Gibberellins and Cytokinins: a Review©

H. William Barnes
Barnes Horticultural Services LLC, 2319 Evergreen Ave. Warrington, Pennsylvania 18976, USA
Email: BHS16@verizon.net

INTRODUCTION

Gibberellins and cytokinins are considered to be part of the five major key hormones in plants. The others are auxins, ethylene and absisic acid (Chen and Shepley, 1975). All five interact with each other to directly affect cell systems and indirectly by signaling pathways to maintain balanced ratios (Perilli et al., 2010; Perniosavá et al., 2011). Gibberellins (GAs) and cytokinins, are instrumental in many growth processes (Bernier 1988; Chen and Shepley, 1975; Heldt et al., 2011) such as initiation of floral parts, flowering itself, fruiting, leaf and stem morphology, and seed germination. Changes in the ratios of GAs and cytokinins to each other and to the other hormones often result in distinct and divergent morphological features such as dwarfism, contorted or twisted growth, weeping forms, fastigate, and columnar forms and unique leaf forms (Figs. 1 and 2).

Fig. 1. Pendulous form of *Picea abies*.

Fig. 2. *Picea abies* normal form.

With respect to GAs there are over 125 known forms, 100 are found in plants and the remaining others are found in fungi and bacteria (Crafts and Miller, 1974; Crozier et al., 2000). In general they are all derivations of a central chemical structure with varying side chains.

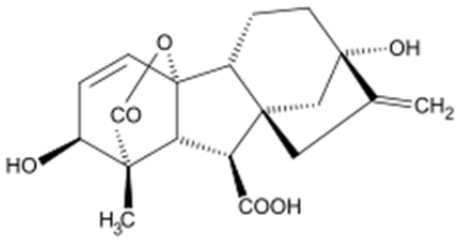

Fig. 3. Gibberellic acid 3.

Cytokinins occur in plants and fungi (Crozier et al., 2000; Heldt et al., 2011) and a rather unique form, kinetin (Barciszewski et al., 2000) which occurs in both humans and plants. All of the natural forms of cytokinins are derived from purine (isoprenoid structures) however, there is another class of chemicals known as phenyl ureas which are structurally different but on many occasions behave strongly as cytokinins (Barciszewski et al., 2000). The best known and most frequently encountered of the phenyl ureas is thidiazuron (Devlin et al., 1989), a totally synthetic cytokinin which has numerous applications in horticulture and agriculture. Another class of chemicals totally distinct from either isoprenoids and phenylureas is the nitroquanidines (Wang, 1996). To date the nitroquanidines are not available except for research use.

NATURALLY OCCURING CYTOKININS IN PLANTS

- N6-(Δ 2-isopentenyl)adenine, 2iP (isoprenoid) (Fig. 4: isoprenoid structure)
- Trans-zeatin, zeatin (isoprenoid)
- Dihydrozeatin (isoprenoid)
- Cis-zeatin (isoprenoid)
- Methoxytopolin, meo T, plus 3 other toplin types (aromatic isoprenoids)
- Kinentin, 6-furfuryladenine (Bernier, 1988)

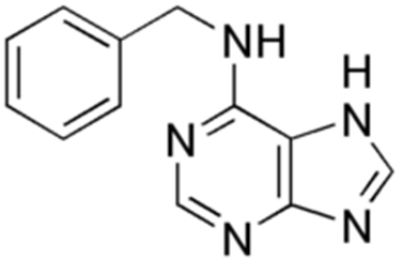

Fig. 4. Isoprenoid structure.

Phenylureas, general formula, side chains vary creating novel forms (Fig. 5).

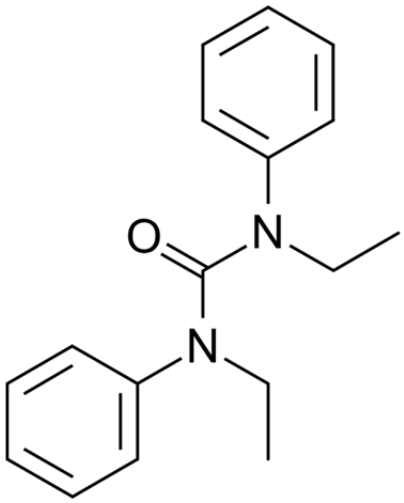

Fig. 5. Phenylurea structure.

Nitroquanidines, general formula (Fig. 6), side chains can vary considerably with significant changes in activity as synthetic cytokinins.

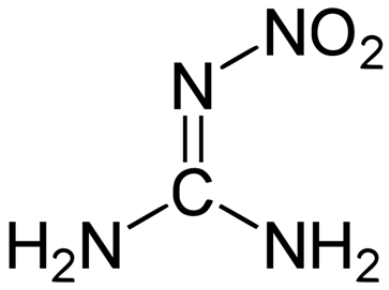

Fig. 6. Nitroquanidine general formula.

In general zeatin is considered to be a natural cytokinin with the highest biological activity followed by 6-benzyladenine (BAP), and 2iP (N6-(Δ 2-isopentenyl)adenine. But derivatives have been made with activities greatly exceeding the conventional natural forms. Research has shown that the formate, acetate, propionate, and indole acetate esters of 2-chlorozeatin have activity 2× that of zeatin. Also 8-methyl benzyl adenine, 8-methyl kinetin and 6-(3-methyl-2-butenylamino)-8-methyl purine are all more active than the naturally occurring forms (Varner and Ho, 1976).

ACTIVITY OF GIBBERELLINS

Of the natural plant forms of gibberellins GA_1, GA_3, GA_4, and GA_7 are the most common (Heldt et al., 2011). While there are other GAs to be found in plant tissues they are most commonly converted to one of the more prominent four types. While the four common GAs (Anonymous, 2013; Al-Juboory-Karim and William, 1990; Barciszewski et al., 2000; Bessler, 1997) are frequently encountered they almost always have different roles in plant tissues. Most important of the four is GA_1 and GA_4. When GA_4 and GA_7 are applied to pines flowering is the primary affect, where as when GA_3 is utilized there is no flowering response. Applied GA_3 in some other plants results in suppression of flowering (Waring and Philips, 1982), whereas GA_4 promotes flowering. In *Lolium* sp. (perennial rye grass) GA_{32} is very active, GA_5 moderately active, GA_3 less so and GA_1, not at all (Bernier, 1988). In some cases the GAs responsible for flowering are essentially ineffective for promotion of stem elongation. Some GAs are known to cause normally female flowering plants to give rise to male flowers (Al-Juboory-Karim et al., 1990; Waring and Philips, 1982).

In some cases GAs are effective only when coupled with altered environmental stresses such as flooding or root pruning. The timing of GA applications is sometimes critical and in dwarf *Pharbitis*, GA_3 is promotive when applied just prior to short day conditions but it is inhibitory after that (Waring and Philips, 1982). In grapevines GA_3 is promotive for flowering at the early stage of floral development but becomes inhibitory once cytokinins take over the process (Bernier, 1988). Oddly the reverse sequence is found in *Fushia* and *Solanum lycopersicum*. Haissig (1972) concluded that GAs play an active role in regulating the actions of auxins in plants and can have an antagonism to auxins affecting the rooting of cuttings. Epicormic shoots are also a result of the interaction of GAs and cytokinins influencing the formation of buds in unusual places (Sachs and Thimann, 1967).

Insects, diseases and environmental damage can alter the GA/cytokinin ratios with each other and with the other hormones (Figs. 7 and 8) (Diener, 1981). When such conditions exist different growth forms are frequent. Some butterfly larvae excrete cytokinins with their saliva to prevent the senescence of the leaves that they are feeding on (Heldt et al., 2011).

Fig. 7. Rose rosette disease causing tissue malformation in *Rosa* 'Radler', Knock Out™ rose.

Fig. 8. *Solidago rugosa* adversely affected by boring insects laying eggs in the stem, cytokinin production and gibberellins balances disturbed resulting in witches broom of the stem.

The main function of GAs is to induce cell growth and stem elongation. They are also influential in perennials and biennials for the formation of rosettes in preparation for flowering the following spring (Heldt et al., 2011; Varner and Ho, 1976) (Fig. 9).

Fig. 9. Rosette formation in *Silphium connatum*, a gibberellin induced response brought on by short days.

Gibberellins are known to induce cone formation in conifers and can be used to initiate flowering in some plants regardless of photoperiodic signals or vernilization treatments (Barciszewski et al., 2000; Bernier, 1988). They also affect seed maturation, fruiting and seed germination (Chen and Shepley, 19757). They can also retard leaf senescence (Barciszewski et al., 2000).

Gibberellins and cytokinins interact with each other and the other major growth hormones to create specific morphological features (Schoene and Yeager, 2005). The weeping forms of *Picea* (Fig. 1) and *Pinus* are but two examples of these types of changes. Graft unions can alter or inhibit either basally or acropetally the transfer of these chemicals in plant systems resulting in the dominance of one type of tissue over another (Figs. 10 and 11).

Since graft unions can alter the flow of these chemicals within plants, suckering can be exacerbated by a flux of bud initiation below the graft union.

Fig. 10. Understock of *Cornus kousa* exhibiting dominance over a grafted portion of the weeping form.

Fig. 11. Graft union increasing the cytokinin balance beneath the union causing a proliferation of buds that can lead to extensive suckering.

Physical changes to plants such as severe browsing, mechanical cutting back, fire, or flooding can cause altered morphological features such as extra large leaves or elongated stems, extensive shoot proliferation (Figs. 12 and 13).

Fig. 12. Epicormic shoots on *Sequoia sempervirens* due to fire damage.

Fig. 13. Extra large leaves on *Paulownia tomentosa* due to gibberellin/cytokinin imbalance caused by stubbing back to the ground.

SEED GERMINATION

Gibberellins are naturally found in high concentrations in immature seeds. As seeds age the ratio of GAs to cytokinins and to absisic acid change so that absisic acid controls germination so that seeds do not germinate at inappropriate times. Cold moist stratification reverses this process and allows the GAs to resume control. However, cold

moist stratification is not the only mechanism for changing gibberellin levels in seed. Dry storage is common in grasses such as *Andropogon virginicus* which often will germinate much better after a prolonged dry storage than when sown fresh.

The use of applied GAs to offset the requirements for cold moist or dry storage is well documented (Schoene and Yeager, 2005; Deno, 1994; Chen and Shepley, 1975; Dunand, 1989; Waring and Philips, 1982; Yan and Dilday, 1993). Gibberellins often will circumvent the need for cold moist stratification but they will also offset dry storage in grasses, requirements for darkness (Dybing and Westgate, 1989) in *Delphinium* and light in Grand Rapids lettuce (Chen and Shepley, 1975). Dunand and others (Dunand, 1989; Yan and Dilday, 1993) found that applied GAs substantially improved the seedling germination and vigor in semi-dwarf rice cultivars.

Gibberellins terminate seed dormancy presumably by changing seed coat permeability (Varner and Ho, 1976) and also by turning on specific enzymes such as the amylases. Commercial brewing practices use the induction of amylases in barley by applied GAs to convert starches to sugars as part of the malting process (Heldt et al., 2011).

Cytokinins too have some role in seed germination (Srinivasan et al., 2006). Kinetin positively affects the germination of *Acer psuedoplatanus*, carpet grass (*Axonopus fissifolius*), clover (*Trifolium* sp.), and light sensitive lettuce (*Latuca* sp.) Kinetin and GA_3 and combinations can overcome absisic acid retardation of the germination in *Latuca* sp. and excised embryos of *Fraxinus* (Chen and Shepley, 1975). Commercially, cytokinins are added to grains germinating for the malting process (Chen and Shepley, 1975) and are used to stimulate germination of rice to insure a more uniform stand in field planting (Heldt et al., 2011; Yan and Dilday, 1993).

Naturally occurring GAs and cytokinins interact on a number of levels. In brief, cytokinins initiate cell division and the creation of lateral bud formation as previously discussed. In concert with GAs new growth is promoted. Principally this is to induce flower formation and flowering structures (Bernier, 1988). In combination with auxins and GAs other types of tissues and plant structures are formed such as roots, fruits, and seeds. Both GAs and cytokinins will alter (Field et al., 1989) the number of flower buds on *Solanum lycopersicum* and *Nicotiana* spp.(Chen and Shepley, 1975). When *Nicotiana tabacum* was treated with a range of cytokinins, kinetin showed tremendous potential at promoting flower formation (Cousson and Tram, 1980). Cytokinins also regulate senescence of fruits, flowers, and leaves by being antagonistic to the effects of ethylene (Heldt et al., 2011). Cytokinins are responsible for the “cancerous” tumors caused by crown gall formation due to the infection from *Agrobacterium tumifaciens* (Heldt et al., 2011). It is interesting to note that cytokinins have an absolute requirement in plants and inspite of massive surveys no plant mutant has been found that is totally deficient in cytokinins, whereas there are examples of the other major hormones being absent (Varner and Ho, 1976).

ROLES FOR EXOGENOUSLY APPLIED GIBBERELLINS AND CYTOKININS

Tissue Culture

One of the most important and prevalent roles of applied cytokinins is in tissue cultures, i.e., without cytokinins there would be no tissue culture of plants (Heldt et al., 2011). In detail cytokinins are known to increase DNA synthesis in the tissue culture process. They also increase the formation of specific enzymes necessary for cellular metabolism (Wang, 1996). Research has shown that BA in tissue culture increases RNA and both soluble and insoluble proteins (Perilli et al., 2010). In general plant tissue culture makes use of the natural occurring cytokinins, benzyladenine, 2iP, kinetin, and zeatin (Heldt et al., 2011). Under some circumstances thidiazuron is used but it is totally synthetic and can alter internal chemical balances. Using cytokinins in plant tissue culture can lead to alteration in the resulting plantlets leading to mutations collectively known as somalclonal variation. Thidiazuron has been implicated as a causal agent in somalclonal variation (Varner and Ho, 1976). In plant tissue culture two plant hormones are of critical

importance, cytokinins and auxins. When the auxin/cytokinin levels are high, root formation is the immediate result. When the reverse is true and auxin/cytokinin levels are low, shoot formation is the probable result.

While GAs are not normally used in tissue culture as it is presumed that tissues will make sufficient amounts for necessary functions, however Varner (Varner and Ho, 1976), found that high cytokinin/gibberellin ratios in tissue cultures results in shortened dark green plants. Tissue culture plants with the reverse, high gibberellin/cytokinin levels result in slender plants with pale long narrow leaves.

Also, work by Geetha et al. (1998) an exception to the general rule found that GAs in tissue culture did have some positive aspect in shoot growth after multiplication.

Other Aspects of Cytokinin Applications

Cytokinins have uses other than tissue culture in a variety of plant production and post harvest systems. Regardless of the photoperiod applied cytokinins can alter flowering times and other morphological changes in plants (Bernier, 1988; Gonzales and Prokakis, 1989; Heldt et al., 2011; Wang, 1996). Benzyladenine (BA) and other isoprenoid cytokinins are used to reduce flower abortion in soybeans (*Glycine max*) (Dybing and Westgate, 1989).

Since cytokinins are known to be inhibitors or antagonistic to the actions of ethylene they are vastly important in the production of cut flowers, cut-flower storage, and potted plants by prolonging the shelf life during transit (Jiang et al., 2009).

Another important aspect of applied cytokinins is the propensity for cytokinins to release lateral buds from dormancy and to initiate new lateral buds (Bessler, 1997; Findley et al., 1994; Garner et al., 1997; Keever, 1994; Keever and Brass, 2013; Kuminek et al., 1987; Tamas, 1988; Varner and Ho, 1976; Yang and Reid, 1992). This technique can be used to create new tissue for cuttings and for clean shoots useful for tissue culture (Yang and Reid, 1992). Tran (Tran and Kiem, 1981) mentions that buds can be initiated in *Begonia* leaves by treatment with the cytokinin 6-(γ,γ-dimethylallylamino)purine (an isoprenoid) (DMAAP).

Sometimes the use of cytokinins is insufficient to create enough secondary shoots and research by Keever and others (Al-Juboory-Karim et al., 1990; Al-Juboory-Karim and Williams, 1990; Foley and Keever, 1993; Schoene and Yeager, 2005; Yang and Reid, 1992) found that combining cytokinins with GAs results in significant increases of secondary buds suitable for propagation.

It should be noted that different cytokinins have different responses in plant tissues (Heldt et al., 2011). Waring and Philips (1982) showed in mosses that benzyl adenine induced bud formation at 1 μM, whereas 2iP caused changes at 100 nM and zeatin acted at 5 nM. The synthetic cytokinin, thidiazuron, is used to break bud in apples (*Malus*) and the natural cytokinins when applied to dormant roses releases buds from dormancy and increases flower numbers over the long haul (Halevy, 1986).

Thidiazuron, a synthetic cytokinin, is known to be especially active (Varner and Ho, 1976) when compared to the natural forms but this activity level makes it ideal for field conditions. Oddly it sometimes behaves in a completely different manner and it is used as a defoliant in cotton plants (*Gossypium* sp.) (Devlin et al., 1989). The synthetic phenyl ureas which have cytokinin activity are under scrutiny as herbicides (Srinivasan et al., 2006). The natural cytokinins, such as BA, 2iP, and kinetin do not initiate such changes in plants.

While cytokinins are generally considered to be antagonists of auxin (Haissig, 1972; Heldt et al., 2011) especially in the rooting of cuttings, Davis and Haissig (1990) suggest that improved rooting of cuttings can be achieved by the use of cytokinins as a foliar application after auxin has been applied to the cuttings. The reasons appear to be enhanced leaf retention and delayed senescence during the rooting process.

Cytokinin manipulation can be achieved by means other than applied chemicals. Transgenic tobacco plants have been developed to have natural excess cytokinins thereby

delaying the otherwise natural senescence of the fully mature leaves (Heldt et al., 2011). This is of great benefit in allocating harvesting sequences.

Exogenous Applications of Gibberellins

Previously mentioned has been the use of GAs for seed germination. In commercial usage GA_3 is ubiquitous (Heldt et al., 2011). However, the use of GAs in seed germination has some cautionary elements. Gibberellins are known all too well to influence the internodal distances in stems (Heldt et al., 2011). Work by this author showed that should GA use in seed germination exceed 1000-2000 ppm excessive stem elongation can occur which results in tall spindly seedlings that cannot support their own weight.

Aside from seed germination, GAs have many practical applications. When applied to adult forms of *Hedera helix* (English Ivy) the adult form can be reverted back to the juvenile form and cease flowering (Varner and Ho, 1976). Gibberellins are often utilized for the production of seedless fruits such as grapes (*Vitis* sp.) (Halbrooks and Crovetti, 1989; Heldt et al., 2011) with substantial increases in fruit size. They can be used to offset the biennial bearing tendencies in apples (*Malus domestica*) and convert them to annual bearing. Golden delicious apples are prone to russetting and GA applications can be used to prevent that disorder. Citrus can be treated with GAs to retard rind -aging and as with grapes to increase fruit size They are also used in cherry (*Prunus avium*) fruit production and in increasing yield in sugar cane (*Saccharum officinarium*) (Sponsel, 2010). The use of GAs to promote flowering has been discussed previously in this paper.

Gibberellin Inhibitors

Because GAs are so ubiquitous in many aspects of plant growth some natural forms of plant growth do not always fit into prescribed production practices. Interference with the actions of auxins as Hassig (1972) mentioned is but one example. Other include excessive growth and flowering initiation that retards the rooting process in cutting production. Sometimes excessive plant growth stymies efforts to maintain control over specific crops and a disproportionate amount of plant growth can make handling and shipping difficult. Gibberellin inhibitors have been developed, such Paclobutrazol, Uniconazole, and CCC (Heldt et al., 2011). These anti-GA chemicals are used both in agriculture and horticulture to great effect to reduce stem growth, stem height, and the growth status of a range of plants. They are also used to increase the yield in crops such as soybeans (*Glycine max*). Inhibitors such as CCC and diaminozide increase flower set in some species by limiting GA expression whereas in other species GAs are utilized to promote flowering. This further implies that GA responses in plants are often on a species-to-species basis and generalities cannot necessarily be assumed (Field et al., 1989).

COMMERCIAL SOURCES OF GAS AND CYTOKININS

The agricultural and horticultural industries have closely followed the research considering the use of GAs and cytokinins (Table 1).

Table 1. Commercial preparations of gibberellins and cytokinins.

Valent:	Fascination, a BA/GA_{4-7}, combo, 1.8% each ProGibb T&O, GA_3, 4% ProGibb, 40% GA_3
Fine Americas:	Fresco, BA/GA_{4-7}, 1.8% each Configure, 2% BA Florgib, 4% GA_3
Norac Concepts Inc., Canada:	Falgro, 4% GA_3, 1.0 g tables

IN CONCLUSION

It should be generally understood that the subject of plant hormones and hormonal interactions is an on-going process and as research progresses new understanding and developments continue to add to the utilization of plant growth regulators for the production of nursery, greenhouse, and agricultural crops. This paper is by no means definitive and new information is all but continuous. It is hoped that this at least brings some attention to how these natural materials can be used for the plant production industry.

SPECIFIC QUESTIONS COME TO MIND

1) With the knowledge of thidiazuron as an agent to break dormancy in buds of *Malus* (Heldt et al., 2011) could that chemical be used to treat dormant bare-root *Crataegus* in order to break dormancy as *Crataegus* is notoriously difficult to resume growth from a dormant bare-root plant?
2) In light of the fact that GAs when used for seed germination an over dose can cause extensive stem elongation resulting in weak stemmed plants, could cytokinins be used instead so that the stem elongation problem is no longer present?
3) Could the suggestion presented by Davis and Haissig (1990) that applied cytokinins after the application of auxins have merit in delaying or eliminating the senescence affiliated with mist production of cuttings?

Literature Cited

Anonymous. 2013. <www.plant-hormones.info/cytokinins.htm>.

Al-Juboory-Karim, H., Splittstroesser, W.E. and Skirvin, R.M. 1990. Proc. Plant Growth Regul. Soc. Amer. Quarterly 18(2):67-72.

Al-Juboory-Karim, H. and William, D. 1990. Stimulation of lateral branch development in Algerian ivy (*Hedera canariensis* L) with GA 4+7, BA and Promalin. Proc. Plant Growth Regul. Soc. Amer. Quarterly 18(4):194-202.

Barciszewski, J., Mielcarek, M., Stobiecki, M., Siboska, G. and Clark, B.F. 2000. Identification of 6-furfuryladenine(kinetin) in human urine. Biochem. Biophysics Res. Commun. 279(1):69-73.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39:175-219.

Bessler, B. 1997.The use of 6-benzylaminopurine for rapid multiplication of tillandsias. HortScience 32(2):256-258.

Chen, S. and Shepley, C. 1975. Role of gibberellins in dormancy and seed germination. p.91-100. In: H.N. Krishnamoorthy (ed.), Gibberellins and Plant Growth.

Crafts, C.B. and Miller, C.O. 1974. Detection and identification of cytokinins produced by mycorrhizal fungi. Plant Physiol. 54(4):586-588.

Crozier, A., Kamiya, Y., Bishop, G. and Yokota, T. 2000. Biosynthesis of hormones and elicitor molecules. p.850-929. In: B.B. Buchanan, W. Gruissem and R.L. Jones (eds.), Biochem. Mol. Biol. Plants. Amer. Soc. Plant Physiol. Rockville, Maryland.

Cousson, A. and Tram Thanh Van, K. 1980. Invitro control of de novo flower differentiation from tobacco thin cell layer on a liquid medium. Physiol. Plant. 51:77-84.

Davis, T.D. and Haissig, B.E. 1990. Chemical control of adventitious root formation in cuttings. Proc. Plant Growth Regul. Soc. Amer. Quarterly 18(1):1-17.

Deno, N.C. 1993. Exogenous Chemical Effects and Stimulation of Germination by Gibberellins. Seed Germination and Practice. 2nd ed. Self-published. Norman C. Deno. State College, Pennsylvania.

Deno, N.C. 1994. Seed germination. Comb. Proc. Intl. Plant Prop. Soc. 44:530-532.

Devlin, R.M., Zbiee, I.I. and Nowicka, S.E. 1989. Effects of thidiazuron on some plant growth systems. p.99-103. In: A.R. Cooke (ed.). Proc. Plant Growth Regul. Soc. Amer.

Diener, T.O. 1981. Viroids: abnormal products of plant metabolism. Ann. Rev. Plant

Physiol. 32:313-325.
Dunand, R.T. 1989. Improvement in seedling vigor of semi-dwarf rice with gibberellic acid. p.14-15. In: A.R. Cooke (ed.). Proc. Plant Growth Regul. Soc. Amer.
Dybing, C.D. and Westgate, M.E. 1989. Genotype, environment and cytokinin effects on soybean flower arbortion. p.29-30. In: A.R. Cooke (ed.). Proc. Plant Growth Regul. Soc. Amer.
Field, R., George, J., Hill, D. and Attiya, H.J. 1989. Improved yields and harvest index in field bean (*Vicia fara*) with paclobutrazol. p.23-28. In: A.R. Cooke (ed.). Proc. Plant Growth Regul. Soc. Amer.
Findley, D.A., Keever, G.J. and Gilliam, C.H. 1994. BA induced offset formation in hosta. Proc. South. Nurs. Assn. Res. Conf. 39:33-34.
Foley, J.T. and Keever, G.J. 1993. Chemically induced branching of *Vinca minor*. J. Environ. Hort. 11(4):149-152.
Garner., J.M., Keever, G.J., Eakes, D.J. and Kessler, J.R. 1997. Sequntial BA application enhance offset formation in hosta. <www.ag.auburn.edu/hort/landscape/gary5.html>.
Garner., J.M., Keever, G.J., Eakes, D.J. and Kessler, J.R. 1997. BA-Induced offset formation in hosta dependant on cultivar. HortScience 32:91-93.
Geetha, N., Venkatachalam, P., Prakash, V. and Lakshmisita, G. 1998. High frequency induction of multiple shoots and plant regeneration from seedling explants of pigeon pea (*Cajanus cajan* L.) Curr. Sci. 75(10):1036-1041.
Gonzales, A.R. and Prokakis, G. 1989. Use of gibberellic acid (GA_3) p.180. In: A.R. Cooke (ed.). Fall spinach production. Proc. Plant Growth Regul. Soc. Amer.
Haissig, B.E. 1972. Meristematic activity during adventitious root primordia development: Influence of endogenous auxin and applied gibberellic acid. Plant Physiol. 49:886-892.
Halbrooks, M.C. and Crovetti, A.J. 1989. Gibberellic acid increases berry size and reduces seed traces in 'Orlando Seedless' grapes. p.34-39. In: A.R. Cooke (ed.). Proc. Plant Growth Regul. Soc. Amer.
Halevy, H.A. 1986. Recent advances in use of growth substances in ornamental horticulture. In: M. Bopp (ed.), Proceedings of the 12th International Conference on Plant Growth Substances. Springer Verlag, Berlin.
Heldt, H.-W., Piechulla, B. and Heldt, F. 2011. Plant biochemistry. Academic Press. Burlington, Massachusetts.
Jiang, G. Z., Wu, L., Macnish, A.J., King, A., Yi., M. and Reid, M.S. 2009. Thidiazuron, a non-metabolized cytokinin, shows promise in extending the life of potted plants. Acta Hort. 847:59-65.
Keever, G.J. 1994. BA-induced offset formation in hosta. J. Environ. Hort. 12:36-39.
Keever, G.J. and Brass, T.J. 2013. Presence of offsets reduces hosta's response to benzyladenine. <www.ag.auburn.edu/hort/landscape/gary7.html>.
Kuminek, M., Vanek, T., Kalendova-Kulasova, A. and Pilar, J. 1987. The effect of two cytokinins on production of stem cuttings by stock plants of *Euphorbia pulcherrima* Willd. and *Gerbera jamesonii* Hook. Scienta Hort. 33:281-289.
Morrone, D., Chambers, J., Lowry, L., Kim, G., Anterola, A., Bender, K. and Peters, R.J. 2009. Gibberellin biosynthesis in bacteria: Separate ent-copalyl diphophate and ent-kaurene synthases in *Bradyrhizobium japonicum*. FEBS Lett. 583(2):475-480.
Nailo, K.N., Furuak, S. and Suzuki, H. 1981. Effects of benzyladenine on RNA and protein synthesis in intact bean leaves at various stages of ageing. Physiol. Plant. 52:343-348.
Perilli, S., Moubavidin, L. and Sabatini, S. 2010. The molecular basis of cytokinin function. Curr. Opinion in Plant Biol. 31(1):21-26.
Perniosavá, M., Kuderová, A. and Hejátko, J. 2011. Cytokinin and auxin interactions in plant development: Metabolism, signaling, transport, and gene expression. Curr. Protein Pept. Sci. 12(2):137-147.
Sachs, T. and Thimann, K.V. 1967. The roles of auxins and cytokinins in the release of buds from dominance. Amer. J. Bot. 54:136-144.

