## ABSTRACT

**GREER, LANE**. Improving Postharvest Life, Defoliation, and Stem Length of Woody Cut Stems. (Under the direction of John M. Dole.)

In the first study, the postharvest life and optimum handling procedures were determined for *Buddleia davidii* 'Royal Red', *Hydrangea quercifolia*, *Viburnum tinus*, *Ilex* 'Nellie R. Stevens', *Ligustrum sinense*, *Pyracantha coccinea*, *Buxus sempervirens, Ilex crenata*, and *Myrica cerifera*. In Experiment 1, we investigated pre-treatments of 1-methylcyclopropene (1-MCP), a 10% sucrose pulse, 50°C distillled (DI) and tap water, and 20°C DI and tap water. 1-MCP and 50°C DI water produced the longest vase life for *Buddleia.* The 10% sucrose pulse and 1-MCP gave the longest vase life for *Viburnum*. No pre-treatment extended the vase life of *Hydrangea, I.* 'Nellie R. Stevens', *Ligustrum*, *Pyracantha*, *Buxus, I. crenata,* or *Myrica*. In Experiment 2, stems were stored wet or dry at 5°C for 0, 1, 2, or 3 weeks in light or dark. Optimal storage conditions by species were: *Buddleia*– dark, wet (1 week); *Hydrangea*– cannot be stored; *Viburnum*– light or dark, wet (1 week); *I*. 'Nellie R. Stevens' – light, wet (3 weeks); *Ligustrum* – dark, wet (3 weeks); *Pyracantha* – any condition (3 weeks); *Buxus* – any condition (3 weeks); *I. crenata* – light or dark, wet (3 weeks); and *Myrica* – light or dark, wet (3 weeks). For Experiment 3, stems were held in foam or without foam, with 0, 2, or 4% sucrose + 8-HQS. *Buxus* was the only species not adversely affected by foam. *Myrica* and *Buxus* performed best in 0% sucrose; *Ligustrum* performed best in 2% sucrose; and *Viburnum* and *I.*

'Nellie R. Stevens' performed best in 4% sucrose. Sucrose concentration had no effect on *Buddleia, Hydrangea, Pyracantha*, or *I. crenata*.

In the second study, six defoliants were applied to *Ilex verticillata*, *Celastrus scandens,* and *Salix matsudana*. Defoliants included acetic acid, chelated copper, crop oil concentrate (COC), ethephon, dimethipin, and pelargonic acid. No treatment resulted in defoliation of *Ilex*. Chelated copper at 800 $mg \cdot L^{-1}$ provided 100% defoliation of *Celastrus*. Chelated copper at 400 $mg \cdot L^{-1}$, ethephon at 1000 $mg \cdot L^{-1}$, and dimethipin provided up to 80% defoliation of *Celastrus*. Dimpethipin provided 75% defoliation of *Salix*. In a second experiment conducted with containerized *Salix*, irrigation was stopped for 0, 3, or 6 d before defoliants were applied. Dimethipin promoted 90% defoliation, with no subsequent adverse effects on plants.

Cut stems of *Callicarpa americana* and *S. matsudana* 'Tortuosa' were held in DI water at 5, 20, or 35ºC. No treatment promoted defoliation of *Callicarpa*. Holding cut stems of *S. matsudana* at 20°C promoted 68% defoliation, compared to 53 or 28% for 5 or 35°C, respectively.

In the third study, containerized *Viburnum trilobum* and *Ilex verticillata* were (a) inoculated with a commercial formulation containing 7 species of vesicular-arbuscular mycorrhizae (VAM), (b) inoculated with *Glomus etunicatum,* or (c) allowed to remain uninoculated. Phosphorus (P) was applied daily at 0, 25, or 50 $mg \cdot L^{-1}$. Neither plant species was colonized by any VAM species. Phosphorus supplied at 50 $mg \cdot L^{-1}$ was optimal for increasing stem number and

length of *Ilex*. For *Viburnum*, 25 mg·$L^{-1}$ was optimal for stem growth, but root area, length, and weight were not affected by P concentration.

# IMPROVING POSTHARVEST LIFE, DEFOLIATION, AND STEM LENGTH OF WOODY CUT STEMS

by

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## Dedication

To Bonnie

## Biography

Lane Greer was born and raised in Monroe, Louisiana, but moved to Fayetteville, Arkansas as a teenager. There she earned her Bachelor of Science in Business Administration, with an emphasis in marketing, from the University of Arkansas. She worked for Wal-Mart as an assistant manager and for the University of Arkansas as a cooperative education coordinator before returning to school in horticulture. She graduated with a Master of Science in Horticulture and went to work for Appropriate Technology Transfer for Rural Areas (ATTRA), a USDA program that provides information to growers around the country about sustainable production techniques. While working for ATTRA, she also owned a cut flower farm, which sparked her interest in working with cuts. In 2000 she began her Ph.D. program at North Carolina State University.

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## Introduction

Commercial field grown cut flower production encompasses an incredible array of plant materials including fresh and dried/preserved materials, flowers, stems and berries. Enterprising growers are constantly looking for new species and cultivars, such as woody cut stems, to provide their customers with different products and enhance sales. In addition, woody plant species with decorative stems and berries can be harvested in the fall, winter and early spring when other plants are not available and provide growers with sales during the off season.

Woody plants allow growers to offer unique products. Woody cut stems are grown for four purposes: flowers, foliage, fruit, and decorative stems. Woody plants grown for their fruit include hollies (*Ilex* spp.) and bittersweet (*Celastrus scandens*). Desirable traits in a berried plant that make it suitable for cutting include fast growth, long vase life, and long stems that hold their fruit. The fruit should also be relatively dry, as soft, juicy fruit can stain consumers' furniture, floors, or tablecloths. Additionally, since most berried cuts are not purchased for their foliage, it is desirable to have stems that drop their leaves easily.

*Postharvest*. If woody species are to be accepted by growers and florists, more information on postharvest handling is needed. Air temperature, water temperature, pre-treatments, storage, and holding solutions are all crucial to increased vase life. Nine species were chosen for postharvest evaluation because of their current use in the industry or because of their potential as cut stems. Three species were chosen for their flowers (*Buddleia davidii* 'Royal Red',

*Hydrangea quercifolia,* and *Viburnum tinus* ‘Spring Bouquet’), three for their fruit (*Ilex* ‘Nellie R. Stevens’, *Ligustrum sinense,* and *Pyracantha coccinea*), and three for foliage (*Buxus sempervirens, Ilex crenata,* and *Myrica cerifera*).

*Buddleia davidii* is a good plant for use as a cut flower because it grows fast and produces stems with fragrant, terminal inflorescences in shades of lilac, pink and white. Deadheading and pruning encourage summer-long bloom.

*Hydrangea quercifolia* is a native plant that grows well in the Southeast. It bears long terminal panicles on the previous year’s growth and should yield 15 to 20 stems per plant at maturity.

The Feb. to Mar. flowering time of *Viburnum tinus* ‘Spring Bouquet’ make this an excellent candidate for early spring sales. *V. tinus* is a hardy evergreen shrub with dark green, glossy leaves and dark pink buds that open to white flowers borne in a cyme, usually 8 cm in diameter. The plant is also prolific, producing 30 to 40 stems at maturity.

*Ilex* ‘Nellie R. Stevens’ is a vigorous, commonly grown plant in the Southeast. The large red berries produced late in the year make this a practical cut stem for holiday or winter sales. The fast growth rate, ease of pollination, and lack of insect and disease problems make it a good choice for cut stems.

*Ligustrum sinense* is a common plant, even considered invasive in some areas of the United States. The fragrant flowers are produced in early summer and yield blue-black fruits in fall.

*Pyracantha coccinea* bears orange-red fruit in fall and winter, has a medium-fast growth rate, and can be pruned throughout the year. These characteristics make pyracantha a candidate for cut fruit production.

Although a slow growing plant, the foliage of *Buxus sempervirens* is often used in floral arrangements and wreaths. *Ilex crenata* has the same small-leaved look as *Buxus sempervirens*, but grows faster and without the odor often associated with *Buxus*. *Myrica cerifera* is an evergreen shrub with glossy green, fragrant foliage. This shrub also has a medium-fast growth rate.

*Defoliation*. Woody plants valued for their fruit or stem are usually sold without foliage. Removal of foliage prior to harvest results in less postharvest debris and more aesthetically pleasing stems. Currently, woody cut stems are defoliated either by hand or by pre-harvest spraying of defoliants or desiccants.

Defoliants and desiccants are contact herbicides of variable phytotoxicity. There are two types of defoliants and desiccants: non-hormonal and hormonal. Non-hormonal defoliants cause extensive damage to the plant resulting in foliar chlorosis, necrosis, and desiccation. Injury caused by non-hormonal defoliants can stimulate wound-induced ethylene production, reduce auxin levels, and promote water loss and abscisic acid (ABA) synthesis, all of which expedite leaf abscission. Hormonal defoliants such as 2-chloroethylphosphonic acid (ethephon) mimic the natural process of defoliation or leaf abscission by directly increasing ethylene levels or production.

Defoliants have numerous disadvantages: they are potentially toxic to plants and humans, have unpleasant odors, are expensive, and produce

inconsistent defoliation. Thus, there is a need to improve the consistency while reducing the hazards and costs of defoliants and desiccants. Less toxic herbicides may provide an alternative to defoliants. The less toxic herbicides that show promise in defoliating woody species include pelargonic acid, acetic acid, chelated copper, and ethephon. *Ilex verticillata* 'Winter Red'*, Celastrus scandens,* and *Salix matsudana* 'Tortuosa' are species that could be harvested earlier in the growing season rather than waiting for natural leaf fall to occur.

Numerous woody plant species are valued as cut stems but require long establishment periods and might benefit from VAM inoculation. *Ilex verticillata* 'Winter Red' has bright red berries that ripen in September. *I. verticillata* is deciduous and drops its leaves in late fall, after the berries are mature. Growers often use cut branches of this plant in Christmas arrangements. Although it is a slow-growing plant, the growth rate can be increased with adequate fertilizer and water.

American bittersweet (*Celastrus scandens*) is a fast-growing vine that is often grown or wild-collected for use in fresh and dried floral arrangements. Vines are usually cut during September, before the three-lobed yellow capsules containing crimson seeds ripen in October, and can be sold with or without foliage.

The contorted branches of curly willow (*Salix matsudana* 'Tortuosa') have prompted florists to use this woody plant in fresh or dried arrangements. This shrubby tree is an excellent choice for cut stems because of its fast growth rate,

profusion of branches, and winter color, which can be green, red, yellow, or brown.

Environmental changes such as frost or drought can induce leaf abscission. High or low temperatures applied to harvested stems in a controlled environment may induce defoliation without encouraging fruit drop. *Callicarpa americana* and *Salix matsudana* 'Tortuosa' were used to determine the effects of postharvest temperatures on defoliation.

American beautyberry (*Callicarpa americana)* has larger fruit than other members of the genus. The metallic purple drupes are borne in clusters and begin to color in late September, beginning at the base of the stem. Large, tomentose leaves often do not fall until after the first freeze, which discolors fruits. *Callicarpa americana* is an excellent choice for cut stems because of its fast growth rate, ease of cultivation, and unusual fruit coloration.

*Vesicular-arbuscular mycorrhizae*. Vesicular-arbuscular mycorrhizae (VAM) can increase plant establishment and shoot length, both of which are desirable characteristics in woody cut stems. Vesicular-arbuscular mycorrhizae (VAM) are naturally occurring, soilborne symbiotic fungi that colonize plant roots, forming either vesicles (sack-like structures) or arbuscules (tree-like structures) within and between the plant's root cells (Gerdemann, 1968). Plant root exudates provide mycorrhizae with photosynthetically derived organic carbon (Harris and Paul, 1987). In return, mycorrhizae decrease the effects of environmental stress in several ways.

While roughly 80% of plants form VAM associations, they do so on an inconsistent basis (Trappe, 1987). VAM are usually considered non-specific, so that 150 VAM species infect thousands of plant species.

There are numerous difficulties in testing VAM colonization, including availability of sources, application of VAM to plant roots, media fertility status, and measurement of VAM effects. VAM inoculation has inconsistent effects on plant growth, and this unpredictability may be based on seasonal variation, daylight, and light intensity.

Inoculation with VAM requires labor inputs and establishment of application protocols. These can only be justified if VAM provide some benefit, such as increased height or diameter, faster plant establishment, improved transplant survival, or superior postharvest life. Numerous woody plant species are valued as cut stems but require long establishment periods and might benefit from VAM inoculation, particularly if VAM allow growers to use less fertilizer and pesticides.

*Viburnum trilobum* has bright red berries that ripen in September and a medium growth rate. It has a wide native range (USDA Hardiness Zones 2-7) and can be used in late fall or Thanksgiving arrangements.

*Objectives*. 1) The objective of the postharvest research was to determine the optimum pre-treatment, storage, and holding solutions to increase vase life for nine species of woody cut stems.

2) The overall objective of the defoliation experiments was to find effective, least-toxic methods for early leaf abscission in woody cut stems

through chemical application or environmental manipulation, without concurrent fruit drop or plant necrosis. The objectives of the greenhouse study were: (1) to assess spring vs. fall defoliation using five defoliants; (2) to investigate interactions between irrigation cessation and defoliants; (3) to determine the effects of defoliants on regenerative leaf ability. The objective of the postharvest study was to determine the effect of temperature and storage on defoliation of woody cut stems.

3) The objectives of the VAM research were to inoculate two woody plant species in the greenhouse with vesicular-arbuscular mycorrhizae to enhance root growth, plant establishment, and shoot length.

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## CHAPTER ONE.

