation (Smith 2008). The vine grows along streams and rivers as well as in early successional fields, edges, pastures, railroad and utility rights-of-way, and in waste places where there are moist soils and partial to full sun (Mehrhoff et al. 2003; Smith 2008). Porcelain berry seeds are dispersed in the droppings of birds and small mammals and by berries floating downstream (Robertson et al. 1994).

Little published information is available regarding control options for porcelain berry. Young (2005) recommends hand pulling of vines for small infestations, and hand pulling combined with the herbicides triclopyr or glyphosate are said to be effective. Young (2005) recommends triclopyr amine for foliar or cut stem application or triclopyr ester with oil for basal bark application. Smith (2008) recommends a late-season foliar treatment of 2% triclopyr amine with 0.5% nonionic surfactant, or basal bark treatment of 25% triclopyr ester plus 75% mineral oil.

Aminocyclopyrachlor (formerly DPX-MAT28) is the first member of a new class of herbicides called pyrimidine carboxylic acids, which are thought to be synthetic auxins similar to pyridine carboxylic acid herbicides such as aminopyralid, clopyralid, triclopyr, and picloram. Aminocyclopyrachlor is effective on many broad-leaved weeds, with some selectivity on grasses (Anonymous 2009). It is translocated to roots and shoots and is thought to be active in meristems. Symptoms include leaf and stem epinasty, a common sign of auxin herbicidal activity (Bukun et al. 2010). Aminopyralid is a synthetic auxin in the pyridine carboxylic acid herbicide family and is registered for use on pasture, non-cropland, and natural areas, as well as on grass crops (Senseman 2007). Bukun et al. (2010) found that aminocyclopyrachlor and aminopyralid showed similar patterns of absorption and translocation on Canada thistle and required similar dose rates. Glyphosate is a disruptor of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) pathway and is widely used in agriculture and non-cropland weed control (Senseman 2007). Triclopyr is a pyridine carboxylic acid and a growth regulator that is frequently used on woody species. Richardson et al. (2009) found that triclopyr was at least 93% effective in controlling two other members of the Vitaceae, Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch.] and wild grape (*Vitis* spp.). Imazapyr is an acetolactate synthase (ALS) inhibitor often used on woody species. Imazapyr prevents the biosynthesis of branched-chain amino acids (Tu et al. 2001).

Because no formal comparative studies have been performed to assess the response of porcelain berry to herbicides that are commonly used in natural area invasive plant management programs, the objective of this research was to examine the response of porcelain berry to cut stem and basal bark applications of these five herbicides at varying rates.

Materials and Methods

Field Study. Cut stem and basal bark herbicide trials were initiated in May of 2010 on a population of porcelain berry at Rocky Branch Creek in Raleigh, North Carolina. We repeated the study in June of 2010 in an adjacent location along the same stream. For all trials, woody-stemmed plants were tagged randomly without regard for size. Each treatment was performed on experimental units consisting of three plants, with four replications per treatment. Cut stem treatments included an untreated control, aminocyclopyrachlor[1] at 5% v/v with 95% v/v basal oil, aminopyralid[2] at 5% v/v, glyphosate[3] at 50% v/v, imazapyr[4] at 1% v/v with 99% v/v basal oil, triclopyr amine[5] at 30% v/v, and triclopyr ester[6] at 30% v/v with 70% v/v basal oil. Basal bark treatments included triclopyr ester at 3% v/v with 97% v/v basal oil and aminocyclopyrachlor at 1% v/v with 99% v/v basal oil. Cut stem treatments were applied by cutting the stem 5 cm above ground level and immediately painting the cut surface with herbicide. Control plants were cut in the same manner but not treated. Basal bark treatments were applied by painting the stem from approximately 5 cm above ground level to 30 cm up the stem. Visual estimates of percent control were measured 4 months after treatment (MAT). Chlorosis, necrosis, and length and condition of new growth were rated on a scale from 0% to 100%, with 0% being no apparent injury and 100% being plant death.

Greenhouse Study. Porcelain berry cuttings were made from several populations in Raleigh, North Carolina in 2009 and potted individually in 10-cm^2 pots with a peat-based potting mix[7]. Plants were watered daily and fertilized[8] weekly. When plants were approximately 30 cm tall cut stem treatments were performed, in which the plants were cut just below the first node and immediately painted with herbicide. Basal bark treated plants were painted with herbicide between soil level and the first node. Herbicide concentrations were as discussed above for field trials. The experimental unit consisted of one plant per pot. Each treatment included four replications and the trial was repeated. Treatments were arranged in a completely randomized design. Visual ratings of percent control were estimated weekly as described for the field study. At 2 MAT plants were cleaned, dried at 50 C to constant moisture, and weighed.

Statistical Analysis. Visual percent control data from all trials and dry weights from greenhouse trials were subjected to analysis of variance and means were separated using Fisher's Protected Least Significant Difference ($P \leq 0.05$) in SAS[9]. Data from each trial were pooled as there were no treatment- by- repetition interactions. Data were not transformed. Control treatments were not included in statistical analysis of visual ratings of control, but were included in analysis of dry weight. Multivariate analysis of variance was performed to determine whether or not stem diameter affected treatment efficacy in field trials.

Results and Discussion

In the field trials at 4 MAT, cut stem treatment with aminocyclopyrachlor 5% v/v with 95% v/v basal oil controlled porcelain berry 95% (Table 1). Similar results were observed with aminopyralid 5% v/v at 85% control, and all triclopyr treatments at 89% or greater. Glyphosate and imazapyr provided only 67 and 63% control, respectively. The basal bark application of aminocyclopyrachlor performed poorly, yielding only 34% control. Stem diameter did not significantly affect efficacy of any treatment.

In the greenhouse trials, both cut stem triclopyr treatments provided 100% control and no porcelain berry biomass was measurable (Table 2). Aminopyralid and glyphosate also controlled porcelain berry well at 93%, and dry weight from aminopyralid treatment did not differ from dry weight with triclopyr treatment. Control with imazapyr was similar at 83%. Basal bark application of triclopyr ester gave marginal control at 69% with a dry weight of 6.95 g and both values differed from the excellent control achieved with cut stem triclopyr treatments. Control with aminocyclopyrachlor cut stem treatment was 44% with a dry weight of 6.11 g. Aminocyclopyrachlor basal bark treatment resulted in the lowest control at 26% and resulted in dry weight of 11.14 g, which did not differ from the control.

Aminopyralid and triclopyr provided the best control of porcelain berry under both field and greenhouse conditions. Glyphosate has been recommended for control of porcelain berry and is commonly used in non-cropland situations, but it provided only moderate control in this study. This is consistent with findings by Richardson et al. (2009), who reported that at 11 MAT, triclopyr formulations provided 93% or better control of Virginia creeper and wild grape, whereas glyphosate provided 63% control at most. Similarly, Tworkoski et al. (1988) determined that 1.1 kg/ha of triclopyr ester provided 90% control of Virginia creeper in the first year, although by the second year it had recovered substantially. Further research is needed to determine the best long-term control options for porcelain berry, which may include determination of the most effective rates of these herbicides, efficacy of prepackaged herbicide mixes, and the most cost effective herbicides for natural area weed control. A common recommendation (Young 2005) suggests that regardless of treatment, porcelain berry infestations often require treatment through multiple growing seasons.

Because porcelain berry spreads so readily by seed, it is important to control plants early in the growing season to prevent berry formation to limit the spread of this weed.

Sources of Materials

[1] DPX-MAT28, DuPont Crop Protection; Wilmington, DE

[2] Milestone, Dow AgroSciences; Indianapolis, IN

[3] Touchdown Pro, Syngenta Crop Protection, Inc.; Greensboro, NC

[4] Stalker, BASF Corporation; Research Triangle Park, NC

[5] Garlon 3A, Dow AgroSciences; Indianapolis, IN

[6] Garlon 4, Dow AgroSciences; Indianapolis, IN

[7] Fafard 2 Mix, Conrad Fafard; Agawam, MA

[8] Champion® 17-4-17, The Scotts Company; Marysville OH

[9] SAS V. 9.1, SAS Institute Inc.; Cary, NC

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Table 1. Visual porcelain berry control 4 MAT following field cut stem or basal bark herbicide application.[a,b]

Herbicide	Rate	Application	Control [c]
	% v/v		%
Aminocyclopyrachlor + basal oil	5 + 95	C	95 a
Aminopyralid	5	C	85 ab
Glyphosate	50	C	67 bc
Imazapyr + basal oil	1 + 99	C	63 c
Triclopyr amine	30	C	93 a
Triclopyr ester + basal oil	30 + 70	C	92 a
Aminocyclopyrachlor + basal oil	1+ 99	B	34 d
Triclopyr ester + basal oil	3 + 97	B	89 ab

[a] Visual control rating on a scale between 0 and 100%, where 0 is no visible injury and 100% is total plant death.

[b] Abbreviations: MAT, months after treatment; B, basal bark treatment; C, cut stem treatment.