Schoene, G. and Yeager, T. 2005. Effects of benzyladenine applied alone or in combination with gibberellic acid in sweet viburnum. SNA Research Conference 50:114-122.
Sponsel, V. 2010. Commercial uses of gibberellins. Plant Physiol. Online 5th ed. <http://5e.plantphys.net/article.php?ch=20&id=372>.
Srinivasan, M., Nachiappan, V. and Rajasekharan, R. 2006. Potential application of urea derived herbicides as cytokinins in plant tissue culture. J. Biosci. 31(5):599-605.
Subbiah, V. and Reedy, K.J. 2010. Interacations between ethylene, abscisic acid and cytokinin during germinaton and seedling establishment in *Arabidopsis*. J. Biosci. 35:451-458.
Tamas, I.H. 1988. Hormonal regulation of apical dominance. p.393-410. In: P.J. Davis (ed). Plant hormones and their role in plant growth and development. Kluwer Academic Publishers. Boston.
Tran, T., Van, K. and Kiem, M. 1981. Control of morphogenesis in in-vitro cultures. Ann. Rev. Plant Physiol. 32:291-311.
Varner, J.E., and Ho, D.T.-H. 1976. Hormones. p.714-765. In: J. Bonner and J. Varner (eds.), Plant Biochemistry. Elsevier, Ltd.
Wang, Y.-T. 1996. Cytokinin and light intensity regulate flowering of easter lily. HortScience 31(6):976-977.
Waring, P.F. and Philips, I.D.J. 1982. Mechanisms of actions of plant growth hormones, p.49-104. In: Growth and Differentiation in Plants, 3rd ed. Pergamon Press. New York.
Werner, T., Motyka, V., Strnad, M. and Schmülling, T. 2001. Regulation of plant growth by cytokinin. Proc. Nat. Acad. Sci. (U.S.A.) 98:10487-10492.
Yan, W.G. and Dilday, R.H. 1993. Influence of temperature, gibberellic acid and plant stature on germination of rice. Plant Growth Regul. Soc. Amer. Quarterly 21(3):122.
Yang, G. and Reid, P.E. 1992. Plant growth regulators in the forcing solution enhance budbreak and in-vitro shoot proliferation of *Acanthopanax sieboldianus*. Plant Growth Regul. Soc. Amer. Quartley 20(3):141.

Nurseries in Japan: a 20 Minute Tour©

Sharee Solow
7914 Park Avenue, Elkins Park, Pennsylvania 19027, USA
Email: slsolow@comcast.net

INTRODUCTION

For the month of February 2012, my time at the Kosugi Garden Seminar, Atami City, Japan, was well spent in an intensive learning experience that I would recommend to professionals interested in Japanese gardening history and techniques. Partnering with the European Landscape Association, the "in English" session drew participants from around the world. Dr. Andreas Hamacher developed this course which he conducts fluidly by moving between in English, German, Japanese, and Chinese. The third week focused on hands-on demonstrations of maintenance, nursery operations, and nursery tours which will be the focus for this 20 min photography tour. <http://kosugi-zohen.co.jp/seminar_top.htm>.

WHOLESALE YARDS IN SAITAMA

While driving through the region, it seems that everyone for miles has trees being trained for resale in their yards for a Dr. Seuss-like view (Fig. 1). Wholesale yards in Saitama carry a wide range of woody plants that would be familiar to Americans in bare-root, B&B, and containers but they might be characterized as having extreme forms. Bare-root trees of 2½-in. caliper are stacked in houses with only 12 in. of root. Perfect root balls are artistically wrapped with the number of rope passes around relating to the value of the tree. Huge containers may hold 100-year-old black pines (*Pinus thunbergii*) for years as they continue to be meticulously trained into specific forms before sale. Shipping these trees overseas requires dipping the root balls in a proprietary mix for nematodes. These burlapped root balls are then packed into a plastic container with sterile soil for 1 month and tested for nematodes presence again before shipping.

Fig. 1. A typical wholesale yard in Saitama.

ŌMIYA BONSAI VILLAGE

Ōmiya Bonsai Village (大宮盆栽村 *Ōmiya Bonsai-mura*) is the nickname for the bonsai nursery precinct in Bonsai-chō (盆栽町 *Bonsai-chō*), Kita-ku, Saitama, Japan.

I was there to visit the Omiya Bonsai Art Museum, opened in 2010, but when the demonstration went a bit long for a book signing, we were left with only 20 min to visit just one nursery. There were rows of tables with hundreds of trees both on the tables and crowding the ground below. Two tables displayed heaps of small pots for shohin, literati and other variants below 8 in. while larger pots were primarily stacked on the ground. Unlike the garden bonsai nurseries, this urban, walled, display yard of maybe 2,000 SF was completely set on paving and crushed stone allowing for a very clean environment. The Omiya Bonsai Nursery, Mansei-en is shown below (Fig. 2)

Fig. 2. Omiya Bonsai Nursery, Mansei-en.

VIEWING PRUNING WORK FROM A CAR WINDOW

It was hard to drive along and not stop everywhere to admire the pruning work (Fig. 3) but there was one section of road with particularly impressive trees. We were delighted to find that it was our next demonstration nursery stop where we would see decades of expertise displayed in the field. This third generation pruning master showed us a very extreme method of garden bonsai involving driving a sharp tool through a branch in order to rotate it 180°. Large tree branches can be trained in a shorter time using the technique to achieve the desired form thereby increasing its resale value. He also trained hundreds of S-curved trees with a bamboo and rope system-something we would spend a couple hours doing ourselves. Learning his knot-tying system proved the most difficult aspect for some while visualizing the required pruning before attaching the structure eluded others.

Fig. 3. Viewing nurseries from the car window.

SAIEN GARDEN AND AUCTION WAREHOUSE

Saien Garden and Auction Warehouse (Fig. 4) (across the street) was definitely the place we were all curious to visit because this type of supply system to landscapers is not common to other countries. The massive warehouse buildings might seem familiar but the display yard is another story. Deciduous trees, pines, stone, sculptures, and anything required to complete a Japanese garden is held here for auction. As some trees are developed before resale so very experienced professionals are at hand to work the pieces and create displays. Some unusual techniques were sculpting deadwood from a crapemyrtle with a chainsaw and ridging the crane lift of a three-ball garden bonsai into place for a display garden. For example, the gentleman giving us a black pine pruning lesson worked here as a fun thing to do after retiring from teaching bonsai.

Fig. 4. The wholesale auction yard.

NURSERY AT SHIBAMICHI HONTEN IN SAITAMA

Last, we will focus on the nursery and grafting work at Shibamichi Honten in Saitama (Fig. 5). It was a torrential downpour but we did our best to keep up with him and our translator between the Japanese, German, English, and Swiss explanations. Everything in the yard and the houses was interesting but his immediate work included grafting magnolias, including some shipments from the Raulston Arboretum. His planting style for these was to pack a plastic crate with about 200 bare root trees into a seed-starter type mix, moisten it, and wrap it with plastic before sliding it under the table. Grafted pieces are on the tables, tagged, plastic bagged, and individually potted. Many tags in the nursery are in English as well as Japanese. The sales yard of popular conifers was joined by shrubs that were thought lost to the trade. He wishes to save historic garden specimens even though they may not be profitable. When sticking cuttings, planting medium is mixed and potting trays are filled by hand. Mr. Shibamichi stops to point out a recent project, the Raulston's "Connoisseur Plant" grown from seed, *Magnolia fordiana*. A current project involves multiple crosses to create a *Mahonia* for the cut-flower market. To better understand his life's work we have some green tea and biscuits in a meeting room to dry off perusing stacks of photographs with his decades of plant introductions.

Fig. 5. Hybridizer Akira Shibamichi.

North Dakota State University Cold Climate Breeding©

Todd P. West
North Dakota State University, Plant Sciences, Loftsgard Hall 266E, Fargo, North Dakota 58102, USA
Email: Todd.P.West@ndsu.edu

Climate and soil conditions present a challenge in growing landscape plants in the northern Great Plains. Only a small percentage of woody plant genotypes may perform satisfactorily as a result of insufficient winter hardiness; pest susceptibility; and lack of resistance to drought, desiccating winds, and unfavorable soil conditions [e.g., alkaline (pH) and saline soils]. Historically, there has been a deficiency of adapted, winter hardy, pest resistant woody plants for shelter and landscape uses in the northern Great Plains. As a result of this deficiency, there is a need to breed, evaluate and introduce adapted woody plants to increase plant diversity for this region and avoid monoculture disasters in the future.

The northern Great Plains is a diverse intercontinental environment with limited woody plant species that have been evaluated for use in U.S.D.A. Hardiness Zones 3 and 4. Dr. Dale E. Herman developed the North Dakota State University (NDSU) Woody Plant Improvement Program in the 1970s. Over the years, the Program has introduced 51 superior woody plants for production with increased winter hardiness and cultural tolerances for landscapes throughout the northern Great Plains.

The NDSU Woody Plant Improvement Program has eight objectives:

1) Obtain potential winter-hardy germplasm for evaluation and/or breeding efforts.
2) Replicate evaluations at several sites within North Dakota.
3) Narrow evaluations to most promising selections.
4) When possible, potential final selections are observed by researchers from regional universities and nursery industry personnel to provide additional input.
5) New name cultivar and select potential trademark name.
6) Distribute propagation material to wholesale nursery propagators.
7) License wholesale nursery firms to commercially produce the new woody plant.
8) Publish information to make known the new introductions at all levels.

Germplasm is obtained from multiple sources including: foreign and domestic seed sources including local and regional plant collections of potentially superior native species and specimens. Seedling populations are typically grown out and individuals with superior landscape attributes are selected for potential future releases as well as breeding efforts. Plant breeding was not a focus of the NDSU program and recently has been added as an effort for plant improvement. Both traditional breeding as well as mutagenic breeding is being conducted. Breeding efforts are being developed with multiple genera including *Acer*, *Magnolia*, *Prunus*, and others (Fig. 1). Plants are also evaluated through cooperative evaluation programs with Bailey Nurseries, Inc., St. Paul, Minnesota; J. Frank Schmidt & Son, Inc., Boring, Oregon; Iseli Nursery, Boring Oregon; U.S.D.A. (North Central Regional Plant Introduction Station) NC-7 program; and other firms.

Evaluations are conducted primarily at the NDSU Horticulture Farm, which includes the NDSU Dale E. Herman Research Arboretum. The farm is located near Absaraka, North Dakota (ND), which is a U.S.D.A. Hardiness Zone 4a. The soil type is a Spottswood sandy loam underlain by a water-bearing gravel layer. Under this gravel layer, which extends from 1-1.5 m below the soil surface, is a deep layer of medium loam. This research location provides ideal horticultural soil for evaluation efforts and typifies much of the soil types found in ND. Several other sites are utilized for replication of evaluations, which include city and park planting trials in Bismarck and Fargo, ND and evaluation plots in Carrington, ND (USDA hardiness zone 4a) and Langdon, ND (U.S.D.A. Hardiness Zone 3b) at the ND Agricultural Experiment Stations. The NDSU Dale E. Herman Research Arboretum consists of just over 14 ha and contains 150+ genera and over 3,000 accessions. This collection is one of the largest and most diverse collections of

woody plant germplasm in the Northern Great Plains.

Over 50 introductions have been made since the mid-1980s. In the near future, numerous additional woody plant releases are being planned. Some of the most promising selections include: several birch, several maples, a buckeye (Fig. 2), an elm, a thornless honeylocust, and selections from additional genera. These new releases will have gone through numerous years of evaluation along with research involving vegetative propagation methods.

One limiting factor with ornamental woody plant production is propagation. To have a successful introduction to the nursery trade, an efficient and economically feasible protocol for propagation must be established. New introductions require large clonal plant populations that are readily available for licensed nurseries. As a result, research on propagation is conducted along with evaluations so that nursery growers will have the necessary propagation information along with species recommendations. Propagation research focuses on stem cuttings, plant tissue culture, and grafting. All of the techniques result in clonal propagative material and are essential for increases population numbers of current and potential future introductions. Grafting rootstock compatibility studies are being conducted on select species to determine if grafting compatibility will be an issue, if it affects hardiness of the plant, and assists in decreasing production and management issues. Each species that has potential for release through the NDSU Woody Plant Improvement Program will be screened for propagation issues and protocols will be developed and released to nursery growers as well as published in peer-reviewed journals.

Fig. 1. North Dakota State University *Magnolia* breeding to introduce more ornamental traits into hardy late flowering *Magnolia* × *loebneri* 'Ruth', Spring Welcome® loebner magnolia.

Fig. 2. Upright buckeye (*Aesculus glabra*).

Comparison of Physical, Acid, and Hot Water Scarification on Seed Germination in Eastern Redbud©

Kara Mylor, Sarah Holton and Robert L. Geneve[a]
Department of Horticulture, University of Kentucky, Lexington, Kentucky, 40546, USA

Servet Calýskan
Department of Silviculture, Faculty of Forestry, Istanbul University, 34473 Bahcekoy, Sariyer, Istanbul, Turkey

INTRODUCTION

Eastern redbud (*Cercis canadensis* L.) is a common woody legume landscape tree with a hard seed coat that is impermeable to water (Geneve, 1991). Legume seeds are classified as being physically dormant (Baskin and Baskin, 1998). Most temperate woody legumes display only physical dormancy, but eastern redbud also has a physiological dormancy that requires chilling stratification for germination (Geneve, 1991).

Alleviation of physical dormancy in tree seeds usually involves scarification to mechanically abrade the seed coverings or more commonly seeds are treated with concentrated sulfuric acid to scarify the seed surface (Hartmann et al., 2011). Alternatively, redbud seeds respond to hot water treatments to relieve physical dormancy (Geneve, 2009). Young and Young (1992) in the *Seeds of Woody Plants in North America* recommend treating redbud seeds in boiling water (100°C) for 60s. They also indicate that seeds have been placed in 82°C water and allowed to cool overnight, but do not indicate how effective the treatment was for alleviating physical dormancy. Hot water treatment would be preferable for scarification of large quantities of seeds because it avoids safety and disposal issues associated with sulfuric acid scarification. However, it is not known if heat treating redbud seeds to relieve physical dormancy impacts subsequent release from physiological dormancy during chilling stratification or seedling vigor during germination.

The major objective of the current study were to compare the effects of physical, hot water, and acid scarifications on seed germination and embryo growth in eastern redbud prior to and after chilling stratification.

MATERIAL AND METHODS

Four scarification treatments were applied to redbud seeds.

1) Seeds of redbud were acid scarified by emersion in concentrated sulfuric acid for 40 min then rinsed with distilled water.
2) Physically scarification involved nicking the opposite side of the hilum using a sanding attachment to an electric drill.
3) Seeds were hot water treated by placing seeds in boiling water (100°C) for 60 s followed by rinsing in cool water
4) Seeds were hot water treated by placing seeds in hot water (100°C) and allowing them to cool overnight at room temperature.

After physical, acid, or hot water treatments approximately 50 seeds were placed in Petri dishes with two pieces of germination paper and enough water to cover the bottom ¼ of the seeds. These were stratified at 5°C for 4 weeks.

Seeds were evaluated prior to or after stratification for seed germination and excised embryo growth. Four replicates of 25 seeds or five replicates of 10 embryos were placed in single Petri dishes containing two pieces of germination paper wetted with 5 ml of autoclaved, distilled water. Each Petri dish was sealed with parafilm and

[a] Rgeneve@uky.edu

germination was at 25°C±1 in a lighted germinator. Germination percentages were evaluated after 7 days for intact seeds and daily for 4 days for isolated embryos.

RESULTS AND DISCUSSION

All scarification treatments (physical, acid, and hot water) resulted in a release of physical dormancy (Fig. 1). Embryos removed from non-stratified seeds resulted in germination above 75% following physical, acid, or hot water soak scarification (Fig. 2). However, seeds immersed in boiling water for 1 min germinated at less than 5%. The hot water soak initially had comparable germination to physical and acid treatments, but showed slower embryo germination and reduced germination following stratification (Fig. 3).

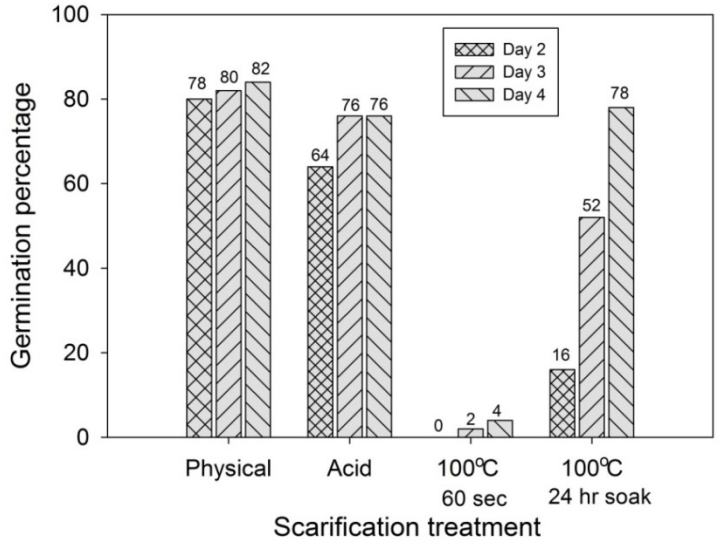

Fig. 1. Germination in isolated embryos of eastern redbud following physical, acid, and hot water scarification without chilling stratification.

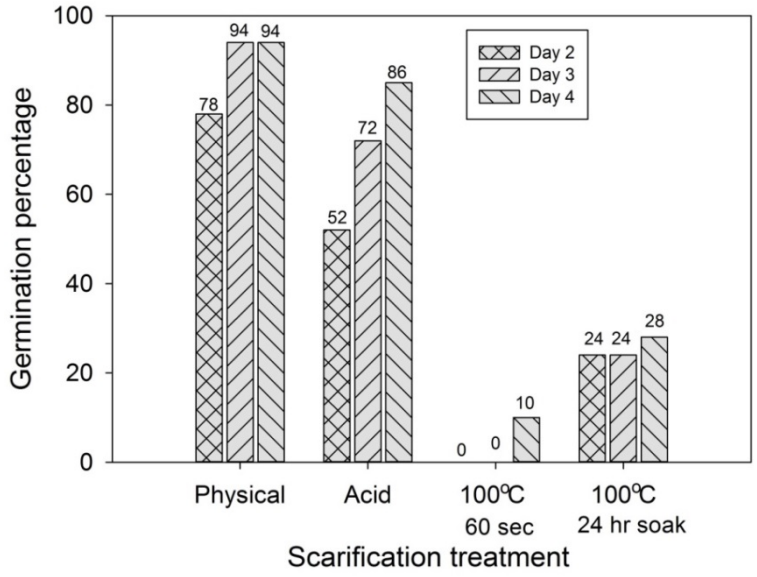

Fig. 2. Germination in isolated embryos of eastern redbud following physical, acid, and hot water scarification after 4 weeks of chilling stratification.

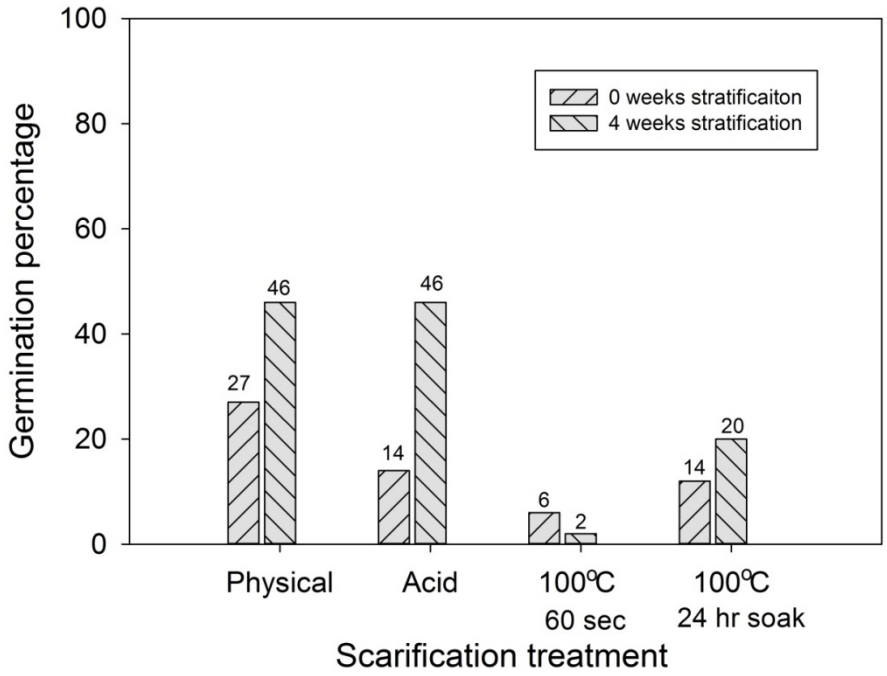

Fig. 3. Germination in intact seeds of eastern redbud following physical, acid, and hot water scarification after 0 or 4 weeks of chilling stratification.

Hot water is an effective, alternative scarification treatment to relieve physical dormancy in eastern redbud seeds. However, contrary to previous reports (Young and Young, 1992), the current results demonstrate that prolonged exposure to 100°C can damage the redbud embryo (Fig. 2) and therefore cannot be recommended as a viable scarification treatment. It is also apparent that these heat treatments predispose the seeds (embryos) to additional damage during stratification (Figs. 2 and 3). Preliminary evidence from additional ongoing research indicates that brief exposure (<15 min.) to hot water below 80°C can be effective at physical dormancy release, while not inducing embryo damage. Until this work is completed care should be taken when using hot water as an alternative to physical or acid scarification.

Literature Cited

Baskin, C.C. and Baskin, J.M. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, New York, USA.

Geneve, R.L. 1991. Seed dormancy in eastern redbud (*Cercis canadensis*). J. Amer. Soc. Hort. Sci. 116:85-88.

Geneve, R.L. 2009. Physical seed dormancy in selected caesalpinioid legumes from eastern North America. Prop. Ornamental Plants 9:129-134.

Young, J.A. and Young, C.G. 1992. Seeds of Woody Plants in North America. Dioscorides Press, Portland, Oregon.

Micropropagation of *Uncaria rhynchophylla* – a Medicinal Woody Plant©

Katsuaki Ishii, Naoki Takata and Toru Taniguchi
Forest Bio-research Center, Forestry and Forest Products Research Institute, Ishi 3809-1, Jyuo, Hitachi, Ibaraki-ken, 319-1301, Japan
E-mail: katsuaki@ffpri.affrc.go.jp

INTRODUCTION

Uncaria rhynchophylla (kagikazura or the cat's claw herb) is a plant species used in traditional Chinese medicine and also kampo (Japanese study and adaptation of traditional Chinese medicine), and is a woody plant found widely in Japan and China. It contains alkaloids rhynchophylline (Fig. 1), iso-rhynchophylline, hirstine, and others (Shi et al., 2003) which are good for treating high blood pressure and dementia. In addition (+)-Catechin and (-)-epicatechin are also found in the plant (Hou et al., 2005). It is in four of the 148 Kampo medicine formulae. Kampo herbal medicines are regulated as pharmaceutical preparations and their ingredients are exactly measured and standardized. Access to Kampo herbal medicines is guaranteed as part of Japan's national health plan for each of its citizens. For the purpose of micropropagation and development of a basis for useful substance production by breeding and cell culture, a tissue culture procedure was developed for this species.

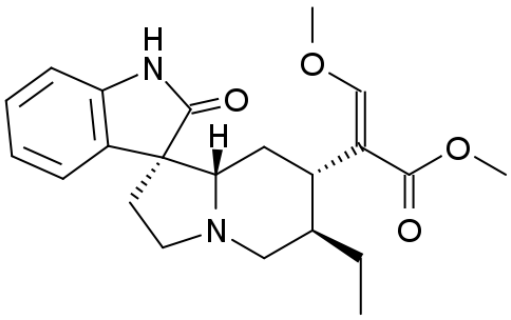

Fig. 1. Rhynchophylline.

MATERIALS AND METHODS

Branch stem segments containing hooks (formed from reduced branches) from wild *U. rhynchophylla* were collected from the forest in Kochi prefecture, Shikoku island, Japan (Fig. 2B). Surface sterilization of stem segments was done using 70% ethyl alcohol for 1 min, 5% hydrogen peroxide for 10 min, then washed well twice with sterile water for eliminating surface microorganism. For initial culture, MS (Murashige and Skoog, 1962), ½ DCR (Gupta and Durzan, 1985), SH (Schenk and Hildebrandt, 1972), and ½ LP (Quiolin and Lepoivre, 1977) media plus different hormonal combinations of 6-benzylaminopurine (BAP), kinetin, Zeatin, and NAA were compared. For subculture and rooting of the shoots, ½ LP and ½ MS medium containing 1 μM IBA were used. For habituation a small scale controlled environment plant culture system [Terrace® System (MKB Dream Co., Japan)] was used. Culture condition was maintained at the constant temperature of 25°C under 16 h photoperiod of 70 $\mu M \cdot m^{-2} s^{-1}$ provided by cold-cathode fluorescent lamp. Propagated plantlets were first cultured in the greenhouse then planted out to the field.

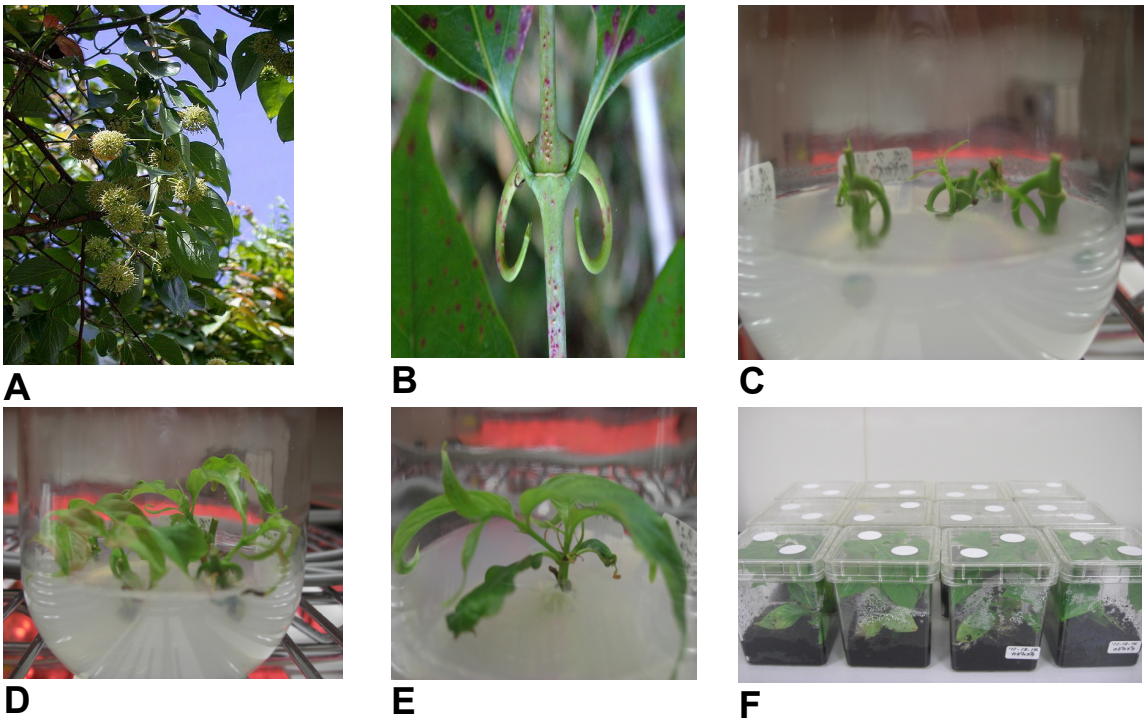

Fig. 2. Process of in vitro propagation of *Uncaria rhynchophylla*. (A) *Uncaria rhynchopylla*, (B) stem spine, (C) shoot induction from spine, (D) shoot elongation, (E) Rooting, (F) Regenerated in vitro plantlets in plant boxes.

RESULTS AND DISCUSSION

Shoots were induced from stem hooks of kagikazura in the ½ MS medium containing BAP or zeatin (Fig. 2C). A ½ MS medium containing 4 µM BAP was the best for shoot induction (Table 1). Callus induced around the stem segments were continuously subcultured in fresh ½ LP medium containing 0.5 µM BAP and 1 µM 2,4-D. These cell lines can be used for the possible secondary metabolite production and for chemical constituents breeding by somaclonal variation or molecular genetics technology. A higher concentration of BAP was better for induction of buds from subcultured stem segments (Table 2). Regenerated plants were obtained by rooting of these shoots on ½ MS medium containing IBA (Fig. 2E, Table 3). A low concentration of IBA was better for rooting (Table 3). Rooted plantlets were cultured in Giffy 7® with 60 ml of 0.1% Hyponex® medium in plant boxes (65×65×100 mm) (Fig. 2F). Each plant box contained one regenerated plantlet. Culture condition were at 25°C constant temperature under a 16-h photoperiod of 50 $\mu M \cdot m^{-2} \cdot s^{-1}$ by cold cathode fluorescent lamps. Then after 2 months, they were habituated in a small scale controlled environment plant culture system [Terrace® System (MKB Dream Co., Japan)] which provides 100% humidity and automatic watering for 1 month (Fig. 3), and then grown in a greenhouse for 6 months. Field planting was successful (Fig. 4). Selection of clones with higher chemical content is planned.

Improving the propagation rate and selection of tree clones with higher contents of rhynchophylline is planned in the future.

Table 1. Shoot induction from stem segments of kagikazura.

Shoot medium	Plant growth regulator (μM)	Shoot induction (%)	Number
½ MS	Zeatin (4)	73 (11/15)	1.2 ± 0.1
½ MS	BAP (4)	85 (11/13)	1.4 ± 0.1
½ MS	Kinetin (4)	10 (1/10)	1
½ MS	Zeatin (4), NAA (0.5)	27 (3/11)	1.3 ± 0.3
½ MS	BAP (4), NAA (0.5)	60 (6/10)	1.2 ± 0.2
½ MS	Kinetin (4), NAA (0.5)	0 (0/10)	0

N=4~16.
Abbreviation: BAP = 6-benzylaminopuring.

Fig. 3. Habituation.

Fig. 4. Field grown *Uncaria rhynchophylla*.

Table 2. Effects of 6-benzylaminopurine (BAP) concentration on bud differentiation from segments of kagikazura (½ LP medium).

BAP (μM)	Average no. of induced buds ± SD
0	0 ± 0
1	0.6 ± 0.25
10	1.6 ± 0.35
50	2.4 ± 0.25

N=10.