## IMPROVING THE POSTHARVEST LIFE OF WOODY CUT STEMS.


### Abstract

The postharvest life and optimum handling procedures were determined for nine species of woody cuts, including three flowering species: *Buddleia davidii* 'Royal Red', *Hydrangea quercifolia*, and *Viburnum tinus* 'Spring Bouquet'; three fruiting species: *Ilex* 'Nellie R. Stevens', *Ligustrum sinense*, and *Pyracantha coccinea*; and three foliage species: *Buxus sempervirens, Ilex crenata*, and *Myrica cerifera*. In Experiment 1, we investigated pre-treatments of 1-methylcyclopropene (1-MCP), a 10% sucrose pulse, 50°C distilled (DI) and tap water, and 20°C DI and tap water. 1-MCP and 50°C DI water produced the longest vase life for *B. davidii* (6.6 and 6.4 d, respectively, compared to the control of 5.6 d). The 10% sucrose pulse and 1-MCP gave the longest vase life for *V. tinus* (15.0 and 13.5 d, respectively, compared to the control of 10.0 d), while vase life of stems placed in 50°C DI and tap water was significantly shorter at 8.8 and 9.0 d, respectively. Ambient (20°C) DI water was optimal for *M. cerifera* (9.9 weeks) while the 10% sucrose pulse experienced the shortest vase life of 2.2 weeks. No pre-treatment extended the vase life of *H. quercifolia, I.* 'Nellie R. Stevens', *L. sinense*, *P. coccinea*, *B. sempervirens,* or *I. crenata*. In Experiment 2, stems were stored wet or dry at 5°C for 0, 1, 2, or 3 weeks in 12-$h \cdot d^{-1}$ (light) or constant dark. Stems that could be stored in light and dry for one week were *V. tinus*; in light and dry for three weeks were *B. sempervirens* and *P. coccinea*. Stems that could be stored in dark and dry for one week included *M.*

*cerifera, L. sinense*, and *V. tinus;* for two weeks were *I.* 'Nellie R. Stevens' and *I. crenata*; for three weeks were *P. coccinea* and *B. sempervirens*. Stems that can be stored in light and wet for one week were *V. tinus*; light, wet for three weeks *P. cocinea. I. crenata*, *I.* 'Nellie R. Stevens', *M. cerifera*, and *B. sempervirens*. Stems that can be stored in dark and wet for one week include *V. tinus and B. davidii*; for two weeks were *I.* 'Nellie R. Stevens'; and three weeks include *L. sinense, P. coccinea, B. sempervirens. I. crenata,* and *M. cerifera*. For Experiment 3, stems were held in foam or without foam, at 0, 2, or 4% sucrose + 8-HQS. The only species that was not adversely affected by foam was *B. sempervirens*. Species that performed best in 0% sucrose included *M. cerifera* and *B. sempervirens*; in 2% sucrose included *V. tinus, L. sinense, I.* 'Nellie R. Stevens, and *B. sempervirens*; and in 4% sucrose included *V. tinus* and *I.* 'Nellie R. Stevens'. Sucrose had no effect on *B. davidii, H. quercifolia, P. coccinea*, or *I. crenata*.

**Introduction**

Temperature is the most important factor affecting postharvest flower quality and vase life (Hardenburg et al., 1986). The recommended temperature range for holding most species of temperate annual and perennial cut flowers is 0 to 4°C; higher temperatures promote senescence by increasing respiration (Nell and Reid, 2001; Çelikel and Reid, 2002b). Other benefits of lower temperatures include reduced endogenous ethylene production, lowered sensitivity to atmospheric ethylene, lowered water loss, and slower development of microorganisms in vase solution (Nowak and Rudnicki, 1990; van Doorn,

1989). High relative humidity (≥95%) is also conducive to longer vase life (Wilkins, 1988).

Water temperature affects vase life by influencing water uptake. Water at 38 to 43°C is recommended for three reasons: preservatives dissolve more easily in it, warm water contains less oxygen, which can plug the cut stem with air emboli (Bridgen, 1997), and warm water is less viscous than cool water (Sacalis, 1993). However, using water temperatures above 40°C for more than a few hours usually shortens vase life (van Doorn, 1997).

Although many cut flowers can be stored wet or dry, dry storage can decrease vase life (Çelikel and Reid, 2002b). Durkin (1980) found that water uptake in roses (*Rosa* hybrids) and chrysanthemums (*Dendranthema* □*grandiflorum*) was inhibited after dry storage, and he suggested this was caused by air emboli in the xylem. Chattaway (1949) and Tyree and Zimmerman (2002) stated that gum deposition in xylem vessels occurs after the vessels have been deprived of water.

In wet storage, water containing high levels of salinity, sodium, or fluoride can damage cut flowers (Waters, 1968). Low pH (between 3.0 and 4.0) is recommended for longer vase life of herbaceous cuts, because acidic water travels faster in the xylem (Han, 2000). Many cut flowers can be stored in the dark for up to two weeks, but low or no light during long storage periods can induce leaf yellowing in some species, including alstroemeria (*Alstroemeria* spp.), lilies (*Lilium* spp.), and chrysanthemum (Nowak and Rudnicki, 1990).

Postharvest handling techniques can be divided into two general categories: pre-treatments and holding solutions. Pre-treatments and pulses are short-term treatments (lasting 1 to 48 hours) conducted immediately after harvest. The theory is to 'load' the stems and leaves with a high concentration of sucrose or other preservative to aid in flower development (Han, 2000). A common pulse uses 5 to 20% sucrose and is applied overnight. Another effective pulse is silver thiosulfate (STS), which blocks the sites of ethylene attachment to plant tissue and is effective against both endogenous and exogenous ethylene. 1-methylcyclopropene (1-MCP) is also effective against endogenous and exogenous ethylene but is applied as a gas. In several studies, 1-MCP was as effective in extending vase life as STS for carnation (*Dianthus barbatus*), alstroemeria, and snapdragon (*Antirrhinum majus*) (Serek et al., 1995), stock (*Matthiola incana*) (Reid et al., 1999), gypsophila (*Gypsophila paniculata*) and delphinium (*Delphinium elatum*) (Skog et al., 1998). 1-MCP may be preferable to STS because it contains no silver, the disposal of which is an environmental concern.

Long-term or holding solutions contain an acidifier (usually citric acid at 350 to 500 $mg \cdot L^{-1}$), sucrose (1 to 2%), and a bactericide (50 to 200 $mg \cdot L^{-1}$) to prevent occlusion of xylem vessels (Han, 2000; Bridgen, 1997). Cuts can remain in holding solutions for their entire postharvest life.

Cut flower growers are constantly looking for new species and cultivars to enhance their product mix. Woody cuts create diversity and extend the production, harvest, and marketing seasons. If woody species are to be

accepted by growers and florists, however, more information on postharvest handling is needed. General recommendations include treating woody stems for a few seconds with hot water at 80 to 90°C, before returning them to cold water (Nowak and Rudnicki, 1990). Kasperski (1956) recommends splitting the stem end of woody cuts for 8 to 10 cm from the base.

The nine species used in this study for postharvest evaluation were chosen because of their current use in the industry or because of their potential as cuts. Three species were chosen for their flowers (*Buddleia davidii* 'Royal Red', *Hydrangea quercifolia,* and *Viburnum tinus* 'Spring Bouquet'), three for their fruit (*Ilex* 'Nellie R. Stevens', *Ligustrum sinense,* and *Pyracantha coccinea*), and three for foliage (*Buxus sempervirens, Ilex crenata,* and *Myrica cerifera*).

*Buddleia davidii* is a good plant for use as a cut because of its rapid growth. It produces stems with fragrant, terminal inflorescences in shades of lilac, pink and white. Deadheading and pruning encourage summer-long flowering (Dirr, 1998). Cut flowers last 5 to 8 days (Kasperski, 1956). White cultivars are not recommended as cuts, as they show petal browning earlier than dark cultivars (Krentz and Behe, 1995). Armitage (2003) recommended conditioning *Buddleia* stems with warm water (temperature unspecified), placing in hydrating solution, and using a preservative in warm water. Byczynski (1997) suggested immersing the stems in 38°C water and leaving them until the water cools. Nowak and Rudnicki (1990) recommended storing *Buddleia* wet at 3 to 4°C.

*Hydrangea quercifolia* is a native plant that bears long terminal panicles on the previous year's growth and should yield 15 to 20 stems per plant at maturity (Armitage, 1993). Recommendations for cutting are to split the stem end and then sear with a flame or by insertion for 30 s in boiling acidic water, before placing the stems in cold water with pH 4.0 overnight (Kasperski, 1956).

The Feb. to Mar. flowering time of *Viburnum tinus* 'Spring Bouquet' make this an excellent candidate for early spring sales. *V. tinus* is a hardy evergreen shrub with dark green, glossy leaves and dark pink buds that open to white flowers borne in a cyme, usually 8 cm in diameter. The plant is also prolific, producing 30 to 40 stems at maturity. To our knowledge, no literature is available on the suitability of this plant as a cut.

*Ilex* 'Nellie R. Stevens' is a vigorous, commonly grown plant in the southeastern United States. The large red berries produced late in the year make this a practical cut for Christmas sales. The fast growth rate, ease of pollination, and lack of insect and disease problems make it a good choice for cut stems. Growers sell many evergreen hollies, but no literature is available on their postharvest life.

*Ligustrum sinense* is a common plant, even considered invasive in some areas of the United States. The fragrant flowers are produced in early summer and yield blue-black fruits in fall. Again, no information is available on using these fruited stems as cuts.

*Pyracantha coccinea* bears orange-red fruit in fall and winter, has a medium-fast growth rate, and can be pruned throughout the year (Dirr, 1990).

These characteristics make pyracantha a candidate for cut fruit production, but we are unaware of any trials that have been performed using pyracantha as a cut.

Although a slow growing plant, the foliage of *Buxus sempervirens* is often used in floral arrangements and wreaths. Nowak and Rudnicki (1990) recommend storing it wet at 2 to 4°C for 1 to 2 weeks.

*Ilex crenata* has the same small-leaved look as *Buxus sempervirens*, but grows faster and without the odor often associated with *Buxus*. *Myrica cerifera* is an evergreen shrub with glossy green, fragrant foliage. This shrub also has a medium-fast growth rate (Dirr, 1998). We are unaware of any literature prescribing the postharvest treatment of cut *Ilex crenata* or *Myrica cerifera* stems. Therefore, the objective of this research was to determine the optimum pre-treatment, storage, and holding solutions to increase vase life for nine species of woody cuts.

**Materials and Methods**

Field grown *Buddleia davidii* 'Royal Red' and container grown *Viburnum tinus* 'Spring Bouquet' stems were harvested when 25 to 50% of the florets were open, with colored buds present. Field grown *H. quercifolia* was harvested when 50% of florets were open and had no discoloration. The three foliage species (*Buxus sempervirens, Myrica cerifera and Ilex crenata)* were harvested from field grown plants when leaves were fully expanded and less than 2% of the foliage was discolored. Field grown fruiting species (*Ligustrum sinense, Pyracantha*

*coccinea*, and *Ilex* 'Nellie R. Stevens') were harvested when the fruit was colored but had not begun to senesce.

Terminal stems were cut 38 cm long between 0800 and 1100 and transported dry to the postharvest treatment area, except for *Buddleia davidii* and *Hydrangea quercifolia* stems, which were transported wet. The foliage was removed from the lower third of each stem, and stems were tagged and weighed before treatment. Stems were randomly assigned to one of 16 postharvest treatments that were grouped into the following three experiments: (1) pre-treatment, (2) cold storage, or (3) holding. Experiments were conducted between Sept. 2001 and March 2003.

Experiment 1 – Pre-treatments. For each species, ten stems were placed in glass vases and treated with one of the following: (1) Ethyl-Bloc 1-methylcyclopropene (1-MCP) (Floralife, Walterboro, S.C.) – Stems were placed into distilled water and sealed in a 0.21 cu m barrel for treatment with 440 $mg \cdot L^{-1}$ 1-MCP; (2) 10% sucrose (Food Lion, Salisbury, N.C.) pulse, pH 4.4; (3) 50˚C (warm) distilled water, pH 4.5; (4) 20˚C (ambient) distilled water, pH 4.5; (5) 50˚C (warm) tap water, pH 6.1; and (6) 20˚C (ambient) tap water, pH 6.1. In all treatments, stems were placed in 300 ml of the indicated solution for 24-h at 5°C and 90% relative humidity. Light was provided at 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with cool white fluorescent bulbs ($12\text{-}h \cdot d^{-1}$). For the water temperature treatments, the temperature was allowed to fall to the cooler temperature (5°C). After 24 hours, the stems were transferred to a lighted area (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) provided by cool white fluorescent bulbs ($12\text{-}h \cdot d^{-1}$) at 20°C and 45 to 60% relative humidity.

Data collection included visual quality ratings of 1 to 5, with 1 being fresh and 5 being dead (Table 1). Vase life was calculated as the total number of days (flowering species) or weeks (fruiting and foliage species) after removal from treatment to termination, the day on which visual quality was rated as 4.

In addition to visual quality, data were collected on solution pH, total amount of solution uptake, and fresh weight of cut stems. For flowering stems, data were collected daily. For fruiting and foliage cuts, the data were collected weekly.

Experiment 2 - Storage. For each species, ten stems were held at 5°C (1) wet in ambient distilled water in glass vases, or dry in a cardboard box lined with a clear polyethylene sheet; (2) in 12-h light or constant dark; (3) 0, 1, 2, or 3 weeks, after which time they were transferred to a lighted area (12-h·$d^{-1}$ at 30 μmol·$m^{-2}$·$s^{-1}$) at 20˚C and 45 to 60% relative humidity.

Experiment 3 - Holding. For each species, ten stems were placed in glass vases and held at 20°C, 45 to 60% relative humidity in a lighted area (30 μmol·$m^{-2}$·$s^{-1}$ for 12-h·$d^{-1}$) in the following treatments: (1) with or without floral foam; (2) in 0, 2, or 4% sucrose + 0.6 g 8-hydroxyquinoline sulfate (8-HQS) per 300 ml distilled water. Oasis floral foam (Smithers-Oasis, Kent, Ohio) was cut into 9 x 5 x 5-cm blocks and saturated with the appropriate holding solution before stems were placed into it. Solutions had a pH of 3.0. For Experiments 1 to 3, data were analyzed by general linear model procedure with means separation by trend analysis (SAS Institute, Cary, N.C.).

Experiment 4. Endogenous ethylene. Fifteen cut stems of each species were placed in sealed glass jars containing 200 ml distilled water. Jars were held at 20ºC under 12-h·d$^{-1}$ light (30 µmol·m$^{-2}$·s$^{-1}$). Ethylene emanating from the cut stems was measured by withdrawing 1 mL air samples from the jar using a syringe and analyzed using a gas chromatograph equipped with an alumina column (Series 1400, Varian Aerograph, Sugarland, TX). Samples were taken at 24 and 48-h after sealing the jars.

**Results**

*Buddleia davidii* 'Royal Red'. The 1-MCP and warm DI water treatments produced the longest vase life, 6.6 and 6.4 d, respectively, compared to the control of 5.6 d (Table 2). Solution uptake was highest in the warm water treatments but lowest in 1-MCP, suggesting no relationship between solution uptake and vase life.

Immediately following removal from storage, stems did not show any visible differences, but vase life at 20°C was affected by storage. Çelikel and Reid (2002a) had similar results with stock. Storage condition was more important than light in determining storage capability of *Buddleia* (Table 3). While *Buddleia* should not be held dry, holding stems wet in the dark for one week did not decrease vase life (Fig. 1). *Buddleia* buds continued to develop and open during wet storage in 5°C.