[c] Means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Table 2. Porcelain berry dry weight and visual control 2 MAT following greenhouse cut stem or basal bark herbicide application.[a,b]

Herbicide	Rate	Application	Control[c]	Dry biomass
	% v/v		%	g
Aminocyclopyrachlor + basal oil	5 + 95	C	44 c	6.11 b
Aminopyralid	5	C	93 a	0.59 ef
Glyphosate	50	C	93 a	1.04 cd
Imazapyr + basal oil	1 + 99	C	83 ab	3.61 bc
Triclopyr amine	30	C	100 a	0 f
Triclopyr ester + basal oil	30 + 70	C	100 a	0 f
Aminocyclopyrachlor + basal oil	1 + 99	B	26 c	11.14 a
Triclopyr ester + basal oil	3 + 97	B	69 b	6.95 b
Non-treated control	---	C	---	10.46 a

[a] Visual control rating on a scale between 0 and 100%, where 0 is no visible injury and 100% is total plant death.

[b] Abbreviations: MAT, months after treatment; B, basal bark treatment; C, cut stem treatment.

[c] Means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Chapter 3

Porcelain Berry, Bushkiller, and Virginia Creeper Growth in Interspecific Competition

Abstract

Porcelain berry and bushkiller are exotic perennial vines in Vitaceae and are considered nuisance invasive weeds of natural and riparian areas in the eastern United States. In order to understand more about the competitive abilities of these aggressive weeds, competition experiments were conducted between porcelain berry, bushkiller, and Virginia creeper, a native member of Vitaceae. Plants grown singly or in combination were monitored for stem growth and biomass production. In this research, porcelain berry and Virginia creeper exhibited similar rates of stem growth, whereas bushkiller grew taller and faster than either other species. Porcelain berry stem growth was reduced in competition with bushkiller. All three species exhibited reduced stem biomass when grown with both other species. Root biomass of porcelain berry and Virginia creeper were not affected by competition, but bushkiller, which produced the heaviest roots, exhibited reduced root biomass when grown with both other species. Porcelain berry root length was reduced by competition with both other species, but neither Virginia creeper nor bushkiller root lengths were affected by competition. These results suggest that bushkiller is the better competitor in ability to accumulate resources. In these experiments, porcelain berry was less aggressive and vigorous than bushkiller but was similar to Virginia creeper.

Introduction

Porcelain berry [*Ampelopsis brevipedunculata* (Maxim.) Trautv.] is an ornamental vine that exhibits invasive behavior in the eastern United States. Porcelain berry's chief attraction is its colorful berries, which are spread by water, birds, and possibly other animals (Mehrhoff et al. 2003). Native to eastern Asia, it has naturalized from New Hampshire south to Georgia and west to Iowa (USDA NRCS 2011). Porcelain berry grows rapidly and blankets surrounding plants, effectively shading out other species and weighing down supporting vegetation (Smith 2008). Once established, plants can grow as much as 15 to 20 feet in a growing season. Large vines will resprout from the vigorous taproot (Mehrhoff et al. 2003). The vine is found along streams and rivers as well as in early successional fields, edges, pastures, railroad and utility rights-of-way, and waste places where there are moist soils and partial to full sun (Mehrhoff et al. 2003).

Bushkiller [*Cayratia japonica* (Thunb.) Gagnep.] is a perennial herbaceous vine that is native to and common in temperate, tropical, and subtropical regions of southeast Asia, Australia, India, New Caledonia, and New Guinea (Hsu and Kuoh 1999; USDA ARS 2011). Its current distribution in the United States includes Alabama (Hansen and Goertzen 2006), Louisiana, Mississippi, North Carolina, and Texas (USDA NRCS 2011). Bushkiller grows rapidly and can shade out other species and smother supporting vegetation. West et al. (2010) report that bushkiller does not produce viable seeds in its invaded habitat in North America, but spreads vegetatively by rhizomatous roots.

Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch.] is a North American native, distributed throughout the east from Canada to Florida and west to Utah (USDA NRCS 2011). It is a component of early to late sere forests, open areas, disturbed sites, and agricultural land and is often cultivated ornamentally (Bush 2002). Virginia creeper is well adapted to tolerate low-light environments and is physiologically plastic, with a high photosynthetic rate at both low and high light levels; however, it is not a dominant canopy plant (Carter and Teramura 1988).

Plants compete with their neighbors for resources, nitrogen being the most limiting (Dillenburg et al. 1993; Tilman 1982; Vitousek and Howarth 1991). In examining the effects of vines on tree growth, Ingwell et al. (2010) posit that vines compete for above- and belowground resources with trees, reduce tree growth rates, and increase tree mortality. Dillenburg et al. (1993) showed that belowground competition for nitrogen was more limiting to tree growth than aboveground competition from vines. The impacts of an exotic species on an invaded community depend on how the invader integrates into the new community, the environmental restrictions imposed, and the biological characteristics of the invader (Vilá and Weiner 2004). One way to predict the behavior of exotic species is to assess their competitive abilities in relation to other plants in the community. Plant interaction studies inform how individual plants affect or suppress the growth of other plants and how individuals respond to the suppression caused by neighboring plants (Goldberg and Werner 1983). Greenhouse interaction studies allow us to examine how plants compete with one another while reducing extraneous competition and other stress variables (Gibson et al. 1999). Measurements of biomass are considered to indicate competitive interactions

(Connolly and Wayne 1996), but changes in growth rates over time indicate the dynamic of species interactions and are better indicators of competitive ability than final yield data alone (Gibson et al. 1999).

We conducted a competition study using a target-neighbor model to examine the competitive effects and responses of porcelain berry, Virginia creeper, and bushkiller. Porcelain berry and bushkiller were selected because they are both aggressive exotic vines that have naturalized in the United States. Virginia creeper is a common native component of natural areas and may occupy similar habitats.

Materials and Methods

Cuttings of porcelain berry and Virginia creeper were collected from several areas around Raleigh, North Carolina in 2009, and root cuttings of bushkiller were obtained from greenhouse-propagated plants derived from West et al. (2010). Interspecific competition trials were conducted using the target-neighbor model of the simple additive design, in which an individual of a "target" species is grown with varying densities of "neighbor" plants which might be the same or different species. Target-neighbor studies with low densities of the target species are useful because they reduce or eliminate intraspecific competition (Gibson et al. 1999). Plants were approximately 40 cm in length at trial initiation. Plants were potted into 30-cm pots with one vertical climbing structure per pot, and were grown in peat-based potting mix[1], watered daily, and fertilized weekly[2]. Greenhouse temperature averaged 30 C, and plants were grown under natural light.

The first experiment was initiated on July 10, 2010, and was repeated on September 20, 2010. Length of the longest stem of each plant was measured weekly for seven weeks. At the termination of the trials, plants were harvested and measurements were taken for number of inflorescences, number of stems, stem mass, root mass, and root length. Plants were oven dried at 50 C for 72 hours and stem and root mass were measured again. Stem lengths at trial initiation were subtracted from final lengths.

Pots included either a single plant, one plant each of two species, or one plant each of all three species, similar to previous research (West et al. 2010). There were four pots for each treatment, and treatments were randomized throughout the greenhouse. Competition design resulted in the following experimental treatments: porcelain berry alone (PB), Virginia creeper alone (VC), bushkiller alone (BK), porcelain berry and Virginia creeper together (PBVC), porcelain berry and bushkiller together (PBBK), Virginia creeper and bushkiller together (VCBK), and porcelain berry, Virginia creeper, and bushkiller planted together (PBVCBK). These treatments resulted in the following statistical slope comparisons for analysis: porcelain berry alone compared with PBVC, porcelain berry alone compared with PBBK, porcelain berry alone compared with PBVCBK, Virginia creeper alone compared with PBVC, Virginia creeper alone compared with VCBK, Virginia creeper alone compared with PBVCBK, bushkiller alone compared with PBBK, bushkiller alone compared with VCBK, and bushkiller alone compared with PBVCBK.

Comparisons of the competitive responses of plant growth rates over time can be examined using slopes of regression curves from regularly collected height measurements

(Gibson et al. 1999). Thus, to evaluate plant growth over time, weekly stem length data were analyzed using the Mixed Procedure in SAS 9.1[3]. A repeated-measures linear mixed-model was fitted with time as a fixed-effect independent variable and treatment as a fixed-effect class variable. The model included a separate slope and intercept for each treatment. Differences in intercepts for the regression lines of growth over time represent treatment effects. Slope estimates represent the mean growth over time for all plants under a particular treatment. Pair-wise comparisons of slopes between treatments were made using the ESTIMATE statement in SAS, and linear regression curves of stem growth over time ($y = y_0 + a_x T$) were performed. Final measurement data were subjected to analysis of variance and means were separated using Fisher's Protected Least Significant Difference ($P \leq 0.05$) in SAS. Trial data were pooled, as each trial was considered a random sample of environment, which allows for comparisons of treatment means and interactions over multiple environments (Carmer et al. 1989).