Table 3. Effects of indole-3-butyric acid (IBA) on rooting of kagikazura (½ Murashige and Skoog medium).

IBA (μM)	Rooting percentage ±SD
0.1	93 ± 5
0.5	80 ± 9
2.5	0 ± 0
12.5	0 ± 0

N=15.

ACKNOWLEDGMENTS
This study was financially supported by Grants-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology of Japan.

Literature Cited

Gupta, P.K. and Durzan, D.J. 1985. Shoot multiplication from mature trees of Douglas-fir (*Pseudotsuga menziesii*) and sugar pine (*Pinus lambertiana*). Plant Cell Rep. 4:177-179.

Hou, W.C., Lin, R.D., Chen, C.T. and Lee, M.H. 2005. Monoamine oxidase B (MAO-B) inhibition by active principles from *Uncaria rhynchophylla*. J. Ethnopharmacol. 100(1-2):216-20.

Jain, A.K. and Nessler, C.L. 1996. Clonal propagation of *Camptotheca acuminate* through shoot bud culture. Plant Cell Tissue Organ Cult. 44:229-233.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Quiolin, M. and Lepoivre, P. 1977. Etude de milieu adaptes aux cultures in vitro de *Prunus*. Acta Hort. 78:437-442

Schenk, R.U. and Hildebrandt, A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell culture. Can. J. Bot. 50:199-202.

Shi, J.S., Yu, J.X., Chen, X.P. and Xu, R.X. 2003. Pharmacological actions of Uncaria alkaloids, rhynchophylline and isorhynchophylline. Acta Pharmacol. Sin. 24(2):97-101.

Soil Conditioner FFC-Ace Effects on Growth and Quality of Berries of Wine Grapes©

Kazuhiro Ichikawa and Tadao Fujimori
Institute of Biological Process Research, Akatsuka Garden Co., Ltd., 1868-3 Takanoo-cho, Tsu, Mie 514-2293, Japan
E-mail:kazu.ichikawa@akatsuka.gr.jp

INTRODUCTION

We have focused our attention on the behavior of certain ions, especially iron ion in water or interactions of water molecules with them. Since 1984, Akatsuka Garden Company has continued research on various solutions to not only accelerate plant growth, but also activate physiological functions of plants. Based on this research, we have developed FFC materials such as FFC-Ceramics (a water improvement device), FFC-Ace (a soil conditioner), and others. In addition, many agricultural producers in Japan have been utilizing FFC materials to rejuvenate plants and increase profits. Those producers have also explored many other original methods for using FFC materials, and consequently found good ways to fit them into their actual production sites. As a result, they have obtained many advantages over the years of use, such as, productivity enhancement, cost reduction, decreased amount of agricultural chemicals required, and others. In addition, it is reported that FFC-Ace enhances the growth of plants under laboratory conditions, improves disease resistance, and drought and salt stress tolerance of plants (Ichikawa et al., 2013; Fujita et al., 2010; Hasegawa et al., 2006; Konkol et al., 2012; Shiraishi et al., 2010; Toyoda et al., 2010). In this paper, we will report a part of the results on the effectiveness of FFC-Ace on wine grape vines under field conditions.

Yamanashi Prefecture is Japan's top producer of grapes. However, the high quality grape berries for brewing are not cultivated easily in the cultivation environment of Japan (Asai, 1993; Nakayama, 1993). Therefore, we started a study on the promotion of the growth of Chardonnay grape vines and quality improvement of the berries by using the FFC materials. In addition, we also examined an additional wine grape, *Vitis vinifera* 'Koshu'. 'Koshu' grapes are widely cultivated in central Japan, particularly Yamanashi Prefecture. 'Koshu' is an indigenous grape cultivar that is used to produce Koshu wine, which is a special product of Yamanashi Prefecture.

MATERIALS AND METHODS

Field experiments of FFC-Ace using Chardonnay and 'Koshu' grapevines were undertaken.

Eighteen Chardonnay grapevines (guyot-style cultivation) were tested. Two holes of approximately 50 cm depth were dug approximately 0.5 m away from the main trunk of the grapevines. The required amount of FFC-Ace was put into the holes and also scattered on the ground surface approximately 1 m in diameter from the main trunk of the grapevines.

Six 'Koshu' grapevines [(shelf-style cultivation (overhead trellis)] were treated. Four holes of approximately 50 cm depth were dug at approximately 2 m away from the main trunk of the grapevines. The required amount of FFC-Ace was put into the holes and scattered on the ground surface approximately 4 m from the main trunk of the grapevines. The FFC-Ace was annually applied in the experimental fields every February from 2008 to 2011. Table 1 shows the amount of FFC-Ace which was applied each year. Twenty berries were randomly sampled from each grapevine to measure grape berry compositions, and divided into two groups. Each fresh berry's weight, the average total soluble solids (expressed as degrees Brix), the average of pH, the average of titratable acidity, and the average total phenolics concentration in juice extracted from the two groups were measured.

Table 1. The weight of FFC-Ace© provided to each grape each year.

'Chardonnay' grapes.

	2009	2010	2011
No FFC-Ace	–	–	–
FFC-Ace 3.7 kg-1	3.7 kg	–	–
FFC-Ace 3.7 kg-2	3.7 kg	3.7 kg	3.7 kg
FFC-Ace 23 kg-1	23 kg	–	–
FFC-Ace 23 kg-2	23 kg	23 kg	23 kg

(per 1 tree)

'Koshu' grapes

	2008	2009	2010	2011
No FFC-Ace	–	–	–	–
FFC-Ace treated	3.7 kg	3.7 kg	45 kg	7.5 kg

(per 1 tree)

Pot Experiments by Using Young Plants of Chardonnay and 'Koshu' Grapes

Young one-year-old plants of Chardonnay and 'Koshu' grapes were tested. We cultivated the young plant in Wagner pot (1/2000a) holding field soil mixed with FFC-Ace (3% w/w) or in untreated field soil. Growth of the young plants was estimated by measuring the length of shoots, the number of leaves, and chlorophyll content (SPAD-unit) in the shoot leaves.

RESULTS AND DISCUSSIONS

Degrees Brix in Chardonnay berries were increased by using FFC-Ace (Fig. 1). Because sugars in berries change to alcohol by fermentation an increase of degrees Brix in berries enhances the value of the wine grapes. Titratable acidity of Chardonnay berries during early growth in the field treated with the FFC-Ace declined earlier than without FFC-Ace. This result for Chardonnay grapes is similar to that of 'Koshu' grapes. Therefore, it was suggested that the application of FFC-Ace promoted maturation of both kinds of berries. Fresh berry weights, pH values, and total phenolics concentrations of both kinds of grapes were not influenced by the application of FFC-Ace. The young plants (1 year old) of Chardonnay and 'Koshu' grapes were transplanted in Wagner pot (1/2000a) holding FFC-Ace treated soil or untreated field soil. Growths of the young plants were observed (Fig. 2). The length of the young shoots of 'Koshu' plants in the soil treated with 3% of FFC-Ace was significantly longer than without FFC-Ace during early growth (105 days after the planting). In addition, the 'Koshu' young plants treated with FFC-Ace had a larger number of leaves than without FFC-Ace, and the SPAD unit of the grape leaves treated with FFC-Ace is higher than without FFC-Ace. The results for Chardonnay were also similar to those of 'Koshu', although no statistically significant difference was observed. These results indicate that the application of FFC-Ace to young grape plants might stimulate plant growth in the early stages, accelerate maturation of berries, and increase degrees Brix in the juice of berries.

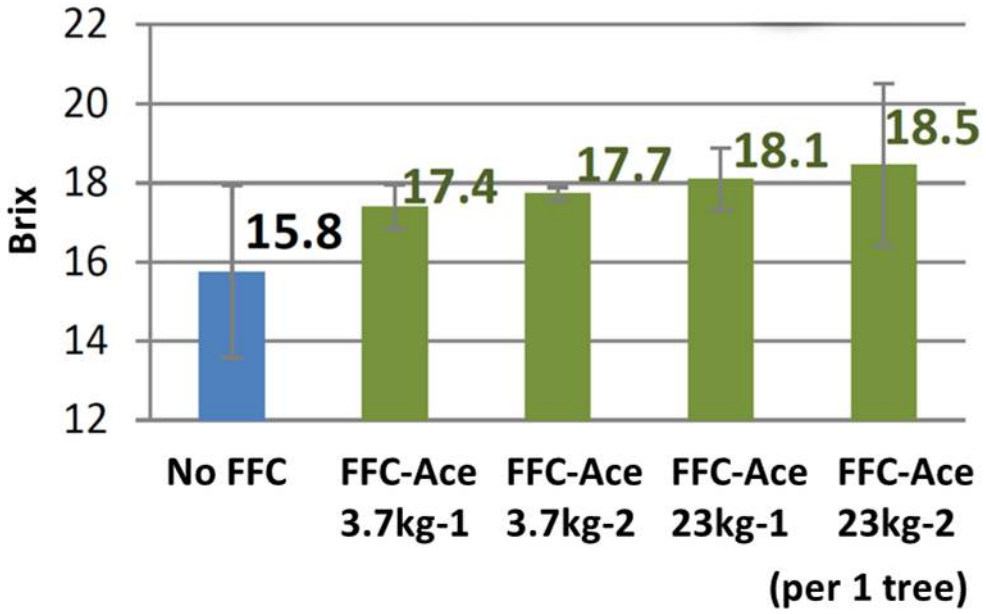

Fig. 1. Degrees Brix in Chardonnay berries were increased by using FFC-Ace.

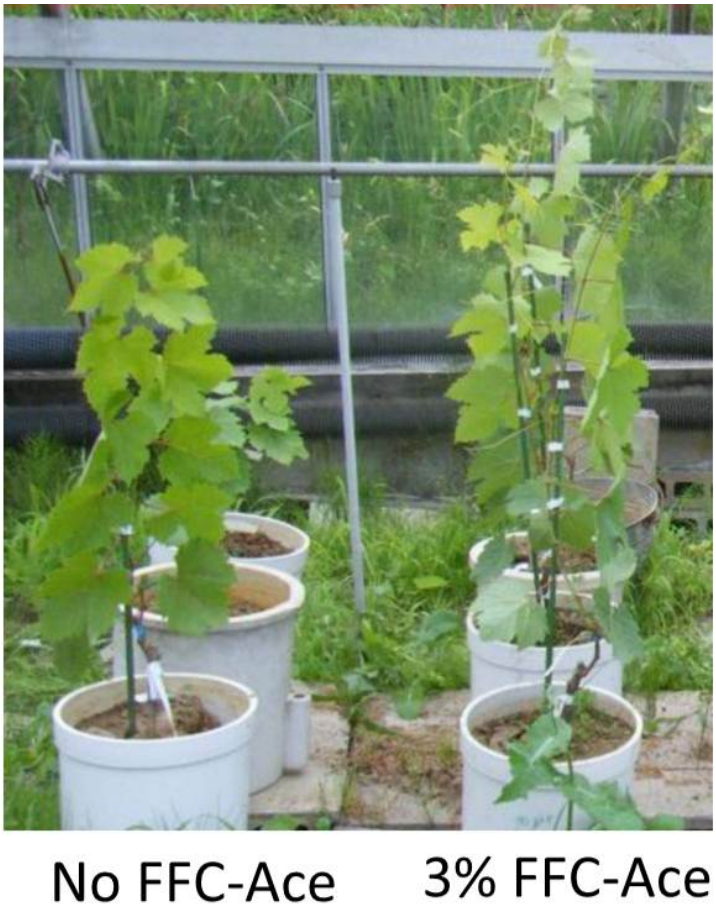

Fig. 2. The length of the young shoot of 'Koshu' plant in the soil treated with 3% of FFC-Ace.

ACKNOWLEDGMENTS
This research is collaborative investigation with Dr. Shunji Suzuki (The Institute of Enology and Viticulture, University of Yamanashi, Yamanashi, Japan). We thank Dr. Suzuki for supplying experimental facilities and helpful advice.

Literature Cited

Asai, S. 1993. Thinking about wine grapes used in Japanese winery. J. Brewing Soc. Japan 88(5):338-343.

Fujita, K., Suzuki, T., Hasegawa, S., Meguro, A., Sugiura, S.H., Toyoda, K., Shiraishi, T., Sakaguchi, E., Nishimura, T. and Kunoh, H. 2010. Enhancement of growth and yield of barley by the soil conditioner FFC-ace. Scientific Reports of the Faculty of Agriculture Okayama University 99:13-20.

Hasegawa, S., Meguro, A., Shimizu, M., Nishimura, T. and Kunoh, H. 2006. The ceramic bead that is suitable for a carrier of plant-rooting accelerator, *Streptomyces* sp. MBR-52. Actinomycetologica 20:23-29.

Ichikawa, K. and Fujimori, T. 2013. Effects on growth of plants by the soil conditioner FFC-Ace©. Comb. Proc. Int. Plant Prop. Soc. 62:459-462.

Konkol, N.R., McNamara, C.J., Bearce-Lee, K.A., Kunoh, H. and Mitchell, R. 2012. Novel method of micronutrient application increases radish (*Raphnus stivus*) and shirona (*Brassica papa* var. *pekinensis*) biomass. J. Plant Nutr. 35(3):471-479.

Nakayama, M. 1993. Wine grape growing in Japan. J. Brewing Soc. Japan 88(9):654-659.

Shiraishi, T., Toyoda, K., Suzuki, T., Meguro, A., Hasegawa, S., Nishimura, T. and Kunoh, H. 2010. Effect of FFC ceramic water on the infection process of a fungal pathogen. Scientific Reports of the Faculty of Agriculture Okayama University 99:27-34.

Toyoda, K., Matsuoka, S., Meguro, A., Hasegawa, S., Nishimura, T., Kunoh, H. and Shiraishi, T. 2010. FFC ceramic water™ enhances plant apyrase activity. Scientific Reports of the Faculty of Agriculture Okayama University 99:21-26.

The Report of IPPS Exchange Program of New Zealand Region and Japan Region©

Takumi Hidaka
Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-Nishi, Miyazaki, 889-2192, Japan
Email: tetsumur@cc.miyazaki-u.ac.jp

From 12 to 29 April, 2013, I participated in the IPPS exchange program between New Zealand and Japan Regions. During my staying, many IPPS New Zealand (NZ) Region members welcomed me as hosts and treated me graciously. I had a profitable time and I gained inspiration and experiences that I had not gained in Japan. Additionally, I attended the New Zealand Annual Conference which was convened during my staying as a member of a 4-Pack. I enjoyed the various presentations and discussions, and gained an understanding of part of horticulture in NZ. I will present here what I experienced during my staying in NZ.

I first encountered feijoa (*Acca sellowiana* syn. *Feijoa sellowiana* Berg.), a myrtaceous fruit, during this journey (Fig. 1). New Zealand is the world's largest feijoa producer and all nurseries I visited dealt in feijoa. The fruit has a moderate sour and crunchy texture, and I loved it very much (Fig. 2). On the west coast of NZ, an area of high rainfall, I visited Tree Top Walk, which was the bridge threading through the trees in a deep forest. On this walk I saw a lot of NZ native plants and was impressed by their beauty and grandeur (Fig. 3). A nursery I visited in Nelson dealt in not only NZ native plants but also various fruit trees including persimmon (*Diospyros kaki* Thunb.) (Fig. 4), which I use for my research. At a nursery I visited in Palmerston North, I learned practical cutting propagation methods. The pots were immersed in warm water to grow callus at the base of cuttings and cool water was used for the mist to improve rooting (Fig. 5). At the end of the journey, I deepened exchanges with a lot of NZ Region's members at the annual conference, and it was a valuable experience when I talked with them about similarities and differences between Japan and NZ.

All host families kindly took me around to places of interest. For example, I watched a colony of fur seals, saw Parliament House (beehive shape) (Fig. 6) which is a building that symbolizes the capital, Wellington, and visited Christ Church Cathedral in Nelson. I enjoyed NZ very much.

I would like to express my gratitude to IPPS Japan regional members who gave me the chance and provided me a valuable experience and to NZ IPPS Region's members who took care of me. I want to make use of the meaningful experiences for my life in the future. I hope to see an expansion of this exchange program and deepening exchanges between the two regions, so that a lot of members will have valuable experiences in both countries.

Fig. 1. A feijoa tree in Jeff Elliott's Nursery (Christchurch).

Fig. 2. Tasting feijoa in Jeff Elliott's house (Christchurch).

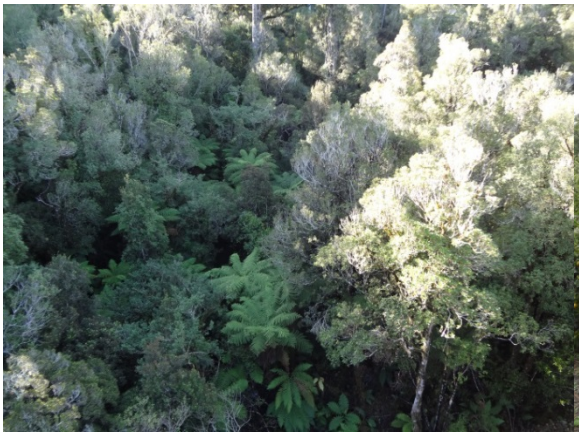

Fig. 3. New Zealand's native trees; a view from "Top Tree Walk" in Hokitika.

Fig. 4. Persimmon trees in Waimea Nursery (Nelson).

Fig. 5. Cutting propagation house in Plantlife Propagators (Palmerston North).

Fig. 6. Beehive parliament house (Wellington).

The Micropropagation *Begonia boliviensis* Crackling Fire© Series©

Fang Li, Masaki Ochiai and Hirokazu Fukui
Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Ryuichi Tachibana
Suntory Flowers Ltd, 863-1 Iketani, Omori, Higashiomi, Shiga 527-0063, Japan
Email: fukui@gifu-u.ac.jp

In this research we used TDZ (0.1, 1.0, 10 μM), 4CPPU (0.1, 1.0, 10 μM), BAP (0.1, 1.0, 10 μM) with NAA (0.0, 0.1 μM) to test the formation ability of adventitious buds, and we found that NAA (0.1 μM) is essential for the formation of adventitious buds. For the explants, stem segment, petiole section, leaf lamina and pedicel section were chosen, and we found that stem segment had the highest formation ability of adventitious buds. Comparing different cytokinins and those concentrations with NAA, we found the index of adventitious bud formation in ½N MS medium containing TDZ (10 μM) and NAA (0.1 μM) was the highest, so we considered that TDZ (10 μM) and NAA (0.1 μM) is optimal for the formation of adventitious buds for *Begonia* Crackling Fire® series.

INTRODUCTION

The *Begonia* genus which contains about 1400 different species is a perennial herb with soft succulent stems. *Begonia* 'Crackling Fire®' series (*Begonia boliviensis* var. *sunjiraore*) bred by Suntory Flowers Ltd., contains short internodes, showy and naturally compact flowers, with a much higher flower count. However, most axillary buds differentiate into flower buds in this series. Vegetative propagation, such as cuttings, cannot provide high propagation efficiency and seed propagation cannot maintain phenotypic stability. In this study, we tried to develop an efficient micropropagation system for *B. boliviensis* Crackling Fire® series.

MATERIALS AND METHODS

Plant Materials and Sterilization

Plants of *B. boliviensis* Crackling Fire® Orange, Creamy Yellow, Pink, and White growing in pots were collected in May 2013 from Suntory Flowers's greenhouse.

Stem segment (2 mm), petiole section (2 mm), leaf lamina (2×2 mm), and pedicel section (2 mm) were used as explants. After washing with neutral detergent and rinsing, the explants were surface sterilized with ethanol (70%, 60 s) and then sterilized by 10 min immersion in 1% sodium hypochlorite solution (NaClO) with a drop of Tween 20 in 100 ml NaClO solution. They were rinsed four times with sterile water then transferred to the culture media.

Media Preparation

Murashige and Skoog (MS) medium was chosen as the inorganic formulation with half strength nitrogen content of the original recipe (½N MS). The media were supplemented with sucrose (30 $g \cdot L^{-1}$) and gelling agent (agar, 7 $g \cdot L^{-1}$), and pH was adjusted to 5.8 by NaOH and HCl. Growth regulators were added and then the media were autoclaved (120°C, 15 min).

Experiment 1: Formation of Adventitious Buds by Different Explants on Various Propagation Media

Explants were placed in the ½N MS medium with different concentrations and combination of thidiazuron (TDZ; 0.1, 1.0, 10.0 μM), N-(2-chloro-4-pyridyl)-N'-phenylurea (4CPPU; 0.1, 1.0, 10.0 μM), and 6-benzylaminopurine (BAP; 0.1, 1.0, 10.0 μM) with

α-naphthaleneacetic acid (NAA; 0.0, 0.1 μM). Each treatment involved 10 replications. There are 20 treatments in all (Table 1). Observation was conducted 6 weeks later after culturing.

Table 1. The growth regulators and concentrations for treatments.

Treatment	TDZ (μM)	4CPPU (μM)	BAP (μM)	NAA (μM)
A	–	–	–	–
B	0.1	–	–	–
C	1.0	–	–	–
D	10.0	–	–	–
E	–	0.1	–	–
F	–	1.0	–	–
G	–	10.0	–	–
H	–	–	0.1	–
I	–	–	1.0	–
J	–	–	10.0	–
K	–	–	–	0.1
L	0.1	–	–	0.1
M	1.0	–	–	0.1
N	10.0	–	–	0.1
O	–	0.1	–	0.1
P	–	1.0	–	0.1
Q	–	10.0	–	0.1
R	–	–	0.1	0.1
S	–	–	1.0	0.1
T	–	–	10.0	0.1

Experiment 2: Formation of Adventitious Buds, Leaf Number, and Maximum Leaf Length on Various Propagation Media

Multiple shoots obtained in Expt. 1 were cut into blocks (4 × 4 mm). All explants were placed in the ½N MS medium with TDZ (10 μM) or 4CPPU (0.01, 0.1 μM) with NAA (0.1 μM), and we set the number of treatments as follows: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM). Each treatment involved 15 repetitions. After 6 weeks observation was conducted.

RESULTS AND DISCUSSION

Experiment 1: Formation of Adventitious Buds by Different Explants on Various Propagation Media

For observation we set the indexes for counting adventitious buds, "0" means no buds, "1" means few, "2" means some, "3" means many, "4" means a large number of buds.

Almost no adventitious buds were observed on media without NAA (Fig. 1). The effectiveness of TDZ (10 μM) with NAA (0.1 μM) was remarkable, and index of adventitious bud formation reached 0.79. Reducing the TDZ concentration reduced adventitious buds formation. High cytokinin concentration with NAA was more effective than cytokinin used alone. In vitro regeneration of four *Begonia* genotypes, *B.*

Semperflorens Cultorum Group, *B. rex*, *B. elatior*, and a hybrid of *Begonia* with unknown parents 'Tiger' was carried out from leaf and petiole segments as explants (Espino et al. 2004). It was mentioned that shoot regeneration was preferentially induced on media containing BAP; quantitative differences being observed among explants and genotypes. However in our research, BAP was not effective for the formation of adventitious buds (Fig. 1). We feel that the reason may be related to genetic differences. Mendi et al. (2009) mentioned that cytokinins are often used to stimulate growth and development. Cytokinins promote cell division when it added together with auxins. With the decreasing concentration of 4CPPU, the formation of adventitious buds was increasing, so we think lower concentration of 4CPPU can induce better formation of adventitious buds.

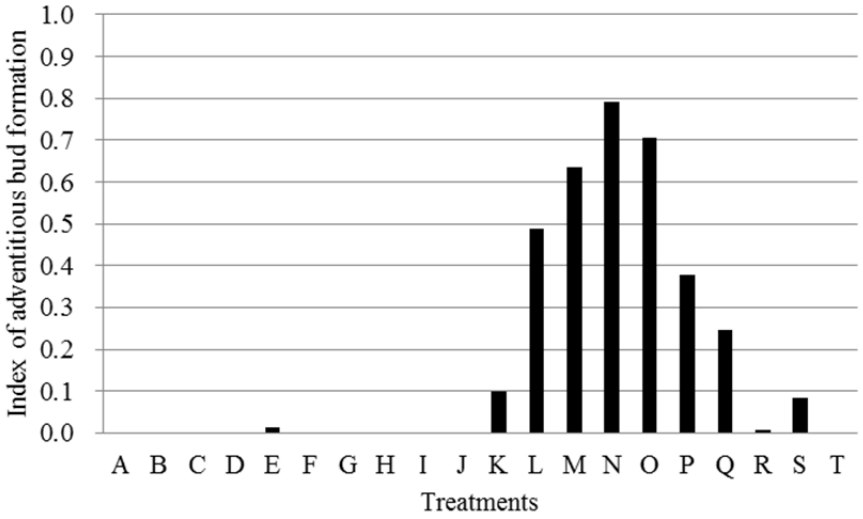

Fig. 1. Formation of adventitious buds on various treatments.

It is clear that only stem segments and petiole sections could produce adventitious buds (Fig. 2). Stem segments especially showed a higher index than petiole sections and the index reached 0.48, when we compared only stem segments and petiole sections in different concentration of cytokinins. Stem segments on medium containing with TDZ (10 μM) and NAA (0.1 μM) showed highest index of adventitious bud formation (Fig. 3). Therefore, we considered stem segment to be the optimal explant for the formation of adventitious buds in the *B. boliviensis* Crackling Fire® series.

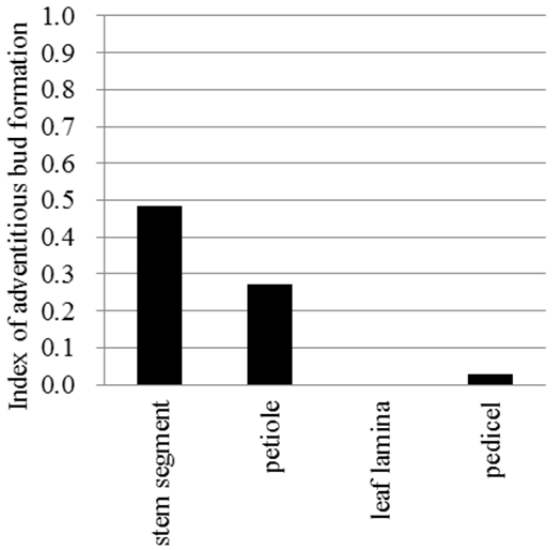

Fig. 2. Formation of adventitious buds from different explants.

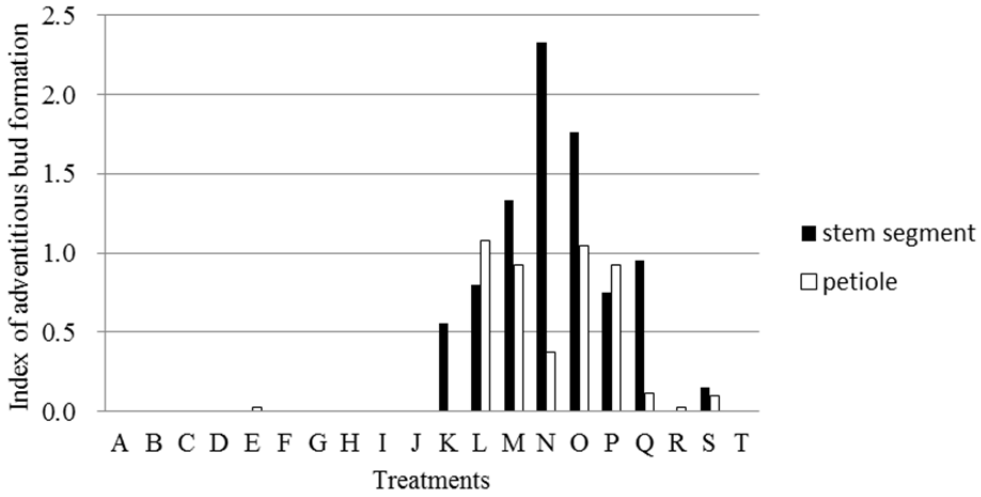

Fig. 3. Formation of adventitious buds from stem segment and petiole section on various treatments.

Experiment 2: Formation of Adventitious Buds, Leaf Number and Maximum Leaf Length on Various Propagation Media. Considering the results from Expt. 1, in Expt. 2 we chose three media to confirm the hypothesis of Expt. 1 that lower concentration of 4CPPU may promote adventitious bud formation. In comparison with Treatment C, the index of adventitious bud formation in Treatment B was higher (Fig. 4), so lower concentration of 4CPPU (0.01 μM) could not induce higher formation of adventitious buds. In the medium containing TDZ (10 μM) and NAA (0.1 μM), explants differentiated many adventitious buds with small leaves (Figs. 5, 6, and 7), and it was clear that that medium was the most effective for the formation of adventitious buds and was a suitable medium for micropropagation of *B. boliviensis* Crackling Fire® series.