Stems held in foam had a significantly shorter vase life than no foam (2.8 d vs. 4.1 d, respectively) (Table 4). Sucrose concentration had no effect on vase life, but stems in higher sucrose concentrations had lower solution uptake. Of

the three flowering species, *Buddleia* had the lowest endogenous ethylene levels (Table 5).

*Hydrangea quercifolia*. No pre-treatment had any effect on vase life, which averaged 2.1 to 3.9 d (Table 2). Although not significantly different from the control, the 50°C treatments yielded the longest vase life. The warm water treatments also had the highest solution uptake (Table 2), suggesting that treatments that increase solution uptake may also extend vase life.

*H. quercifolia* could not be stored under any condition without significantly decreasing vase life (Fig. 1). Foam is not recommended, yielding a 2.0 d shorter vase life than no-foam (Table 4). In addition, sucrose concentration did not affect vase life. As sucrose concentration increased, solution uptake decreased regardless of the presence of foam (data not shown). All stems had moderately high endogenous ethylene levels (42 $\mu L \cdot L^{-1} \cdot gfw^{-1}$) (Table 5).

*Viburnum tinus* 'Spring Bouquet'. The 1-MCP and 10% sucrose pulse pre-treatments produced a 13.5 and 15.0 d vase life, respectively (Table 2), but foliage of the stems pulsed with sucrose was discolored and unattractive, similar to rose foliage that has taken up too much sucrose (Sacalis, 1993). Both warm water treatments produced significantly shorter vase life (8.8 and 9.0 d) than the control (10.0 d) (Table 2). Solution uptake for stems in 1-MCP was highest (Table 2), suggesting that treatments which increase solution uptake may also extend vase life.

Light, storage condition (wet or dry), and storage time affected vase life of *Viburnum tinus* (Table 3). Storing *V. tinus* at 5°C for any period of time

significantly decreased vase life, however, stems stored for one week yielded a week-long vase life (Fig. 1). For longer storage, stems should be held wet, although storage should not exceed 2 weeks under any condition.

Stems held in foam had a significantly shorter vase life (9.0 d) compared to no-foam (14.2 d) (Table 4). Sucrose at 2 or 4% increased vase life by 3.0 and 4.8 d, respectively, compared with the control (9.0 d). Holding in 4% sucrose did not cause discolored foliage, and this treatment yielded a vase life of 13.8 days (Table 4). *Viburnum tinus* had moderately high ethylene levels (41 $\mu L \cdot L^{-1} \cdot gfw^{-1}$) (Table 5).

*Ilex* 'Nellie R. Stevens'. No pre-treatment extended the vase life of *Ilex* 'Nellie R. Stevens' (Table 6). The 2 and 4% sucrose, no-foam holding treatments yielded a vase life of 5.4 and 5.0 weeks, respectively, compared to the 4.1-week control of 0% sucrose (Table 8).

Postharvest life of *Ilex* 'Nellie R. Stevens' was most strongly impacted by storage condition and time (Table 7). Storing stems wet did not decrease vase life (Fig. 2). Storing stems dry in the dark for two weeks did not decrease vase life, and *Ilex* 'Nellie R. Stevens' could be stored in any condition for one week with no decr ease in vase life. Endogenous ethylene levels were very low (Table 5).

*Ligustrum sinense.* No pre-treatment extended the vase life of *Ligustrum sinense*, which ranged from 2.4 to 3.2 weeks (Table 6). Similarly, no differences in solution uptake occurred.

Light, storage condition, and storage time affected the subsequent vase life of *Ligustrum* (Table 7). *Ligustrum* could be held wet for one week without decreasing vase life (Fig. 2). Stored wet in the dark, *Ligustrum* was held for 3 weeks with no subsequent decrease in vase life.

Stems in foam had a shorter vase life (1.6 weeks) than those not in foam (2.7 weeks) (Table 8). Sucrose concentration also affected vase life, with the 2% sucrose solution yielding a 2.5-week vase life, compared to a 1.8-week vase life for the control (Table 8). Interestingly, higher sucrose concentrations increased solution uptake (data not shown). Like the other fruiting species, endogenous ethylene levels were very low (9 $\mu L \cdot L^{-1} \cdot gfw^{-1}$) (Table 5).

*Pyracantha coccinea.* No pre-treatment or sucrose concentration extended the vase life of *Pyracantha coccinea*, which ranged from 1.0 or 1.4 weeks (Tables 6 and 8). Stems pulsed with sucrose had lower solution uptake (13.0 ml) compared to all other treatments (ranging from 48.5 to 59.0 ml).

The interaction of light and storage time was the only significant factor governing storage of *Pyracantha* (Table 7). Holding *Pyracantha* at 5°C for 2 weeks did not reduce vase life (Fig. 2). Holding for 3 weeks in dark did not reduce vase life. Stems in foam had a significantly shorter vase life (1.0 weeks) than those not in foam (1.4 weeks), but sucrose concentration did not affect vase life. Endogenous ethylene levels were lower for this species than any other tested (Table 5).

*Buxus sempervirens*. No pre-treatment or holding solution extended the vase life of *Buxus sempervirens*, which ranged from 5.4 to 8.1 weeks (Tables 9 and 11).

Light and storage condition during storage did not affect *Buxus sempervirens* (Table 10). For the storage experiment, vase life controls varied between 6.0 and 8.1 weeks (data not shown). Storing stems wet in light did not diminish vase life (Fig. 3).

Vase life decreased as sucrose concentration increased, with 0% sucrose yielding a 7.5-week vase life compared with 5.4 weeks for 4% sucrose (Table 10). Endogenous ethylene levels were very low (Table 5).

*Ilex crenata*. No pre-treatment extended the vase life of *Ilex crenata,* which ranged from 6.6 to 10.9 weeks (Table 9). Light, storage condition, and storage time affected the vase life of *Ilex crenata* (Table 10). Storing stems dry in the dark for up to two weeks did not decrease vase life (Fig. 3). Stems can be stored wet in light for up to two weeks, and wet, dark for three weeks without decreasing vase life.

Foam decreased vase life by 2.3 weeks, and sucrose holding solutions did not extend vase life (Table 11). Endogenous ethylene levels were high compared to other species (85 $\mu L \cdot L^{-1} \cdot gfw^{-1}$), but still low overall.

*Myrica cerifera*. The optimum pre-treatment for *Myrica cerifera* was 20°C DI water, yielding a 9.9 week vase life compared to 7.8 weeks for the control (Table 9). The 10% sucrose pulse proved detrimental, decreasing vase life to

2.2 weeks. As with *Viburnum tinus*, foliage was discolored by the sucrose pulse treatment.

Light, storage condition, and storage time affected the vase life of *Myrica cerifera* (Table 10). Although storing in dark, dry conditions for more than 1 week significantly decreased vase life, the vase life was still 8.0 weeks (Fig. 3). Even after three weeks of dark, dry storage, *Myrica* had a vase life of almost 5 weeks. Furthermore, wet storage for up to 3 weeks did not diminish vase life. The vase life of *Myrica* was longest when no sucrose was added to the holding solution.

**Discussion**

*Ethylene*. The relatively low levels of endogenous ethylene for all nine species suggest that natural senescence of woody cuts may not be affected by endogenous ethylene production (Table 5). Fjeld et al. (1995) found very low levels of endogenous ethylene in *Ilex aquifolium* and determined that this was not the reason for postharvest leaf drop. However, 1-MCP application increased vase life of 3 species, *Buddleia davidii* 'Royal Red', *Viburnum tinus* 'Spring Bouquet', and *Myrica cerifera*, suggesting an ethylene role in stem senescence.

*Storage*. Çelikel and Reid (2002b) found significant differences between wet- and dry-stored gerberas only at temperatures higher than 5ºC, but stems were stored for only 5 d. Storing stems in water became more important with increasing storage time. No symptoms of chilling injury were noted with any species, indicating that decreased postharvest life was most likely a result of desiccation.

*8-hydroxyquinoline sulfate (8-HQS)*. Four species, including *Buddleia davidii, Ilex crenata, Ilex* 'Nellie R. Stevens', and *Myrica cerifera*, experienced decreased vase life when stored in 8-HQS. The difference in vase life may be due to two factors. Stems for the two experiments were harvested on different days (Morisot et al., 1998). Systema-Kalkman (1991) found that *Syringa vulgaris* stems harvested at the end of the season had higher resistance to water flow. The second factor may be that the low pH of 8-HQS had a negative impact on vase life. Stamps (1985) saw similar results with cut stems of English ivy (*Hedera helix)* and croton (*Codiaeum variegatum*) placed in floral preservative with a pH of 2.8. The preservative decreased vase life by 51% and 69% for ivy and croton, respectively. Similar results were seen using Rogard® (Gard/Rogard, Carpentersville, Ill.) which contains 8-HQS, on tulips (Staby et al., 1978) and roses (Greer et al., 2004). Jones and Hill (1993) found that the vase life of *Alstroemeria aurantiaca* 'Mona Lisa' and *Tulipa hybrida* 'Apeldoorn' was significantly reduced by sodium dichloroisocyanurate (DICA), a germicide commonly used in floral preservatives.

Contrary to species such as roses (Durkin, 1979; van Doorn and Perik, 1990), *Gypsophila paniculata* (D'Hont and Langeslag, 1995), and *Gloriosa rothschildiana* (Jones and Truett, 1992), higher solution pH did not decrease vase life for any of the species tested. Similarly, Han et al. (1990) saw no relationship between low pH and cut brodiaea (*Tritelexia laxa* 'Queen Fabiola') flowers. According to Vamos-Vigyazo (1981), enzymes involved in polymerization processes leading to deposition of lignin and suberin are inhibited

at low pH. Since lignin and suberin are already present in great amounts in woody stems, low pH may not be necessary to extend the vase life of woody cuts.

Flowering stems. For *Hydrangea quercifolia* and *Viburnum tinus*, treatments that caused higher solution uptake also had longer vase life. The 10% sucrose pulse decreased solution uptake for *Buddleia davidii* and *Hydrangea quercifolia*, and darkened the foliage of *Viburnum tinus*. Durkin (1980) found that normally sluggish lateral movement of water in woody tissues is retarded even further after desiccation. Sucrose also slows rehydration of cut roses if stems are placed into a sucrose solution immediately after cutting. Durkin (1980) found that adding sucrose to acidified water significantly decreased solution uptake, and he surmised that sucrose slows the lateral movement of water through vessel walls. Therefore, we do not recommend pulsing with 10% sucrose for any of the flowering species tested.

Foam significantly reduced the vase life of all flowering species, similar to results with *Campanula medium* (Bosma, 2001). Although none of the woody stems exhibited cold injury, dry cold storage in light significantly reduced vase life for all species. The stomata may have closed fully in the dark, as was the case with cut roses in a study conducted by Slootweg and van Meeteren (1991). We recommend storing stems wet in the dark rather than holding them wet in light.

*Buddleia davidii* 'Royal Red'. Our findings were similar to those of Krentz and Behe (1995), who found that stems held in preservative yielded a 6.8 d vase

life. We agree with Nowak and Rudnicki (1990), who recommended holding *Buddleia* wet at 3 to 4°C.

Some species such as *Gladiolus* (Serek et al., 1994) experience a climacteric rise in respiration and ethylene production. Redman et al. (2002) found that applying exogenous ethylene did not affect vase life of *Buddleia*, confirming that senescence in *Buddleia* is ethylene-insensitive. However, data herein showed that the 1-MCP treatment yielded a 1.0 d longer vase life than the control. It is possible, therefore, that endogenous ethylene levels are important in determining vase life.

Regardless of treatment, all flowers in the panicle bloomed, indicating that stems may have been harvested too late. Cutting immediately after the first floret opens might allow all florets to open and produce a longer vase life.

*Hydrangea quercifolia.* Although stems in the warm water treatments did not have significantly longer vase life from the control, they did have longest vase life. This agrees with Kasperski (1956), who recommended searing or boiling the cut stem ends of *H. quercifolia*, and Byczynski (1997), who recommended immersing stems in 38°C water for extended vase life. A 38ºC temperature may have yielded better results than 50°C. Warm water treatments may have adversely affected stems by causing protein and membrane disintegration at the base of the stem.

It is uncertain whether endogenous ethylene was due to aging, wounding, pollination, or a combination. In many species ethylene levels rapidly rise just prior to abscission and senescence, referred to as the climacteric peak. *H.*

*quercifolia* did not respond to 1-MCP, suggesting that ethylene may have already peaked before harvest.

The large petal area of *H. quercifolia* may have contributed to its short vase life. The number of stem stomata may have also influenced water uptake and vase life, since water uptake is affected by number of stomata in rose stems (with few stomata and low uptake) and carnation stems (with many stomata that account for 40% of water uptake) (Carpenter and Rasmussen, 1974).

*Viburnum tinus*. The 10% sucrose pulse may have supplied carbohydrates above the level that would be provided by the plant if the stem were still attached, and this oversupply resulted in foliage discoloration. Carbohydrates may be accumulating in cell tissues rather than causing membrane disintegration (Bieleski et al., 1992). For flowers kept in high light situations, discoloration might be avoided. *Protea eximia* cut stems exhibited leaf spotting when oversupplied with sucrose, but spotting was not seen on plants with high endogenous carbohydrate levels due to high-light conditions during growth (Bieleski et al., 1992). Another remedy is to hydrate flowers in a solution that does not contain sucrose before placing them in a sucrose-containing solution (Sacalis, 1993).

Fruiting species. Postharvest life ranged from 1 week for *Pyracantha coccinea* to 11.6 weeks for *Ilex* 'Nellie R. Stevens'. No pre-treatment extended the vase life of any of the fruiting species. Although not significantly different from the control, the 10% sucrose pulse had highest uptake and vase life in *Ilex* 'Nellie R. Stevens' but caused extremely low solution uptake in *Pyracantha*

*coccinea*. In the sucrose holding solutions, higher concentration of sucrose led to increased solution uptake in *L. sinense* but caused the opposite in *P. coccinea*. The *P. coccinea* fruit may have been more mature than the other species, thus demanding fewer carbohydrates for additional fruit development and ripening. Fruit on *I.* 'Nellie R. Stevens' appeared to be less mature than fruit on the other two species, and the vase life of the *Ilex* stems was significantly increased by sucrose holding solutions. The additional carbohydrate supplied by the sucrose may have aided the fruit in final ripening. Thus, stems cut before fruit are fully mature may benefit from sucrose holding solutions. The 2% and 4% sucrose holding treatments provided the longest vase life overall for fruiting species. Although more research needs to be conducted, we recommend sucrose pulsing or holding solutions for fruiting species, especially when fruit is less mature. Stems held in foam, regardless of sucrose concentration, had shorter vase life compared to no-foam treatments. Interestingly, ethylene levels were very low for all fruiting species, suggesting that these species are non-climacteric.