Results and Discussion

Porcelain berry and Virginia creeper exhibited similar stem growth (Figure 1). Porcelain berry stem growth was not affected by competition with Virginia creeper or both species, but was affected when grown with bushkiller (Table 1). Porcelain berry was the most floriferous species, with up to 48 inflorescences per plant; however, competition with bushkiller or with both bushkiller and Virginia creeper reduced the number of porcelain berry inflorescences by 55 to 70% (Table 2). Number of stems produced was not affected by competition as porcelain berry produced only one or two stems per plant. Porcelain berry stem biomass was 20.8 g in monoculture and was negatively affected by competition with bushkiller or both bushkiller and Virginia creeper. Root biomass was not affected by competition. Porcelain berry root length was only reduced in competition with both Virginia creeper and bushkiller.

Virginia creeper stem length was only significantly reduced by competition with both porcelain berry and bushkiller (Table 1). In this study, Virginia creeper produced only one or two inflorescences per plant and competition did not affect floral production (Table 2). Virginia creeper produced only one stem per plant and this variable was not affected by competition. Virginia creeper stem weight was 23.4 g when grown alone and was reduced in competition with bushkiller or with both other species. Competition did not affect Virginia creeper root biomass or root length.

Bushkiller exhibited the greatest stem growth (Figure 1), and its growth rate over time was not affected when plants were grown in competition (Table 1). Bushkiller inflorescences did not exceed seven per plant and flower production was not affected by competition (Table 2). Bushkiller produced an average of six stems when grown with porcelain berry, significantly more than any other treatment. Bushkiller stem biomass was decreased 25% by competition with both other species but not affected when grown with a single competitor. Root mass was greater than that of porcelain berry or Virginia creeper (Figure 2) but was reduced when grown with both species. Bushkiller root length did not differ between treatments.

Bushkiller exhibited the fastest growth rate and accumulated more stem and root biomass than the other species when grown in monoculture (Figure 1; Table 2). Bushkiller was also less affected by competition than the other species, indicating that bushkiller is likely the better competitor in ability to accumulate resources. Similar results were noted by West et al. (2010) in a study of bushkiller in competition with trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bur.] and wild grape (*Vitis* spp.). In that study, bushkiller was a significantly better competitor than either trumpetcreeper or wild grape, and the authors suggest that it may cause significant detrimental effects to infested communities. Porcelain berry does not appear to have had negative competitive effects on Virginia creeper, and based on biomass measurements both species may allocate resources to their varying parts in much the same manner, with the exception of flower production. Dynamics among the three species may have changed over time and in a natural setting. Literature suggests that porcelain berry is a common but not dominant component in its native habitat (Anonymous 1993; Zhiduan and Wen 2007); thus it may exist in a similar niche as that of Virginia creeper and may not be as invasive in the United States as is bushkiller. However, porcelain berry's capacity for prodigious seed production and use by birds may allow it to spread farther and more quickly in its invaded habitat. Porcelain berry appears to be more proliferative and aggressive than native Vitaceae in some areas, and additional research would be helpful to confirm this observation as well as to determine its value and/or detriment to wildlife.

Sources of Materials

[1] Fafard 2 Mix, Conrad Fafard; Agawam, MA

[2] Champion® 17-4-17 fertilizer, The Scotts Company; Marysville, OH

[3] SAS Institute, Inc.; Cary, NC

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Table 1. Slope estimates and pairwise slope comparisons of porcelain berry, Virginia creeper, and bushkiller in interspecific competition over 7 weeks[a].

Treatment/ Species	Slope Estimate	Slope Comparison	P value
PB	8.86 ± 1.38	----	----
PB with VC	10.06 ± 1.38	-1.21	NS
PB with BK	3.22 ± 1.38	5.64	0.0043
PB with all	5.01 ± 1.38	3.84	NS
VC	12.44 ± 2.27	----	----
VC with PB	11.43 ± 2.12	1.02	NS
VC with BK	12.90 ± 2.12	-0.46	NS
VC with all	8.29 ± 2.12	4.16	0.0001
BK	34.96 ± 2.69	----	----
BK with PB	28.48 ± 2.70	6.48	NS
BK with VC	31.37 ± 2.69	3.59	NS
BK with all	30.58 ± 2.69	4.38	NS

[a] Slope comparisons calculated via pairwise comparisons of slopes of each species grown in monoculture versus its slope in competition. A significant P-value indicates that the growth rate of a species in monoculture differs from its growth rate with competition. NS, not significant.

Table 2. Inflorescence number, stem number, stem biomass, root biomass, and root length from porcelain berry (PB), Virginia creeper (VC), and bushkiller (BK) after seven weeks of growth alone and in interspecific competition[a].

Treatment	Inflorescence	Stems	Stem biomass	Root biomass	Root length
	#	#	g	g	cm
PB	46 a	2 cd	20.8 bcd	7.1 d	59 abc
PB with VC	48 a	1 d	15.2 de	7.2 d	57 bcd
PB with BK	21 b	2 cd	8.3 ef	3.9 d	46 cd
PB with all	14 bc	2 cd	7.5 f	3.7 d	43 d
VC	2 c	1 d	23.4 bc	4.0 d	52 bcd
VC with PB	2 c	1 d	18.6 cd	3.5 d	58 bc
VC with BK	1 c	1 d	10.9 ef	3.9 d	53 bcd
VC with all	1 c	1 d	9.6 ef	3.4 d	62 ab
BK	4 c	3 bc	31.4 a	16.6 a	66 ab
BK with PB	7 bc	6 a	24.6 abc	16.3 a	74 a
BK with VC	4 c	2 cd	25.9 ab	12.9 ab	65 ab
BK with all	3 c	3 bc	23.4 bc	11.1 bc	63 ab

[a] Means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

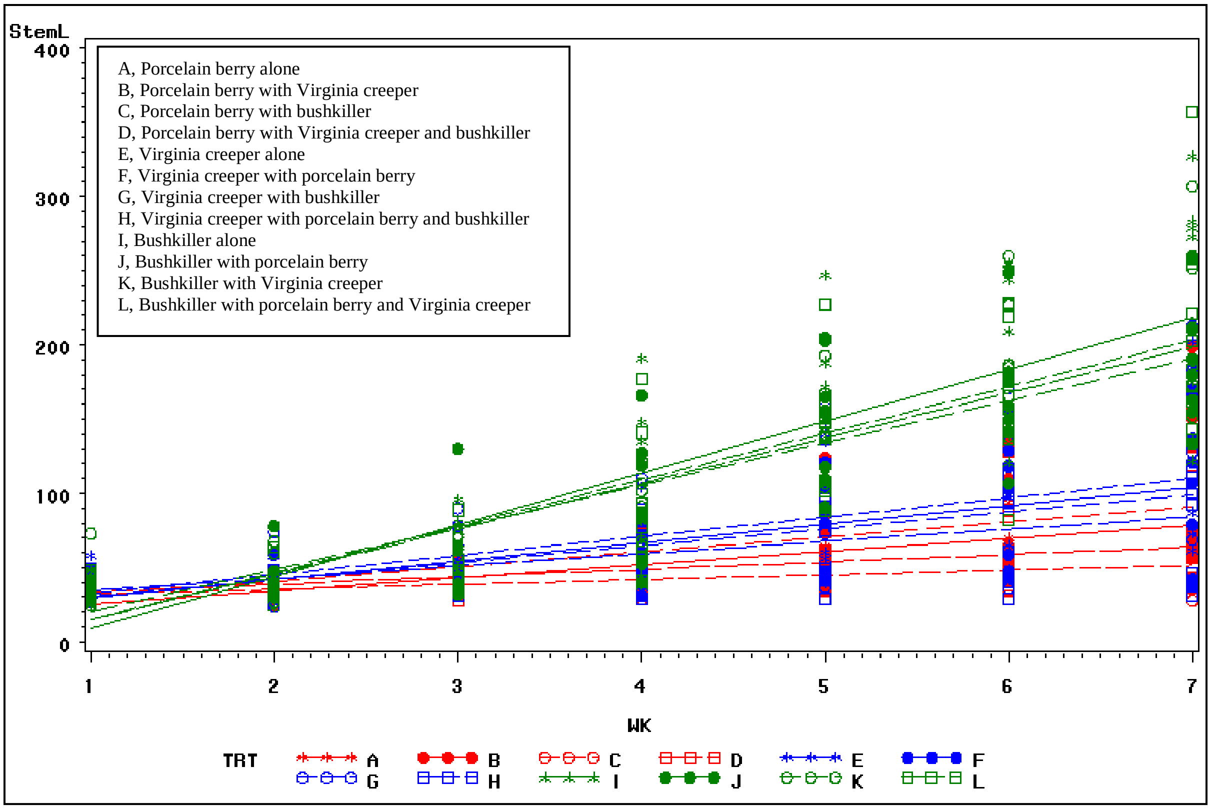

Figure 1. Rate of growth ($y = y_0 + a_x Time$) for porcelain berry, Virginia creeper, and bushkiller grown alone and in interspecific competition.