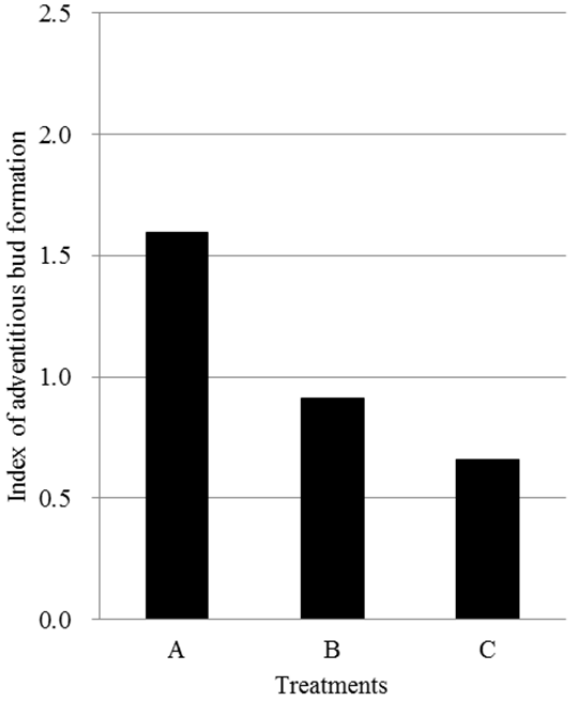

Fig. 4. Formation of adventitious buds on different treatments: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), and (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

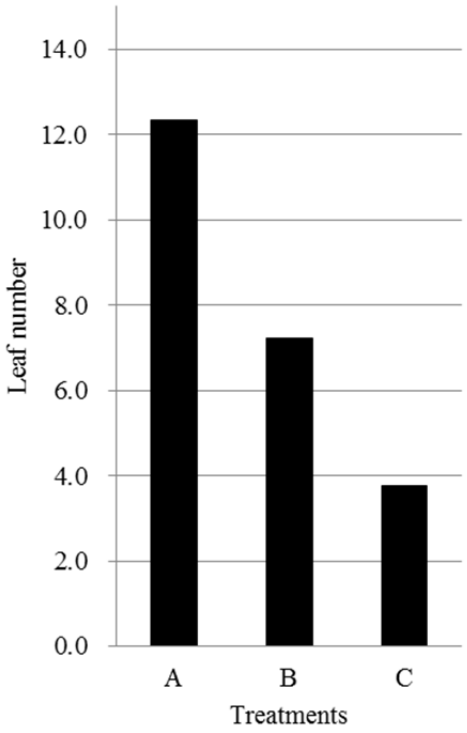

Fig. 5. Leaf number in different treatments (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

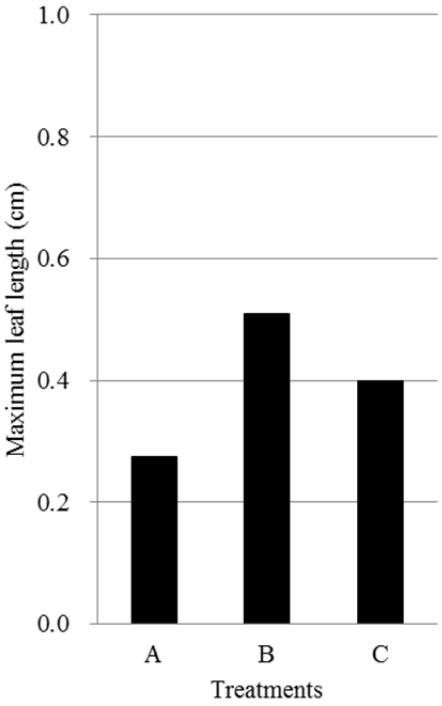

Fig. 6. Maximum leaf length in different treatments: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

Fig. 7. Growth situation of Experiment 2 after 6 weeks: (A) TDZ (10 μM) with NAA (0.1 μM), (B) 4CPPU (0.1 μM) with NAA (0.1 μM), and (C) 4CPPU (0.01 μM) with NAA (0.1 μM).

Although explants on media containing 4CPPU (0.1, 0.01 μM) and NAA (0.1 μM) developed a few adventitious shoots, leaves were larger than those on Treatment A (TDZ (10 μM) with NAA (0.1 μM)). Especially the maximum leaf length was the longest in medium contained 4CPPU (0.1 μM) (Fig. 6), and there was a large number of roots in media containing 4CPPU (Fig. 7). So, micropropagated bud on medium containing with TDZ (10 μM) and NAA (0.1 μM) were able to make elongate shoots, leaves, and roots by transplanting to medium containing 4CPPU (0.1 μM) and NAA (0.1 μM), and it was able to make plantlets for acclimation.

Literature Cited

Espino, F.J., Linacero, R., Rueda, J. and Vazquez, A.M. 2004. Shoot regeneration in four begonia genotypes, Biol. Plant 48(1):101-104.

Mendi, Y.Y., Curuk, P., Kocaman, E., Unek, C., Eldogan, S., Gencel, G. and Cetiner, S. 2009. Regeneration of begonia plantlets by direct organogenesis. Afric. J. Biotechnol. 8(9):1860-1863.

Micropropagation of Ornamental Aquatic Plants, *Glossostigma*, *Microcarpaea*, and *Limnophila*©

Toru Niki and Wakanori Amaki
Department of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 246-0034, Japan
Email: amaki@nodai.ac.jp (corresponding author)

Three aquatic plants, *Glossostigma elatinoides* (Benth.) Hook.f., *Limnophila* sp. (unidentified), and *Microcarpaea minima* (K.D. Koenig ex Retz.) Merrill. were examined to clarify the optimal culture medium and cultural conditions for their micropropagation. Murashige and Skoog (MS) medium strength (1/1, 1/2, 1/4 and 1/8), sucrose concentration (0, 10, 20, 30, and 40 g·L^{-1}), initial pH of medium (4.5, 5.0, 5.5, 6.0, and 6.5), and the kind and concentration of gelling agent (6, 7, 8, 9, 10 g·L^{-1} for agar and 3 g·L^{-1} for gellan gum) were examined. The optimal medium conditions for all of the examined plants was ½ MS, 20 g·L^{-1} sucrose, and 3 g·L^{-1} gellan gum. The optimal medium pH was 5.0 for *G. elatinoides* and 6.0 for *M. minima* and *Limnophila*. Water supplement during culture was effective on the growth and acclimatization of multiplied plants. The optimal timing of supplement was after 20 days for *G. elatinoides* and 30 days for *M. minima* and *Limnophila* from the explant inoculation.

INTRODUCTION

In recent Japan, the enjoyment of aquariums is booming, and the commercial demand for aquatic plants has greatly increased. In vitro tissue culture has been identified as an effective technique for large scale multiplication of elite plants. Several reports have demonstrated that aquatic plants can be multiplied by in vitro propagation through proliferation from pre-existing bud in *Cryptocoryne*, *Anubias*, *Myriophyllum*, and *Potamogeton* (Kane et al., 1990, 1999; Huang et al., 1994; Zhou et al., 2006; Kanchanapoom et al., 2012), through adventitious shoot formation in *Limnophila* and *Aponogeton* (Rao and Mohan Ram, 1981; Carter and Gunawardena, 2011) or embryogenesis in *Scirpus* and *Nymphoides* (Wang et al., 2004; Myung et al., 2010). However, the most suitable micropropagation method for each species which are commercially common in Japan is still uncertain. That is, the present conditions for the mass production of high-quality aquatic plants for aquarium is not known. Therefore, we started a series of experiments to clarify the basic culture conditions for mass production of aquatic plants by micropropagation. At first, we selected three aquatic plants for experiment materials because those plants are used for the aquarium frequently in Japan. We chose the pre-existing bud culture method because we wanted to propagate clone plants without somatic variations. In this reports, we tried to clarify the most suitable culture medium and the effect of water supplement during in vitro culture for the acclimatization to aquarium conditions.

MATERIALS AND METHODS

Preparation of Materials

Shoot tip explants (about 1 cm long) were prepared from in vitro mother plants, *Glossostigma elatinoides* (*Phrymaceae*), *Microcarpaea minima* (*Plantaginaceae*) and *Limnophila* sp. (unidentified; *Plantaginaceae*) which were provided gratis by EARTH ONE Ltd. (Tokyo, Japan). The explants were placed on the multiplication medium [half strength of Murashige and Skoog (1962) medium (MS) + 20 g·L^{-1} sucrose + 8 g·L^{-1} agar, pH 5.8] for maintenance and multiplication of stock plants for the following experiments.

Experiments for the Optimal Medium Constitution (Experiment 1)

Murashige and Skoog medium strengths (1/1, 1/2, 1/4 and 1/8), sucrose concentrations (0, 10, 20, 30, and 40 g·L^{-1}), initial pH values of the media (4.5, 5.0, 5.5, 6.0, and 6.5), and the kind and concentration of gelling agents [6, 7, 8, 9, 10 g·L^{-1} for agar (Kanto Chemical Co.

Inc., Japan) and 3 $g \cdot L^{-1}$ for gellan gum (Wako Pure Chemical Industries, Ltd., Japan)] were examined.

Effects of Water Supplement during Culture (Experiment 2)
Fifty milliliters of autoclaved pure water with the pH adjusted to 5.8 using 0.1 N NaOH just before autoclaving was poured into each test tube on the gelled media at 10, 20, 30 days after the inoculation of the explants. The growth after water supplement was compared with the cultures without water supplement (control).

Culture Conditions and Measurements
Twenty milliliters of each medium was poured into a φ40×130 mm flat-bottomed glass test tube and autoclaved at 120°C for 15 min before explant inoculation. All cultures were incubated under 23±1°C and 16-h light with cool white fluorescent lamps (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) with an 8-h dark period. Total fresh weight of multiplied plants in each test tube was measured at 40 days for Experiment 1 and 50 days for Experiment 2 after the inoculation of explants.

RESULTS AND DISCUSSION
The results of media strength are presented in Figure 1. On the MS medium used as the standard medium for plant tissue culture abnormal growth (fading and death of leaves, and growth retardation of roots) was observed. The cause of the abnormal growth seems to be high osmotic pressure stress. All three species showed the best growth on the ½ MS medium. Figure 2 shows the results of sucrose concentration. The optimal concentration was 20 $g \cdot L^{-1}$ for *Glossostigma elatinoides*. The highest value of fresh weight in *Microcarpaea minima* and *Limnophila* was obtained on the medium supplemented with 40 $g \cdot L^{-1}$ of sucrose. However, more than 30 $g \cdot L^{-1}$ of sucrose caused the abnormal growth and early fading and death of leaves. The best concentration for the normal growth was also 20 $g \cdot L^{-1}$ in *M. minima* and *Limnophila*. The optimal pH was 5.0 for *G. elatinoides* and 6.0 for *M. minima* and *Limnophila* (Fig. 3). The best gelling agent for all of three species was 3 $g \cdot L^{-1}$ of gellan gum (Fig. 4).

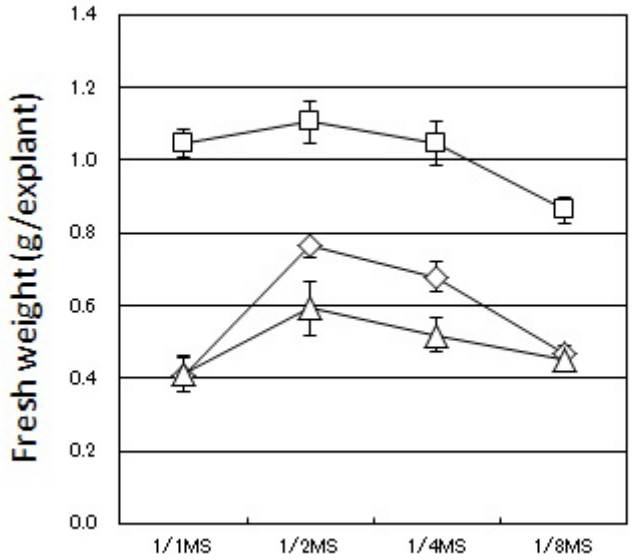

Fig. 1. The effects of medium strength on the growth of three aquatic plants, *Microcarpaea minima* (□), *Glossostigma elatinoides* (◇), and *Limnophila* sp. (△).

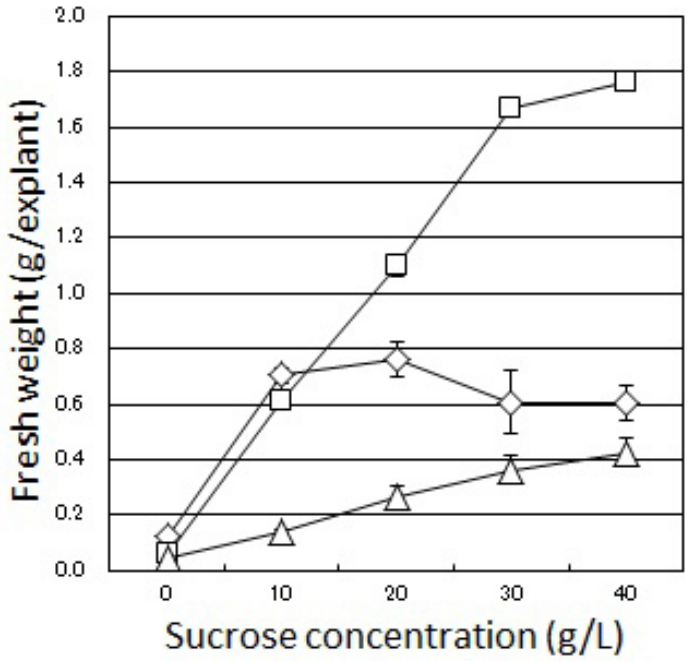

Fig. 2. The effects of sucrose concentration on the growth of three aquatic plants, *Microcarpaea minima* (□), *Glossostigma elatinoides* (◇), and *Limnophila* sp. (△).

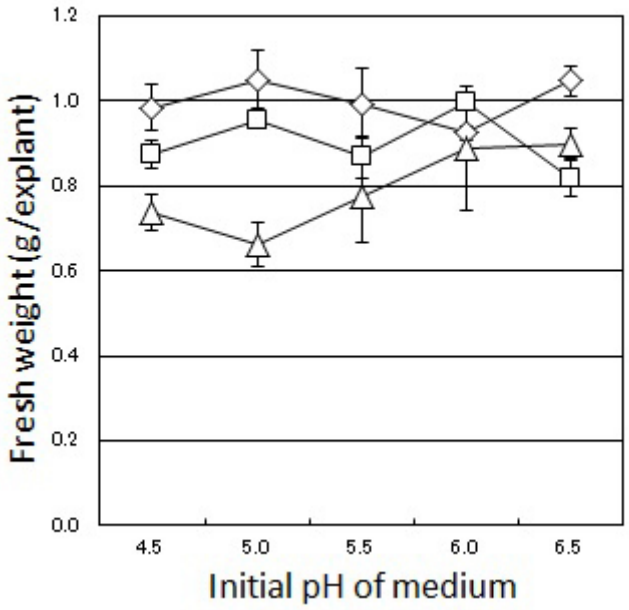

Fig. 3. The effects of initial pH of medium on the growth of three aquatic plants, *Microcarpaea minima* (□), *Glossostigma elatinoides* (◇), and *Limnophila* sp. (△).

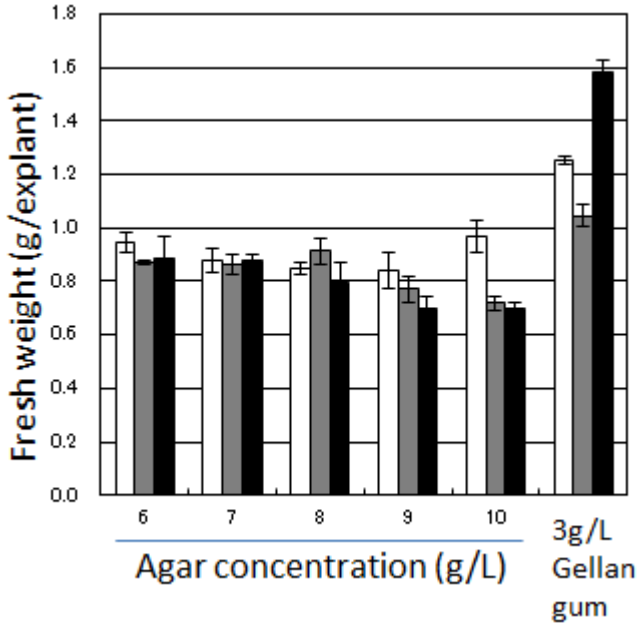

Fig. 4. The effects of gelling agents on the growth of three aquatic plants, *Glossostigma elatinoides* (□), *Microcarpaea minima* (▒), and *Limnophila* sp. (■).

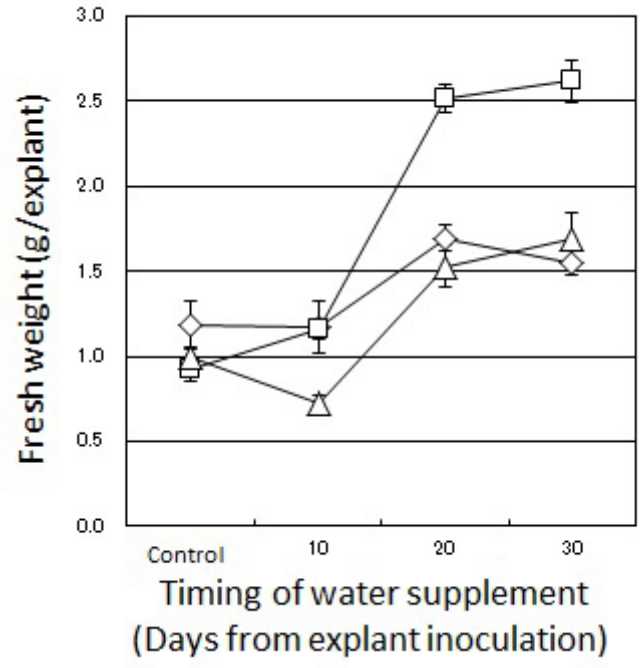

Fig. 5. The effects of water supplement and its timing on the growth of three aquatic plants, *Microcarpaea minima* (□), *Glossostigma elatinoides* (◇), and *Limnophila* sp. (△).

Water supplement during culture was effective on the growth and acclimatization of multiplied plants (Fig. 5). All three plants investigated are amphibious-respondors (amphibious-respondor refers to plant which can live under water and in the ground. It is a term mainly used for the plant groups which can live also at the place where a water level is

fluctuated in the field of ecology) which are able to grow in water presence or absence condition. After water supplement, leaves of the plants were morphologically changed from aerial (emerged) leaves to submerged leaves (leaves in the water) which were characterized with the linear and thin thickness. Therefore, multiplied plants on the gelled media with gellan gum could be acclimatized to aquarium conditions by water supplement during culture. The optimal timing of supplement was after 20 days for *G. elatioides* and 30 days for *M. minima* and *Limnophila* from the explant inoculation (Fig. 5).

Literature Cited

Carter, J. and Gunawardena, A.H.L.A.N. 2011. Regeneration of the aquatic monocot *Aponogeton madagascariensis* (lace plant) through callus induction. Aquat. Bot. 94:143-149.

Huang, L., Chang, Y. and Chang, Y. 1994. Rapid in vitro multiplication of the aquatic angiosperm, *Anubias barteri* var. *undulata*. Aquat. Bot. 47:77-83.

Kanchanapoom, K., Chunui, P. and Kanchanapoom, K. 2012. Micropropagation of *Anubias barteri* var. *nana* from shoot tip culture and the analysis of ploidy stability. Not. Bot. Horti. Agrobo. 40:148-151.

Kane, M.E., Gilman, E.F., Jenks, M.A. and Sheehan, T.J. 1990. Micropropagation of the aquatic plant *Cryptocoryne lucens*. HortScience 25:687-689.

Kane, M.E., Davis, G.L., McConnell, D.B. and Gargiulo, J.A. 1999. In vitro propagation of *Cryptocoryne wendtii*. Aquat. Bot. 63:197-202.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Myung, J.O., Hye, R.N., Hong-Keun, C., Jang, R.L. and Suk, W.K. 2010. High frequency plant regeneration system for *Nymphoides coreana* via somatic embryogenesis from zygotic embryo-derived embryogenic cell suspension cultures. Plant Biotech. Rep. 4:125-128.

Rao, S. and Mohan Ram, H.Y. 1981. Regeneration of whole plants from cultured root tips of *Limnophila indica*. Can. J. Bot. 59:969-973.

Wang, J., Seliskar, D.M. and Gallangher, J.L. 2004. Plant regeneration via somatic embryogenesis in the brackish wetland monocot *Scirpus robustus*. Aquat. Bot. 79:163-174.

Zhou, C., An, S., Jiang, J., Yin, D., Wang, Z., Fang, C., Sun, Z. and Qian, C. 2006. An in vitro propagation protocol of two submerged macrophytes for lake revegetation in east China. Aquat. Bot. 85:44-52.

Possible Use of Plant Peptide Hormone in Horticulture©

Takuya Tetsumura
Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-Nishi, Miyazaki 889-2192, Japan
E-mail : tetsumur@cc.miyazaki-u.ac.jp

Plant hormones, phytohormones, are chemicals acting as signal molecules. They are produced in plants and occur in extremely low concentrations. They shape the plants and are environmentally responsive signal molecules. We accept that there are five major classes of plant hormones: abscisic acid, auxins, cytokinins, ethylene, and gibberellins. Recently, brassinosteroids, jasmonates, florigen (FT protein), plant peptide hormones, salicylic acid, and strigolactone have been identified as plant hormones by plant physiologists. However, I am sure that most people involved in horticulture do not know about plant peptide hormones and strigolactone.

Plant growth regulators(PGRs) are extensively used in horticulture. They are plant hormones, derivatives of plant hormones, and chemicals controlling and enhancing natural plant growth processes. One PGR, prohydrojasmon, is a skin color accelerator for apples and table grapes and a rind puffing inhibitor for satsuma mandarins. Prohydrojasmon is a derivative of jasmonates which promotes the production of defense proteins, signal molecules, which are used to fend off invading organisms. If prohydrojasmon is used more in agriculture and horticulture, we will recognize jasmonates as plant hormones. Brassinosteroids have been intensively investigated for three decades and used on farms with poor environment to improve crop production, but they are not used in advanced countries because their agricultural land is fertile. Hence, most farmers do not know brassinosteroids. It will take a long time for most agriculturists and horticulturists to utilize brassinosteroids as plant hormones. New signal molecules found by plant physiologists may be recognized as plant hormones after an extensive use of them or their derivatives in agriculture and horticulture.

Peptides are short amino acid chains and peptide hormones such as growth hormone, insulin, and vasopressin are well known as important hormones in animals. Peptides also exist in plants and, in recent years, many studies have demonstrated that peptide signaling plays a great role in various aspects of plant growth and development. Plant physiologists have found over 15 plant peptide hormones. Systemin is an 18 amino acid peptide and its main function is to coordinate defensive responses against insect herbivores through the production of jasmonic acid. Phytosulfokine is 5 amino acid peptide and promotes proliferation of plant cells. Stomagen is 102 amino acid peptide and controls differentiation of stomata. LUREs are 83 and 93 amino acid peptides that were identified as pollen-tube attractants.

A substantial proportion of these peptides are secretory and act as local signals mediating cell-to-cell communication. Specific receptors for several peptides were identified as being membrane-localized receptor kinases (Matsubayashi and Sakagami, 2006). These results were found in a model plant, *Arabidopsis thaliana*, and most of the basic research on plant peptide hormones were conducted with *Arabidopsis thaliana* by plant physiologists. However, we can find no report about their use in applied research, although basic research has indicated that they could be explored as PGRs in agriculture and horticulture.

Arabidopsis thaliana has 32 CLE peptides, named for the CLAVATA3 (CLV3)/ESR-related peptide family, including CLV3 (Betsuyaku et al., 2011). The CLE peptides are thought to regulate cellular differentiation activity in the shoot and root apical meristem, and synthetic CLV3 peptide reduced the size of shoot and root meristems (Kondo et al., 2006). We identified five *CLV3*-like genes from grape vine (Tominaga-Wada et al., 2013). These *CLV3*-like genes encode peptides containing 43 - 128 amino acids. Expression analyses showed that the five grape *CLV3*-like genes are expressed in leaves, stems, roots, and axillary buds with significant differences in their

levels of expression.

Callus cultures of persimmon, citrus, and lychee were cultured on differentiation media supplemented with 1 μM synthetic CLV3 peptide, which killed buds on mango shoots, but they grew as well as those on the control media. One of CLE peptide family, CLE25, is thought to be more biologically active than CLV3. For rooting of persimmon shoots, 100 nM CLE25 was added to the root inducing medium, and inhibited their rooting and decreased the number of roots compared to the control. Moreover, 100 nM CLE25 in the root development medium, which was used 10 days after the root induction in the root inducing medium, did not prevent the shoots from rooting. These results suggested that the differentiation of root primordia was suppressed by CLE25. Similarly, CLE25 may control root meristems, because the root meristems in the root development medium with 100 nM CLE25 seemed to stop growing 40 days after the rooting culture. The CLE peptide family is known to control differentiation of epidermal cells and further experiments are needed.

Synthetic CLE peptides are very expensive to produce. In addition, the biological activities of peptides are drastically changed by glycosylation and phosphorylation. It will take a very long time to use plant peptide hormones in horticulture. I hope for rapid progress of studies in this field.

ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grant Number 24658032.

Literature Cited

Betsuyaku, S., Sawa, S. and Yamada, M. 2011. The function of the CLE peptides in plant development and plant-microbe interactions. Arabidopsis Book 9:e0149.

Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H. and Sakagami, Y. 2006. A plant peptide encoded by CLV3 identified by in situ MALDI-TOF MS analysis. Science 313:845-848.

Matsubayashi, Y. and Sakagami, Y. 2006. Peptide hormones in plants. Annu. Rev. Plant Biol. 57: 649-674.

Tominaga-Wada, R., Nukumizu, Y., Wada, T., Sawa, S. and Tetsumura, T. 2013. CLAVATA3-like genes are differentially expressed in grape vine (*Vitis vinifera*) tissues. J. Plant Physiol. 170:1379-1383.

Pollen Germination Ability of Acerola in Relation to Fruit Set©

Naomi Amari, Yuuki Nakano and Naoto Iwasaki
School of Agriculture, Meiji University, Kawasaki, Kanagawa 213-8571, Japan
Email: iwasaki@isc.meiji.ac.jp

The fruit of acerola (*Malpighia*) are known for their extremely high vitamin C content. However, the rate of fruit set by open pollination is generally low. Therefore, factors that might affect pollen germination rate such as temperature, humidity, and time of flowering during the day were investigated using several cultivars. The pollen germination rate varied depending on the time sampled, while treatment with humid air after flowering was found not to influence the pollen germination rate. Flower buds were sampled from acid-type trees about 3 days before bloom. They were maintained in an incubator at 25, 30, 35, and 40°C. For buds that bloomed after 2 or 3 days of incubation, the pollen grains were sown on agar medium and maintained at 27°C for 5 h. The pollen germination rate decreased significantly when the buds were placed under high-temperature conditions at 35 or 40°C. It can be inferred that the nutritional conditions of the tree, such as photosynthetic products during flower bud development, affect the pollen germination rate at flowering time.

INTRODUCTION

Acerola, an evergreen shrub in the family *Malpighiaceae*, is regarded as having originated in Central America. The fruit is extremely rich in vitamin C. Their contents are almost 3,000-4,000 mg per 100 g for immature fruit or about 2,000 mg per 100 g for mature fruit. Therefore, the fruit has attracted interest not only for its nutritional function but also for medical applications. The trees continue growth at 15°C or more, and bloom 3-4 times each year. Nevertheless, the rate of fruit set is generally low (Ishihata and Ito, 1994; Yonemoto, 2009). Yonemoto (2009) noted that trees in the open field set fruit well by open pollination, but that the rate of fruit set by open pollination is low under greenhouse culture in Okinawa. Acerola is entomophilic, and can set fruit by either self-pollination or cross-pollination. Acerola anthers produce many imperfect pollen grains, which is regarded as one reason for its lower rate of fruit set. Reportedly, the pollen germination rate is low (Handa et al., 2003) because the anther does not dehiscence automatically in Japanese cultivars or in those recently introduced from overseas (Handa et al., 2005). However, more than 50% of open pollinated flowers reportedly set fruit in Jamaica (Raw, 1979), and 51.7% of hand-pollinated flowers set fruit in Hawaii (Yamane and Nakasone, 1961). Freitas et al. (1999) reported that 30% of open pollinated flowers, 23.8% of cross-pollinated flowers, and 17.3% of self-pollinated flowers set fruit in Brazil. Therefore, the rate of fruit set in acerola can vary greatly among cultivars and according to environmental conditions.

In this study, factors that might affect pollen germination rate such as temperature, humidity, and time of flowering each day were investigated using several acerola cultivars.

MATERIALS AND METHODS

Plant Material

Experiments were conducted in 2012 with trees growing in a greenhouse at Meiji University. The cultivars used in the experiments included two cultivars of sweet-type and one cultivar of acid-type (cv. unknown). One sweet-type cultivar was 'Hawaiian Queen' (Sweet type B). The other was unknown (sweet-type A). These trees were obtained from Kagoshima University in 2006 and were planted in 29 or 60-L pots with humus soil.

Fruit Set by Hand Pollination

Pollen of the acid-type cultivar was hand-pollinated to flowers of the sweet-type cultivars at 9:00, 12:00, and 15:00 on 20 July and 21 July. The anthers of acid-type cultivar were

collected one day before hand pollination, with dehiscence at 28°C for 24 h. Hand pollination of flowers of sweet-type cultivars was performed using a small paintbrush. The pollinated flowers were assigned a label to record the flowering date and time. The fruit set percentages were determined 2 weeks after pollination.

Pollen Germination Rate

The pollen germination rate was evaluated on agar medium containing 1% agar, 0.01% of H_3BO_3, and 20 or 30% of sucrose. In addition, the pH of the medium was adjusted to 6.0. The pollen germination percentage was determined under a digital microscope (VH-8000C; Keyence Co.) as the ratio of the number of germinated grains per field of view to the total number of grains per field of view. A pollen grain was regarded as germinated when the pollen tube length was greater than the grain diameter. On 20 July and 21 July, flowers of acid type were sampled at 9:30, 12:30, and 15:30. Then the pollen grains were sown on the agar medium immediately and were incubated under dark condition at 27°C for 24 h.