Foliage species. Pre-treatments did not extend the vase life of *Ilex crenata* or *Buxus sempervirens*. For *Ilex crenata* and *Buxus sempervirens*, the 10% sucrose pulse decreased solution uptake but had no effect on vase life. For *Myrica cerifera*, solution uptake was not affected by pulsing but vase life was short due to foliage discoloration. For foliage species, we do not recommend sucrose pulsing or holding treatments. Foam had less detrimental effects on vase life of foliage cuts than with flowering and fruiting species. We agree with

Nowak and Rudnicki (1990), who recommended storing *Buxus sempervirens* wet at 2 to 4°C for 1 to 2 weeks.

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Table 1. Visual quality ratings of cut stems from nine species of woody plants.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| | | ***Buddleia davidii* 'Royal Red'** | | |
| 25-50% of florets open, colored buds | 51-100% of buds opening, <20% of florets brown | Wilting of stem tip, 20-49% of florets brown | Wilting of stem, 50-89% of florets brown | >90% of florets brown |
| | | ***Hydrangea quercifolia*** | | |
| Up to 50% of florets open, no discoloration | 51-100% of florets open, <10% of florets with brown spots | Lower florets wilted, brown, or spotted | Significant spotting or wilting of florets (50%) | >90% of florets brown or wilted |
| | | ***Viburnum tinus* 'Spring Bouquet'** | | |
| 25-50% of florets open, colored buds and no florets dropped | 25-100% of florets open, 1-10% of florets dropped, no discolored buds | Buds opening, 11-50% of florets dropped, slight wilting of cyme | Significant wilting of cyme, 51-90% of florets dropped | >90% of florets brown or dropped |

Table 1 (continued)

| | | | | |
|---|---|---|---|---|
| ***Ilex* 'Nellie R. Stevens'** | | | | |
| Fruit partially to fully colored with 0-4% brown discoloration | 5-10% of fruit discolored or dropped | 10-30% of fruit discolored or dropped | 31-50% of fruit discolored or dropped | >50% of fruit discolored or dropped |
| ***Ligustrum sinense*** | | | | |
| Fruit fully colored with 0-4% of fruit desiccated, discolored, or dropped | 5-10% of fruit desiccated, discolored, or dropped | 11-30% of fruit desiccated, discolored, or dropped | 31-50% of fruit desiccated, discolored, or dropped | >50% of fruit desiccated, discolored, or dropped |
| ***Pyracantha coccinea*** | | | | |
| Fruit fully colored with 0-4% of fruit desiccated, discolored, or dropped | 5-10% of fruit desiccated, discolored, or dropped | 11-30% of fruit desiccated, discolored, or dropped | 31-50% of fruit desiccated, discolored, or dropped | >50% of fruit desiccated, discolored, or dropped |

Table 1 (continued)

| | | | | |
|---|---|---|---|---|
| ***Buxus sempervirens*** | | | | |
| 0-2% of leaves yellow or stippled | 3-10% of leaves yellow or stippled | 11-30% of leaves yellow or stippled | 31-50% of leaves yellow or stippled | >50% of leaves yellow or stippled |
| ***Ilex crenata*** | | | | |
| 0-2% of leaves desiccated or dropped | 3-10% of leaves desiccated or dropped | 11-30% of leaves desiccated or dropped | 31-50% of leaves desiccated or dropped | >50% of leaves desiccated or dropped |
| ***Myrica cerifera*** | | | | |
| 0-2% of leaves brown or dropped | 3-10% of leaves brown or dropped | 11-30% of leaves brown or dropped | 31-50% of leaves brown or dropped | >50% of leaves brown or dropped |

Table 2. The effects of pre-treatments on post-treatment vase life (days) and solution uptake (ml) of three flowering species.

| | *Buddleia davidii* 'Royal Red' | | *Hydrangea quercifolia* | | *Viburnum tinus* 'Spring Bouquet' | |
|---|---|---|---|---|---|---|
| Pre-treatment | Vase life | Uptake | Vase life | Uptake | Vase life | Uptake |
| 1-MCP | 6.6 a [z] | 22.0 d | 2.8 | 49.0 bcd | 13.5 a | 110.0 a |
| 10% sucrose pulse | 6.1 ab | 28.5 cd | 2.1 | 32.5 d | 15.0 a | 70.0 bc |
| 50ºC DI water | 6.4 a | 47.5 a | 3.8 | 65.0 b | 8.8 c | 77.0 bc |
| 20ºC DI water | 5.5 b | 36.0 bc | 2.2 | 57.0 bc | 11.3 b | 80.5 b |
| 50ºC tap water | 6.1 ab | 43.0 ab | 3.9 | 89.0 a | 9.0 c | 67.5 c |
| 20ºC tap water | 5.6 b | 32.0 c | 2.1 | 44.5 cd | 10.0 b | 54.5 d |
| Significance | * [y] | *** | NS | *** | *** | *** |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Table 3. F-test probability (*P*) for the effects of 0, 1, 2, or 3 weeks 5°C storage on flowering stems in 12-h·d$^{-1}$ light or 24-h·d$^{-1}$ dark, in distilled water or packed in cardboard boxes lined with clear polyethylene sheeting.

| | *Buddleia davidii* 'Royal Red' | *Hydrangea quercifolia* | *Viburnum tinus* |
|---|---|---|---|
| Light (L) | 0.2865 | 0.0947 | 0.0067 |
| Storage condition (C) | 0.0001 | 0.0445 | 0.0001 |
| Storage time (W) | | | |
| Linear | 0.0001 | 0.0001 | 0.0001 |
| Quadratic | 0.0001 | 0.0001 | 0.1656 |
| Residual | 0.1985 | 0.0140 | 0.9330 |
| L*C | 0.1364 | 0.3666 | 0.1895 |
| L*W | 0.2465 | 0.4820 | 0.0001 |
| C*W | 0.0001 | 0.6708 | 0.0001 |
| L*C*W | 0.3813 | 0.9386 | 0.0016 |

Table 4. The post-treatment vase life (days) of three flowering species as affected by foam and sucrose holding solutions.

| Holding solutions | *Buddleia davidii* 'Royal Red' | *Hydrangea quercifolia* | *Viburnum tinus* 'Spring Bouquet' |
|---|---|---|---|
| No foam | 4.1 a[z] | 3.1 a | 14.2 a |
| Foam | 2.8 b | 1.1 b | 9.0 b |
| *Significance* | *** [y] | *** | *** |
| 0% suc. + 8-HQS | 3.7 | 2.5 | 9.0 b |
| 2% suc. + 8-HQS | 3.4 | 1.9 | 12.0 a |
| 4% suc. + 8-HQS | 3.4 | 1.9 | 13.8 a |
| *Significance* | NS | NS | *** |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Foam x sucrose interactions were nonsignificant.

Table 5. Endogenous ethylene production by nine species of woody cut stems after 48 hours. Means are an average of 15 stems.

| Species | Ethylene ($\mu L \cdot L^{-1} \cdot gfw^{-1}$) |
|---|---|
| Flowering species | |
| *Buddleia davidii* 'Royal Red' | 24 |
| *Hydrangea quercifolia* | 42 |
| *Viburnum tinus* | 41 |
| Fruiting species | |
| *Ilex* 'Nellie R. Stevens' | 13 |
| *Ligustrum sinense* | 9 |
| *Pyracantha coccinea* | 5 |
| Foliage species | |
| *Buxus sempervirens* | 3 |
| *Ilex crenata* | 85 |
| *Myrica cerifera* | 16 |

Table 6. The effects of pre-treatments on post-treatment vase life (weeks) and solution uptake (ml) of three fruiting species.

| | *Ilex* 'Nellie R. Stevens' | | *Ligustrum sinense* | | *Pyracantha coccinea* | |
|---|---|---|---|---|---|---|
| Pre-treatment | Vase life | Uptake | Vase life | Uptake | Vase life | Uptake |
| 1-MCP | 10.5 | 230.0 c[z] | 2.8 | 84.0 | 1.3 | 58.0 a |
| 10% sucrose pulse | 11.6 | 308.0 a | 3.0 | 63.0 | 1.0 | 13.0 b |
| 50ºC DI water | 8.8 | 257.5 bc | 2.9 | 64.5 | 1.2 | 59.0 a |
| 20ºC DI water | 10.4 | 290.0 ab | 2.5 | 64.5 | 1.2 | 55.5 a |
| 50ºC tap water | 9.0 | 229.5 c | 2.4 | 55.0 | 1.0 | 48.5 a |
| 20ºC tap water | 10.1 | 219.5 c | 3.2 | 79.0 | 1.0 | 50.5 a |
| *Significance* | NS [y] | ** | NS | NS | NS | *** |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Table 7. F-test probability (*P*) for the effects of 0, 1, 2, or 3 weeks 5°C storage on fruiting stems in 12-h·d$^{-1}$ light or 24-h·d$^{-1}$ dark, in distilled water or packed in cardboard boxes lined with clear polyethylene sheeting.

| | *Ilex* 'Nellie R. Stevens' | *Ligustrum sinense* | *Pyracantha coccinea* |
|---|---|---|---|
| Light (L) | 0.0515 | 0.0007 | 0.1523 |
| Storage condition (C) | 0.0001 | 0.0001 | 0.3058 |
| Storage time (W) | | | |
| Linear | 0.0001 | 0.0001 | 0.4094 |
| Quadratic | 0.0009 | 0.0002 | 0.1523 |
| Residual | 0.7410 | 0.0606 | 0.7831 |
| L*C | 0.0003 | 0.0362 | 0.3058 |
| L*W | 0.0520 | 0.0260 | 0.0339 |
| C*W | 0.0001 | 0.0008 | 0.6124 |
| L*C*W | 0.0010 | 0.0218 | 0.1630 |

Table 8. The post-treatment vase life (weeks) of three fruiting species as affected by foam and sucrose holding treatments.

| Holding solutions | *Ilex* 'Nellie R. Stevens' | *Ligustrum sinense* | *Pyracantha coccinea* |
|---|---|---|---|
| No foam | 5.7 a[z] | 2.7 a | 1.4 a |
| Foam | 4.0 b | 1.6 b | 1.0 b |
| *Significance* | ***[y] | *** | ** |
| 0% suc. + 8-HQS | 4.1 b | 1.8 c | 1.3 |
| 2% suc. + 8-HQS | 5.4 a | 2.5 a | 1.2 |
| 4% suc. + 8-HQS | 5.0 a | 2.2 b | 1.2 |
| *Significance* | ** | *** | NS |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Foam x sucrose interactions were nonsignificant.

Table 9. The effects of pre-treatments on post-treatment vase life (weeks) and solution uptake (ml) of three foliage species.

| | *Buxus sempervirens* | | *Ilex crenata* | | | *Myrica cerifera* | | |
|---|---|---|---|---|---|---|---|---|
| Pre-treatment | Vase life | Uptake | Vase life | Uptake | | Vase life | | Uptake |
| 1-MCP | 7.0 | 58.5 | 8.4 | 314.0 | a[z] | 8.8 | ab | 94.5 |
| 10% suc pulse | 7.8 | 38.5 | 9.2 | 98.0 | d | 2.2 | d | 95.0 |
| 50ºC DI water | 8.1 | 44.0 | 7.6 | 237.5 | b | 7.5 | bc | 77.5 |
| 20ºC DI water | 6.9 | 52.0 | 9.3 | 325.0 | a | 9.9 | a | 86.0 |
| 50ºC tap water | 6.6 | 48.5 | 6.6 | 174.5 | c | 6.0 | c | 66.0 |
| 20ºC tap water | 6.6 | 42.0 | 10.9 | 258.5 | b | 7.8 | bc | 101.0 |
| *Significance* | NS[y] | NS | NS | *** | | *** | | NS |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Table 10. F-test probability (*P*) for the effects of 0, 1, 2, or 3 weeks 5°C storage on foliage stems in 12-h·d$^{-1}$ light or 24-h·d$^{-1}$ dark, in distilled water or packed in cardboard boxes lined with clear polyethylene sheeting.

| | *Buxus sempervirens* | *Ilex crenata* | *Myrica cerifera* |
|---|---|---|---|
| Light (L) | 0.4293 | 0.0045 | 0.0001 |
| Storage condition (C) | 0.7252 | 0.0001 | 0.0001 |
| Storage time (W) | | | |
| Linear | 0.0001 | 0.0001 | 0.0001 |
| Quadratic | 0.1610 | 0.0002 | 0.0001 |
| Residual | 0. 7832 | 0.0011 | 0.3618 |
| L*C | 0.8604 | 0.0001 | 0.0001 |
| L*W | 0.2682 | 0.0002 | 0.0347 |
| C*W | 0.0281 | 0.0001 | 0.0001 |
| L*C*W | 0.0407 | 0.0001 | 0.0075 |

Table 11. The post-treatment vase life (weeks) and solution uptake (ml) of three foliage species as affected by foam and sucrose holding treatments.

| Holding solutions | *Buxus sempervirens* | *Ilex crenata* | *Myrica cerifera* |
|---|---|---|---|
| No foam | 6.5 | 4.2 a [z] | 3.5 a |
| Foam | 6.3 | 1.9 b | 2.9 b |
| *Significance* | NS [y] | *** | * |
| 0% suc. + 8-HQS | 7.5 | 3.2 | 4.1 a |
| 2% suc. + 8-HQS | 6.5 | 3.2 | 2.8 b |
| 4% suc. + 8-HQS | 5.4 | 2.9 | 2.7 b |
| *Significance* | NS | NS | *** |

[z] Means with different letters are significantly different at $P<0.05$.

[y] NS, *, **, *** Not significant, significant at $P=0.05$, 0.01, and 0.001, respectively.

Foam x sucrose interactions were nonsignificant.