Figure 2. Porcelain berry, bushkiller, and Virginia creeper (left to right) roots when grown in competition.

Chapter 4

Porcelain Berry Seed Germination and Seedling Perennation

Abstract

Environmental factors such as light and temperature are known to affect seed germination. An understanding of seed germination of weedy species can help to predict potential spread and inform effective control measures. We conducted numerous trials on porcelain berry to determine temperature requirements to break seed dormancy, soil depths most conducive to germination, and the ability of seedlings to resprout after being clipped at ground level. Fresh seeds and those stored in-fruit at 5 C for four months did not germinate when subjected to a range of constant temperatures. Physically scarified fresh seeds did not germinate at alternating temperatures of 15 and 25 C. Temperature studies showed that up to 96% of thermally stratified seeds under lights germinated when exposed to alternating temperatures, whereas 52% or less germinated under constant temperature treatments. Seeds did not germinate in dark conditions at constant temperatures, but up to 96% germinated in the dark if exposed to alternating temperatures. Perennation tests showed that after being cut at soil level, 25% of plants with six or fewer leaves resprouted. The greatest rate of resprouting occurred in plants with 9 leaves or more. However, up to 10% of porcelain berry seedlings with only two true leaves were able to resprout. Porcelain berry germinated poorly on the soil surface or deeper than 6 cm, but at depths between 1 and 4 cm, mean germination of up to 80% was observed. These results indicate that porcelain berry seed germination may be limited in areas with low temperature variation or where seeds become buried deeply in the

soil. Porcelain berry's ability to regenerate after clipping has significant implications for common management techniques such as mowing or use of contact herbicides.

Introduction

Porcelain berry [*Ampelopsis brevipedunculata* (Maximowicz) Trautvetter] is an exotic invasive woody perennial vine in Vitaceae. In the United States it is sold as an ornamental and its chief attraction is the colorful berries that form in cymes along most nodes. Seeds are disseminated by birds and possibly other animals, and seeds may be spread by water because the species often grows in riparian habitats (Robertson et al. 1994). Because seasonal variation in temperature and moisture are directly related to seedling survival, the ability of seeds to remain dormant until the appropriate conditions are present likely is very important (Washitani and Masuda 1990). Seed dormancy is the condition in which a seed will not germinate even in circumstances which typically would be expected for optimum germination. Dormancy may be physiological, morphological, physical, or a combination of these (Baskin and Baskin 2003). Bouwmeester and Karssen (1992) suggest that seed dormancy is regulated predominantly by temperature, as evidenced by the behavior of seed with seasonal changes. When dormancy is relieved but suitable environmental conditions are not met for plant viability, the seed enters secondary dormancy.

Washitani and Masuda (1990) tested porcelain berry seed after moist and cold storage to determine conditions necessary to break dormancy. They found that storage using dry chilling at 4 C for four months followed by moist chilling at 4 C for 1 month resulted in greater germination than either treatment alone, and that chilling increased the range of temperatures at which the seeds would germinate compared to controls. Tsuyuzaki and Miyoshi (2009) found that cold temperature doubled porcelain berry germination, and interestingly, that the addition of smoke to some temperature treatments increased germination. However, little other information is available regarding porcelain berry's temperature requirements for germination once dormancy is broken, nor for optimal seed depth. In this study, we examined several basic biological factors to gain a better understanding of porcelain berry's germination and seedling behavior to inform best management practices for this weed.

Materials and Methods

Seed germination. Berries from wild porcelain berry plants that had been keyed to species were collected in Raleigh, North Carolina in 2009 and 2010.

Seed Dormancy. A trial to test dormancy of seed was performed using unscarified and untreated seeds from dry berries stored at 5 C for 4 months. Seeds were placed on 70 mm blotter paper[1] in 10 cm deep glass petri dishes containing 240 mL of clean pea gravel, to which 115 mL of deionized water was added. Five seeds were added to each of three dishes. The trial was repeated once. Dishes were placed on a temperature gradient table at 15, 20, 25, 30, 35, and 40 C. Same-temperature water was added as needed. Seeds were checked daily for germination, the criterion for which was radicle protrusion of at least 1 mm. The trial was discontinued after 21 days, at which time ungerminated seeds were nicked and soaked in tetrazolium chloride (TZ) for 26 hours to determine viability. An additional test was performed on seeds from fresh berries that were subjected to temperatures of 10, 16, 21, 25, 29, and 33 C.

A scarification trial was performed to examine physical dormancy in porcelain berry. Fresh seeds were scarified with sandpaper and were placed in two 100 x 15 mm petri dishes containing blotter papers to which 10 mL of deionized water had been added. There were twenty-five seeds per petri dish. The seeds were placed in a germination chamber at alternating temperatures of 15 and 25 C. They were checked daily for germination using the criterion previously described. The trial was discontinued after 21 days and was repeated once.

An imbibition trial was also performed to determine whether physical dormancy was a factor in the non-germinability of fresh seed. Six freshly collected, cleaned seeds were weighed individually and soaked in deionized water for 1, 6, 12, 24, 36, 48, 60, 72, and 87 hours. Seeds were weighed at each interval and then returned to water until the next interval to determine if and when they imbibed water. This trial was not repeated, as its purpose was solely to determine whether or not seeds imbibe water, which would normally indicate the condition of physical dormancy (Baskin and Baskin 2003).

Temperature Requirements. A study of temperature requirements for porcelain berry germination was initiated. Petri dishes containing two blotter papers were wetted with 10 mL of deionized water. Twenty-five seeds that had been stored at 5 C were added to each of three petri dishes per temperature treatment. Additional tests for germination in darkness were initiated with exactly the same protocols, but dishes were wrapped in two layers of heavy aluminum foil. Dishes were placed in incubators at 20 or 25 C at constant temperature, or at 25/15, 30/20, or 35/25 on alternating 16 and 8 hour cycles. The light condition experiment was repeated twice. The darkness condition portion was discontinued after the first trial, as all seeds not exposed to light only germinated in alternating temperature conditions. Seeds not in dark conditions were checked daily for germination. Trials were concluded when germination slowed significantly or after three weeks. TZ tests were performed on non-germinated seeds from the first experiment to determine viability.

Depth of planting. A trial was initiated to assess the ability of porcelain berry seeds to germinate from varying depths. Wild-collected, thermally stratified porcelain berry seeds were planted in a greenhouse in foam cups containing a locally supplied sandy loam soil. A drainage hole was made in the bottom of each cup and coffee filters were placed over the holes to prevent soil loss and shifting of seeds. Treatments consisted of planting seeds at 0, 1, 2, 3, 4, 6, or 8 cm. Each cup contained five seeds, at one depth per cup. There were five cups per treatment, and the experiment was repeated once. Cups were watered as needed. Temperature in the greenhouse averaged 28 C.

Perennation. Perennation studies were initiated to determine the ability of porcelain berry to resprout after having all aboveground biomass removed. Wild-collected, thermally stratified seeds were planted in a greenhouse in 15- cm round pots containing peat-based potting mix[2]. The experiment was established using a completely randomized block design. Pots were watered daily and greenhouse temperature averaged 28 C for both experiments. Four seeds were planted per pot and were thinned to two plants per pot. There were ten pots per treatment. The experiment was repeated once. Treatments consisted of clipping plants at soil level when they reached 2, 4, 6, 7, 8, 9, or 10 true leaves with minimum leaf width of 2.5 cm. Date of clipping was noted and plants were allowed to continue to grow to determine whether or not they would recover from removal of all aboveground tissue. Plants were monitored for regrowth for at least 17 days. Regrowth length, number of shoots, and number of leaves, if any, were noted at 7 days after resprouting.

Data from the trials were pooled as there were no treatment by repetition interactions. Data from germination trials were subjected to analysis of variance[3] and means were separated using Fisher's Protected Least Significant Difference ($P \leq 0.05$). Data from the depth of planting trial were subjected to regression analysis[4] with germination (y) as a function of depth (x), according to the cubic equation $y= y_o + ax + bx^2 + cx^3$. Data from the perennation trial were subjected to regression analyses with number of resprouts and number of days required to resprout (y) as a function of number of true leaves at clipping (x), according to the linear equation $y= y_o + ax$.

Results and Discussion

Seed germination. *Seed Dormancy.* No dried or fresh seeds germinated from the initial germination tests on the temperature gradient table. TZ testing indicated that 63% of these seeds were viable. Because the dried seeds were stored in-fruit, substances in the fruit tissue may have resulted in embryo quiescence, as is sometimes observed in other species (Osborne 1981; Toole et al. 1956). Fresh, scarified seeds did not germinate at 15/25 C. The lack of germination after scarification suggests that physical or chemical treatment as would occur during animal digestion may not be critical for porcelain berry germination.

Imbibition trials on fresh seed showed that seeds increased approximately 25% in weight after soaking in water, with most water being absorbed by 36 hours (data not presented). It would be expected that seeds would germinate after imbibing water, indicating that physical dormancy is broken (Baskin and Baskin 2003). However, the porcelain berry seeds used in fresh-seed experiments did not germinate despite the abundant availability of water.