On 8 August flowers were sampled immediately after blooming from two sweet-type trees and one acid-type tree. Then anthers were collected from each flower. The anthers of each cultivar were divided into two groups. Those of one group were allowed to open in the usual manner after being put into a drying oven maintained at 28°C for 24 h. Another group of anthers was then wrapped with the paraffin paper and put into the 100 ml beaker with a small quantity of water for treatment with humid air for 24 h. The pollen grains were sown on the agar medium after treatment. They were then incubated at 27°C for 5 h.

Flower buds at 1-3 days before blooming were sampled from acid-type trees in August, September, and November. The floral axis of the bud was dipped into water (August) or properly diluted floral preservative solution (September and November) in a small container (ca. 50 ml). They were maintained at 25, 30, 35, and 40°C in an incubator (LH-30-8CT; Nippon Medical and Chemical Instruments Co. Ltd.). The day length in the incubator was fixed at 14.5 h. The buds bloomed after 1 or 2 days. Then the pollen grains collected from bloomed flowers and sown on the agar medium and maintained at 27°C for 5 h.

Pollen Morphology

The diameters of germinated and the non-germinated pollen were determined under a digital microscope.

RESULTS AND DISCUSSION

Fruit set by the cross pollination at different times after blooming are shown in Table 1. The flowers of 'Hawaiian Queen' set no fruit by pollination at any time. Although the sweet type B set fruit by pollination at 9:00 AM and 15:00 PM, the rates were 5% at both times. As noted also in previous reports (Handa et al., 2003; Yonemoto, 2009), the rate of fruit set of acerola trees used for this study was very low.

Table 1. Differences in the fruit set by cross-pollination among the times of pollination in sweet type cultivars.

Cultivar	Time of pollination	No. flower	No. fruit set	Rate of fruit set (%)
Sweet-type A	9:00	40	2	5.0
	12:00	20	0	0.0
	15:00	40	2	5.0
Sweet-type B	9:00	15	0	0.0
	12:00	10	0	0.0
	15:00	15	0	0.0

The pollen germination rate was generally higher in the acid-type cultivar than in the sweet-type cultivar in these experiments: the germination rate of acid-type cultivar was about 20%, although it was invariably less than 10% in sweet-type cultivars. However, the germination rates of the pollen collected from the flowers after bloom showed no consistent tendency, even in the acid-type cultivar. The pollen germination rate differed depending on the time at which the pollen was sampled (Fig. 1). Matsuda et al. (2009) reported that the pollen of passion fruit maintained germination ability for a longer period under humid conditions (about 80% RH) in comparison with dry condition (about 13% RH). However, in acerola, the relative humidity of the air was also considered not to influence the germination rate of pollen collected from the flowers after blooming (Fig. 2). Therefore, the daily variation in the pollen germination rate seemed to be unaffected by relative humidity of the air.

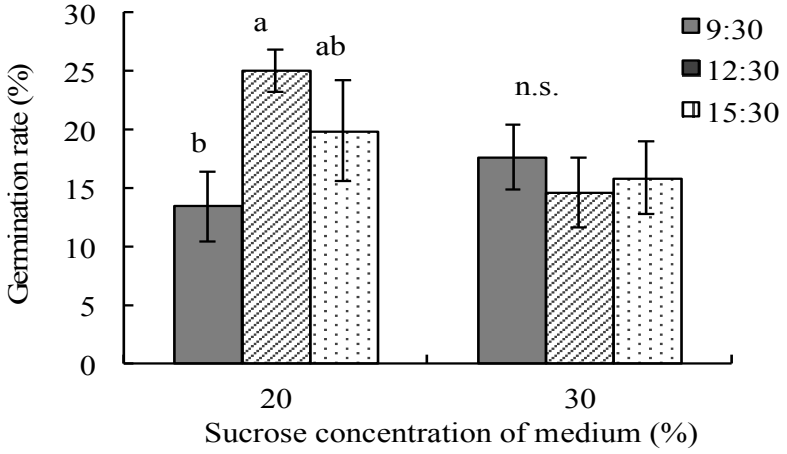

Fig. 1. Changes in the germination rates after flowering. Values with different letters are significantly different by Fisher's PLSD at 5% level.

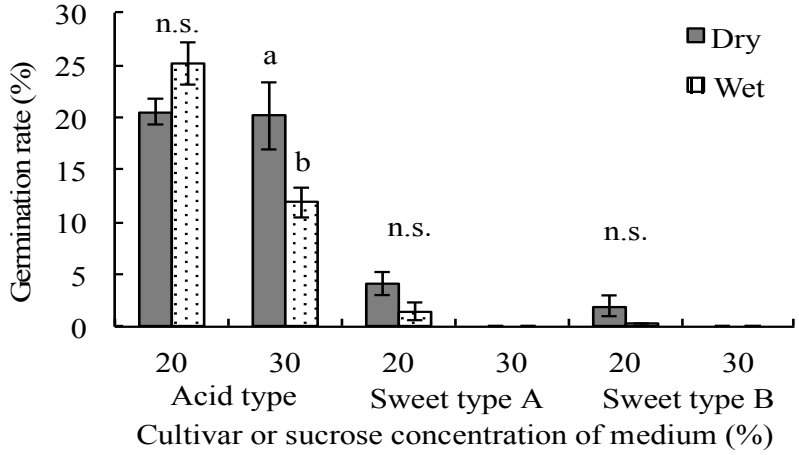

Fig. 2. Differences in germination rates of the pollen treated with dry or wet air. Values with different letters are significantly different by Fisher's PLSD at 5% level.

When buds before flowering were placed under high temperature conditions for 24-48 h, the pollen germination rate decreased significantly (Figs. 3 and 4). Decreased pollen germination rate with the rise of treatment temperatures was similar, but the sucrose

contents of the medium changed. Therefore, high temperatures immediately before flowering are thought to reduce pollen germination after flowering. The influence of high temperature was apparently greater in September than in November. In September, no difference was found in the pollen germination rate between 25 and 35°C, although it was significantly higher at 25°C than at 35°C in November. The effect of a difference in the temperature on pollen germination was apparently affected by the temperature in the greenhouse during flower-bud development (Table 2). Moreover, the germination rate of pollen treated with 25°C was highest in September and lowest in November, suggesting that the daily minimum temperature also affects the germination rate (Fig. 5).

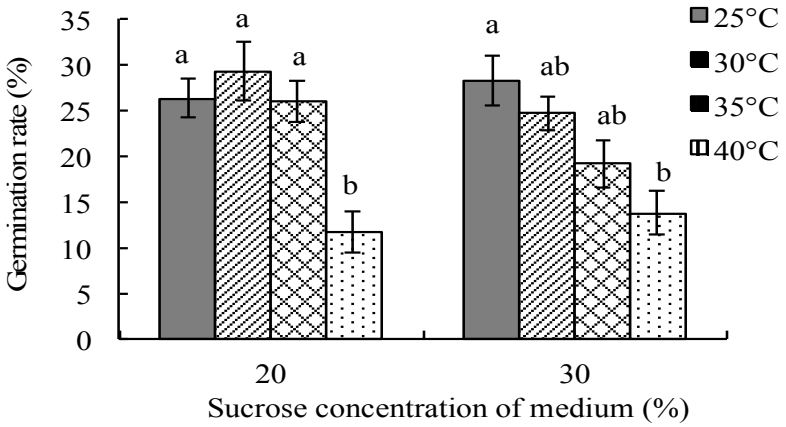

Fig. 3. Differences in the pollen germination rates of the flower treated with temperatures just before flowering (Sept.). Values with different letters are significantly different by Fisher's PLSD at 5% level.

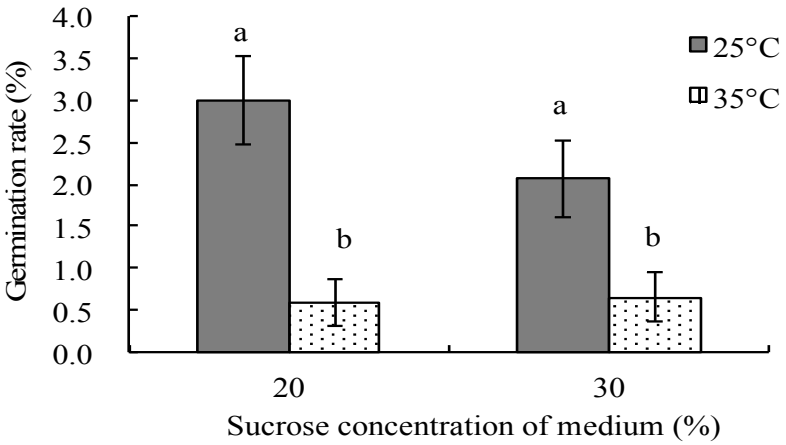

Fig. 4. Differences in the pollen germination rates of the flower treated with temperatures just before flowering (Nov.). Values with different letters are significantly different by Fisher's PLSD at 5% level.

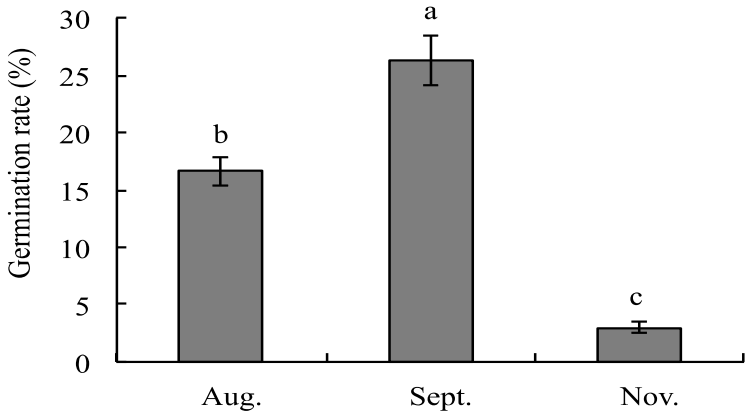

Fig. 5. Differences in the pollen germination rates of the flowers treated with 25°C just before flowering. Different letters indicate significant difference by Fisher's PLSD at 5% level.

Table 2. Maximum, mean, and minimum temperatures in the greenhouse during 5 days just before flower sampling in each month.

	Max. (°C)	Mean (°C)	Min. (°C)
Aug.	41.3	30.6	24.4
Sept.	37.4	27.7	23.1
Nov.	27.2	19.0	15.4

The germinated pollen diameter was significantly larger than that of non-germinated pollen (Fig. 6). Therefore, development of pollen was thought to be inferior at high temperatures and the number of germinated pollen decreased. In general, the rate of pollen germination decreases under high temperature conditions (Yasutomi, 1994; Yonemoto et al., 1999). Nevertheless, the results obtained in this study indicate clearly that high temperatures during pollen development also decrease the rate of germination. It can be inferred that the nutritional conditions of the trees, such as the photosynthetic products during flower bud development, affect the pollen germination rate at flowering time.

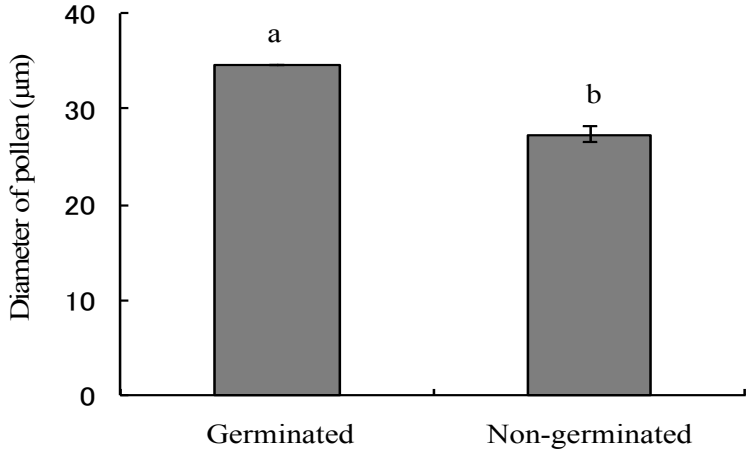

Fig. 6. Difference in diameters between germinated and non-germinated pollen. Different letters indicate significant difference by Fisher's PLSD at 5% level.

Literature Cited

Freitas, B.M., Alves, J.E., Brandao, G.F. and Araujo, Z.B. 1999. Pollination requirements of West Indian cherry (*Malpighia emarginata*) and its putative pollinators, *Centris* bees, in NE Brazil. J. Agric. Sci. Cambridge 133:303-311.

Handa, K., Ishihara, K. and Ootsubo, T. 2003. Studies on the fruit set of acerola 1. Flower organ, ability of pollen, several treatments of fruit set. Hort. Res. (Japan) 72(Suppl. 2):141 (in Japanese).

Handa, K., Ootsubo, T. and Ishihara, K. 2005. Studies on the fruit set of acerola 2. Pollen germination and fertility. Hort. Res. (Japan) 74(Suppl. 1):93 (in Japanese).

Ishihata, K. and Ito, S. 1994. Effects of growth regulators applied at blooming time on fruit quality of acerola, *Malpighia emerginata* DC. Jpn. J. Trop. Agr. 38:113-118.

Matsuda, N., Shimabukuro, S., Matsumura, M., and Ichi, R. 2009. Development of techniques for cultivating passion fruit in greenhouse. 5. Effect of male parent and pollen storage on fruit set in the purple passion fruit (*Passiflora edulis* Sims). J. Okinawa Agric. 43:11-19.

Raw, A. 1979. *Centris dirrhoda* (Anthophoridae), the bee visiting West Indian cherry flowers (*Malpighia punicifolia*). Revista de Biologia Tropical 27:203-205.

Yamane, G.M. and Nakasone, H.H. 1961. Pollination and fruit set studies of acerola *Malpighia glabra* L. in Hawaii. Proc. Amer. Soc. Hort. Sci. 78:141-148.

Yasutomi, T. 1994. Tropical fruit tree – Problems in horticulture under plastic house. Jpn. J. Trop. Agr. 38:162-169.

Yonemoto, Y., Higuchi, H., Nakanishi, T. and Tomita, E. 1999. Conditions of artificial media for pollen germination and tube growth of cherimoya (*Annona cherimola* Mill.). Jpn. J. Trop. Agr. 43:260-264.

Yonemoto, Y. 2009. Acerola. p.138-141. Nettaikaju no saibai: kanjukuka wo tsukuru, tanoshimu 28shu. Nousangyosonbunka Kyoukai, Tokyo.

Cut Rose Production under Supplemental Lighting with Super Bright White Light Emitting Diodes©

Sumihisa Furufuji
Stanley Electric Co., Ltd., 2-14-1 Eda-nishi, Aoba, Yokohama 225-0014, Japan
Email: sumihisa_furufuji@stanley.co.jp (corresponding author)

Wakanori Amaki
Department of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 246-0034, Japan

Hirokazu Fukui
Graduate School of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan

Effect of supplemental lighting with a super-bright, white-light emitting diode (LED) on the productivity and the quality of cut flowers in rose (*Rosa* 'Tint') were examined and compared with that of a high pressure sodium (HPS) lamp. The LED lighting has an advantage that lighting is able to perform during rainy season in Japan when the weather is very cloudy but also relatively high air temperature because of the markedly low heat generation compared with HPS lamp. As the results of the experiment from June 2012 to March 2013, the LED supplemental lighting was shown to be remarkably effective in improving the productivity and maintenance of the high quality in cut flower production, especially during the June-July rainy season.

INTRODUCTION

The central part of Japan usually has large seasonal weather variations. For example, in winter heavy cloudy skies and relatively low air temperatures exist on the Sea of Japan side of the country and changes into sunny days with considerably higher air temperatures in summer. Furthermore, during the rainy season from June to July, relatively high temperatures and high humidity conditions continue and result in the lack of sunshine. The rose is a crop requiring a lot of light irradiation for growth and production of high quality cut flowers and pot plants. The shortage of sunshine in winter and rainy season are the most important reasons for decreased yield and quality drop of rose products. Therefore, recently, we have undertaken experiments on the effects of supplementary lighting with a super-bright, white-light emitting diode (LED) on rose production. Up to now, the promotional effects of supplemental night lighting using various LEDs from cutting propagation to just before shipment in winter on pot-rose production has been clarified (Furufuji et al., 2013). The advantages of LED lighting are the low heat generation and the ability to momentarily switch the lights ON/OFF (Watanabe, 2011) compared with conventionally used high pressure sodium (HPS) lamps. Therefore, LED lights would be the best for supplemental lighting in winter and rainy seasons. In winter, it seems that the momentary switching ON/OFF of LED lighting depending on changes of solar radiation will result in some energy saving. In the rainy season which is cloudy but also with a relatively high temperature it is impossible to use HPS lamps as a light source under greenhouse cultivation because of the high heat generated. Thus, the effectiveness of LED supplemental lighting through all seasons in the rose cut-flower production was examined.

MATERIALS AND METHODS

The Experimental Site and Facility Conditions

This experiment was carried out in the greenhouses at the Noda Rose Nursery (Godo, Gifu Prefecture, Japan) from 14 June 2012 to 31 March 2013. This nursery already had experience with supplementary light cultivation using HPS lamp (GAN 400AL; 400W, GAVITA, Norway) for cut-flower rose production in a hydroponic system using rock-wool

medium, in the arching cultivation system (Ohkawa and Suematsu, 1999). Their facility had installed several environmental control facilities including: pad and fan, combustion-type heating, heat pump, and CO_2 enrichment system.

Plant Material and Experimental Procedures

In this experiment, rose plants (*Rosa* 'Tint') which were 1 year old were used. This cultivar known to show low productivity under low light conditions was convenient to judge the effect of supplemental lighting on the amount of cut flower production. A part of the HPS supplemental lighting greenhouse was turned off and the super bright white LED lamp unit (LLM0311A; 31W, Stanley Electric Co., Ltd., Japan) was installed. The LED supplemental lighting was double controlled by a timer (turned on at 23:00-17:00) and an illuminance meter (turn on at less than 10,000 lx in the greenhouse). The light intensity of the LED plots during night was 80-100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD on the surface of bent shoots. The LED supplemental lighting was performed from the start of the experiment. The HPS supplemental lighting started from 25 Oct. 2012. The lighting time was 0:00-7:00 and the light intensity of the HPS plot was 50-70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD on the surface of bent shoots. A no supplemental lighting plot, the control, was prepared in another greenhouse.

RESULTS AND DISCUSSION

Sellable cut flower numbers per month of the respective plots through the examination period are shown in Figure 1. The accelerated effect of supplemental lighting on the cut flower production was obvious when compared with the no supplemental lighting plot. Just after the start of the experiment, in July to September, the harvest number of cut flowers in the HPS supplemental lighting plot increased. The increment of yield numbers in the HPS lightning plot was caused by the supplemental lighting of the previous season (from Oct. 2011 to Mar. 2012). However, the number in the LED lighting plot was more than that of HPS lighting plot (Fig. 1). From our observation, it took about 2-3 months from the bud break of flowering shoot to harvest in this experiment. The LED supplemental lighting during the rainy season was effective on flowering shoot growth during August to September (Fig. 1). When yield number of the LED lighting plot from January to March 2013 was compared with that of the HPS lighting plot, both of them were almost equal (Fig. 1). However, it seemed that the LED lighting was more effective rather than the HPS lighting for rose cut flower production when the effects were estimated through the entire experimental period.

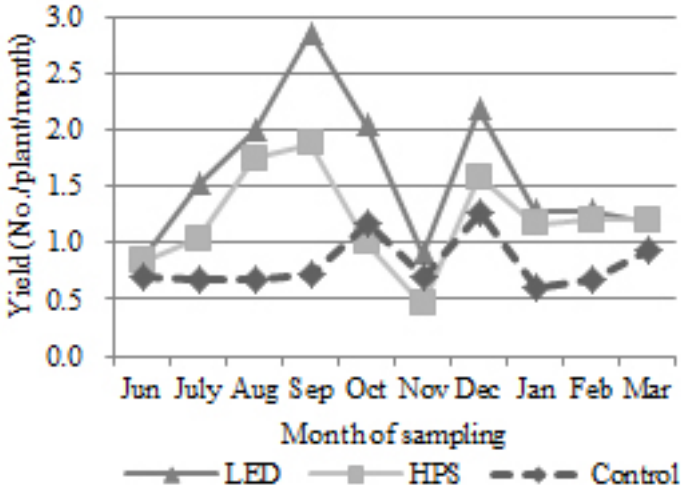

Fig. 1. Effects of supplemental lighting and light source on the yield of rose cut flowers.

The quality of cut flowers produced in respective plots was compared. The quality of cut flowers was evaluated by the length (Fig. 2) and fresh weight (Fig. 3) of respective cut flowers and the height (Fig. 4) of respective flower (nearly equal the length of the longest petal). The average length of the cut-flower stems increased from 64 cm to 79 cm. However, it made little difference between the experimental plots (Fig. 2). The same tendency was observed on the height of flowers and fresh weight of cut flowers. There were some seasonal changes on both of the values, but remarkable differences were not seen between experimental plots (Figs. 3 and 4). Consequently, it may be said that the quality of harvested cut flowers did not have obvious differences between experimental plots.

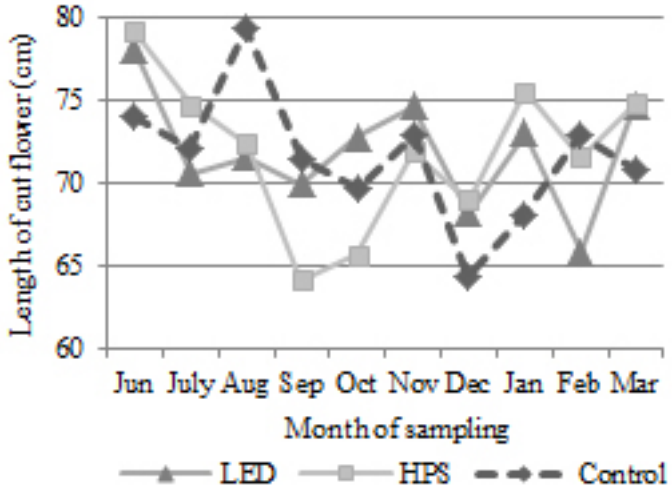

Fig. 2. Effects of supplemental lighting and light source on the length of rose cut flowers.

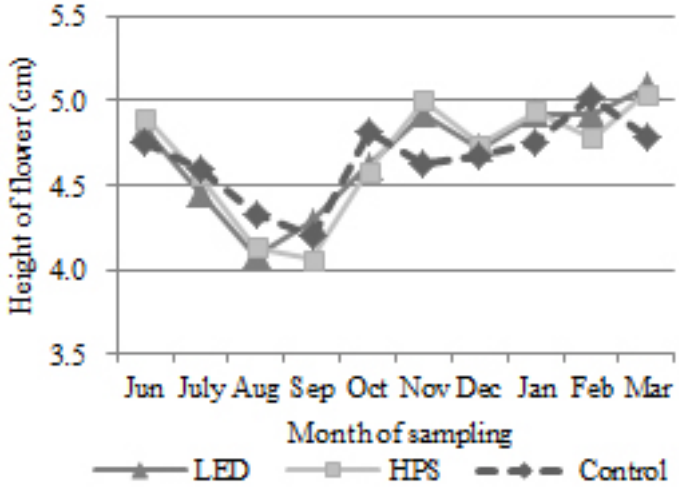

Fig. 3. Effects of supplemental lighting and light source on the height of rose flowers.

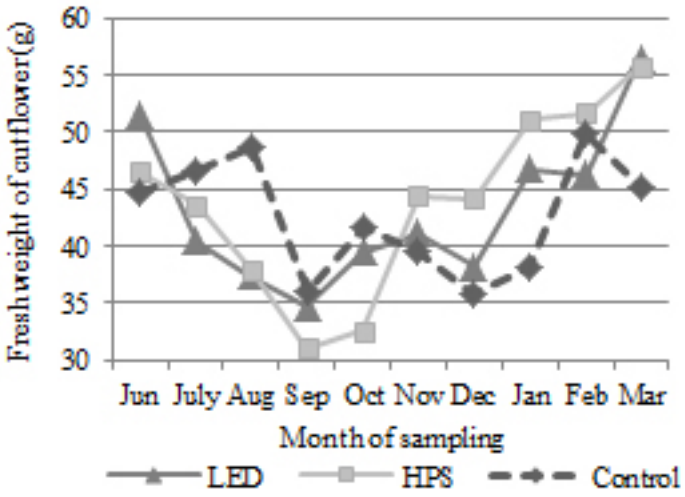

Fig. 4. Effects of supplemental lighting and light source on the fresh weight of rose cut flowers.

Though the experimental period was about 9 months, the estimation of the annual production number from the results mentioned above was performed (Table 1). The average number of cut roses harvested per square meter per year was estimated to be about 100 in Japan using the arching cultivation system. However, the cultivar 'Tint' used in this experiment is an inherently low productive type and thus the estimated number stayed at 83.3 in the no supplemental lighting (control) plot. The number of HPS lighting plot was 126.4, that is, 1.5 times of the control plot. The number of LED lighting plot was 166.4 which is twice the control and 1.3 times the HPS lighting plot. The value of the LED lighting plot was thought to be a value that equaled the amount in the Netherlands. In conclusion, the improvement of cut flower productivity without reducing the quality of cut flowers was noticeable when the super bright white LED was used as the supplemental light source throughout the year. In addition, from the viewpoint of energy saving, the LED unit has a bigger merit than HPS lamps.

Literature Cited

Furufuji, S., Ajiki, S., Amaki, W., Kunii, M. and Ohnishi, M. 2013. Effects of light quality in supplemental night lighting on the growth and flowering of pot miniature rose just after cutting. Hort. Res. (Japan) 12(Suppl. 1):448 (in Japanese).

Ohkawa, K. and Suematsu, M. 1999. Arching cultivation techniques for growing cut-roses. Acta Hort. 482:47-52.

Watanabe, H. 2011. Light-controlled plant cultivation system in Japan — Development of a vegetable factory using LEDs as a light source for plants. Acta Hort. 907:37-44.

Characteristics of New Granular Rockwool©

Toru Tanibe, Daisuke Ikezaki and Osamu Sakamoto
Taiheiyo Materials Corporation, 2-4-2 Osaku, Sakura, Chiba 285-0802, Japan

Masaki Ochiai and Hirokazu Fukui
Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
Email: fukui@gifu-u.ac.jp

INTRODUCTION

Rockwool has been developed as a nutrient culture medium in the Netherland. The rockwool culture system was introduced to Japan in 1983 and has been expanding in tomato and rose culture. Rockwool as a culture medium has been used in a slab bed system. In this system the rockwool fibers are mixed with a binder and formed into a rectangular shape. In contrast to the rockwool slab, granular rockwool has not been popular, although granular rockwool for potted plant media is effective for improving of physical soil conditions. We believe the reason for granular rockwool's lower popularity is high price. On the other hand, granular rockwool has attracted attention as an alternative construction material to asbestos and every year has been used at 200,000 tons in Japan. So we converted rockwool used in the construction field to the agricultural field, and developed a new granular rockwool for horticulture.

MATERIALS AND METHODS

Four new rockwool types were used; fine granular rockwool (R210), roughly granular rockwool (rough rockwool), medium granular rockwool (medium rockwool), and grainy granular rockwool (grain rockwool) along with two types of commercial granular rockwool; Ryu-jou-men (Nippon Rockwool Co.) and 012-519 (Grodan), were used as controls.

Granular rockwools were mixed at 0, 10, and 20% into a commercial peat moss mix (BM-2, Berger). The pH (KCl), electrical conductivity (EC), and cation exchange capacity (CEC) were measured.

To investigate growth effects of these granular rockwools, the mixed media with these rockwools were used for cultivation of *Spathiphyllum* 'Fairy Wing'. Micropropagated plantlets were transplanted to plug tray with 200 cells filled with BM-2 on 29 October 2012, and acclimated. After 2 months, the plants were transplanted to 6-cm pots filled with the mixed potting media consisting of BM-2 and 10 or 20% granular rockwool. On 19 April 2013 the plants were transplanted to 9-cm pots filled with the same potting media. There were 10 plants per treatment and maximum leaf length and leaf numbers were measured.

RESULTS AND DISCUSSION

The CEC of R210 was lowest and was 1.11 me/100 g and CEC of grain rockwool was next at 1.78 me/100 g (Fig. 1). Ryu-jou-men had a high CEC of 6.84 me/100 g. The mixed media with BM-2 had the same CEC as rockwools. Although pH values of the rockwools were high at around 9.0, the mixed media with BM-2 were 6.5 to 7.0. The EC of all rockwools was around 0.1 $mS \cdot cm^{-1}$. Electrical conductivity increased by mixing with BM-2, and all media mixed with 20% BM-2 were around 0.3 $mS \cdot cm^{-1}$.

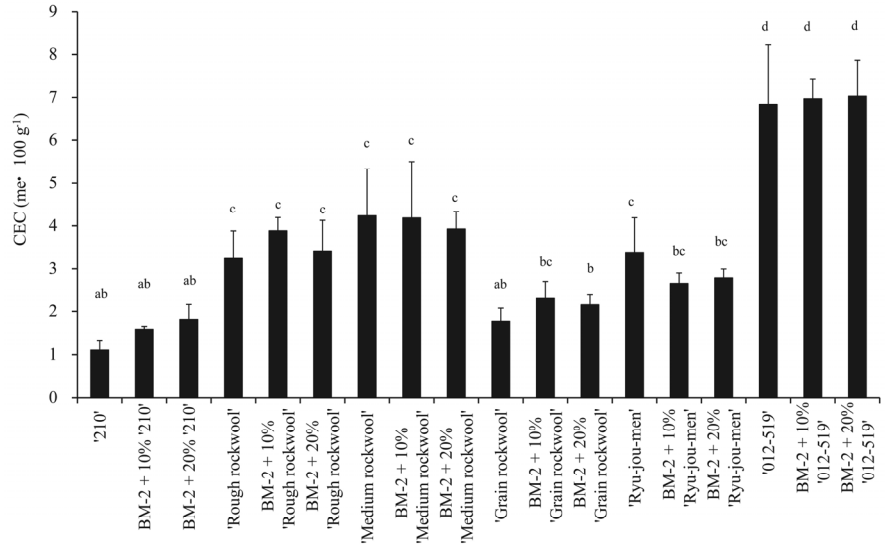

Fig. 1. Cation exchange capacity of mixed media with BM-2.