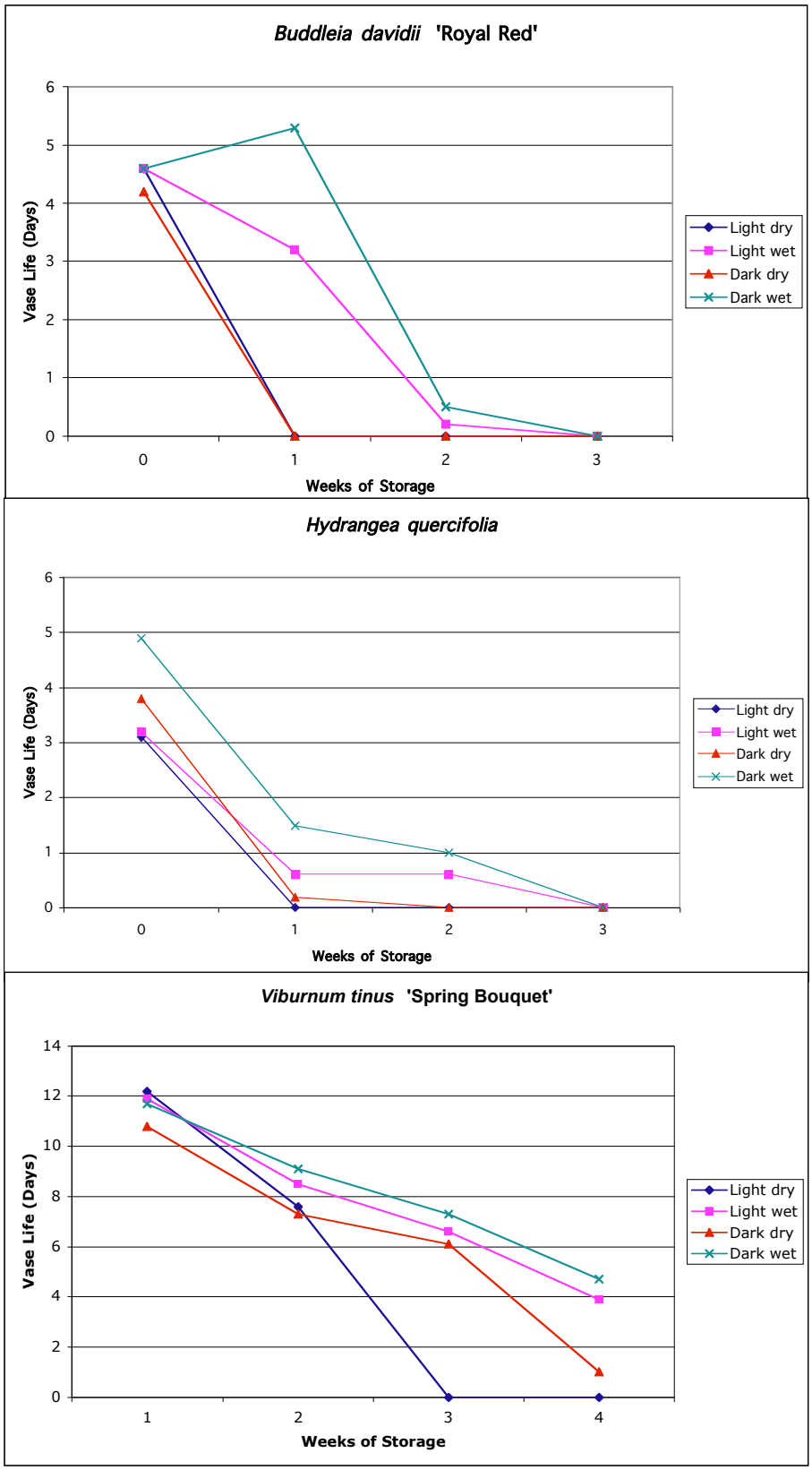


Fig. 1. Vase life of three flowering species *Buddleia davidii* 'Royal Red'; *Hydrangea quercifolia,* and *Viburnum tinus* 'Spring Bouquet' after 0 to 3 weeks storage at 5ºC.

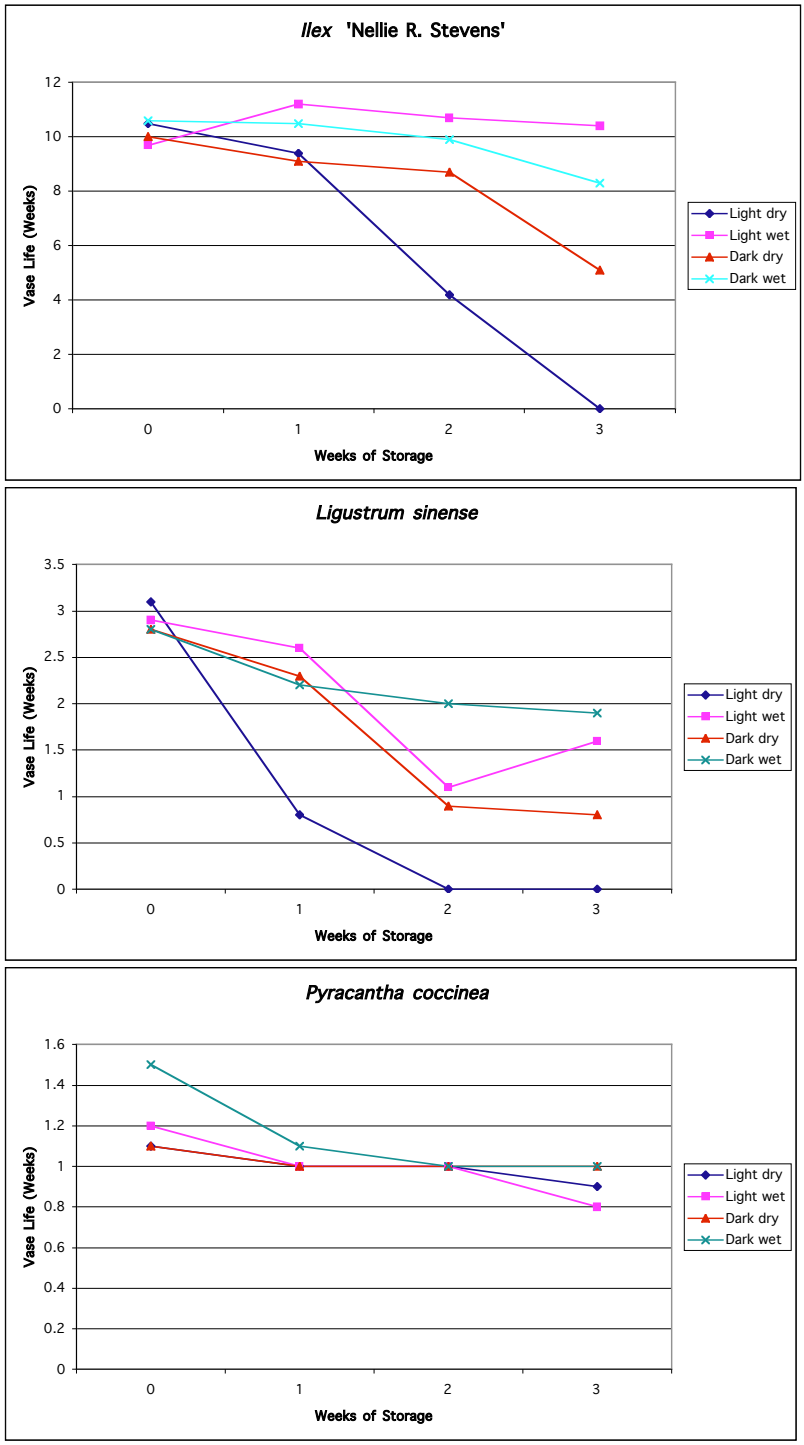


Fig. 2. Vase life of three fruiting species *Ilex* 'Nellie R. Stevens'; *Ligustrum sinense,* and *Pyracantha coccinea* after 0 to 3 weeks storage at 5ºC.

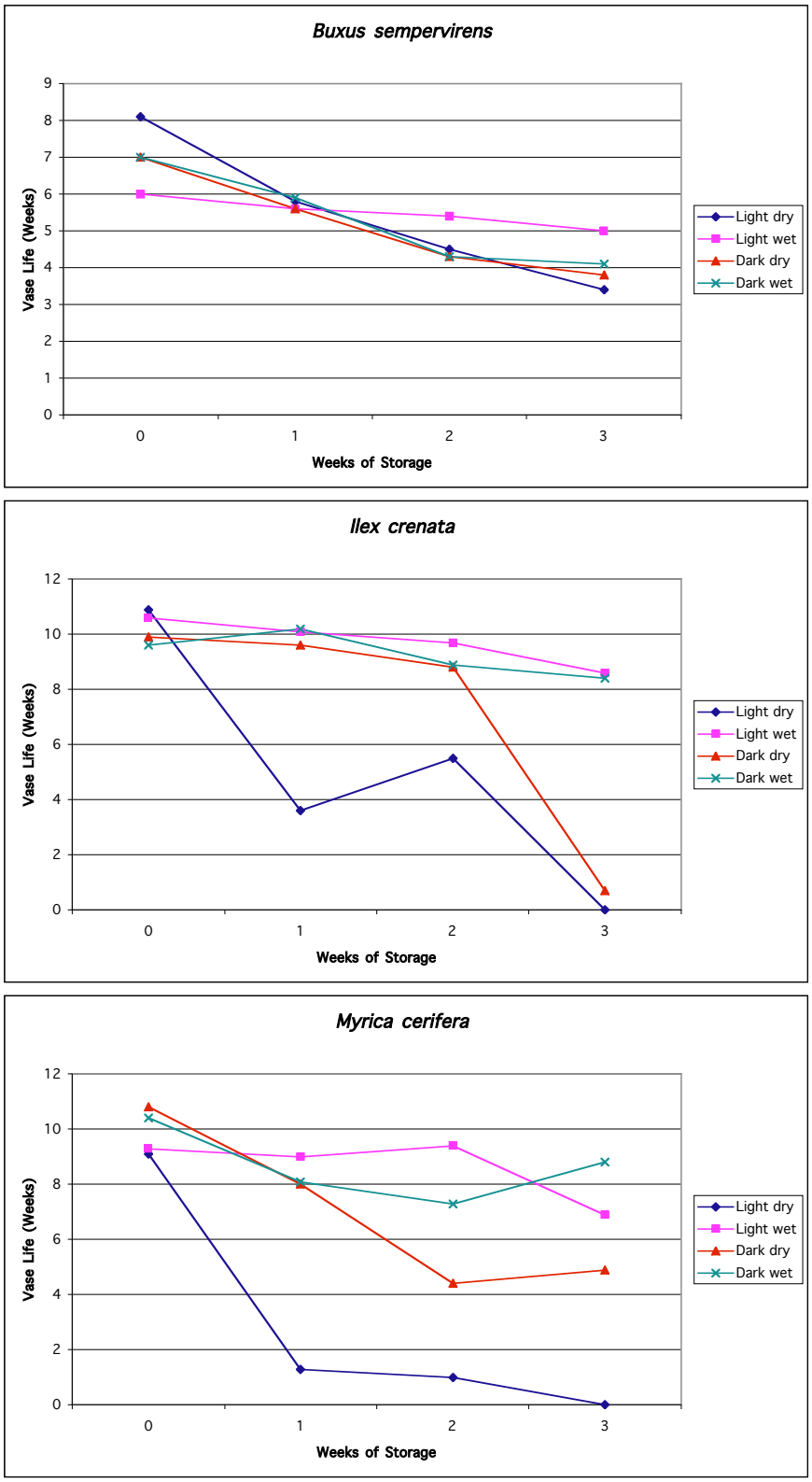


Fig. 3. Vase life of three foliage species *Buxus sempervirens, Ilex crenata,* and *Myrica cerifera* after 0 to 3 weeks storage at 5ºC.

## CHAPTER TWO.

## PRE-HARVEST CHEMICAL AND POSTHARVEST ENVIRONMENTAL DEFOLIATION OF WOODY CUT STEMS.


### Abstract

Six defoliants were applied in fall and tested for their efficacy in pre-harvest defoliation of field-grown *Ilex verticillata* 'Winter Red', *Celastrus scandens,* and *Salix matsudana* 'Tortuosa'. Defoliants included acetic acid (Burnout) at 1500, 2000, 3000, or 4000 mg·L$^{-1}$; chelated copper (Captain) at 100, 200, 400, or 800 mg·L$^{-1}$; crop oil concentrate (COC) at 1% (v/v); ethephon (Florel) at 250, 500, 1000, or 1500 mg·L$^{-1}$; dimethipin (Harvade) at 1600 mg·L$^{-1}$ plus 1% (v/v) COC; pelargonic acid (Scythe) at 100, 250, 500, 1000, or 1500 mg·L$^{-1}$; and a tap water control. Captain at 800 mg·L$^{-1}$ provided 100% defoliation of *C. scandens* with no fruit drop. Captain at 400 mg·L$^{-1}$, Florel at 1000 mg·L$^{-1}$, and Harvade provided up to 80% defoliation of *C. scandens* with <10% fruit drop. No treatment resulted in defoliation of *I. verticillata*. Harvade provided 75% defoliation of *S. matsudana*. In a second experiment conducted in the spring with containerized *S. matsudana*, irrigation was stopped for 0, 3, or 6 d before defoliants were applied. Harvade was effective in promoting 88% leaf drop, but none of the irrigation treatments promoted defoliation. No treatment adversely affected plants. Postharvest defoliation of *S. matsudana* 'Tortuosa' and *Callicarpa americana* was also examined. Cut stems were held in distilled (DI) water at 5, 20, or 35ºC for 1, 3, 5, or 7 days. Holding cut stems of *S. matsudana*

at 20°C promoted 68% defoliation, compared to 53 or 28% for 5 or 35°C, respectively. Neither holding time nor temperature promoted defoliation of *C. americana*.

**Introduction**

Woody cuts valued for their fruit or stem are usually sold without foliage. Removal of foliage prior to harvest results in less postharvest debris and more aesthetically pleasing stems. Currently, woody cuts are defoliated either by hand or by pre-harvest spraying of defoliants or desiccants used to facilitate mechanical harvesting of crops such as cotton (*Gossypium hirsutum*) and grapes (*Vitis vinifera*) (Yang, 1986). Defoliants are also used to facilitate the loosening of fruit from trees prior to mechanized harvesting or to synchronize fruit ripening (Cooper et al., 1968; Edgerton and Blanpied, 1968). Defoliation of cut stems can extend vase life by reducing water loss (Carpenter and Rasmussen, 1974).

Defoliants and desiccants are contact herbicides of variable phytotoxicity (Morgan, 1985). There are two types of defoliants and desiccants: non-hormonal and hormonal. Non-hormonal defoliants cause extensive damage to the plant resulting in foliar chlorosis, necrosis, and desiccation. Injury caused by non-hormonal defoliants can stimulate wound-induced ethylene production, reduce auxin levels, and promote water loss and abscisic acid (ABA) synthesis, all of which expedite leaf abscission (Morgan, 1985). Hormonal defoliants such as 2-chloroethylphosphonic acid (ethephon) mimic the natural process of defoliation or leaf abscission (Morgan, 1985) by directly increasing ethylene levels or production (Morgan, 1969; Suttle, 1985).