Temperature Requirements. In trials examining temperature requirements for germination, porcelain berry seeds germinated much more quickly and at higher rates when exposed to alternating temperatures than when exposed to constant temperatures (Table 1). Gardner (1921) noted that seeds of many species exhibit this phenomenon. In our study, light was a requirement for germination with constant temperatures, but up to 96% of seeds germinated in the dark when exposed to alternating temperatures. Many species are known to germinate in dark conditions when exposed to alternating temperatures (Gardner 1921; Válio and Scarpa 2001). Tsuyuzaki and Miyoshi (2009) also report that porcelain berry germination was significantly lower in dark treatments than in control, cold, and heat treatments.

Depth of planting. Seeds germinated poorly on the surface of sandy loam soil, with only 6% germination (Figure 1), and most were observed to decay. The highest mean germination rate of 80% occurred at depths between 1 and 4 cm, with a marked reduction at 6 cm, and no seeds germinated at 8 cm. This corresponds with previous research suggesting that most seeds emerge from the top one to four cm of soil (Cousens and Moss 1990). Of the seeds that did not emerge from 6 cm deep, 88% decayed, 5% appeared viable but did not germinate, and 7% sprouted but did not reach the soil surface before dying (data not presented). Of the seeds which did not emerge from 8 cm deep, 50% were observed to decay, 14% appeared viable but had not germinated, and 36% had germinated but died before reaching the soil surface. This may indicate that porcelain berry seeds are not persistent in soil. Haywood (1994) and Lambers et al. (2005) observed that *Vitis* spp. seeds were viable for up to five years in forest litter. However, few studies on porcelain berry seed germination have been performed and seed persistence and longevity under field conditions are unknown.

Perennation. The ability of porcelain berry seedlings to resprout after clipping was correlated with leaf number (Figure 2). Seedlings with only two true leaves were able to resprout at a rate of 10%. Regression analysis predicts that 25% of plants with six leaves could resprout, and about 50% of plants with ten leaves or more are predicted to resprout. The amount of time required for the plants to resprout after cutting also was dependent on plant size at clipping, with larger plants resprouting more quickly than smaller plants (Figure 3). These results reflect Wilson's (1979) observation that 8% of Canada thistle [*Cirsium* *arvense* (L.) Scop.] plants were able to resprout after clipping plants with only two true leaves. Additionally, the ability and speed of thistle plants to resprout increased as plants increased in age and leaf number before clipping. The ability of porcelain berry to sprout after removal of all aboveground tissue has implications for control of this species by mowing, grazing, or contact herbicides, which do not translocate to roots. As expected, larger plants are more likely to resprout and in less time than smaller plants. Further studies of physical disturbance, such as the use of fire, would be helpful to determine control protocols for porcelain berry.

These trials suggest that porcelain berry seeds could germinate in much of the United States, as the climate exhibits the annual variation in temperature likely to break seed dormancy and to trigger germination. The regenerative capability exhibited by small seedlings suggests that this species would be very tolerant of a single exposure to cutting, grazing, contact herbicides, or similar control measures, although this should be confirmed with further research.

Sources of Materials

[1] Thermo Fisher Scientific; Waltham, MA

[2] Fafard Superfine Germinating Mix, Conrad Fafard, Agawam; MA

[3] SAS 9.1, SAS Institute; Cary, NC

[4] SigmaPlot, Systat Software, Inc; San Jose, CA

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Table 1. Germination of wild-collected, thermally stratified porcelain berry seed under five temperature regimes.

Temperature	Light Germination[a]	Dark Germination
C	%	%
20	43.7 c	0 d
25	39.3 c	4 d
15/25	96.0 a	96 a
20/30	94.0 a	92 a
25/35	92.3 a	64 b

[a] Means with the same letter are not significantly different, according to Fisher's Protected LSD ($P \leq 0.05$).

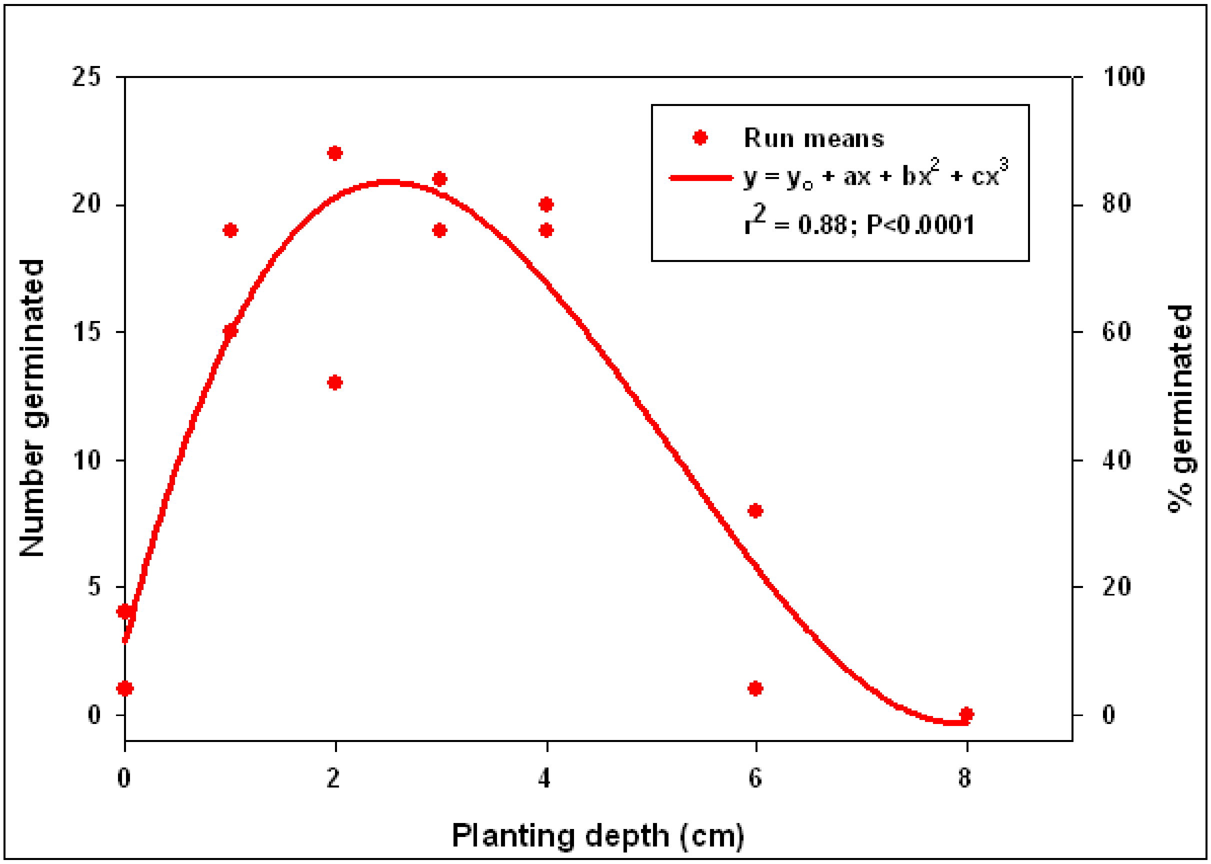

Figure 1. Germination of wild-collected, thermally stratified porcelain berry seed as affected by planting depth.