Effects of mixed potting media containing of BM-2 and 10 or 20% granular rockwool on growth of *Spathiphyllum* 'Fairy Wing' is shown in Figures 2 and 3. In BM-2 medium containing no rockwool, maximum leaf length was 7.7 cm on 19 April, and was 15.6 cm on 3 September. Maximum leaf length in all mixed potting media containing rockwools was larger than that of BM-2 mix and the effectiveness on plant growth by mixed rockwool was observed. The leaf length of the plants on medium rockwool was 21.3 cm compared to Ryu-jou-men and 012-519 at 19 and 18 cm respectively; medium rockwool therefore had a significant promotive effect of plant growth.

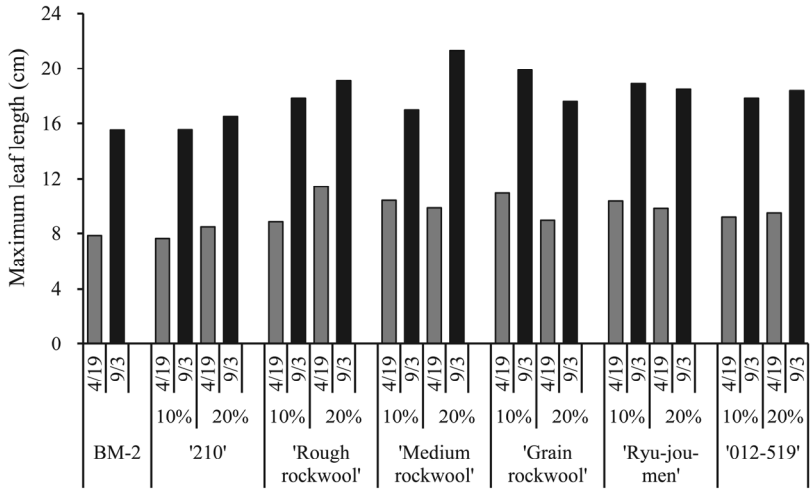

Fig. 2. Effect of mixed culture media containing of BM-2 and 10 to 20% granular rockwool on growth of *Spathiphyllum* 'Fairy Wing'.

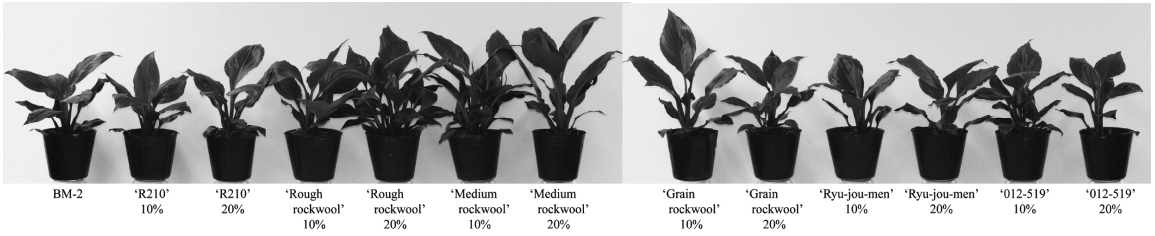

Fig. 3. Growth of *Spathiphyllum* 'Fairy Wing' in 29 October 2012.

From the above results, the newly developed granular rockwools (rough, medium, grain and R210) had no different physicochemical properties compared to the commercial rockwools used to grow plants. In addition, these rockwools had the same growth property effects for the cultivation of *Spathiphyllum* 'Fairy Wing' as the commercial granular rockwools with medium rockwool having a greater promotive effect on plant growth.

It is believed that rockwool improves the rhizosphere environment by increasing the gaseous phase ratio and soil water retention, and plant growth was promoted by these effects. We would like to promote the more wide spread use of these granular rockwools in pot-plant production in the future.

Wild Roses and Rose Industry in Iran©

Yoshihiro Ueda
Gifu International Academy of Horticulture, 1094-8 Shio, Kani-shi, Gifu 509-0251, Japan
Email: ueda-yoshihiro@horticulture.ac.jp

Iran is the original place of oil-yielding roses and the roses were cultivated in the mountains of southern Persia (Iran) for religious ceremonies in B.C. 12 century. I visited Iran in 2010 to research the long history of rose oil and rose water production, and explore wild rose species native to Iran (Fig. 1).

Fig. 1. Map of Iran, (A) The place of field research, (B) Meymand, and (C) Darab.

In Iran, there are about 14 species of genus *Rosa* reported to exist. Among them, *Rosa persica* Michx. is one of the most famous species and recently, the breeding using *R. persica* or the persica hybrids has been becoming popular. We went out into the field to observe wild plants which were located in 300 km west from Mashhad. During the observation I found *R. percica* and *R. hemisphaerica* Herrm. *Rosa persica* was growing in the dry slope. *Rosa moschata* Herrm. was used as a hedge plant and was planted lining a street.

Damask rose (*R. damascena* Mill.) is widely cultivated throughout Iran and there are many local cultivars. An Iranian researcher suggests that Iran is the center of diversity of Damask rose and gave us some clues about the unknown center of origin of this rose. Fars Province is located in the southern part of Iran and Shiraz is capital city of Fars. Shiraz and surrounding areas have a long history in production of rose water. I visited Meymand near Shiraz which is an ancient city and one of the famous production areas of rose water and rose oil. In Meymand (Fig. 2), I also saw a store specializing in rose water.

In Darab, there is a huge production area of Damask rose and the total area in and around Darab is about 4,000 ha (Fig. 3). In Fars province including Meymand and Drab, total production area of Damask rose is about 4,500 ha.

Low air humidity, bright sunshine, continuous and considerable temperature fluctuations, rather warm winter and ample irrigation are suitable conditions to grow Damask rose. Therefore, the climate of Fars Province might provide such conditions.

Fig. 2. Still for rose oil and rose water production.

Fig. 3. Production field of Damask rose in Darab. (Photograph by courtesy of Dr. A. Karami of Shiraz University).

The main product using Damask rose is rose water in Iran. They are use for drinking water, cosmetic, and cooking.

In Iran the musk rose is also an important rose for extracting rose water and rose oil.

Technical Sessions©

Tom Saunders
Saunders Bros., Inc., 2717 Tye Brook Highway, Piney River Virginia 22964, USA
Email: tom@saundersbrothers.com

Robert Lee
Transcend Nursery, 52063 Ridgecrest Drive, Independence, Louisiana 70443, USA
Email: buddyazaleas@yahoo.com

MONDAY MORNING, 4 NOVEMBER, 2013
The 38th Annual Meeting of the International Plant Propagators' Society-Southern Region of North America convened at 7:45 am at the University of Georgia Hotel and Conference Center, Athens, Georgia with President Tom Saunders presiding.

PRESIDENT TOM SAUNDERS
President Saunders welcomed everyone to Athens, Georgia, for the 38th Annual Meeting of the International Plant Propagators' Society-Southern Region of North America. He thanked Local Site Committee Chair, Matt Chappell and his committee and volunteers for the long hours in arranging the excellent tours, hotel, other planning activities and all their attention to detail. He welcomed students, first time attendees and new members, asking them to stand and be recognized. Saunders thanked the Executive Committee, and Maarten van der Giessen's Sponsorship Committee, which raised $42,000 in cash sponsorships; this was outstanding with the challenging economic times. He asked sponsors to please stand and be recognized for their support. Saunders encouraged the membership to visit and show their support of our sponsors during the meeting. He encouraged all members to make new members and first-time attendees feel welcome — share with them and seek from them. He encouraged good questions and enthusiastic participation at the Tuesday night question box. He requested members to fill out the membership questionnaire to help improve the IPPS-SRNA. Saunders announced that this year we participated with Great Britain & Ireland in the *Young Propagator Exchange* program between the two regions. He asked Josie Leech from Great Britain & Ireland (GB&I) who visited our region and was hosted by Lancaster Farms, and Judson Lecompte from the Southern Region of North America who was our designee to GB&I to stand and be recognized. Both of these young professionals had an incredible exchange experience in our respective regions. This is the first year we are doing the Vivian Munday Young Horticultural Professional Scholarship Work Program. We currently have a "4-pack" of four young professionals (Ben Ford, Seth Allen, Diana Cochran, and Jeremiah Devore) who are making a strong contribution to this year's program. Saunders thanked Program Chair and 1st Vice-President, Buddy Lee, for the excellent program and slate of speakers he assembled.

PROGRAM CHAIR BUDDY LEE
Program Chair Buddy Lee welcomed all members, guests and students. He thanked the membership for the opportunity to serve them, and then reviewed the scheduled program. The Question Box, scheduled for Tuesday evening, was to be co-chaired by Jeff Howell and Bob Black. He then introduced the first moderator, Barbara Stump.

West Meets East on Ornamentals©

Donglin Zhang
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: donglin@uga.edu

INTRODUCTION

When talking about ornamentals, we did introduce the *Magnolia grandiflora* to Asian countries (East) more than 120 years ago and the plant has been widely cultivated in Asian gardens and landscape. But, until today, only two cultivars were developed in China, while we (USA) had at least 85 cultivars on the market. If comparing the natural species of *Magnolia* (narrow-sense), China has 38 species while only eight are native to the USA (Wu and Raven, 1994). *Nandina domestica* was introduced to the West in 1804 and more than 36 cultivars have been developed and marketed in USA (Dirr, 2009). In China, all *N. domestica* have been marketed as the species, with no cultivar development (Table 1). Obviously, the natural resource of ornamental plants is much richer in East, than the West. In term of new plant breeding, we are more advanced than that of the East. The marriage of East and West is imperative in the field of ornamental horticulture. We can fully utilize natural ornamental plant resource (East) and traditional and advanced plant breeding technology (West) for breeding better ornamental plants for our nursery industry and gardens.

Table 1. Taxa of common woody ornamental genera, species, and cultivars in USA and China.

Taxa	China	USA
	Number of indigenous genera	
Magnoliaceae	13	2
Theaceae	12	3
	Number of indigenous species	
Callicarpa	42	1
Ilex	210	16
Magnolia (narrow-sense)	38	8
Magnolia	105	8
Rhododendron	571	25
Viburnum	74	15
	Number of released cultivars	
Lagerstroemia indica	4	88
Magnolia grandiflora	2	85
Nandina domestica	0	36
Osmanthus fragrans	38	0

PLANT INTRODUCTION

Ornamental plants from Asian countries were introduced to the USA indirectly from Europe until the direct collection trips of E.H. Wilson of the Harvard University Arnold Arboretum in the late 1800s (Cox, 1986; Foley, 1969). Although the Arnold Arboretum grew and distributed a lot of E.H. Wilson's plants, we still do not know the fate of plants that were not hardy in Boston and adjacent areas (Zhang et al., 1998). In 1940s, special collections (such as for fiber and food, as well as the live fossil plant *Metasequoia glyptostroboides*) also brought a lot of ornamental plants back to the USA (Janick and Simon, 1993). Plant hunters from botanical gardens and arboreta and nursery growers have since made many trips to China and enriched the diversity of ornamental plants

tremendously, i.e., *Loropetalum* and *Distylium* were the results of these trips and plant exchange programs (Dirr, 2011). With the awareness of invasive plant species, we hope to bring back plants with greater ornamental potential for our gardens and landscapes.

FLORAL SIMILARITY

The East and the West once joined based on "continental drift" by Alfred Wegener (1922) and proved by many similar plant and animal fossils around different continents. Fossil of dawn redwood was reported from all continents and today it is successfully cultivated on all continents. Climatic and floristic similarities between West and East could be easily understood by comparing meteorological data and disjunctive distribution of plant species, such as *Liriodendron chinense* (East) and *L. tulipifera* (West). Both species, as well as their hybrids, grow well in both regions. Actually, more than half of our USA ornamental plants are from the East. Some common climatic and environmental conditions allow us to successfully introduce many ornamental plants between the East and the West. Plant explorers are constantly seeking more plants with greater ornamental potential; there are more than 14,000 Chinese woody species — 3-fold more species than in the USA.

PLANT COLLECTION

The challenges of collecting plants from the East and bringing them back to the West should be shared by all plant collectors. After collaborating with Chinese, Japanese, and Korean horticultural professionals for the last 12 years, I was finally able to bring a few plants back this year. For plant collecting in the East, you must be prepared for:

Eating and Drinking

Regardless of your plant knowledge, you have to learn the local culture. How to eat and drink is very important to the people in the East. If you know how to eat and drink in China (with many dishes on a Lazy Susan and moutai), Japan (sushi, tempura, and sake), Korea (gogigui, kimchi, and soju), and other eastern countries, your trip to collect plants is much more enjoyable. Compared with the USA, eating and drinking customs are more complicated in the East. You have to learn the eating and drinking culture and be sure to present your collection trip details at the dinner table.

Culture on Ornamental Plants

Because of limited living space, ornamentals such as grafted cacti are used as balcony decorations in majority of Asian countries. Only a few government nurseries produce ornamental plants for public gardens and landscapes. However, in the past 15 years, the private nursery industry in both China and Korea has developed rapidly and commercially introduced new and exciting ornamental plants. However, the natural germplasm of potential ornamental plants is rich and one has to make collections from remote mountains. If you collect in Japan, you should be able to get a lot of usual ornamental plants from the nursery trade.

Ornamental Plant Solution

To prioritize plants you want to collect from the East, you should determine your need and/or nursery industry demand. Recently, boxwood blight caused significant damage to our boxwood cultivars. We needed to collect new boxwood clones with better disease resistance, and boxwood substitutes such as *Syzygium buxifolium*. If you like purple leaves of *Vitex trifolia* there are opportunities to collect new *Vitex* germplasm from the East. New plant selections of evergreen *Viburnum*, *Anneslea fragrans*, *Symplocos tetragona*, and *Phoebe zhennan* should be collected for further evaluation. While exploring for wild tulips in Xijiang, China, I was overwhelming impressed by natural ornamental plant resources. The success of tulip breeding and the diversity of tulip cultivars in today's market should encourage us to explore and collect more new plants from the East for our nursery markets and breeding lines.

Edible Landscape Plants
Many fruit plants from the East are also beautiful landscape plants. One of my Ph.D. students collected 51 ornamental peach cultivars from around the world, especially discarded selections from fruit peach breeding programs (Hu et al., 2005). Other species such as *Diospyros cathayensis*, *D. lotus*, *Hovenia acerba*, *Lycium chinense*, *Myrica rubra*, and *Ziziphus jujuba* should be further trialed and selected for both edible fruits and desirable landscape features. Potential ornamental plants should be targeted for other benefits such as medicinal uses, fragrance, and beverage characteristics.

Targeted Groups
Everyone knows *Camellia*, but not too many people know the new species (*C. azalea*) with bright red flowers and rhododendron-like leaves which was discovered in 1986 (Wei, 1986). Ma et al. (2008) listed 35 golden camellia species in the world and many breeding work had been done, especially crossing them with much more cold hardy species. When I visited Hunan Academy of Forestry last year, their collection of 450 cultivars of *C. oleifera* was very impressive. We have a long way to go in introducing new and better camellias to our gardens. *Hydrangea* — do we need more? Yes, we do need double flowered *H. paniculata* and other interspecific hybrids. Obviously, we do need to actively collect plants with greater ornamental potential for breeding lines and nursery markets.

PRESERVATION AND RETURN OF ORNAMENTAL PLANT RESOURCE

Training Ornamental Horticulturalists
The development of ornamental plant industries is highly associated with the economic development. With rapid economic development in the East, especially China, the demand for better ornamental plants is much higher. We should not just collect plants from the East, but also collaborate with them on ornamental research and plant exploration. The training of ornamental horticulturists for the East is a very important step to better utilize natural plant resources and we should actively support this mission.

Preservation of Ornamental Germplasm
Plant collection is not just for profit, but also for plant conservation. No wild Ginkgo trees can be found in China today. But it is a popular ornamental plant and widely cultivated around the world. *Davidia* (dove tree) has a limited wild population. However, you can cultivate it in majority of USA gardens. One Japanese nursery collected seven cultivars of *Davidia*, which extended its diversity way beyond its native gene pool. Ornamental plants beautify our landscapes and preserve our natural plant resources.

Exchange and Return of Ornamental Germplasm
Collection of ornamental plants is not a one way street from the East to West. Many native plants from the USA, such as *Chamaecyparis thyoides*, *Cornus florida*, *Hydrangea quercifolia*, *Ilex verticillata*, *Kalmia latifolia*, and *M. grandiflora*, are doing well in the East. When I found *Lagerstroemia* 'Black Magic' in the Chinese nursery trade, I was sure it was the same plant that was bred in USA and returned to China; *L. indica* is native to China and I do not know how this cultivar was introduced. Recently, several ornamental cultivars developed in Japan were lost. Fortunately, Japan shared them with USA horticulturists so plant material was shipped back to Japan. There are many cultivars developed in USA from introduced plants from the East that have been introduced to the Asian nursery trade (Table 1).

MODERN TECHNOLOGY
The development of DNA technology has significant impact on all fields of science, including ornamental horticulture. Based on traditional hybridization, we can employ modern DNA technique and embryo rescue/embryogenesis and develop a rapid woody

plant breeding system (Fig. 1). Seeds of *Ilex* usually take 2-3 years to germinate and some cross-hybrid embryos may not be able to produce viable seeds. However, we can take the embryos after the cross hybridization and germinate them in the culture room (embryo rescue). We worked on *I. crenata* last July and the seedling could be transplanted in October (only 3-4 months). We are working on the trait-associated markers now and hope to screen the hybrid seedlings with these markers. If we can rescue hybrid embryos in 3-4 months and check their seedling DNA with identified trait-associated markers, we can shorten our woody plant breeding cycle.

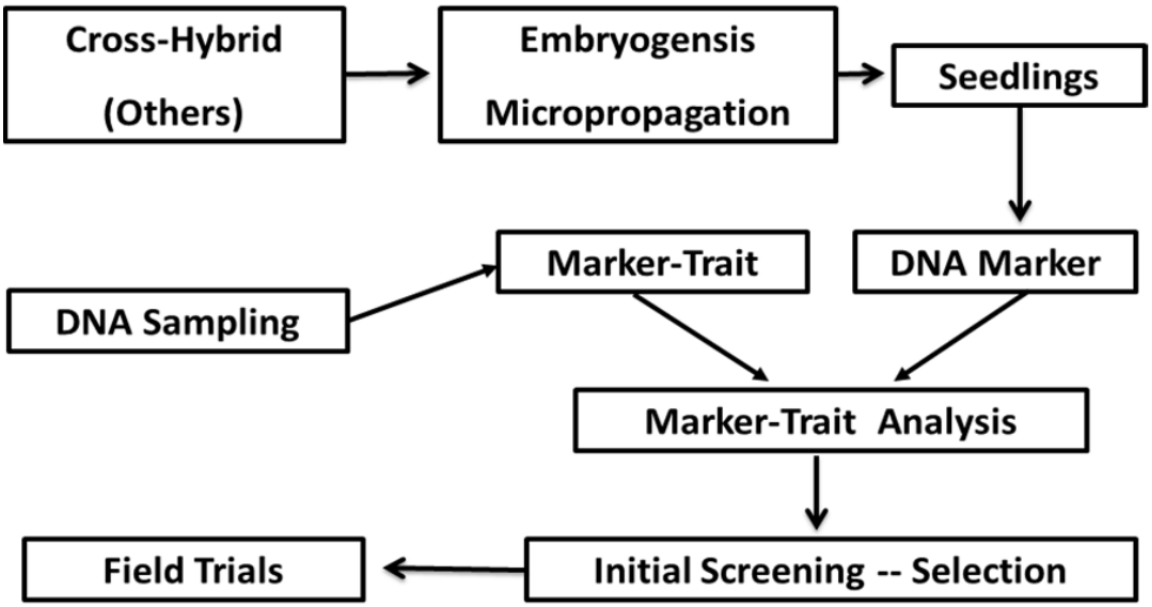

Fig. 1. Rapid woody plant breeding system.

ACKNOWLEDGEMENT
I thank the faculty of the University of Georgia (UGA) for appointing me as the Michael A. Dirr Endowed Chair Professor and allowing me to better explore the wonderful world of ornamental plants. Funding from the University of Georgia Research Foundation made it possible to collect plants from the East. Help and support from the faculty, staff and students in the UGA Horticulture Department are greatly appreciated. I thank Buddy Lee and IPPS for the opportunity to present my research program. I look forward to working with IPPS members and hope to develop better ornamental plants to our nursery trade and gardens.

Literature Cited

Cox, E.H.M. 1986. Plant-Hunting in China. Oxford University Press, Warwick House, Hong Kong.
Dirr, M.A. 2009. Manual of Woody Landscape Plants. 6th ed. Stipes Pub. Co., Champaign, Illinois.
Dirr, M.A. 2011. Dirr's Encyclopedia of Trees and Shrubs. Timber Press, Portland and London.
Foley, D.J. 1969. The Flowering World of "Chinese" Wilson. The MacMillan Company, Collier-MacMillan Ltd., London, U.K.
Hu, D.Y., Zhang, Z.S., Zhang, D.L., Zhang, Q.X. and Li, J.H. 2005. Genetic relationship of ornamental peach determined using AFLP markers. HortScience 40(6):1782-1786.

Janick, J. and Simon, J.E. (eds.). 1993. New Crops. Wiley, New York, NY, USA.
Ma, J.L., Zhang, D.L., Zhang, R.Q., Li, J.Y. and Jiang, Y. 2008. Golden camellias from Guangxi, China. Proc. South. Nurs. Assn. Res. Conf. 53:169-173.
Qi, C. and Sun, X. 2002. A Survey of Hunan Seed Plants. Hunan Science and Technology Publishing House, Changsha, China.
Wei, Z. 1986. A new *Camellia* species from China. Bull. Bot. Res. 6(4):141-143.
Wu, Z.Y. and Raven, P.H. (eds.). 1994. Flora of China. Science Press, Beijing, China and Missouri Botanical Garden, St. Louis, Missouri.
Zhang, D.L., Lasseigne, F.T. and Dirr, M.A. 1998. A survey of Chinese native plants of potential ornamental and economic value for the Southeastern United States. J.C. Raulston Arboretum Newsletter 1998(3):3-6, 27-35.

What's Cookin' with Southern Rhododendrons?©

Stephen Krebs
The Holden Arboretum, 9500 Sperry Road, Kirtland, Ohio 44094, USA
Email: skrebs@holdenarb.org

INTRODUCTION

Root rot caused by the invasive soil fungus *Phytophthora cinnamomi* is a major source of mortality in *Rhododendron* and many other popular ornamental genera (Benson and Broembsen, 2001). The pathogen may also restrict the natural occurrence or horticultural use of *Rhododendron* species and cultivars in the Southern USA. *Phytophthora cinnamomi* is more problematic in warmer climates because it is susceptible to frost and thrives in warm, wet soils (Brasier, 1996; Marcais et al., 1996). Epidemiologists predict that global warming will increase both the activity and northward migration of the pathogen (Anderson et al., 2004; Bergot et al., 2004).

Genetically-conferred host resistance to *P. cinnamomi* offers an additional and sustainable method of disease management in additional to existing cultural and chemical controls. Among some *Rhododendron* subgenera — notably *Tsutsusi* (evergreen azaleas) — resistance is found at relatively high frequency (Benson, 1980), which may explain why this group of plants thrives in the warmer regions of the USA (e.g., the Gulf South). In contrast, resistance among large-leafed, elepidote rhododendrons (subgenus *Hymenanthes*) occurs at less than 3% frequency (Hoitink and Schmitthenner, 1974; Krebs and Wilson, 2002), and garden use of this group is restricted to more northern, cooler regions of the USA. A notable exception is the elepidote species *R. hyperythrum* from Taiwan — it is resistant to root rot and both the species and hybrids derived from it perform well in southern Louisiana (Thornton, 1990).

Recently, a new breed of rhododendrons has been introduced with the potential to overcome some of the limitations posed by root rot disease and warm climates to successful plant culture. Plant Development Services, Inc. has introduced five *R. hyperythrum* hybrids from Dr. John Thornton's Louisiana breeding program (www.azaleachapter.com/gulf_south.htm) into their Southern Living Plant Collection® under the Southgate™ brand. These rhododendrons have novel heat tolerance and are targeted for U.S.D.A. hardiness Zones 6-9. Because *R. hyperythrum* is also known to be resistant to *P. cinnamomi*, it is possible that the hybrids derived from it have some resistance. However, formal tests of root rot resistance were not part of the original field evaluations conducted by Dr. Thornton.

A rhododendron breeding program for root rot disease resistance was started at The Holden Arboretum in the late 1990s, with the goal of producing plants that were easier to grow for both producers and consumers. At that time, heat tolerance was not an objective, and the breeding strategy was based on transferring resistance from a small group of cold-hardy, resistant cultivars to a broader ornamental group of plants (Krebs and Wilson, 2002). However, the cultivar parent material proved to be marginally effective in producing the desired results, primarily due to sterility in some cultivars, poor breeding value for the resistance trait in others, or poor offspring (F_1) quality. By 2005, the main source of resistance for breeding shifted from rhododendron cultivars to the species *R. hyperythrum*, primarily due to the success of the Thornton hybrids in the USA. Gulf South, and the realization that the heat tolerance trait was commercially more valuable than root rot resistance because of the potential for an expanded geographic market. The association of heat tolerance and disease resistance in *R. hyperythrum* suggests that they may be functionally interdependent traits. Our working hypothesis for the breeding program maintains that disease resistance is a key component of heat tolerance (because *P. cinnamomi* pressure increases in warm, wet conditions) and that root rot resistant rhododendrons are more adapted to southern climates.

RESISTANCE BREEDING WITH *RHODODENDRON HYPERYTHRUM*

Although it is native to Taiwan, *R. hyperythrum* occurs at high enough elevations [1000-2000 m (3281-6562 ft)] to be considered a hardiness Zone 6 species (Cox, 1990). At Holden Arboretum's David G Leach Research Station in Madison, Ohio (Zone 5b), *R. hyperythrum* grows well in the field but exhibits some flower bud damage at winter temperatures below -13°C (8°F). In addition to root rot resistance, this species possesses a number of desirable ornamental attributes — thick, deep green glossy foliage, a mounded, dense growth habit, and a floriferous nature. It frequently covers its foliage with blooms, more like an evergreen azalea than a typical rhododendron (Fig. 1).

Fig. 1. Vegetative (left) and floral (right) characteristics of *Rhododendron hyperythrum.*

The tendency to set a high number of flower buds is due to an unusually high number of axillary shoots or "breaks" that are formed below the current year's flowers. This also helps maintain a dense habit. Flower color in *R. hyperythrum* is invariably pink in bud opening to white, and the inflorescence or "truss" is usually somewhat open or lax due to long peduncles.

The main breeding strategy with *R. hyperythrum* has been to cross it with cultivars that have more cold hardiness, more saturated flower colors (pink, red, purple, and yellow), and a well-formed inflorescence (typically a ball-shaped or pyramidal truss). However, this usually means crossing the species with a plant that is susceptible to root rot, because there are very few resistant, hardy cultivars with deep flower colors (Hoitink and Schmitthenner, 1974; Krebs and Wilson, 2002). Fortunately, another key attribute of *R. hyperythrum* is its high breeding value, an ability to transmit a high level of root rot resistance to F_1 progeny in a resistant × susceptible cross. Numerous breeding experiments using disease screens have demonstrated large average gains in resistance in F_1 progeny compared to the susceptible parent (Fig. 2). Large single generation gains are important in woody plant breeding because the time from seed to flowering can be prolonged (average of 4 years in rhododendrons).

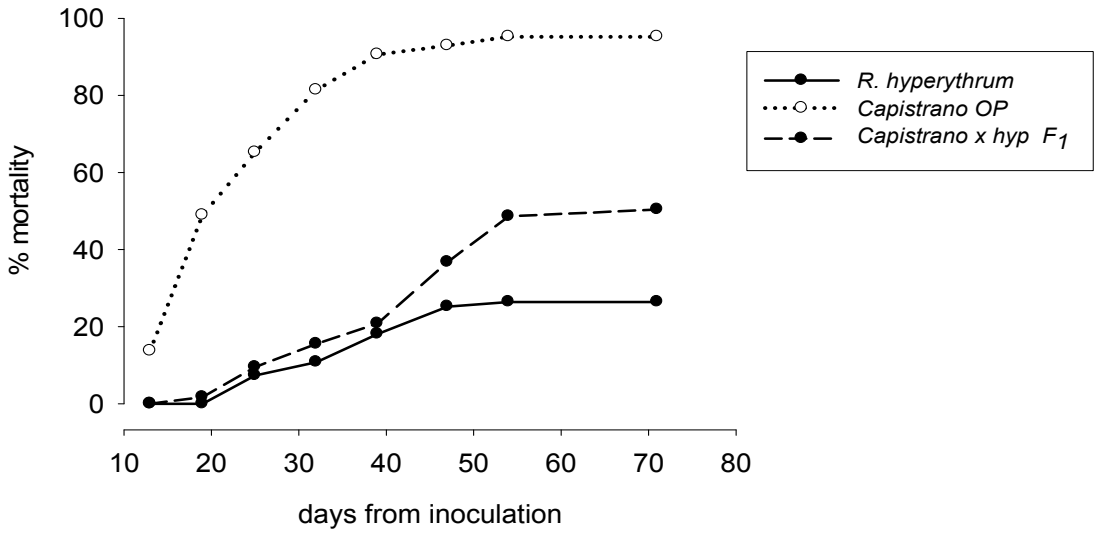

Fig. 2. Disease progress plots resulting from inoculations of *R.* 'Capistrano' open-pollinated seedlings (S=susceptible seed parent), *R. hyperythrum* species seedlings (R=resistant parent), and F_1 seedlings from the cross *R.* 'Capistrano' × *R. hyperythrum*. The plots are used to calculate the area under each curve (AUC), and gain from selection is estimated as $[AUC_S - AUC_{F1}] / [AUC_S - AUC_R] * 100 = 83\%$ in this example.