Defoliants have numerous disadvantages: they are potentially toxic to plants (Morgan, 1985) and humans (Scarborough et al., 1989), have unpleasant odors and high costs (Morgan, 1985), and produce inconsistent defoliation (Cathey, 1980). Thus, there is a need to improve the consistency while reducing the hazards and costs of defoliants and desiccants (Morgan, 1985). Less-toxic herbicides may provide an alternative to defoliants.

Pelargonic acid (Scythe®, Mycogen Corp., San Diego, Calif.) is a contact, nonselective, non-residual foliar herbicide that utilizes a fatty acid found in soil and numerous foods. Pelargonic acid causes extremely rapid desiccation of green tissue. Disruption of the cell membrane results in cell leakage and death of all contacted tissues (Biconet, 1998). Depending on plant size and species, some re-growth may occur. Pelargonic acid is labeled for control of immature annual and perennial dicotyledons and monocotyledons (Biconet, 1998). Pline et al. (2000) observed that a 3% (v/v) concentration of pelargonic acid was phytotoxic to common lambsquarters (*Chenopodium album*), sicklepod (*Senna obtusifolia*), and giant foxtail (*Setaria faberi*). As with glyphosate (Roundup®, Monsanto, St. Louis, Mo.), another non-hormonal defoliant, pelargonic acid is most effective when air temperatures are above 27°C (Neal, 1997) and should not be applied within 2 h of rain or irrigation (Biconet, 1998). The recommended concentration for use of Scythe (57% a.i., pH 3.75) as an herbicide is 1:20 or 1:10 (concentrate: water). Pelargonic acid is also used as an herbicide synergist. The addition of 3% (v/v) pelargonic acid to glufosinate improved control of yellow nutsedge (*Cyperus esculentus*) (Pline et al., 2000).

Acetic acid (vinegar) is the active ingredient in Burnout (St. Gabriel Labs, Gainesville, Virginia), another contact herbicide. Extracts from lemon juice are included as an inert ingredient. Acetic acid is a postemergent, nonselective, foliar herbicide recommended for use on immature annual and perennial dicotyledons and monocotyledons. The recommended concentration for use of Burnout (23.15% a.i., pH 1.85) as an herbicide is 1:3 (concentrate:water).

Ethephon (Florel®, Aventis, Bridgewater, N.J.) is applied to foliage, enters the leaf tissue, and degrades under alkaline conditions to produce ethylene, phosphate, and chloride ions (Warner and Leopold; 1969 Yang, 1969). In cotton, ethephon induces defoliation and enhances boll opening. Rasmussen and Cooper (1968) found that ethephon promoted citrus (*Citrus* spp.) fruit abscission but caused “excessive” defoliation. Although ethephon will induce defoliation without injuring the crop, it has limitations. For example, low temperatures inhibit abscission activity, presumably because degradation of ethephon is temperature dependent (Olien and Bukovac, 1978). This is problematic for fall-harvested crops because of decreased air temperatures in the field. In addition, ethephon does not inhibit regrowth after defoliation, and its activity is not consistent. The recommended concentration for use of Florel (3.9% a.i., pH 2.58) as a fruit eliminator is 500 $mg \cdot L^{-1}$ for sweetgum (*Liquidambar styraciflua*), if it is applied during flowering (Banko and Stefani, 1995), but 750 $mg \cdot L^{-1}$ was more effective for defoliation of apple (*Malus* spp.) trees (Larsen, 1973).

Dimethipin (Harvade® 5F, Uniroyal Chemical, Middlebury, Conn.) (2,3-dihydro-5,6-dimethyl-1,4-dithiin-1,1,4,4-tetraoxide), is used as a cotton defoliant

and is a weak inhibitor of regrowth (Brecke et al., 2001). Because dimethipin causes leaf cells to lose water slowly (Cothren et al., 2001), it can be considered a mild desiccant. The recommended concentration for Harvade (48% a.i., pH 8.0) as a cotton defoliant varies with time of year, age of crop, and environmental conditions. Two applications of dimethipin at 200 $mg \cdot L^{-1}$, combined with 2% Dupont-WK surfactant, successfully defoliated *Ficus carica* nursery stock (Dozier et al., 1987). Dimethipin applied at 1600 $mg \cdot L^{-1}$ gave 71% leaf drop and 1% berry drop of *Ilex verticillata* (Banko and Stefani, 1999).

Chelated copper is the primary ingredient of Captain™ (SePro Corp., Carmel, Ind.), registered as an aquatic algaecide. Captain (9.09% a.i., pH 10.1) has no guidelines for use as a defoliant or herbicide. Cupric ethylenediaminetetracetic acid (CuEDTA), another form of chelated copper, at a concentration of 2.3 $mM \cdot L^{-1}$ was effective in increasing ethylene in citrus fruit, producing three times more ethylene than FeEDTA or ascorbic acid (Rasmussen and Cooper, 1968). As with ethephon, CuEDTA provided greater leaf abscission than fruit abscission (Rasmussen and Cooper, 1968).

Environmental changes such as drought can also induce leaf abscission (Osborne, 1989). Reduced carbohydrate levels and water stress induced leaf abscission in several plant species including *Euphorbia pulcherrima* (Dole and Wilkins, 1999) and *Rosa* spp. (Morgan et al., 1990). Postharvest defoliation through manipulation of a controlled environment may be possible.

The overall objective of this project was to find effective, least-toxic methods for early leaf abscission in woody cuts through chemical application or

environmental manipulation, without concurrent fruit drop or plant necrosis. The objectives of the greenhouse study were: (1) to assess spring vs. fall defoliation using five defoliants; (2) to investigate interactions between irrigation cessation and defoliants; (3) to determine the effects of defoliants on regenerative leaf ability. The objective of the postharvest study was to determine the effect of temperature and storage on defoliation of woody cut stems.

**Materials and Methods**

*Pre-harvest defoliation*. In the first experiment, eleven treatments were applied to *Salix matsudana* 'Tortuosa' plants in the field in Raleigh, N.C. The treatments included: Burnout at 1500 or 2000 $mg \cdot L^{-1}$, Captain at 100 or 200 $mg \cdot L^{-1}$, ClawEl crop oil concentrate (Brandt Consolidated, Pleasant Plains, Ill.) (COC) (a surfactant) (1% v/v), Florel at 250 or 500 $mg \cdot L^{-1}$, Harvade at 1600 $mg \cdot L^{-1}$ plus 1% v/v COC), Scythe at 100 or 250 $mg \cdot L^{-1}$, and a tap water control. All plants were sprayed to runoff with a backpack sprayer. Application was made on 2 Sept. 2002, after foliage matured and stem elongation was complete. Air temperatures ranged from 19 to 24°C, relative humidity ranged from 75 to 100%, and no precipitation occurred for 12 d after defoliant application. Data collected included dates of 10% of foliage yellowing, first dropped leaf, and 50%, 75%, and 90% defoliation. The experimental design was a randomized complete block with five replications per treatment.

For the second experiment, twelve treatments were applied to *Celastrus scandens* and *Ilex verticillata* 'Winter Red' in the field in Wisconsin in Oct. 2002, before fruits had fully colored. Plants were sprayed to runoff. Air temperatures

ranged from 5.5 to 17°C; relative humidity was 90 to 100%. Chemicals and concentrations used were: Burnout at 2000, 3000, and 4000 $mg \cdot L^{-1}$; Captain at 200, 400, and 800 $mg \cdot L^{-1}$; Florel at 1000 $mg \cdot L^{-1}$; Harvade at 1600 $mg \cdot L^{-1}$ plus 1% (v/v) COC; Scythe at 250, 500, and 1000 $mg \cdot L^{-1}$; and a tap water control. Data collected included percent defoliation and fruit drop. The experimental design was a completely randomized block with five replications per treatment.

For the third experiment, a greenhouse study was conducted in the spring of 2003 using *Salix matsudana* 'Tortuosa'. Curly willow cuttings were rooted and grown for 5 months in the greenhouse in 13-cm. standard plastic pots containing Fafard 4P potting media (Fafard, Inc., Anderson, S.C.). Plants were hand watered and fertilized weekly with 150 $mg \cdot L^{-1}$ N of Peters 20N-4.4P-16.6K (J.R. Peters, Inc., Allentown, Pa.). Experimental design was completely randomized with 9 defoliant concentrations, 3 irrigation timings, and 8 replications per treatment. Defoliants included Captain at 400 or 800 $mg \cdot L^{-1}$, 1% (v/v) COC, Florel at 1000 or 1500 $mg \cdot L^{-1}$, Harvade at 1600 $mg \cdot L^{-1}$ plus 1% (v/v) COC, Scythe at 1000 or 1500 $mg \cdot L^{-1}$, and a tap water control. For the three irrigation timings, plants were irrigated continuously (control), or irrigation was stopped 3 or 6 d before treatment to simulate mild or severe water stress, respectively. All defoliants were sprayed to runoff using backpack sprayers. Plants were observed daily for two weeks, and again 6 weeks after treatment. Data collected included dates of first dropped leaf, 50% defoliation, and 90% defoliation. At the termination of the experiment, quality ratings of 1 to 5, with 1 having all leaves present and 5 having no leaves present, were made.

*Postharvest defoliation*. In Oct. and Nov. 2002, cut stems *of Callicarpa americana* and *Salix matusdana* 'Tortuosa' were placed for 1, 3, 5, or 7 days at 5, 20, or 35°C and held in distilled (DI) water under cool-white fluorescent lights (30 □mol $m^{-1}$ $s^{-2}$) for 12 h·$d^{-1}$. After treatment, stems were placed in distilled water at 20°C. Ten d after treatment, jars containing stems were tapped three times on an extruded metal bench and percent defoliation recorded. For *Callicarpa,* the number of dropped fruit per stem was also recorded. The experimental design was a 3 x 4 factorial in a completely randomized block design with 10 replications. Data were analyzed by general linear model procedure with means separation by trend analysis (SAS Institute, Cary, N.C.).

**Results**

*Preharvest defoliation*. In the first experiment, only the ethephon and dimethipin treatments produced chlorosis or necrosis (data not shown). Dimethipin applied at 1600 mg·$L^{-1}$ produced significant leaf drop ($P$=0.001), inducing 75% necrosis and desiccation 2 d after treatment (DAT). Slight agitation of the stems resulted in leaf drop. Ethephon applied at 500 mg·$L^{-1}$ produced 10% foliage chlorosis 6 DAT, but the foliage did not exhibit further chlorosis, necrosis, or drop.

In experiment two, no treatment defoliated *I. verticillata* (data not shown). For *Celastrus scandens*, three treatments produced significant ($P$=0.001) defoliation: chelated copper at 800 mg·$L^{-1}$ (100% defoliation), or 400 mg·$L^{-1}$ (50% defoliation), and ethephon at 1000 mg·$L^{-1}$ (36% defoliation) (Fig. 1). Ethephon

encouraged fruit ripening and opening, but no treatment significantly increased fruit drop.

In experiment three, dimethipin (Harvade) promoted 88% defoliation ($P$=0.05) (Fig. 2). No irrigation treatment promoted defoliation (data not shown). There was no interaction between herbicide and irrigation treatments. The oldest leaves dropped first. Next to abscise were the youngest leaves, and middle leaves were last to drop or did not drop.

*Postharvest defoliation*. Holding *Salix* stems at 20°C significantly increased defoliation to 68% ($P$=0.05), compared to 53% for stems held at 5°C and 28% for stems held at 35°C (data not shown). There was a significant temperature by time interaction: stems held at 35°C for 1 d lost 44% of foliage, those held for 3 d lost 48%, those held for 5 d lost 11%, and those held for 7 d lost 8% of their foliage. However, no treatment promoted 90% defoliation. Holding cut *Callicarpa* stems at 5, 20, or 35ºC did not promote leaf drop (data not shown).

**Discussion**

*Pre-harvest defoliation*. In the first experiment, concentrations of all defoliants except dimethipin may have been too low to produce defoliation. The selected concentrations were chosen because no literature was available on concentrations appropriate for defoliation, as opposed to foliar necrosis. Pelargonic acid and acetic acid were formulated to kill annual and perennial weeds, but their effects on woody plants were not known. Similarly, chelated copper has never been used as an herbicide. Chemicals that cause mild injury

to the leaf blade or petiole are usually the most effective defoliants; extremely caustic, toxic, and desiccating types result in rapid tissue inactivation and death (Hall, 1958). Extremely rapid drying prevents formation of an abscission zone. As a result, “leaf sticking” occurs: leaves are completely necrotic but do not fall, negating the purpose of the treatment.

For the second experiment, several factors may have contributed to the lack of results. The effectiveness of a defoliant is influenced by weather conditions: temperature, relative humidity, seasonal rainfall, and precipitation before, during and following application (Logan and Gwathmey, 2002), and by plant factors: vigor, water stress, fruit load, variety, and other factors (Cathey, 1980). Logan and Gwathmey (2002) found that seasonal daily minimum temperatures were the dominant weather factor influencing response of cotton to harvest aids. High night temperatures during the growing season promoted susceptibility to defoliation. Weather conditions during and after application mainly influenced defoliation by hormonal defoliants, whereas the contact-type defoliants were less sensitive (Logan and Gwathmey, 2002). Many contact defoliants have lower minimum activity temperatures (12.7 to 15.6ºC) than hormonal defoliants (15.6 to 18.3ºC) (Hake et al., 1996). Low air temperatures (5.5 to 17ºC) during defoliant application were most likely responsible for poor uptake. Rasmussen and Cooper (1968) found that the abscission activity of ethephon was greatly decreased in cold weather (January application) as opposed to a warm November application.

Treatments were applied on an overcast day. Cathey (1986) found that cloudy weather reduced response to some defoliants. High temperatures and sunlight intensity at the time of application make the waxy layer of the leaf more pliable and speed movement of harvest-aid chemicals through the cuticle (Roberts et al., 1996).

A rain event occurred within an hour after spraying. *Celastrus* plants were treated first and experienced more defoliation than *Ilex*. Defoliants applied to *Ilex* may have been washed off before they were absorbed or translocated. Precipitation shortly following application may wash harvest-aid materials from the foliage, reducing efficiency (Elsner and Taylor, 1978).