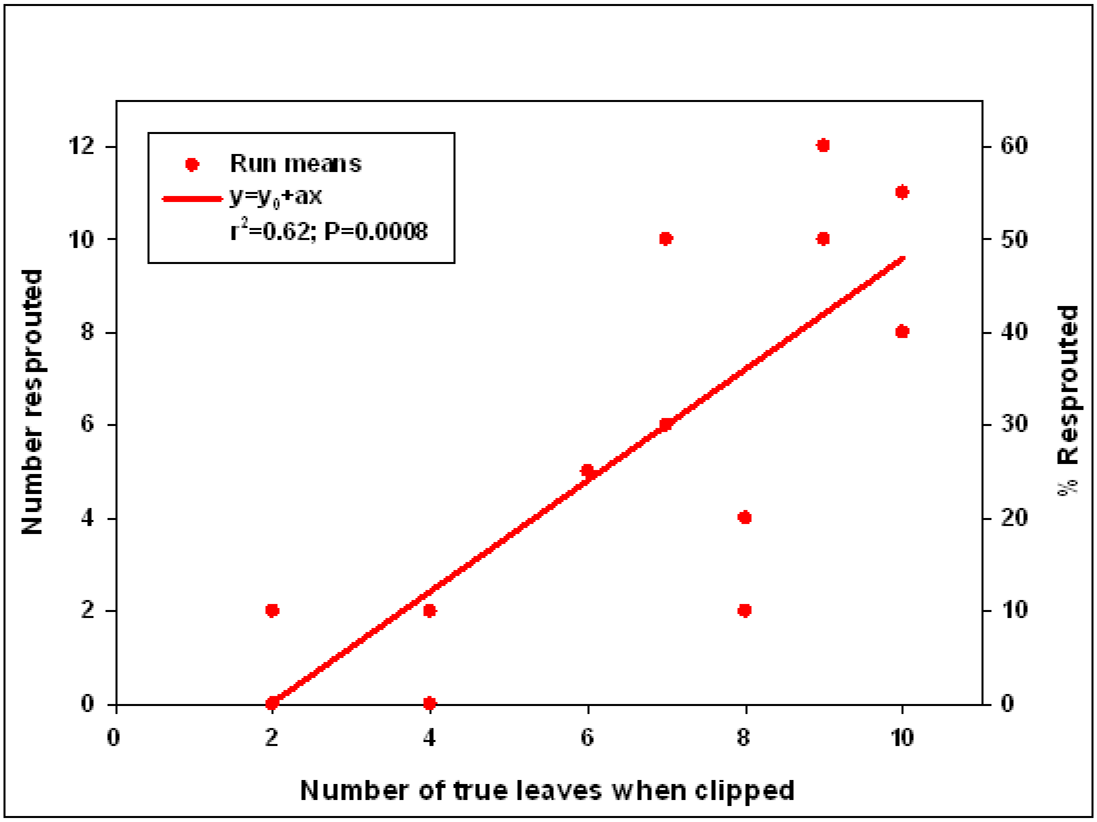

Figure 2. Porcelain berry seedling resprouting as affected by number of true leaves present at time of clipping.

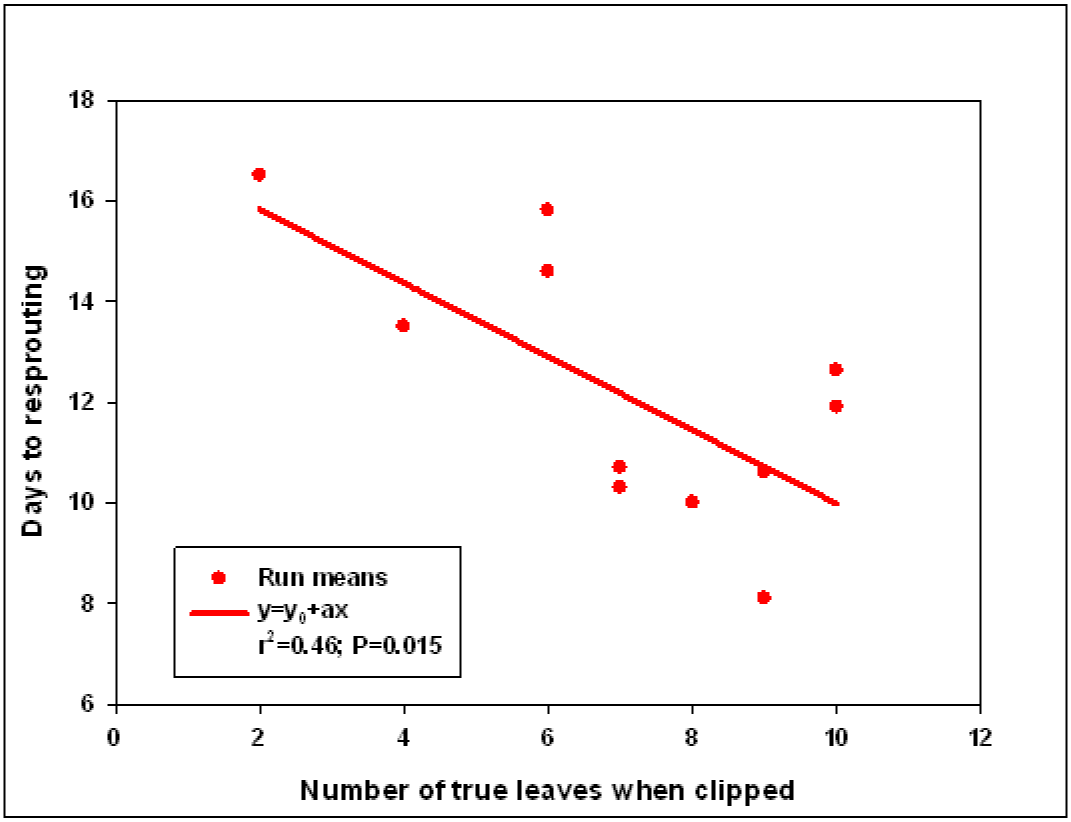

Figure 3. Number of days required for porcelain berry seedling resprouting as affected by number of true leaves present at time of clipping.

Chapter 5

Predicting the Potential Distribution of Porcelain Berry and Bushkiller in the United States Using Two Bioclimatic Models

Abstract

Porcelain berry and bushkiller are exotic invasive vines in Vitaceae that have been shown to outcompete native flora and may reduce biodiversity. Porcelain berry grows in the eastern United States from New Hampshire to Georgia and west to Iowa. Bushkiller is limited to Alabama, Louisiana, Mississippi, North Carolina, and Texas. We used two climate models, CLIMEX and NAPPFAST, to determine the potential extent of invasion of these pest species. Both models indicated that porcelain berry could increase its distribution to some areas of the Midwest and the Pacific Northwest, as well as to continue its southeastern expansion. Both models predicted that the climates of the Pacific Northwest and the Southeast are favorable for invasion by bushkiller, but that most of the interior of the United States is not favorable for growth of this species. The NAPPFAST model indicated that the climate of the upper Midwest is not suitable for bushkiller, whereas CLIMEX predicted that some regions of the northern Midwest may be moderately suitable. The models included the current invaded ranges for both species and predicted similar patterns of potentially suitable regions for invasion by each species.

Introduction

Climate models are used to predict the potential geographic distribution and impacts of plants, animals, and pathogens (Venette et al. 2010). Bioclimatic models are used with the presumption that species distributions are limited by climate. These models incorporate various climate variables from a species' native range and are used to infer its potential geographic distribution. The models also can be used to predict effects of climate change on a given species (Beaumont et al. 2005), and are helpful for policymakers, program managers, and risk analysts, who use them to determine pest risks and form appropriate management policies (Magarey et al. 2007; Venette et al. 2010). Information regarding species' biological and geographic requirements is easily accessible, making the use of these models relatively easy. However, pest risk models should be made and interpreted carefully to avoid incorrect estimates of potential ranges of exotic species. The quality of pest risk maps is dependent on the veracity of knowledge about the biological and environmental needs of the species (Venette et al. 2010). Factors that hinder accurate assessment of potential range include the nonexistence or non-availability of data on home range distribution, lack of climate, weather, and community composition data, and lack of absence data (Venette et al. 2010). Additionally, an organism may thrive in a region that has low climatic similarity to its native range simply because the organism is adaptable or has physiological tolerance to a wider range of conditions than presumed, but the range of climatic conditions would still be within the fundamental niche of the species. Conversely, non-climatic factors may limit a species' presence in regions that experience similar climate to its invaded range (Robertson et al.

2008). Although physical and biological components are important factors in a species' presence or absence, climate is the only factor with widely available data to help us understand the potential range of a given species (Sutherst et al. 2007).

Porcelain berry [*Ampelopsis brevipedunculata* (Maxim.) Trautv.] is an invasive exotic weed that is currently found from New Hampshire south to Georgia and west to Iowa. It is native to eastern Asia from northern Japan to the Philippines (Anonymous 1993; Ohwi 1984; USDA ARS 2011) and tolerates a wide range of temperatures and precipitation conditions. It is known to occur in Heilongjiang Province in northern China, where the mean minimum temperature may be as low as -24 C, to the Philippines, with an average maximum temperature of 33 C (Anonymous 2010a). Additionally, mean annual precipitation varies from approximately 530 mm to 2000 mm in its native range (Anonymous 2010b).

Bushkiller [*Cayratia japonica* (Thunb.) Gagnep.] occurs in temperate, tropical, and sub-tropical regions of Asia, Australia, India, Malaysia, New Caledonia, and New Guinea (Hansen and Goertzen 2006; Hsu and Kuoh 1999). In the United States, it is only known to occur in Alabama, Louisiana, Mississippi, North Carolina, and Texas (Hansen and Goertzen 2006; USDA NRCS 2011). In its native range it experiences mean monthly temperature extremes from -7 C in Hokkaido, Japan to 41 C in northeast India. Mean annual precipitation varies from approximately 600 mm to 3500 mm (Anonymous 2010a, 2010b).

Knowledge of the potential extent of naturalization of these species can help policymakers and land managers determine appropriate regulatory efforts, as well as best early detection/

rapid response, and management practices. This study was undertaken to provide a thorough assessment of the potential distribution of these two invasive weeds in the United States.