Some very high quality plants have been obtained among the variable F_1 progeny. Selection at the Madison, Ohio, site is made for plants that have good vigor and foliage quality in the open field, winter hardiness (U.S.D.A. hardiness Zone 5b), and well-shaped inflorescences with good flower color. Flower color and patterning in F_1s from white × red, white × yellow, or white × purple crosses is variable, often ranging from white, to intermediate colors, to occasional saturated colors. Examples of superior flowers from these populations are shown in Figure 3. Characteristics of an exceptional hybrid include a dense mounded habit, dark green glossy foliage, and saturated red flower color that are most desirable in a commercial plant (Fig. 4).

Fig. 3. Floral attributes of F_1 selections from *Rhododendron hyperythrum* breeding populations.

Fig. 4. An example of an F_1 selection with superior floral and vegetative attributes.

STRESS TESTS ON *RHODODENDRON HYPERYTHRUM*

While greenhouse breeding combined with controlled *P. cinnamomi* inoculations can be used to identify and select for root rot resistance progeny, the actual field resistance of these individuals may vary once planted outside. Abiotic stresses such as salinity, ozone, and extremes of temperature or moisture can predispose plants to disease and reduce resistance. In rhododendrons, for example, it was demonstrated that pre-stressing the resistant cultivar *R.* 'Caroline' with drought or flooding and subsequently inoculating with *P. cinnamomi* resulted in high susceptibility to root rot disease (Blaker and MacDonald, 1981). Similarly, resistant chrysanthemums were rendered more susceptible to root rot by exposing their roots to higher soil temperatures prior to inoculation (MacDonald, 1991).

In 2011, we completed a field flooding trial of rhododendrons that included root rot susceptible checks, several resistant cultivars (benchmarks), *R. hyperythrum*, and a group of *R. hyperythrum* derivatives (F_1s) that included the SouthGate™ cultivars. The plants were exposed to repeat flooding and draining episodes throughout one field season, All the resistant benchmark cultivars were dead or severely diseased by the end of the season, while the species *R. hyperythrum* and all of its hybrids survived. However, they exhibited more disease than under non-flooding conditions (Krebs, 2013). Presence of *P. cinnamomi* in symptomatic tissue was confirmed at the end of the field trial. The key finding from this work was that *R. hyperythrum* is significantly less predisposed to root rot disease under flooding conditions (compared to resistant plants, including *R.* 'Caroline', that lack *R. hyperythrum* in their genetic background), and that this trait is heritable and can be transmitted to offspring in a breeding program. This is a valuable characteristic for field growers of rhododendrons and for consumers who often plant into poorly drained soils around their homes.

The predisposing effect of heat stress on root rot disease in rhododendrons is in the process of being determined both in the greenhouse and in the field. In the greenhouse experiment, the root systems of pot-grown plants are immersed in a hot water bath set at a target temperature (30 min exposure), then cooled down, inoculated, and assessed for symptoms over a 2-3 month period.

Treatment temperatures ranged from 25-30°C (77-86°F), which span values relevant to hardiness Zone 8 maximum summer soil temperatures [≈40°C (104°F)] or black container mixes exposed to summer sun [≈50°C (122°F)]. Preliminary data from this experiment suggest that *R. hyperythrum* is less predisposed to root rot disease by heat stress than the

resistant cultivar *R*. 'Ingrid Mehlquist', which lacks *R. hyperythrum* in its genetic background.

Additional information on heat stress and disease will be obtained from a field trial of F_1 selections in southern Louisiana managed by Buddy Lee (Plant Development Services, Inc.). Over 160 selections from the Ohio breeding program were propagated and planted in a replicated, randomized trial in Spring 2013. At this time (Fall 2013) there has already been some mortality among plants, and by the end of the 2nd year of southern exposure (Fall 2014), there may be enough information to identify the superior performers and set them on track for commercial introduction. The field being used is an old nursery site, so it is highly likely that *P. cinnamomi* is present. This can be confirmed by isolating the pathogen from symptomatic tissue on affected plants. The results from the Louisiana field trial may also provide a test of our original hypothesis that disease resistance (to root rot in this instance) is a key component of heat tolerance and warm climate adaptation in plants. Because the F_1 progeny vary in resistance, their performance in hardiness zone 8 may vary accordingly. If our hypothesis is correct, the best performing plants in those conditions will also be the most resistant to root-rot disease.

Literature Cited

Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. 2004. Emerging infectious diseases of plants: pathogen, pollution, climate change, and agrotechnology drivers. Trends Ecol. Evol. 19:535-544.

Benson, D.M. 1980. Resistance of evergreen azalea to root rot caused by *Phytophthora cinnamomi*. Plant Dis. 64:214-215.

Benson, D.M. and Broembsen, S.V. 2001. *Phytophthora* root rot and dieback. In: R.K Jones and D.M. Benson (eds.), Diseases of Woody Ornamentals. APS Press, St. Paul, Minnesota.

Bergot, M., Cloppet, E., Perarnaud, V., Deque, M., Marcaiss, B. and Desprez-Loustau, M.L. 2004. Simulation of potential range expansion of oak disease caused by *Phytophthora cinnamomi* under climate change. Global Change Biol. 10:1539-1552.

Blaker, N.S. and MacDonald, J.D. 1981. Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to *Phytophthora* root and crown rot. Phytopathol. 71:831-834.

Brasier, C.M. 1996. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann. For. Sci. 53:347-358.

Cox, P.A. 1990. The Larger Rhododendron Species. Timber Press, Inc. Portland, Oregon.

Hoitink, H.A.J. and Schmitthenner, A.F. 1974. Resistance of rhododendron species and hybrids to *Phytophthora* root rot. Plant Dis. Report. 58:650-653.

Krebs, S.L. and Wilson, M. 2002. Resistance to *Phytophthora* root rot among contemporary rhododendron cultivars. HortScience 37:790-792.

Krebs, S.L. 2013. Resistance to *Phytophthora* root rot varies among rhododendrons subjected to repeated flooding in the field. Acta Hort. 990:243-252.

MacDonald, J.D. 1991. Heat stress enhances *Phytophthora* root rot severity in container-grown chrysanthemums. J. Amer. Soc. Hort. Sci. 116:36-41.

Marcais, B., Dupuis, F. and Desprez-Loustau, M.L. 1996. Modeling the influence of winter frosts on the development of the stem canker of red oak caused by Phytophthora cinnamomi. Ann. For. Sci. 53:369-382.

Thornton, J.T. 1990. Breeding rhododendrons for the Gulf South. J. Amer. Rhod. Soc. 44:91-93.

What's Chillin' with Northern Camellias©

Barry Yinger
Conard-Pyle Star Roses, West Grove, Pennsylvania 19390, USA
Email: byinger@starrosesandplants.com

INTRODUCTION

Camellia japonica is a large shrub or small tree native to a broad band of territory in East Asia, including parts of China (Shandong, Zhejiang), Taiwan, Japan, and Korea. It is found in mountainous areas and frequently on rocky hillsides near the ocean.

Cultivated forms of *Camellia japonica* were introduced to Europe in the early 1700s and to the United States of America in the early 1800s, where they eventually became a familiar feature of southern gardens. The earliest introductions were mostly, if not all, Chinese cultivated cultivars, followed by many introductions from Japan.

Until recently, the outdoor cultivation of camellias in the eastern United States was limited to U.S.D.A. Zones 7 to 9. Washington D.C. was considered to be the northern limit of hardiness, and even there most camellias were killed or severely injured in the coldest winters. Unusually cold weather in the late 1970s and early 1980s killed almost all of the 900 cultivars of camellias at the U.S. National Arboretum. Except for a few plants in the most favorable coastal locations along the Atlantic coast as far north as Martha's Vineyard, camellias were impossible to grow outside the South without protection from winter wind and cold.

ORIGIN OF *CAMELLIA JAPONICA*

Despite its wide range in the wild in East Asia, it is likely that until about 35 years ago, all of the wild *C. japonica* genetics in the USA were of Japanese origin, and all of the cultivated genetics were from China and Japan. Wild Japanese genetics were introduced several times through explorations conducted by the U.S. National Arboretum, but I have found no record of wild genetics being introduced from China or Korea. The 1980 Sino-American Botanical Expedition marked the resumption of the collection of wild genetics of trees and shrubs in China, but *Camellia japonica* was not among its many collections.

CHOLLIPO ARBORETUM, CHOLLIPO, SOUTH KOREA

In 1979, I finished my studies in the Longwood Gardens M.S. program and accepted a job working in Korea, helping to develop the Chollipo Arboretum on the Yellow Sea coast of Korea. The arboretum had been founded by the late Carl Ferris Miller, an expatriate American, at a fishing village called Chollipo in what was then a very remote part of Korea. While I was living there I was given an article written in the early 20th century by the Japanese botanist Ueki. I had studied Japanese language at the University of Maryland, so I was able to read this very interesting account about finding populations of broad-leaved evergreen trees and shrubs such as *C. japonica* growing on islands near what is now the North Korean coast of the Yellow Sea, at and above 38° N latitude. This area is very cold in winter, buffeted by the bitterly cold prevailing winds from Siberia. I became determined to visit these islands and see if those camellias could still be found.

CAMELLIA COLLECTION AT SOCHONG ISLAND, SOUTH KOREA

After 2 years at Chollipo, I arranged to return to the USA, but took some time to investigate the prospect of collecting these northern camellias. At Chollipo I had become friends with Mr. Young June Chang, a young man who was fulfilling his mandatory military service at a remote army camp about 7 miles further up the peninsula. We were helping each other learn our respective native languages, and I earned points by packing a case of beer, on foot, for 7 miles into the army camp on most Friday nights. Young June and I first asked around various government agencies in Seoul, trying to find out if anyone had any information about these plants, and if we could get permission to visit the islands. The islands belong to South Korea, but are within sight of the North Korean

mainland, so only native born residents of the islands and military personnel are permitted to take the ferry that is the only transportation to the islands. Despite the fact that Young June's uncle was a four star general; we were shunted from agency to agency and finally gave up. We decided to take the less formal and more direct approach, and went to the ferry pier at Inchon with our camping gear and a bottle of whiskey, and bribed our way onto the boat. The authorities were very put out when we arrived at Sochong Island after about 20 h at sea, but there was no boat returning for several days so they allowed us to look around with a Korean army baby-sitter.

My first sighting of the grove of old camellias on a slope near the ocean on Sochong Island is a sight I will never forget. It was winter and the landscape was brown and bleak except for a few pine trees and the camellias with their glistening dark green leaves. They were growing on open hillsides completely exposed to the northwest wind. I found a cut stump and counted about 500 growth rings. The seed capsules had opened and seed lay thick on the ground. This grove of old trees at Yedong village was one of the most impressive groups of old plants we saw, although we saw many smaller plants growing along rocky cliffs on Sochong and Taechong islands. We also saw many trees growing in the gardens of villagers who had moved them from the wild, even on Paekryong Island, part of which is north of 38° north latitude.

As the military authorities on the island realized that we were odd but not dangerous, they permitted us to visit the other nearby islands, and Young June was able to arrange for us to return. I returned to the islands again with Young June to collect more seeds and cuttings, most of which went to Dr. Clifford Parks at the University of North Carolina Chapel Hill. Based on a proposal that I wrote, the U.S. National Arboretum developed a plan to visit the islands in 1984, and I returned twice more with several representatives of cooperating public gardens. Altogether a large quantity of seeds and cuttings representing many different genotypes were introduced to the United States. Several cooperating institutions, including Longwood Gardens and the Morris Arboretum in Pennsylvania, germinated wild seed and planted it outdoors for observation.

TRIALING CAMELLIA COLLECTIONS IN U.S.D.A. ZONE 6

In 1992 I started to plant camellias grown from collections from these islands at my farm in south central Pennsylvania in U.S.D.A. Zone 6. I have trialed many camellias there, but none has proved quite sufficiently hardy, including the fall-blooming introductions bred by Dr. William Ackerman. In general, I found that at my location, camellias lost some or all of the top growth at -18 to -12°C (0 to 10°F) and flower buds rarely survived at temperatures lower than -12°C (10°F). In the winter of 1993-94 we experienced the coldest temperatures ever recorded [-36°C (-23°F) night low, with a high temperature of -24°C (-12°F) the following day. A typical low winter temperature for us was -21 to 23°C (-5 to -10°F)]. The Korean camellias suffered little damage, and have thrived ever since, with volunteer seedlings appearing throughout the garden. They have never failed to bloom, with flower bud hardiness of at least -24°C (-12°F). Over about 25 years I have tested many of the camellias said to have enhanced cold-hardiness, and the only selections that keep their wood through cold winters are some of Cliff Parks' April series, but even those sometimes lose some or all of their flower buds.

When I started planting Korean camellias in Pennsylvania, I did not know they were in fact hardier than Japanese or Chinese selections as we know them. As I continued to grow the plants from wild seed, I noticed that some of the morphological characteristics that I saw in the wild — smaller, thicker leaves with a heavy cuticle; shorter internodes — were displayed in cultivation as well. I also noticed that they bloom over an unusually long season. Some buds will open in late winter after a brief warm spell, and others will bloom throughout the spring until some time in May. This will allow an unusually long season for pollination of the Korean genetics.

TRIALING AT ARBORETA AND NURSERIES

At the U.S. National Arboretum in Washington D.C., hundreds of seedlings were planted out in rows for observation. One of these seedlings was selected by Joe Gray of Hines Nurseries in California, planted out at my farm in Pennsylvania, and later named 'Korean Fire'. It has a larger, flatter flower than the typical wild plant, along with excellent U.S.D.A. Zone 6 hardiness. Dr. Cliff Parks, then a professor at the University of North Carolina, selected a white-flowered form from my first seed introductions that has since been named 'Korean Snow'. The Morris Arboretum in Philadelphia, PA, and Longwood Gardens, in Kennett Square, Pennsylvania, have been observing plantings from wild seed for many years, but they are not quite cold enough for a good U.S.D.A. Zone 6 trial.

Cliff Parks' April series, and other hardier forms of camellia, are now widely promoted for U.S.D.A. Zone 6, but often they do not perform well in the middle to colder parts of that zone. Many gardeners who think that they are in U.S.D.A. Zone 6 are now in U.S.D.A. Zone 7. Heavily populated parts of Pennsylvania, New Jersey, New York, and coastal New England that once were considered to be in U.S.D.A. Zone 6 are now shown on the U.S.D.A. hardiness map to be in Zone 7, or even 7b. There is still a great opportunity to enhance hardiness, especially flower bud hardiness.

With the backing of my employer, Conard-Pyle Star Roses, I have initiated a breeding project at my farm in south central Pennsylvania. On the most recent U.S.D.A. hardiness zone map, we are now shown to be in U.S.D.A. Zone 6b, but my farm is in a valley that is consistently 5° to 10° colder than the surrounding area. I have created a 1-acre trial plot in an open field, fenced against deer and rodents. Here we are planting the hardiest known cultivated cultivars along with the wild Korean genetics. So far 129 plants are in place, with perhaps another 50 to be planted next year.

Cultivars to trial were selected based on my own experience, and the experiences of gardeners and nurserymen with long experience growing camellias. Most of the cultivated cultivars are from Dr. Cliff Parks' breeding, plus a few old cultivars that have survived harsh winters in other locations. There are a few from Dr. William Ackerman's breeding program. Additional cultivars will be added as I find a source for plants.

We will transfer pollen between the Korean plants and non-Korean cultivars in spring, and allow bees to pollinate. Seed will be harvested in early fall, germinated at Conard-Pyle, to be grown for 1 year in deep cell pots. These seedlings will be planted out in the same field in their second spring for evaluation. Superior individuals will remain for observation and further breeding.

Any mother plants or seedlings that lose wood or flower buds over winter will be removed from the trial plot. The goal is to develop a series of cultivars with a range of flower forms and colors that are reliably hardy throughout U.S.D.A. Zone 6, and perhaps into Zone 5b. Preliminary selections will be propagated and sent out for trial to sites in U.S.D.A. Zones 5b to mid-6.

Natives: the "In" Word in the Gardening World©

Rick Webb
Louisiana Growers, 63279 Lowery Road, Amite, Louisiana 70422, USA
Email: rick@lanativeplants.com

INTRODUCTION

I was a Pre-Forestry major whose only formal plant materials courses were introductory Dendrology Horticulture classes. After working a summer at a wholesale nursery, my major was changed to Plant Science with a Bachelor of Science awarded in 1979. So Sylvaculture, Soil Science, and Agronomy were as important in my college education as Horticulture.

This was followed by an 8 year "graduate program" with a field-grown and container nursery where I worked in propagation, production, sales, harvesting, and shipping of mainline woody trees and shrubs and groundcovers.

In 1988, after deciding it was time to start my own business, I opened my own nursery and worked with plants that were from my earlier education: trees and shrubs of native woodlands and a few exotics. As I learned more of the landscape uses of these natives, impetus was placed on visiting and studying the diverse habitats and plants of the area and making germplasm collections.

The focus of the nursery is geared to unique plants from Florida Parishes of eastern Louisiana, which entails a slice of the Gulf Coast plain from near-Piedmont conditions on the Upper Terrace through the flat savannahs of the Lower Terrace to the brackish marshes of the Gulf Coast along Lake Ponchartrain. All of this compressed into 30 miles, north to south.

WHAT ARE NATIVE SPECIES?

Any talk on Natives must begin with the first question: Define Native?

- Being of the place or environment in which a person was born or a thing came into being.
- Of, pertaining to, or characteristic of the indigenous inhabitants of a place or country.

But when we talk about inhabitants of a place, movement of the species has to come in to discussion. So Einstein was correct: Everything is about Space and Time.

- If a plant originated on the Southeastern coast of Asia and was relocated to a similar area of the North America, is a native?
- If a plant was relocated 200 years ago, is it native?
- If a plant migrated with the Ice Age and is here now, is it a native?
- If a plant was moved out of its original range by Americans, is it a native?
- If a plant that is native to the Pacific Northwest of North America is planted in Central Park, New York City, is it still native?
- If a species is collected from the northern end of its range and moved to the southern end, is it a native? Are all the seedlings of a given individual that is indigenous to an area optimal, or would a given seed do better in another ecological range – and if so, is it a native?
- If a given individual is collected from a wet bay gall in the pinewoods and is moved 100 yards up slope to a mesic hillside, is it a native? Or is it the same old question: Nurture verses Nature?

DESIGNING LANDSCAPES

So when I give presentations to gardeners and landscapers, it has been my goal to talk about natural forms and species in the design and intent of our landscapes, rather than native verses non-native.

Gardeners are advised to follow a few guidelines:

- Nature's designs are irregular, not random.
- Plants in nature evolve to fill niches. Identify those and fill them appropriately.

- Never design a planting as an even number or a straight line or a pure arc.
- Think about how the native fauna, including humans, use the landscape and how it can change over time.
- Think about how the garden will evolve over time.
- Think of the garden, as a forest, in layers.
- Study your site and surrounding similar stable sites and their species.
- Ask yourself or your clients if the five "F's" of seasonality are important: flowers, form, foragers, fruit, fragrance, and fall color.
- Create woodlands and thickets and get rid of the "manicured lawn look" except for small areas that mimics water.
- Mulch, mulch, mulch.
- Remember: This is not rocket science; it is quite simple.

IMPORTANCE OF NATIVE PLANTS IN NATURAL DESIGNED AND CONSTRUCTED SPACES

So why are native plants in natural designed and constructed spaces important? In part, it helps mitigate the dramatic changes of the last two centuries to our environment. Other benefits include:

- To (re)create habitat for native flora and fauna and co-habitant humans.
- To remind us of our sense of place.
- To manage stormwater.
- To minimize maintenance costs.
- To protect our properties.
- To create a business niche from a national trend.
- To add value to our real estate.
- To beautify our spaces.

PROPAGATION OF NATIVES

Now since this is a meeting of propagators, propagation is essential. The simple answer is that there is a commonality of propagation methods used with other non-native, commercial plants. Whether by seed, cuttings, division, or grafting — there are specific propagation systems for each species.

There are two exceptions that are specific to native plants and designed native landscapes:

- Some clients want a truly diverse garden and will accept only seedling propagated plants. Hence, it is essential that these plants can be propagated by seed. They also use a confusing term to describe clonal selections of native plants: "nativar" — defined as a cultivar or hybrid derived from a native plant; in this way, one can define the native range and describe a cultivar or hybrid that originated from that range a nativar, i.e., an ecotype. Other clients want some predictability when site conditions allow use of cultivated cultivars.
- Location, Location, Location. To many this is second in importance only to the species selected for a garden. To this group the very definition of a weed [an undesirable plant that grows and reproduces invasively outside its native habitat] is expanded to also include incorrectly placing a native in the landscape.

SELECTIONS OF LOCAL NATIVES

Here are a few selections of our local natives:

Clethra

We made trips into *Clethra* habitat in Washington and St. Tammany parishes and took cuttings from individual and mixed source plants. I have been disappointed in its ability to establish successfully. Several stock plants are in the ground around our office and individual plants were sent to the U.S.D.A. in Tennessee. We have not named any cultivars.

Cyrilla
Where we find populations of *Cyrilla racemiflora* on the lower terrace, we noticed its massing habit in wet habitats and the diversity of form. So it is easy to find forms that vary from small foliage shrubby forms, to small trees with large foliage, to layered horizontal forms. Colors range from orange-red fall color to evergreen foliage. I have yet to see any unique flower color other than typically found with the species. We have established five numbered selections.

Ilex
We are interested in *Ilex vomitoria*. One yellow-gold fruited form was selected and named. *I. vomitoria* 'Chesborough', which is an open, small foliage, large shrub / small tree form. Another form has red fruits with a full round habit and normal foliage is *I. vomitoria* 'Lowery Road'. We also have also selected a tight semi-dwarf male with small foliage that will be named 'El Chico'.

Lyonia
Lyonia lucida is common in wet pine flats just south of us. We have two named selections: *L. lucida* 'Lorraine' which grows to 2.1 m (7 ft.) with large glossy foliage with white spring flowers, and *L. lucida* 'Hoover Road' which is a mid-size selection that is deep pink in bud and then pale pink. It is nearly white at anthesis. Two others were selected and numbered.

Viburnum
There is difficulty with the taxonomy of *Viburnum*. In our nursery, we work with two groups: Arrowwood viburnum (*V. dentatum*) and witherod (*V. cassinoides*). From the immediate north shore of Lake Ponchartrain on the lower terrace and up to wet flats in on the upper terrace are populations with small foliage and flower clusters and very shrubby habit. This has been called *V. ashei* in older texts and is now lumped into *V. recognitum* by the Plants Database. Several selections are:
1. *Viburnum dentatum* 'Osceola'. It was the first named cultivar in this Florida Parishes place name series, which I found along a Tangipahoa roadside southeast of Husser, Louisiana. It was the first small foliage arrowwood I had noticed. The red-brown wood color of the new growth is an indicator of the plant's outstanding red fall color. Flowers are great and fruits and thick branching makes it fine bird habitat. The habit is a large round arching shrub to 3 m (10 ft.) tall and 3.7 m (12 ft.) wide with very nice fall color.
2. *Viburnum* 'Lee's Landing'. Its small foliage and size is its namesake, with the smallest foliage [1.9 cm (0.75 in.) wide by 3.8 cm (1.5 in.) length] named form that we have. It has a tight round growth habit that is much smaller in size than the typical species. It has decent flowering and fruit, but with a yellow fall color. The selection was found just 1 mile north of the Tupelo/Cypress swamp along the lake and the Tangipahoa River in poorly drained Flatwoods near Lee's Landing, Louisiana.
3. *Viburnum* 'Chemekete'. This was wild collected from a flat pinewoods 2 miles northeast of Robert, Louisiana. 'Chemekete' comes from Native American that describes the geographic features of the area. It is appropriately named and one of the most arrowwood-like forms that I have found. This viburnum has upright straight growth from the crown that arch after 1.8 m (6 ft.) to half as wide as a tall shrub. It has good flowering in mid-May, summer fruit set and pale reddish, orange, purple fall color. It has a narrow habit for the species.
4. *Viburnum* 'Greensburg'. It is a favorite of mine. A combination of the attributes of 'Osceola' include its red, nearly burgundy fall color, rounded habit, small foliage, and a stature smaller than 'Lee's Landing' – all of what makes it unique. It will grow to 1.8-2.4 m (6-8 ft.) after 10 years.
5. *Viburnum* 'Abita Flatwoods'. The plant was found between Pearl River, Louisiana and Abita Springs, Louisiana along Highway 36 in wet pond cypress - longleaf pine habitat. It is a small foliage form with dark red twigs, rounded habit but less striking fall

color makes it a good shrub.
6. *Viburnum* 'Uneedus'. It was found near Uneedus, Louisiana, and has very linear foliage. Flowers are typical of the species, but it sets loads of fruit and maybe self-fertile. Its mature size and habit have not been determined. The mother plant was growing in a crowded site with poor conditions so we must get them stabilized and field-trialed.
7. The other arrowwood type in my woods is what we have just around us on upland mesic understory sites. This type has large 0.9-1.2 m (3-4 ft.) coarse textures foliage and large flowers clusters. Another difference is its limit to commercially produce as it has few apical growth points to make cuttings of and these root in lower percentages. I have managed to root a few each year, but have been given most away. The last couple of plants have been maintained as propagation stock, but multiplication is slow.
8. *Viburnum* 'Ben's Creek'. It was found between Bogalusa and Franklinton, Louisiana, and its full dense habit was the attraction. Turns out that might have been a function of it being out in the open on a young loblolly pine plantation as it has resumed the expected more-open, tree-like habit of the species when planted in bright understory. It has a rich red fall color, good flowers, fruit, fall foliage, fabulous form, and a good bird attractor.
9. *Viburnum* 'Squirrel Creek'. This is the most local selection in my collection. It was found in the back woods of my property and I moved it up to anchor the corner of my carport. It has a loose upright large shrub to small tree. I use it as the perfect native selection.
10. Witherod (*V. cassinoides*) and *V. nudum*. Witherod (*V. cassinoides*) and *V. nudum* are scattered in our area in wet flats. They have green glossy foliage and a small tree habit with the waxy stems.
11. *Viburnum* 'Chappapeela'. It was found growing at the water line of an upland creek east of Amite, Louisiana. While deciduous with rich red internal fall color, it is nearly evergreen. It is an upright large shrub/small tree.

SUMMARY

1) Although they do not consider themselves as "native plants" people, the vast majority of the members of the nursery/landscape industry propagate, grow, sell, install, and maintain native and non-native plants.
2) Are we willing to make the changes to the nursery industry necessary to promote native plants in naturalized gardens, to encourage regionalization matched with local production demand to raise awareness of the importance of viewing our spaces as shared eco-systems or to address the functional purposes of our landscapes not just the ornamental value?
3) Do we expect the educational and research components of our industry to pursue these same goals?
4) As growers are we willing to expand diversity in our production lines and pot-up a handful of seedlings or cuttings, or do we focus on the latest patented / trademarked gem and produce thousands of them to make a living?
5) And if root systems are naturally wide and shallow, why do we not produce plants in similar-shaped containers, rather than traditional tall, upright containers?
6) Are we as an industry and as individuals willing to take the long road with the hills of change?

From the "Address on the Nation's Space Effort" by John F. Kennedy delivered at Rice University in Houston, Texas on September 12, 1962.

"…and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win, …"

Remember — Diversity Rules!

Irrigation Volume and Fertilizer Rate Influence Growth and Leaching Fraction from Container-Grown *Gardenia jasminoides*©[1]

Amanda Bayer, John Ruter and Marc van Iersel
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: bayer10@uga.edu

INTRODUCTION

Over irrigating is a common problem in container-plant production because of poor uniformity and efficiency of irrigation systems (Fare et al., 1992) and the preference of growers to deal with the consequences of applying too much water vs. too little (Yeager et al., 2010). Along with this, many growers apply large amounts of fertilizer out of concern that lower fertilizer applications could negatively impact growth (Owen et al., 2008; Tyler et al., 1996). The combination often excessive irrigation and high fertilizer rates leads to significant leaching of fertilizers, which has a negative environmental impact as the leachate enters local ecosystems (Lea-Cox and Ross, 2001). Many states now have laws and regulations regarding nutrient runoff from nurseries necessitating that growers better manage the irrigation and fertilization applications (Beeson et al., 2004).

Growers have already adopted more effective irrigation practices including cyclic irrigation, drip irrigation, and grouping similar sized containers (Yeager et al., 2010; Tyler et al., 1996). Better management practices for fertilization and nutrient leaching have also been adopted, including using controlled-release fertilizers that last throughout the production period and monitoring substrate nutrient levels (Yeager et al., 2010). However, to irrigate and fertilize more efficiently more research is needed examining how plant growth is affected by reduced irrigation and fertilization.

It seems likely that fertilizer inputs can be reduced if irrigation is applied more efficiently because more efficient irrigation reduces leaching. With reduced leaching, more fertilizer remains in the container and available to the plant. Our objective was to determine how irrigation volume and fertilizer rate affect growth and leaching fraction of *Gardenia jasminoides*. Our hypothesis is that more efficient irrigation can reduce the fertilizer requirements without impacting plant growth, while reducing the leaching fraction.