The hormone balance model proposes that there are three general phases in a leaf: leaf maintenance, shedding induction, and shedding (Morgan, 1985). During the leaf maintenance phase, auxin, and, to a lesser extent, gibberellins and cytokinins, suppress abscission by maintaining the abscission zone in an immature or juvenile condition and ensuring that the leaf remains attached to the plant (Abeles et al., 1967). During shedding induction, the activity and translocation of these growth-positive hormones from the leaf blade to the petiole decreases, while activity of the senescense-promoting hormones ethylene and abscisic acid (ABA) increases (Morgan et al., 1992). This is followed by the terminal phase, in which various cell wall degrading enzymes cause cell separation in the abscission zone (Campillo and Lewis, 1992). Eventually, with the help of gravitational forces, the leaf is shed (Addicott, 1982; Osborne, 1989).

Leaf age often alters sensitivity to ethylene (Morgan and Durham, 1973; Suttle and Hulstrand, 1991); thus, it is possible that increased sensitivity to ethylene, rather than increased concentrations of ethylene, signals the abscission process. Young leaves do not normally abscise because of their high auxin content. In mature cotton plants, for example, old leaves abscise rapidly and extensively when treated with ethylene (Morgan and Durham, 1973). Older leaves have declining auxin levels and auxin transport capacity. These changes make these leaves more sensitive to a climacteric-like rise in ethylene that occurs just prior to senescence. However, exogenous ethylene applied at high concentrations will cause the abscission of the young terminal leaves of young cotton plants before the mature or fully expanded young leaves (Hall et al., 1957). In *Salix*, the oldest and youngest leaves dropped before middle leaves, which is similar to leaf abscission of *Fagus sylvatica* (Addicott, 1982).

*Postharvest defoliation*. Cold and heat can induce leaf abscission when brought on gradually, but severe or immediate introduction to extreme temperatures can induce the opposite reaction (Addicott, 1982). Cool temperatures, such as a light frost, encourage changes in leaf metabolism and lead to abscission. Extremely low temperatures can injure or kill the abscission zone cells, so that physiological separation cannot occur. Plant response to high temperatures is analogous (Addicott, 1982). Additionally, water stress brought on by high temperatures can induce abscission, but only if the abscission zone cells remain viable. The 5 and 35°C treatments used in this study may have produced more defoliation if the stems had been introduced to the different

environments more gradually. It is possible that stems cut during the middle of the growth season had not formed an abscission zone, or that the extreme temperatures rapidly killed existing abscission zone cells.

**Summary**

From our whole plant studies, we determined that defoliation was dependent on several factors, including plant water stress, air temperature, precipitation following application, type of chemical, concentration of application, season of application, and plant species. Ideally, plant water stress would be avoided by providing sufficient irrigation throughout the growing season. Air temperatures of 24 to 32ºC and a relative humidity of 85 to 95% are ideal for herbicidal uptake. In order for the herbicide to dry slowly but thoroughly, precipitation should not occur for at least 4 h following application of defoliants.

Efficacy of defoliation using harvest-aid chemicals will depend on type of chemical and concentration. The defoliants that showed promise included ethephon, a hormonal defoliant, and dimethipin and chelated copper, both non-hormonal. Regrowth occurred quickly on plants treated with ethephon and dimethipin. Chelated copper at 800 $mg \cdot L^{-1}$ provided 100% defoliation of *Celastrus scandens*, whereas lower concentrations were less effective (data not shown). Season of application affects defoliation by affecting air temperature, leaf age, and regrowth potential: a warm fall day would be ideal. Plant species may affect defoliation, but we were not able to determine to what extent.

Future research should address these issues. Chemicals and concentrations that should be tested include ethephon at 1000, 1500, 2000, and

2500 mg·L$^{-1}$, dimethipin at 1200, 1400,1600, and 1800 mg·L$^{-1}$, and chelated copper at 800, 1000, and 1200 mg·L$^{-1}$. This should provide a regression line to determine optimum concentrations of effective chemicals.

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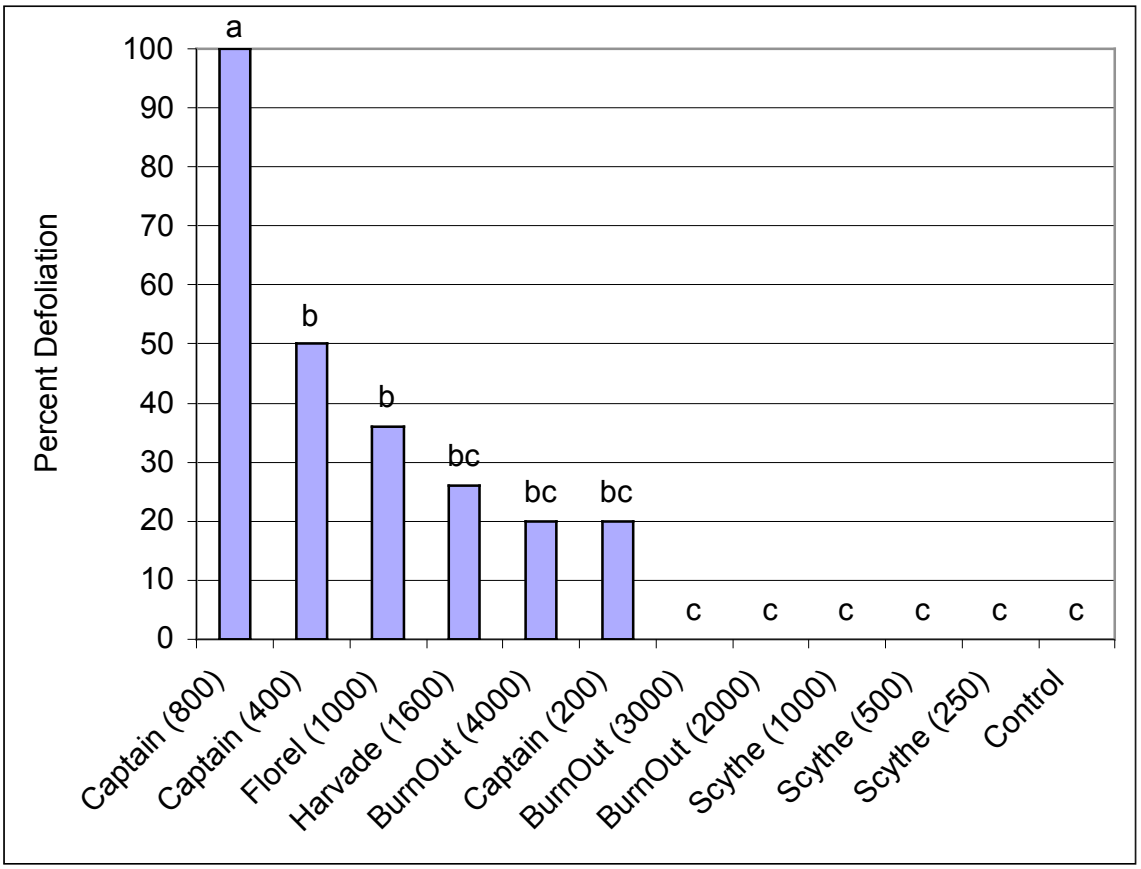


Fig. 1. Percent defoliation of *Celastrus scandens* after herbicides were applied in fall. Means are an average of 5 replications.

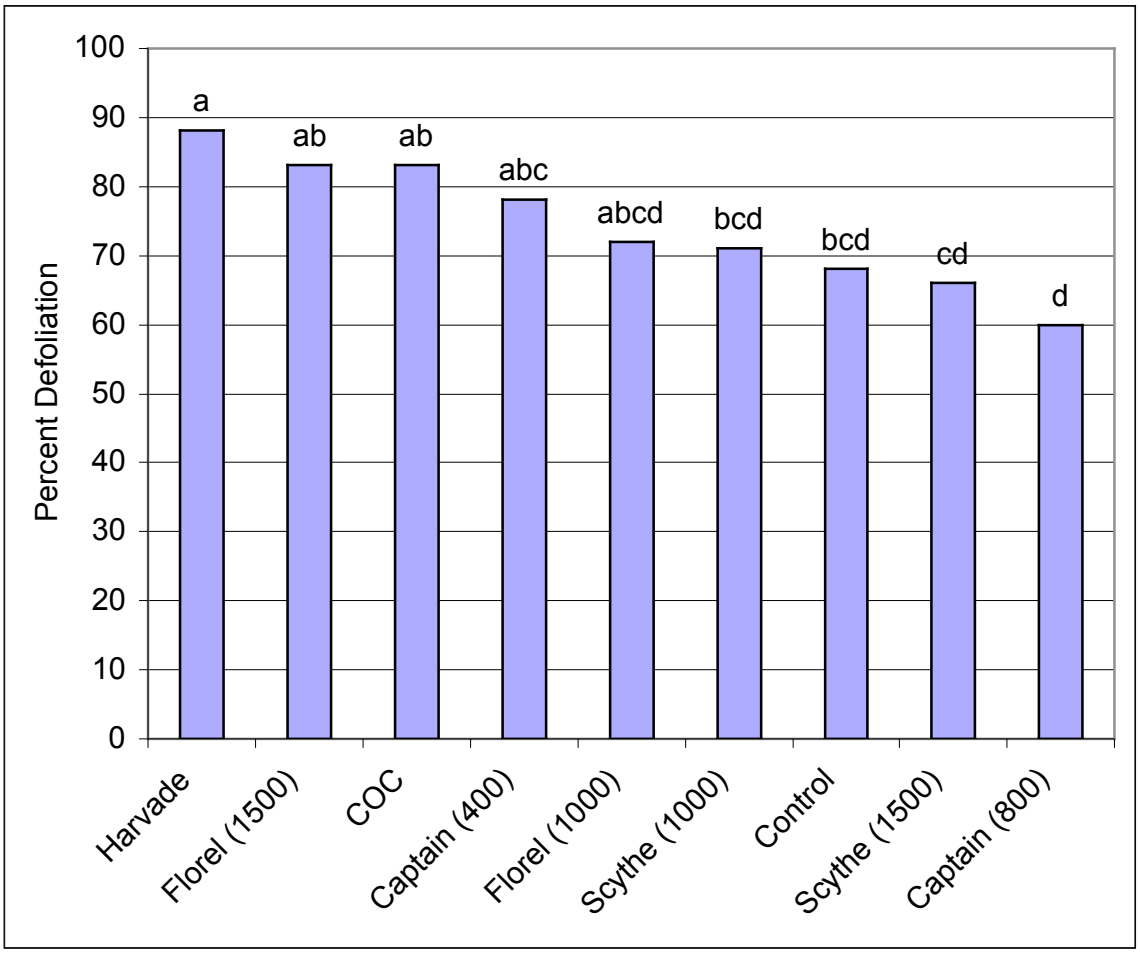


Fig. 2. Percent defoliation of *Salix matsudana* 'Tortuosa' after herbicides were applied in spring. Means are an average of 24 replications.

# CHAPTER THREE.

# COLONIZATION OF *ILEX VERTICILLATA* AND *VIBURNUM TRILOBUM* BY VESICULAR-ARBUSCULAR MYCORRHIZAE.


## Abstract

Vesicular-arbuscular mycorrhizae (VAM) can speed plant establishment and increase shoot length, both of which are desirable characteristics in woody cut stems. Colonization of plant roots by VAM is influenced by plant species, developmental stage, and concentrations of phosphorus (P). Containerized *Viburnum trilobum* and *Ilex verticillata* were inoculated with a commercial formulation containing 7 species of VAM, inoculated with *Glomus etunicatum,* or allowed to remain uninoculated. Phosphorus (P) was applied daily at 0, 25, or 50 $mg \cdot L^{-1}$. Neither plant species was colonized by any VAM species. Phosphorus supplied at 50 $mg \cdot L^{-1}$ was optimal for increasing stem number and length of *Ilex*. For *Viburnum*, 25 $mg \cdot L^{-1}$ was optimal for stem growth, but root area, length, and weight were not affected by P concentration.


## Introduction

Vesicular-arbuscular mycorrhizae (VAM) are naturally-occurring, soilborne symbiotic fungi that colonize plant roots, forming either vesicles (sack-like structures) or arbuscules (tree-like structures) within and between the plant's root cells (Gerdemann, 1968). Vesicles contain oil droplets and serve as temporary storage organs (Gerdemann, 1975). Arbuscules are highly branched and are the site of nutrient exchange between mycorrhizae and plant.

Plant root exudates provide mycorrhizae with photosynthetically derived organic carbon (Harris and Paul, 1987). In return, mycorrhizae decrease the effects of environmental stress in several ways. VAM aid plants in water and nutrient uptake, especially phosphorus, (Gerdemann, 1968; Gerdemann, 1975; Gupta et al., 2000) and may protect plants against pathogens (Gerdemann, 1975).

While approximately 80% of plants form VAM associations, they do so on an inconsistent basis: 50% of species always form mycorrhizal associations, 20% form another type of fungal association such as ectomycorrhizal, and 12% have varying percentages, i.e., they form associations under some conditions but not others (Trappe, 1987). Harley (1959) speculated that anything causing slowed root growth or fewer actively growing roots would increase infection. Thus, vigorous, actively-growing roots are rarely infected until their growth rate decreases.

VAM are usually considered non-specific, so that 150 VAM species infect thousands of plant species. However, numerous VAM species outcompete another. McArthur and Knowles (1993) reported a greater growth response of potato (*Solanum tuberosum*) inoculated with *Glomus intraradices* compared with those inoculated with *G. mosseae*. Schultz et al. (1979) found that four VAM species produced significantly different growth increases on different genetic families of sweetgum (*Liquidambar styraciflua*), although the families were close siblings.

There are numerous difficulties in testing VAM colonization. VAM are ubiquitous in undisturbed soils, and several studies have shown contamination by VAM in non-inoculated crops (Fonseca et al., 2001; Onguene and Habte, 1995; Davies et al., 1987; Bagyaraj and Powell, 1985). Until recently, pure inoculum was difficult to obtain, although commercial sources are now available. An in-depth analysis and evaluation of these inocula are necessary before the practice of applying VAM to containerized plants can be recommended. Correspondingly, little information exists on how to apply these commercial sources. Method of application may affect colonization and efficacy of VA mycorrhizae. Shanmugam et al. (1981) found that inoculum applied to the top 1 to 2 cm of soil provided better results than inoculum applied to the deeper root zone.

Inoculum is most often grown in sterilized sand or a sand mixture, which is dissimilar to native ecosystems. The inoculum is transferred to the plant growing medium used for experimental analysis, again changing the soil environment. Because virtually all native soils have VAM, they must first be sterilized before they can be used. This process changes the biology, chemistry, and physical properties of the soil (Warcup, 1957).