Materials and Methods

The NAPPFAST Model. The United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) uses a web-based mapping system called the North Carolina State University APHIS Plant Pest Forecasting (NAPPFAST) system, developed jointly by APHIS, ZedX, Inc.[1], and North Carolina State University (Magarey et al. 2007). NAPPFAST weather data are supplied by the National Oceanic and Atmospheric Administration broadcast system, NOAAPort, a satellite communication service from the U.S. National Weather Service, and are obtained from about 2000 stations in North America. NAPPFAST incorporates a climate matching program known as Bio-Environmental Appraisal and Mapping Model (BAMM), which can include 71 climate variables and daily weather data sets. The program uses georeferenced observations such as those from Global Biodiversity Information Facility[2] (GBIF). The resulting map shows suitability for invasion as a percentage ranging from zero (least suitable) to 90 (highly suitable). Suitability of 50% or less is considered low, meaning that a species may thrive at a location only in some years or only in part of the year (J. Schlegel, personal communication).

The CLIMEX Model. CLIMEX[3] is a software-based simulation program that consists of two models; *Compare Locations* and *Match Climates*. *Compare Locations* uses the known range of conditions in which the organism thrives to project its potential success in new ranges. CLIMEX uses data from 2400 global weather stations which provide monthly climate variable information. The model calculates an annual growth index to describe the potential for a species' growth based on favorable temperature and soil moisture conditions. Up to eight stress indices (e.g., heat, cold, wet, dry, and combinations of these) provide more information about the most unfavorable conditions the species can tolerate. The growth and stress indices combine to provide a single number, the ecoclimatic index (EI), to describe the suitability of the climate at a particular location for the species in question (Sutherst et al. 2007). The EI ranges from 0 (indicating conditions unsuitable for growth) to 100 (indicating perfect conditions for the species' growth and reproduction), and is assigned on maps with circles representing EI suitability ratings of 0, 25, 50, 75, or 100.

For the NAPPFAST model, 635 observations for porcelain berry were obtained from GBIF, including 82 observations used to train the model. For bushkiller, 426 observations were available, including 90 observations used to train the model. Data that did not include geographic coordinates were not used, nor were observations that were suspected to be cultivated. Maps of favorability for invasion in the continental United States were created with data from the native range only and with data from native and invaded ranges separately to train the model.

For the CLIMEX program the *Compare Locations* model was used. For both species, the minimum and maximum native range temperatures previously described were used to infer climate requirements. Then stress parameters were added to iteratively fit the model so that maps only showed species suitability in the known home range (Bourdôt et al. 2010). For porcelain berry the only stress variables added were soil moisture extremes. Porcelain berry occurs in regions with moderate to high rainfall and warm and cold temperature extremes. The soil moisture index was set at 0.5 for the lower and 2 for the upper thresholds. Bushkiller is also tolerant of extremes of temperature, but occurs mainly in regions with high precipitation, so soil moisture and dry stress variables were used. Soil moisture values for bushkiller were set at 0.75 for the lower and 8 for the upper thresholds. Dry stress was set at 0.8. These values were used to generate maps to predict affinity of each species for the continental United States.

Results and Discussion

The NAPPFAST model for porcelain berry corresponds with its current distribution throughout the northeastern U.S. (EDDMapS 2011; USDA NRCS 2011), and indicates that the Pacific Northwest and northern California are at least 80% favorable for growth (Figure 1). The Southwest and western Plains regions show poor suitability except a small area centered in Colorado that is up to 70% favorable for growth, perhaps because of the high annual snowfall in the Rocky Mountains. The model suggests that the southeastern U.S. is very favorable for growth of porcelain berry, although its current distribution is limited in this area.

CLIMEX provides similar results for porcelain berry, showing that its current range in the Northeast is highly favorable, with an abrupt decrease in suitability west of Iowa, its current westward extent (Figure 2). This model also predicts that porcelain berry could be problematic in the Pacific Northwest with 50 to 75% suitability. Like the NAPPFAST model, CLIMEX indicates that porcelain berry could persist throughout the southeastern U. S. Given the similar appearance of porcelain berry to other Vitaceae, misidentification of this species is possible, and it may still be in an early stage of invasion in the region.

The NAPPFAST model overlaps the current distribution of bushkiller in the U.S. and assigns the mid-Atlantic 80% or greater suitability, with increasing fit southward and west to Texas (Figure 3). The Pacific Northwest and south into California appear to be very favorable for growth of bushkiller. The model predicts that the lower Midwest is moderately suitable, but that the majority of the Midwest, Great Plains, and Southwest are not suitable for bushkiller.

CLIMEX predicts that the southeastern U. S. is most suitable for bushkiller (Figure 4). From southern Virginia southward and west to Louisiana, 75% suitability is predicted. The Pacific Northwest appears moderately suitable at 50% in northern and coastal areas and decreasing southward and inland. In contrast to NAPPFAST, the CLIMEX model predicts that a larger region of the northern Midwest is moderately suitable for bushkiller, with a favorability of 50%. However, both models agree that the majority of the interior of the U. S. is not suitable for invasion by this species.

Porcelain berry inhabits extremely cold regions in its native range, while bushkiller is common in warmer climates with higher levels of precipitation. Both models predict that porcelain berry has the potential to invade colder and drier regions of the U. S. than does bushkiller. The models suggest that porcelain berry may continue its advance into the interior of the continent, while bushkiller may be limited to more humid regions. Both models accurately include the current invaded ranges of porcelain berry and bushkiller and predict that both species have the potential to increase their extent of invasion in the United States. Information gained from these climate models may be useful for state regulatory agencies to implement policies to prevent the introduction of plants or propagules into regions in which these species are absent. Land managers in regions which are at risk for invasion may use this information to train staff in identification of these species, to implement early detection/rapid response programs, and to inform the public about the threats posed by these aggressive invaders.

The NAPPFAST program provides a data quality assessment. Data quality for the porcelain berry model was rated fair due to the low number of observations available from different continents. The quality assessment for bushkiller was rated excellent. The CLIMEX program does not offer a quality assessment.

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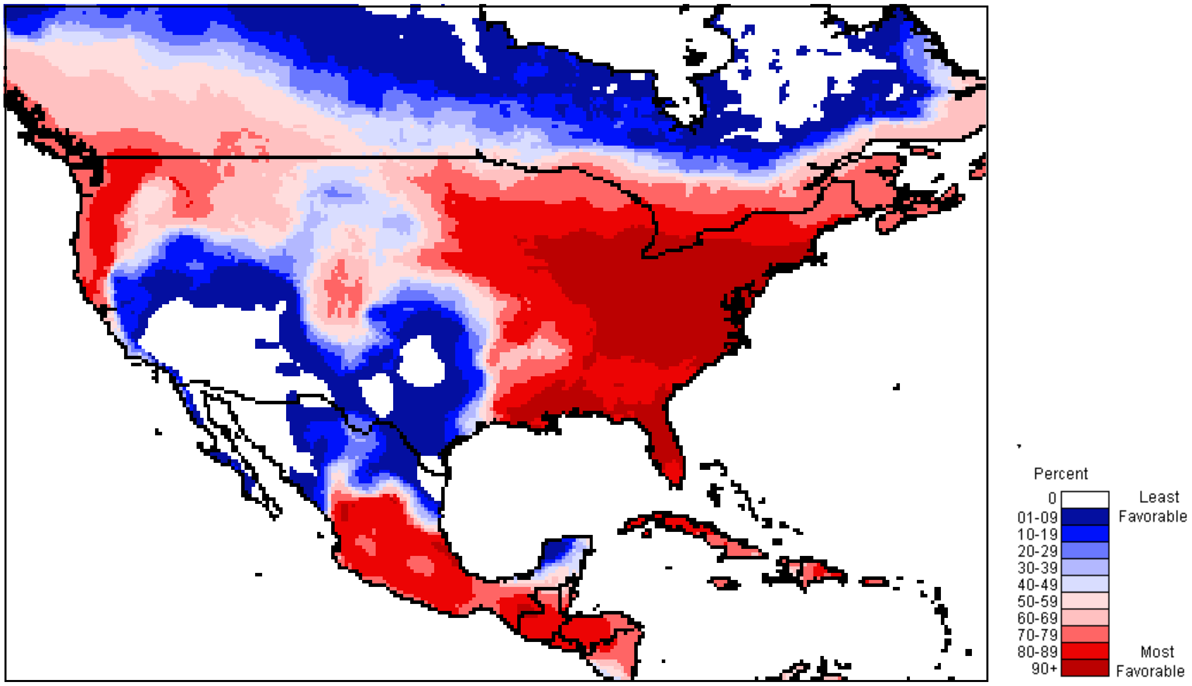

Figure 1. NAPPFAST potential distribution model for *Ampelopsis brevipedunculata*.