Fertility status, especially soluble P concentrations, of the plant growing medium can impact results. Barea (1991) observed that adding P decreased the amount of external mycelium, the density of arbuscular development, and the number of mycorrhizal entry points.

VAM inoculation had inconsistent effects on plant growth, and this unpredictability may be based on transplant timing in relation to growth phenology of the plant species (Kemery and Dana, 2000). Seasonal variation has been reported for wheat (*Triticum* spp.) (Hayman, 1970), grapes (*Vitis vinifera*) (Deal et al., 1972), and four species of prairie plants (Kemery and Dana, 2000), with infection low at the beginning of the growing season and increasing through summer and autumn.

VAM inoculation at the seedling production stage is a relatively low cost practice (Kemery and Dana, 2000), comparable to fertilization with P (Menge, 1983). However, inoculation with VAM does require labor inputs and establishment of application protocols. Inoculation can only be justified if VAM provide some benefit, such as increased height or diameter, faster plant establishment, improved transplant survival, or superior postharvest life.

VAM may allow growers to use less fertilizer and pesticides. In conventional nursery and greenhouse production systems, woody plants are grown in pasteurized, soilless media, incorporating such components as peat, bark, perlite, and vermiculite. These media do not contain VAM, so nutrient (especially P, Cu, and Zn) and water uptake is less than in soils that have VAM (Menge, 1983; Menge et al., 1978). Plants also do not have the resistance to pathogens provided by mycorrhizae (Schenck and Kellam, 1978). Growers have compensated for the absence of mycorrhizae by increasing fertilizer and water rates (Menge, 1983). Not only is this excess fertilizer expensive, it can also contribute to groundwater contamination. Johnson and Menge (1982) found that

P fertilizer could be reduced by approximately 70%, and N, K, and micronutrients reduced by 30 to 40%, by using VAM in a commercial woody plant nursery operation. Increased shoot length due to inoculation with VAM fungi in greenhouse media has been demonstrated for several woody species, including *Juniperus horizontalis* 'Bar Harbor', *Liquidambar styraciflua, Liriodendron tulipifera, Magnolia grandiflora, Pittosporum tobira*, *Podocarpus macrophylla,* and *Viburnum suspensum* (Bryan and Ruehle, 1976; Crews et al., 1978; Davis et al., 1983; Kormanik et al., 1977; Maronek et al., 1980; Schultz et al. 1979).

Numerous woody plant species are valued as cut stems but require long establishment periods and might benefit from VAM inoculation. *Ilex verticillata* 'Winter Red' has bright red berries that ripen in September (Dirr, 1998). *I. verticillata* is deciduous and drops its leaves in late fall, after the berries are mature. Growers often use cut branches of this plant in Christmas arrangements. Although it is a slow-growing plant, the growth rate can be increased with adequate fertilizer and water (Dirr, 1998).

*Viburnum trilobum* also has bright red berries that ripen in September, but it has a medium growth rate (Dirr, 1998). It is not as widely grown as *Ilex verticillata*, but it has a wide native range (USDA Hardiness Zones 2-7) (Dirr, 1998) and can be used in late fall or Thanksgiving arrangements. The objectives of this research were to inoculate two woody plant species in the greenhouse with vesicular-arbuscular mycorrhizae in hope of enhancing root growth, plant establishment, and shoot length.

**Materials and Methods**

In Experiment 1, rooted cuttings of *Viburnum trilobum* 'Wentworth' and *Ilex verticillata* 'Winter Red' were not inoculated (control) or inoculated with Mycorrhizal Root Dip (BioOrganics, LaPine, Oreg.), a commercial formulation containing a minimum of 50 spores/cc of seven species of VAM spores, namely *Glomus brasilianum, G. clarum, G. deserticola, G. intraradices, G. monosporus, G. mosseae*, and *Gigaspora margarita*. The mixture also contained a hydrophilic gel and was applied following the manufacturer's directions. In Experiment 2, the commercial formulation or *Glomus etunicatum* was applied to *Viburnum trilobum* 'Wentworth' and *Ilex verticillata* 'Winter Red'. *G. etunicatum* was prepared using sand-based media. After 6 months of growth, roots and soil were thoroughly mixed to produce the inoculum, containing infected roots, hyphae and spores. This was placed just below the plant's existing root system. Both inocula were examined and found to contained viable spores.

All cuttings were placed in a greenhouse root medium consisting of 40% bark, 40% peat, 10% perlite, and 10% vermiculite and grown for 4 months in a 3.8-L black plastic pot. Phosphorus fertilizer was applied daily at 0, 25, or 50 $mg \cdot L^{-1}$. In experiment 1, plants were placed in ambient light in the greenhouse and grown from May 2001 to May 2002. For Experiment 2, conditions were the same, except that the experiment was conducted from Nov. 2002 to Mar. 2003. In experiment 1, data collected 3 and 12 months after initiation of the study included shoot number, shoot length, and plant diameter (average of two measurements, the second taken perpendicular to the first). In experiment 2, the

above data were collected every month, as well as shoot fresh and dry weight and percent VAM colonization. To assess VAM colonization, root samples were cleared and stained with trypan blue following procedures by Phillips and Hayman (1970). The experimental design was a completely randomized block with 10 replications per treatment. Data were analyzed by general linear model procedure with means separation by trend analysis (SAS Institute, Cary, N.C.).

**Results**

In experiment 1, P increased shoot length and root weight (data not shown). For *Ilex*, 50 mg·L$^{-1}$ P provided longest stems ($P$=0.001) and highest number of stems ($P$=0.01) after both 3 and 12 months. Although 50 mg·L$^{-1}$ provided greatest root length, area, and dry weight of *Ilex* roots, it was not significantly different ($P$=0.05) from 25 mg·L$^{-1}$. For *Viburnum*, 25 mg·L$^{-1}$ P was optimal but not different from 50 mg·L$^{-1}$ for increasing shoot number and length. *Viburnum* root area, length, and weight were not affected by P concentration. In experiment 2, P did not increase shoot length, shoot number, or root weight of either species (data not shown). Neither the commercial formulation nor *Glomus etunicatum* colonized *Ilex verticillata* or *Viburnum trilobum* in either experiment (data not shown).

**Discussion**

*VAM.* The plant-fungus-soil association necessary for colonization demands close inspection. If the plant has access to all its necessary resources, symbiosis will not develop, as the fungus then becomes a carbon drain (Buwalda and Goh, 1982). The fungus continues to attempt establishment as long as it

has adequate energy. Hyphae resulting from spore germination have a limited capacity to grow and will die if they do not encounter a susceptible root within a week or so (Brundrett, 1991).

Edaphic factors such as organic matter content, source and type of peat, and presence of microorganisms play a key role in determining VAM establishment and colonization. Substrates high in organic matter, such as peat, may not be suitable for VAM formation (Biermann and Linderman, 1983; Johnson and Hummel, 1986). Soilless media characteristics such as pH, soluble salts, and particularly P levels, influence mycorrhizal infection and host plant growth response (Johnson and Hummel, 1986). Davis et al. (1983) found that *G. fasciculatum* colonized 25 to 55% of sweetgum (*Liquidambar styraciflua*) roots, while *G. mosseae* colonized only 3 to 15% of roots, and this difference was due to soil pH. Skipper and Smith (1979) established that *Gigaspora gigantea* infected soybean (*Glycine max*) in acid soil (ph 5.1), but *Glomus mosseae* did not. In natural ecosystems, *Glomus etunicatum* occurs in acidic soils (pH 4.5 to 5.3) with low available P (Bhatia et al., 1996). In our studies, pH ranged from 5.6 to 6.1 for both species; thus, pH may have been too high for effective colonization by *G. etunicatum.*

Biermann and Linderman (1983) found that, in soilless media, mycorrhizal fungi did not colonize plants as extensively and did not increase host P concentration as in soil. This phenomenon occurred with three fungal species (*Glomus fasciculatum, G. mosseae*, and *Acaulospora spinosa*) on both geranium (*Pelargonium hortorum*) and subterranean clover (*Trifolium subterraneum*).

Schultz et al. (1981) found that spore production was increased in soil but significantly depressed in soilless media. Peuss (1958) found that adding peat to field soil decreased plant colonization and growth enhancement by VAM, and Gaunt (1978) noted the same reaction when adding vermiculite. Calvet et al. (1992) found that some types of peat and composted substrates had a negative effective on the establishment of VAM symbiosis. The sphagnum peat used may have contained low concentrations of soluble substances or VAM-suppressive microorganisms that inhibited the establishment and functioning of VAM (Peuss, 1958). Shoot length in apple plants inoculated with two *Glomus* species was increased only after disease-causing microorganisms were eliminated by steam-pasteurizing the soil (Taube-Baabe and Baltruschat, 1993). Mosse (1972) believed that host specificity in VAM, which are usually thought to have wide host ranges, may be determined more by interactions between fungal strains and the soil than between the fungus and the host plant.

Several studies have explored compatibility of VAM species with plant species. With incompatible species, deleterious effects may be seen either in the host or the fungus (Bhatia et al., 1996). Pederson et al. (1991) found that fresh weight of asparagus (*Asparagus officinalis*) plants inoculated with four species of VAM was significantly lower when colonized by *Glomus fasciculatum* and *G. monosporum* than by *G. clarum*, *G. intraradix*, and *G. versifomre,* while *G. vesiculiferum* did not colonize asparagus roots at all. The effectiveness of VAM species may depend on the characteristics of the soil from which they were originally isolated (Pederson et al., 1991). Louis and Lim (1988) detected

different responses in VAM development of two isolates of *G. clarum* obtained from sites with different P levels. The ability of a particular VAM species to colonize a particular plant species depends on the physiological, ecological, and genetic variability of with plant and VAM species (Sharma et al., 1986). There is a fungus-plant 'recognition' process with four stages: (1) cell-to-cell contact and appressorium formation; (2) morphological and structural changes within the fungus that allow root colonization; (3) integration of the physiology of both symbionts; and (4) redistribution of enzymatic activities involved in nutrient exchange between plant and fungus (Gianinazzi-Pearson, 1984; Azcón-Aguilar and Bago, 1994).

Although Crews et al. (1978) showed colonization of *Viburnum suspensum* by *Glomus fasciculatum* and *G. mosseae,* no studies have shown colonization of *V. trilobum* by any VAM species. Maronek et al. (1980) noted that the only way to determine the effectiveness of a specific mycorrhizal fungus to a particular plant species under specific conditions is to test it under those conditions. One species of VAM that colonizes one plant species or cultivar may not colonize another. Schenck et al. (1975) found *Glomus macrocarpus* reduced nematode populations in one soybean cultivar but not in another.

The commercial VAM formulation may not have colonized roots because of physical exclusion due to the hydrophilic gel (J. Morton, personal communication). Although the spores were viable, they were smaller than the gel particles. The gel prevented spores from coming into contact with roots. The

root exudates that trigger spore germination and hyphal growth were thereby unable to reach the spores.

Plants with coarse root systems are more dependent on mycorrhizal association for increased nutrient uptake (Brundrett, 1991). Plants generally do not support both high levels of mycorrhizal colonization and root systems with fine roots, because of the high metabolic cost that would result (Brundrett, 1991). Neither *Ilex verticillata* nor *Viburnum trilobum* have coarse root systems. The activity of the root system, the growth rate, and the ability to respond quickly to changing soil characteristics also determine mycorrhizal dependency (Azcón-Aguilar and Barea, 1997).

Climatic factors affect plant growth as well as fungal growth. In a study conducted with four species of prairie forbs, Kemery and Dana (2000) noted that treatment effects were limited by root morphology, vegetative vigor, and developmental phenology. Increasing vegetative vigor led to greater colonization, and treatment with VAM after flowering had no effect (Kemery and Dana, 2000). In wheat, however, infection did not appear until after flowering (Hayman, 1970). This may be influenced by daylength, temperature, and light intensity (Smith, 1974). Long days increased VAM infection in *Citrus sinensis* (Johnson et al., 1982). Furlan and Fortin (1973) found that day/night temperatures of 21/26°C were optimal for infection, sporulation, and growth increases of *Allium cepa*, while Schenck and Smith (1982) found 30°C optimal for infection of six species of VAM on soybean.

Light intensity influences colonization of VAM. A 50% reduction in light intensity lowered infection on tobacco (*Nicotiana tabacum*) from 85 to 31% (Peuss, 1958). Johnson et al. (1982) found that colonization increased with high light (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared with low light (6 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Low light intensity may increase sporulation rather than hyphal growth (Trappe, 1987). Low light intensity depressed growth of mycorrhizal alfalfa (*Medicago sativa*) and maize (*Zea mays*) (Daft and El-Giahmi, 1978). Low light intensity in greenhouses during winter may reduce mycorrhizal infection (Koch, 1961; Meloh, 1963; Winter and Meloh, 1958), as may have been the case in Experiment 2. Tester et al. (1986) noted that reduced photosynthesis levels decreased the rate of VAM entry-point formation in the host root, suggesting that there may be a link between photosynthates and the initial stages of VAM colonization. In mycorrhizal roots, a substantial portion of photosynthates allocated to the roots is required for fungal growth and maintenance. This cost is crucial in plants with sink limitations, with source capacity unable to meet sink demands. It is possible that insufficient allocation of photosynthates to support VAM symbiosis is a limiting factor for colonization.

The developmental stage at which inoculation occurs may affect colonization. Early establishment of VAM is key to improving plant performance. Greater plant response is often seen when inoculation occurs early in the life cycle, or at the beginning of annual root growth, of a plant (Vidal et al., 1992; Azcón-Aguilar et al., 1992). Colonization will only occur on young secondary roots that are actively growing or recently formed (Barea, 1991).

*Phosphorus effects*. In Experiment 1, there was a direct relationship between P concentration and plant growth. *Ilex verticillata* responded very quickly to fertilization with the highest concentration of P and continued this response throughout the year-long study. *Viburnum trilobum* also responded to P fertilization, but a lower concentration was necessary for optimal shoot growth. This difference between species can be attributed to different morphological characteristics of their root systems: *Ilex* has a very fine, fibrous root system, while *Viburnum* roots grow more slowly and have fewer root hairs. The lack of response to P fertilization in Experiment 2 was most likely due to low light intensity during the winter months.

To conclude, numerous environmental, soil, fungus, and plant factors may have contributed to the poor VAM colonization results obtained in these experiments. Although many of these factors could be changed to promote or enhance VAM colonization, the resulting study may not be relevant to the grower. Providing adequate P is less troublesome and provides more certain results than VAM inoculation.

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