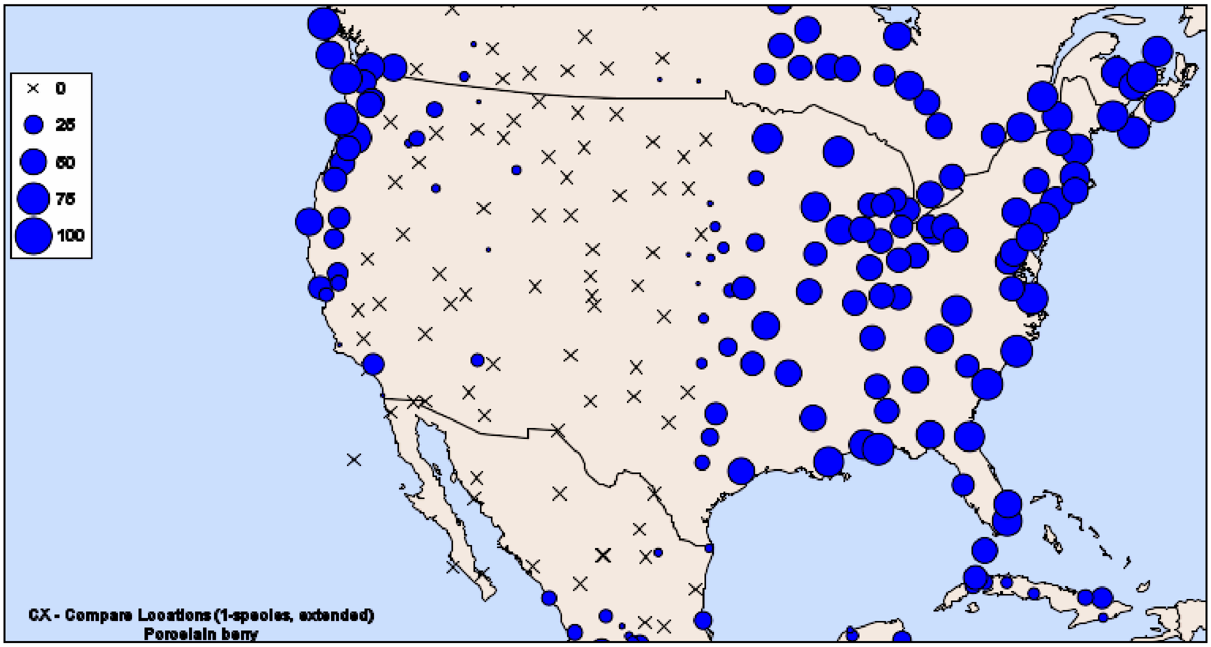

Figure 2. CLIMEX potential distribution model for *Ampelopsis brevipedunculata*.

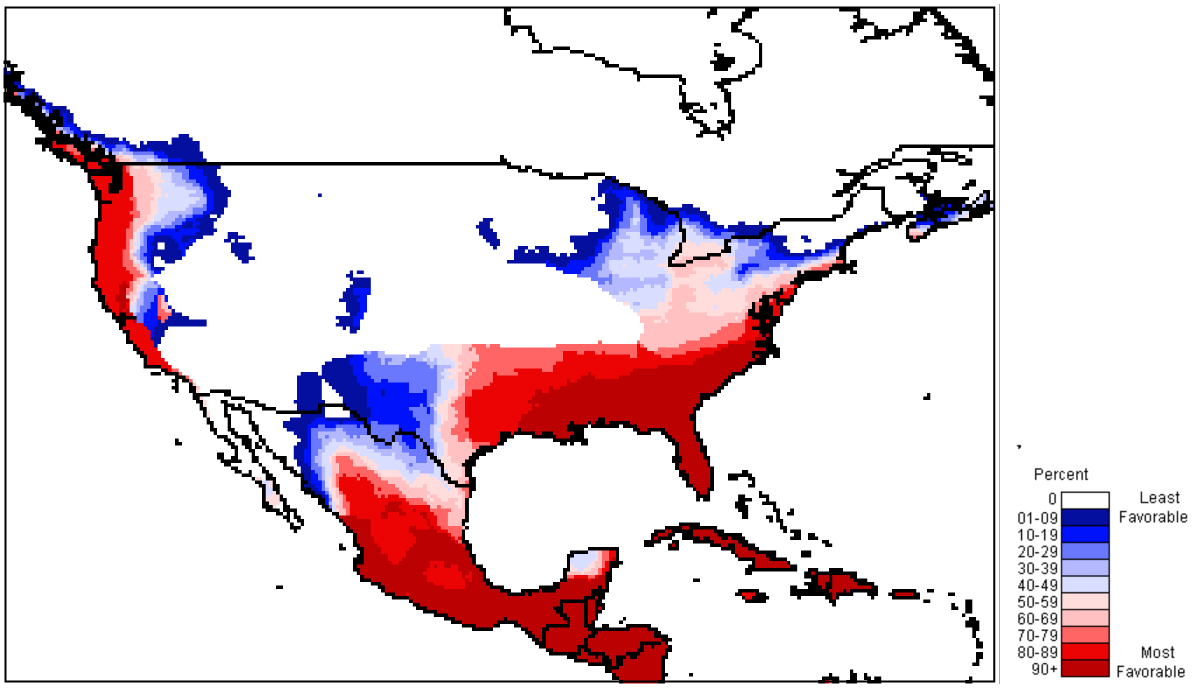

Figure 3. NAPPFAST potential distribution model for *Cayratia japonica*.

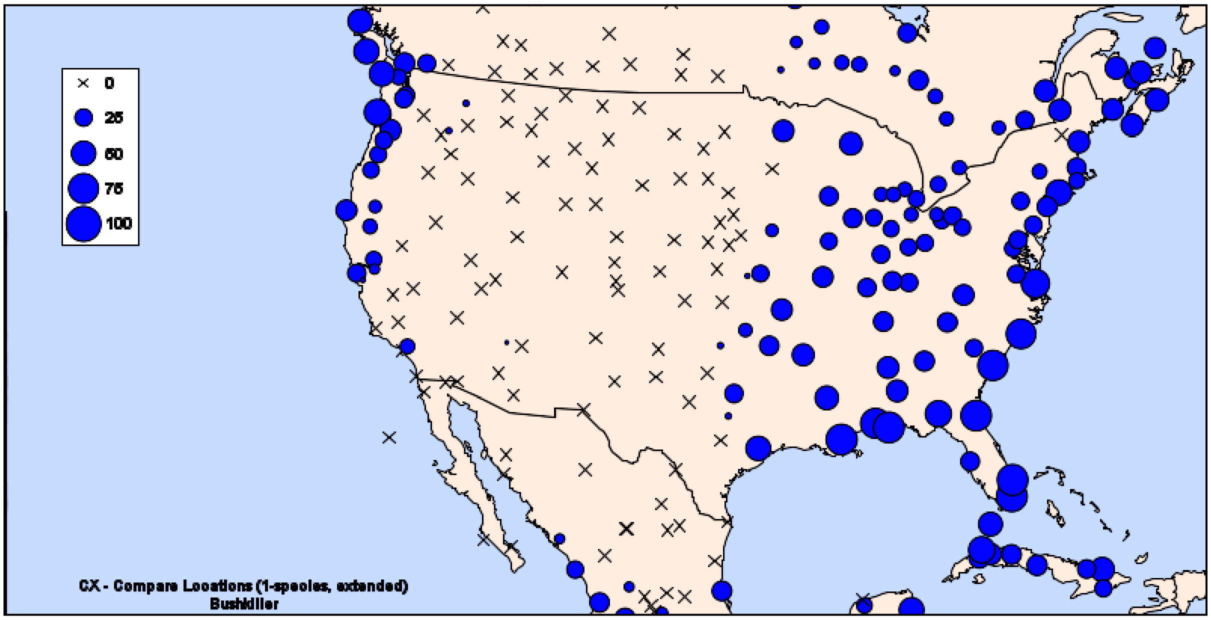

Figure 4. CLIMEX potential distribution model for *Cayratia japonica*.

APPENDICES

Appendix A. Visual porcelain berry control 4 MAT following field foliar herbicide application.[a,b]

Herbicide[c]	Rate	Control[d]
	Kg ae ha^{-1}	%
Aminocyclopyrachlor	0.17	73 a
Aminocyclopyrachlor	0.35	70 a
Aminopyralid	0.12	13 b
Glyphosate	2.02	78 a
Imazapyr	0.14	90 a
Triclopyr amine	0.84	70 a
Triclopyr amine	1.68	67 ab
Triclopyr ester	0.84	60 ab
Triclopyr ester	1.68	95 a

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[a] Visual control rating on a scale between 0 and 100%, where 0 is no visible injury and 100% is complete plant death.

[b] Abbreviations: MAT, months after treatment.

[c] Methylated seed oil at 1% v/ v included with each treatment

[d] Means with the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Appendix B. Visual porcelain berry control 2 MAT following greenhouse foliar herbicide application.[a,b]

Herbicide[c]	Rate	Control
	Kg ae ha^{-1}	%
Aminocyclopyrachlor	0.17	80
Aminocyclopyrachlor	0.35	78
Aminopyralid	0.12	45
Glyphosate	2.02	78
Imazapyr	0.14	48
Triclopyr amine	0.84	75
Triclopyr amine	1.68	80
Triclopyr ester	0.84	95
Triclopyr ester	1.68	100

[a] Visual control rating on a scale between 0 and 100%, where 0 is no visible injury and 100% is complete plant death.

[b] Abbreviations: MAT, months after treatment.

[c] Methylated seed oil at 1% v/ v included with each treatment