MATERIALS AND METHODS

The experiment took place at the University of Georgia horticulture farm in Watkinsville, Georgia, from July to October 2012. Rooted cuttings of *Gardenia jasminoides* 'Madga I', Heaven Scent® gardenia were planted in #2 black plastic containers in a pine bark substrate with micronutrients, gypsum, and lime incorporated. Plants were given time for root establishment before irrigation treatments were applied. Fertilizer treatments were applied at planting.

Treatment combinations included fertilizer rates of 100, 50, and 25% of bag rate and irrigation application rates of 66, 100, 132, or 165 ml per irrigation event for a total of 12 treatment combinations. Controlled release fertilizer (Florikan 18-6-8) was incorporated into the upper part of the substrate at 40 g per plant (100%), 20 g per plant (50%), or 10 g per plant (25%). Irrigation was controlled via a soil moisture sensor automated irrigation system similar to that described by Nemali and van Iersel (2006). Irrigation was applied to maintain a 35% volumetric water content (VWC) for the control treatment (100% fertilizer rate, 66 ml irrigation treatment) and was applied via dribble rings with pressure compensated drip emitters. Substrate moisture readings were taken every 20 min, and when the substrate moisture of the control plants dropped below 35% VWC, all treatments in a replication were irrigated. Thus, all plants within a replication were watered the same number of times, but with different amounts of water each time. The

[1] First Place – Graduate Student Research Paper Competition.

experimental design was a randomized complete block with four replications. There were four plants for each treatment combination in each replication.

Leachate was collected in 10-gal. containers. Rainwater was excluded from the container, so that only rainwater that had moved through the substrate was included in the leachate (Fig. 1). Plant height and width as well as leachate volume were measured biweekly. Irrigation volumes and rainfall were measured daily throughout the experiment. Growth index was calculated as (height + width1 + width2)/3. Leaching fraction was calculated as leachate volume/(irrigation volume + rainfall volume).

RESULTS AND DISCUSSION

Growth index increased quadratically over the course of the experiment (Fig. 2). At the conclusion of the experiment, the growth index of all treatment combinations, except those receiving the 25% fertilizer treatment, was larger than the control (66 ml, 100% fertilizer) (Table 1). Dunnett's multiple comparisons analysis of growth indices at the conclusion of the experiment shows that the only significantly different treatment combinations from the control were the 100% (165 ml irrigation) and 50% (132 ml irrigation) treatment combinations which were 13% greater. The similar growth indices in the 100 and 50% fertilizer treatments for all irrigation volumes shows the potential for using reduced fertilizer applications along with moderate irrigation volumes to grow salable plants of *Gardenia*.

Irrigation volumes were inversely related to rainfall because rainfall increased the VWC of the substrate above the 35% threshold, therefore irrigation did not occur. The impact of irrigation volume and fertilizer rate on leaching fraction differed for the biweekly leachate collections. Large rainfall volumes (2-4 in. total rainfall volumes) during the time between collections created similar leaching fractions for all treatment combinations (Fig. 4, top and middle graphs). The clearest impact of treatments can be seen during the last 2 weeks, during which it did not rain. For this collection time, both irrigation volume ($P<0.001$) and the interaction between irrigation volume and fertilizer rate ($P=0.006$) affected the leaching fraction.

Tyler et al. (1996) found that irrigation applied to reach a high (0.4-0.6) or low (0.0-0.2) leaching fraction did not affect shoot growth of *Cotoneaster* × *suecicus* (syn. *dammeri*) 'Skogholm'. These findings are similar to those in this study with irrigation volume not significantly affecting growth. In contrast to our study, in which growth with the moderate fertilizer applications (50%) was not significantly different than high applications (100% rate) (Fig. 3), Tyler et al. (1996) found that reducing fertilizer application rate by 50% did significantly reduce growth. However, Cabrera (2004) also reported that moderate fertilizer applications can be used without reducing plant quality.

Further research investigating fast vs. slow growing species, high vs. low fertilizer requirements, and high vs. low water use would give a clearer picture of how irrigation and fertilization can be altered in a production environment to reduce inputs while producing salable plants. The results of this study suggest that reduced fertilizer rates can be used in combination with efficient irrigation to produce salable plants. As more states are impacted by laws and regulations regarding nutrient runoff from nurseries and existing laws become more strictly enforced, it will become imperative that growers adopt more efficient fertilization and irrigation practices.

ACKNOWLEDGEMENTS

We thank Sue Dove, Kevin Whitaker, Faustine Sonon, and Kengelle Chukwurah for her help with this research. Funding for this research was provided by USDA-NIFA-SCRI award no. 2009-51181-05768.

Literature Cited

Beeson, R.C., Jr., Arnold, M.A., Bilderback, T.E., Bolusky, B., Chandler, S., Gramling, H.M., Lea-Cox, J.D., Harris, J.R., Klinger, P.J., Mathers, H.M., Ruter, J.M. and

Yeager, T.H. 2004. Strategic vision of container nursery irrigation in the next ten years. J. Environ. Hort. 22:113-115.
Cabrera, R.I. 2003. Nitrogen balance for two container-grown woody ornamental plants. Sci. Hort. 97:297-308.
Fare, D.C., Gilliam, C.H. and Keever, G.J. 1992. Monitoring irrigation at container nurseries. HortTechnol. 2:75-78.
Lea-Cox, J.D. and Ross, D.S. 2001. A review of the federal clean water act and the Maryland water quality improvement act: the rationale for developing a water and nutrient management planning process for container nursery and greenhouse operations. J. Environ. Hort. 19:226-229.
Nemali, K.S. and van Iersel, M.W. 2006. An automated system for controlling drought stress and irrigation in potted plants. Sci. Hort. 110:292-297.
Owen, J.S., Jr., Warren, S.L., Bilderback, T.E. and Albano, J.P. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. HortScience 43:906-2008.
Tyler, H.H., Warren, S.L. and Bilderback, T.E. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. J. Environ. Hort. 14:199-204.
Yeager, T., Million, J., Larsen, C. and Stamps, B. 2010. Florida nursery best management practices: Past, present, and future. HortTechnol. 20:82-88.

Table 1. Growth index of *Gardenia jasminoides* 'Madga I', Heaven Scent® gardenia at the conclusion of the four month study and percent difference in growth index by treatment combination from the control (2 min irrigation, 100% fertilizer) treatment.

		Growth index (mm)	Difference from control (%)	P<0.05
100% Fertilizer	66 ml	188.5		
	100 ml	203.1	8	
	132 ml	204.8	9	
	165 ml	213.1	13	*
50% Fertilizer	66 ml	196.4	4	
	100 ml	208.2	10	
	132 ml	212.2	13	*
	165 ml	197	5	
25% Fertilizer	66 ml	189.9	1	
	100 ml	182.7	-3	
	132 ml	180.6	-4	
	165 ml	134.1	-4	

*Dunnett's multiple comparison (significance at P<0.05).

Fig. 1. The setup for leachate collection/rainfall exclusion.

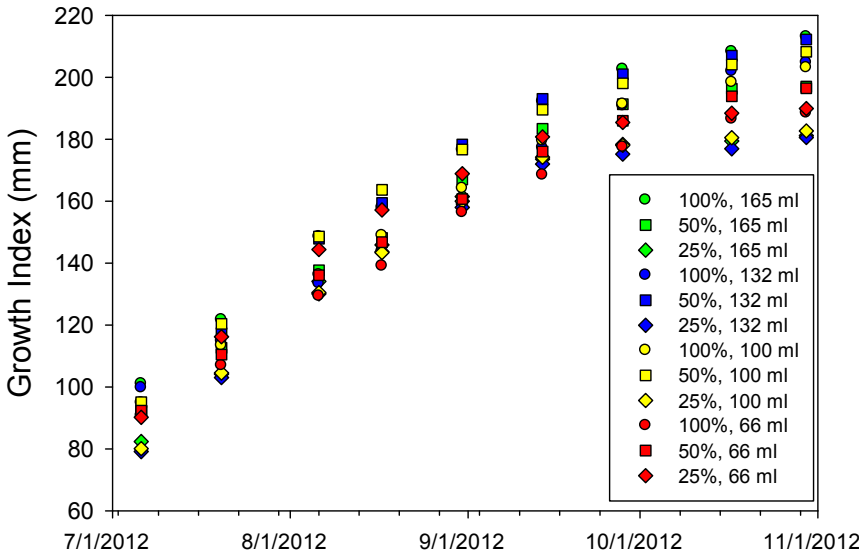

Fig. 2. Growth index (mm) of *Gardenia jasminoides* 'Madga I', Heaven Scent® gardenia over the course of the four month study.

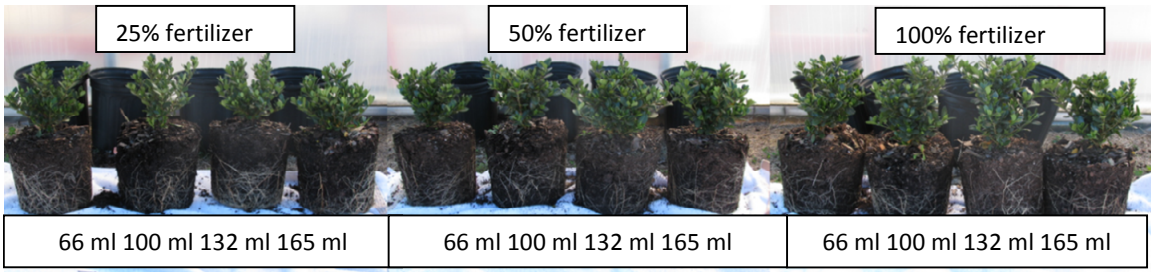

Fig. 3. Photos of plants showing all treatment combinations at the conclusion of the experiment. Treatments are 25% fertilizer rate to the left, 50% in the center, and 100% to the right and irrigation volumes are from 66-165 ml moving left to right in all pictures.

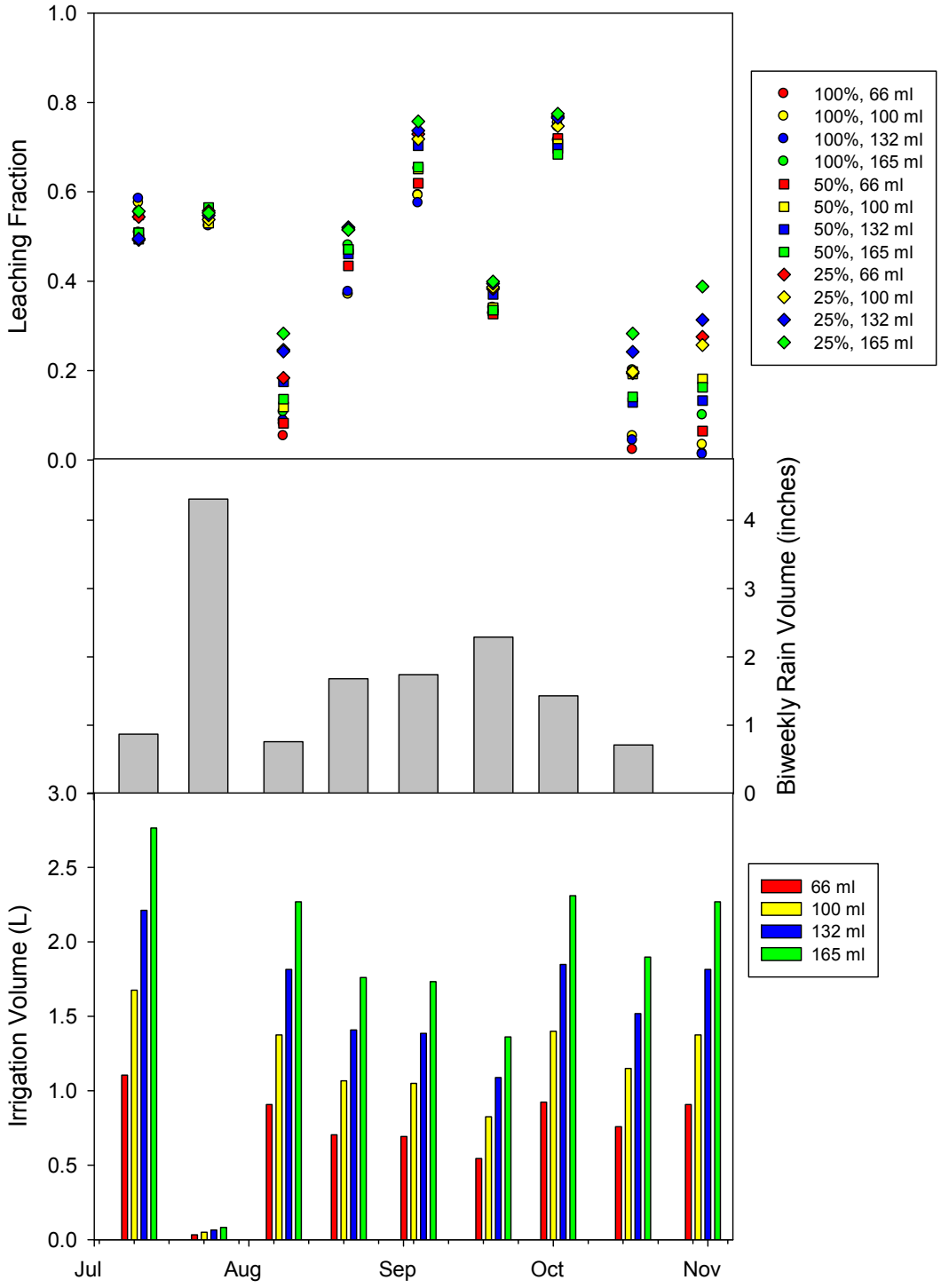

Fig. 4. Leaching fraction (irrigation volume + rainfall/leachate volume) (top graph), biweekly rainfall volume (inches) (middle graph), and biweekly irrigation volume (L) over the course of the 4 month study.

Fertilizer Movement in Nursery Containers: What Happens during Irrigation?©[1]

Tyler C. Hoskins, James S. Owen, Jr., Jeb S. Fields and Julie Brindley
Virginia Tech, Department of Horticulture, Hampton Roads Agricultural Research and Extension Center, 1444 Diamond Spring Road, Virginia Beach, Virginia 23455, USA
Email: jim.owen@vt.edu

Recommendations for the efficient use of fertilizer resources in containerized plant production systems are largely based on season-long nutrient leaching dynamics under the impact of various fertilizer and irrigation management practices. To date, little research has been conducted to understand the principles of water and fertilizer movement through a nursery container during irrigation and how these principles may impact nutrient fate. A saturated solute transport study was conducted by passing a fertilizer solution and deionized water through a saturated pine bark substrate and measuring the electrical conductivity of the drainage. The result is a breakthrough curve, which is helpful to both growers and researchers in understanding the movement of water and fertilizers through the highly porous and relatively inert substrates used in containerized ornamental crop production.

INTRODUCTION

Mineral nutrients are conventionally applied to containerized ornamental crops via irrigation as soluble salts dissolved in irrigation water (i.e., fertigation) or by a plastic-encapsulated fertilizer source (i.e., controlled release fertilizer; CRF) which release nutrients over long periods of time (i.e., months). Water is the medium by which these nutrients are made available to plants. Water applied via irrigation fills substrate pores and either carries dissolved nutrients to roots or hydrates CRF prills, leading to the diffusion of nutrients from inside the prill to the surrounding water. Between irrigation events, roots will absorb a portion of the available water and nutrients. Pore water not absorbed before the next irrigation is displaced by newly applied water, thus being leached from the substrate into the surrounding environment.

Our current understanding of nutrient fate in containerized ornamental crops has been based on theory of CRF nutrient release (Adams et al., 2013), plant absorption (White, 2012), and season-long nutrient release (Newman et al., 2006). However, there is limited experimental research investigating nutrient fate (availability, uptake, and leaching) during irrigation events. To understand the principles of water and solute (i.e., fertilizers dissolved in water) transport we may utilize well-established procedures from the study of soils (Skaggs, 2002). However, the physical properties of soils differ from that of bark-based substrates. Particle sizes for major soil groupings, as classified by the U.S.D.A., are sands (<2-0.05 mm), silts (<0.05-0.002 mm), and clays (<0.002 mm) and porosities may range from 30-60% for most soils (Hillel, 1998) depending on composition. In contrast, the particle sizes of the substrate used in this study are 8.0% > 6.3 mm, 27.6% 6.3-2 mm, 37.9% 2-0.71 mm and 26.5% < 0.71 mm by weight with an average total porosity of 73.5%. Our objective is to understand movement of dissolved nutrients entering and being displaced from a bark-based substrate using traditional methodology from soil science.

MATERIALS AND METHODS

On 19 June 2013 a bark and sand (9:1, v/v) substrate [bulk density (Db) = 0.32 $g \cdot cm^{-3}$] was potted into trade gallon (2.7 L) nursery containers (Myers Industries, Middlefield, Ohio). Containers were placed on an outdoor gravel pad at the Hampton Roads Agricultural Research and Extension Center and were subjected daily to a 15-min overhead irrigation (0.5 in./h, SD = 0.1). Substrate from five containers was pooled into

[1] Second Place – Graduate Student Research Paper Competition.

one composite sample and allowed to equilibrate overnight in a sealed container before use to ensure a more evenly distributed moisture content.

The experimental unit was a 30-cm acrylic column (7.75 cm i.d.; 8.9 cm o.d.; vol. = 1.415 L ≈50% of a trade gallon nursery container) enclosed by polyvinyl chloride (PVC) flat caps at each end. Barbed fittings (3.5 mm i.d.) in each cap created an inlet and outlet point at each end of the column. The vertically oriented column was attached via 5/16-in. Tygon tubing to a three-way valve and two mariotte bottles, one containing deionized (DI) water and the other containing a fertilizer solution comprised of nitrogen (N), phosphorus (P) and potassium (K) with an electrical conductivity (EC) of 0.33 $mS \cdot cm^{-1}$. Mariotte bottles allowed for the maintenance of constant pressure as the fluid level in the bottles decreased. Figure 1 depicts the physical setup of the experiment.

The column was packed with substrate using a modified version of the NCSU porometer procedure (Fonteno, 2003). The sample column was joined to a 15-cm sealed-base column below and a 30-cm column above to create a 75-cm packing apparatus. This apparatus was loosely filled to the top with substrate and dropped 7 times from a height of 17.5 cm to achieve a uniform Db in the middle column. Top and bottom sections of the packing column were removed and the substrate surface at each end was leveled, covered with a circular window screen to minimize sediment loss and capped. Once packed, the column was placed such that the inlet was on the lower end of the column allowing the substrate to be saturated from below with DI water. Saturation was paused for 5 min at the 10 and 20 cm heights to allow for moisture equilibration. The column was then returned to an upright position (inlet at the top) and steady state flow of DI water through the column was established minutes based on the difference in height (z) between the column outflow valve and the base of the mariotte bottle air inlet tube (Δz = 54.45 cm). Prior to initiating the experiment, the steady state flow rate of DI water was determined to be 4.6 $ml \cdot s^{-1}$, SD = 1.5 and effluent (drainage) was confirmed to have no residual salts from the pore water or bark using a HI 9813-6 pH/EC meter (Hanna Instruments, Woonsocket, Rhode Island). Next, a two-step breakthrough experiment was initiated by infiltrating two pore volumes (PV = volume of water held in the pore spaces = 1.04 L) of fertilizer solution into the DI saturated column (Step 1) after which the influent was returned to DI water (Step 2) until the effluent was free of any residual fertilizer solution (EC = 0). The final flow rate was determined to be 4.4 $ml \cdot s^{-1}$, SD = 1.5.

A total of 38 samples were collected in 150-ml increments for each of three repetitions and analyzed for EC. Mean and standard error of EC were calculated and are reported graphically in Figure 2 as the relative EC (C/C_i), where C = actual EC for a given sample and C_i = EC of the input solution. In addition, theoretical piston flow was calculated based on the porosity of the substrate and column dimensions (Skaggs, 2002). Graphs in Figure 2 are breakthrough curves (BTC) overlaid with the theoretical piston flow model.

RESULTS AND DISCUSSION

The BTC in Figure 2 illustrates the dramatic difference in the observed solute (i.e., fertilizer) transport from the piston flow model. Fertilizer solution in Step 1 quickly begins to leach at 0.4 PV demonstrating the rapid movement of water through the column before filling all the pores. Effluent EC was 80% of the fertilizer solution at the first pore exchange and did not reach full concentration until 2 PV. Step 2 produced similar results with the infiltrating DI water reaching the outlet at 0.3 PV. Effluent EC was 10% of the fertilizer solution at 1 PV and did not fully diminish until 1.6 PV.

To understand the BTC deviations from piston flow it is first necessary to understand that in a conceptualized piston flow model, a sharp boundary (commonly called a “front”) exists between the input solution and the solution already in the pore spaces. As the input solution is delivered and effluent is simultaneously drained, the front moves toward the outlet like a piston through a cylinder. The arrival of the front at the end of the cylinder is reflected by a sudden jump in relative effluent concentration from either $C/C_i = 0$ to 1 in Step 1 or $C/C_i = 1$ to 0 in Step 2. This should occur upon the displacement of 1 PV.

It is well known that water transport is affected by pore/channel size and the degree to which they are interconnected (Ma, 1997). Substrates with an array of particle sizes contain an array of pore/channel sizes where water can more easily flow through large pores and arrive ahead of the conceptual piston. Conversely, smaller pores and channels retard the flow of water through allowing water to arrive after the piston. Chemical processes, such as the diffusion of nutrients in the presence of a concentration gradient, may also slow the progression of influent behind the piston. In Step 1, the nutrients from the relatively concentrated input solution diffuse into pore water and may take longer to fully displace, diffuse, and reach full concentration. Step 2 is similar, however the gradient is reversed. Furthermore, the effect of chemical interaction between the fertilizer solution and the bark should not be ignored. Though difficult to quantify using EC measurements, the exchange capacity of bark can interact with both cations (K, NH_4) and anions (PO_4) and retard their movement through the system.

Saturated conditions are rare in containerized nursery production systems. However, analysis of saturated solute transport is an easy tool to help understand some of the principles of water and fertilizer movement during irrigation. These results indicate that even though the physical properties of pine bark substrates may fall outside the spectrum of a typical soil, the fundamental tools used in soil science can be utilized to better understand soilless substrate production systems. Additionally, further research is warranted to explore the dynamic behavior of water and fertilizers during irrigation. The study of wetting front patterns and the transport of fertilizers through an unsaturated system has the potential to provide information that would lead to more informed management decisions in nurseries. Research herein will allow scientists and growers to better understand solute movement in highly porous, relatively inert substrates under the various water flow conditions observed in ornamental crop production.

Literature Cited

Adams, C., Frantz, J. and Bugbee, B. 2013. Macro and micronutrient release characteristics of three polymer-coated fertilizers: Theory and measurements. J. Plant Nutr. Soil Sci. 176:76-88.

Fonteno, W.C. and Harden, C.T. 2003. Procedures for determining physical properties of horticultural substrates using the NCSU porometer.

Hillel, D. 1998. Environmental Soil Physics. Academic Press, San Diego, California.

Ma, L. and Selim, H.M. 1997. Physical nonequilibrium modeling approaches to solute transport in soils. Adv. in Agron. 58:95-150.

Newman, J.P., Albano, J.P., Merhaut, D.J. and Blythe, E.K. 2006. Nutrient release from controlled-release fertilizers in a neutral-pH substrate in an outdoor environment: I. Leachate electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. HortSci. 41:1674-1682.

Skaggs, T.H. and Leij, F.J. 2002. Solute transport: theoretical background. In: J.H. Dane, and G.C. Topp (ed.), Methods of Soil Analysis. Soil Sci. Soc. of Am., Madison, Wisconsin.

White, P.J. 2012. Ion uptake mechanisms of individual cells and roots: short-distance transport. In: P. Marschner (ed.), Mineral Nutrition of Higher Plants. Academic Press, London.

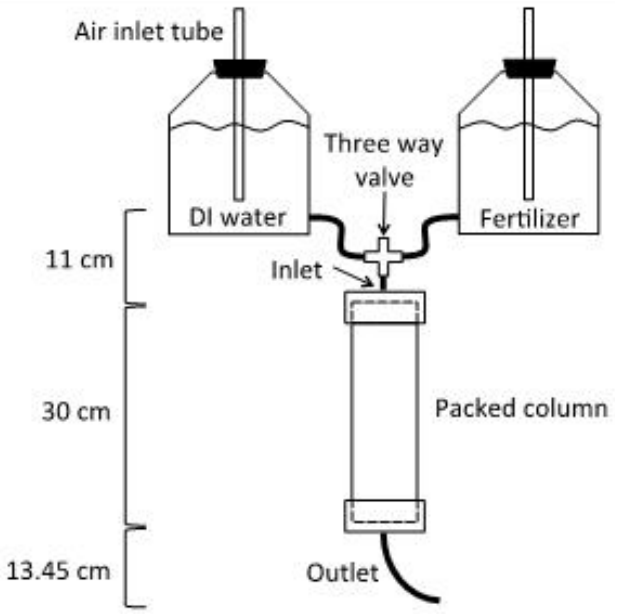

Fig. 1. Physical setup of the saturated column. Mariotte bottles allow for constant pressure through the system as the water level in the container decreases. A three-way ball valve allows for the seamless transition between DI water and fertilizer solution sources.

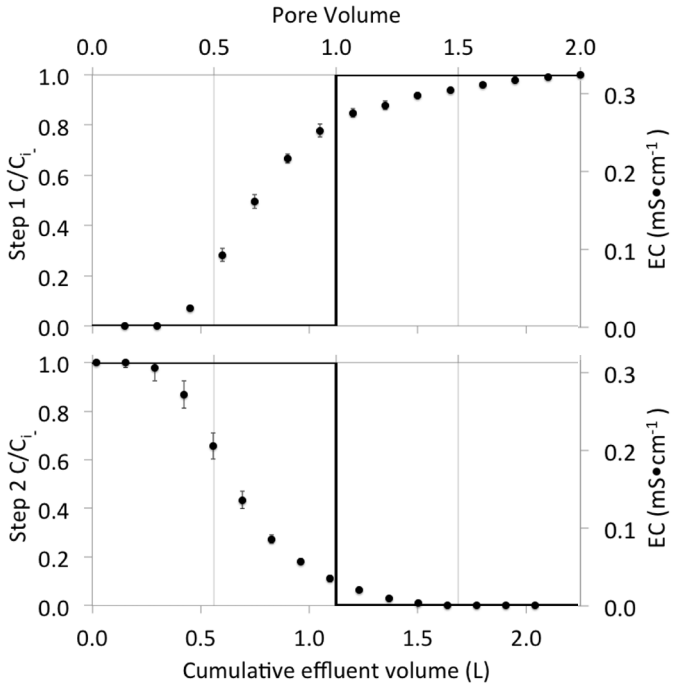

Fig. 2. Breakthrough curves showing the relative concentration (electrical conductivity) of the effluent as a fertilizer solution is infiltrated into a saturated column (Step 1) and as deionized water is infiltrated (Step 2) into the same column immediately after step 1. Solid line represents the piston flow model.

Classic City Garden Awards: Best New Plants from the Trial Garden at UGA©

John M. Ruter and Meg Green
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: ruter@uga.edu

INTRODUCTION

The Trial Gardens at the University of Georgia (UGA) were started in 1982 by Dr. Allan Armitage and Dr. Michael Dirr. Dr. John Ruter took over as Director of the Trial Gardens from co-founder Dr. Armitage in July of 2013. The mission of the UGA includes teaching, research, and new plant introductions. The UGA is an essential trialing site for heat and humidity tolerance for many of the world's breeding companies. During most summers there are 50-60 days reaching ≥32°C (90°F).

Teaching in the garden focuses on two classes taught by Dr. Ruter, HORT 3500 taught during the fall semester which focuses on annuals, vines, and fall-blooming perennials, and HORT 3510, taught in the spring which focuses on bulbs, spring ephemerals, and early-blooming perennials. Both classes are taught as half-semester courses. The Trial Gardens are also utilized by classes from Agricultural Communications, Entomology, Landscape Architecture, Plant Pathology, and other departments from the Arts & Sciences.

As for research and trialing, we work with over 20 of the major breeding companies from around the world. In 2013 we evaluated over 750 annual taxa in ground beds, hanging baskets, and containers. Trials also include numerous perennials and 180 landscape roses. Overall there are approximately 2,000 different taxa growing on less than 0.3 ha (0.75 acre). Several plants have been introduced to the trade via the Trail Gardens over the past 20 years (http://ugatrial.hort.uga.edu/). This tradition will continue in the future as Dr. Ruter releases plants from his breeding program at UGA.

TRIAL ESTABLISHMENT AND DATA COLLECTION

Seed of slower-growing crops such as begonias and geraniums are received and sown during the month of January. Most other seed crops are sown in February and March. Cuttings for propagation are generally received during the first 2 weeks of March. Propagules are shifted into 3.75-in. containers and grown in the greenhouse using recommended protocols. Substrate is a custom mix from OldCastle Lawn & Garden. Hanging baskets made from recycled newspaper (Western Pulp, Oregon) are also planted and grown-out during this time. In-ground trial beds are rototilled in March-April and appropriate soil amendments are added based on soil tests. In-ground beds are irrigated with drip irrigation placed on top of the bed that has been covered with pine straw mulch before planting. Pelargoniums and cold-tolerant annuals such as petunias and calibrachoas are placed out into the garden at the end of April, with all other plants going out during the month of May. Plants are liquid-fed during the growing season using several different fertilizer products.

Data collection begins in early June and is taken every 2 weeks until the end of September. All data is taken by Meg Green, Trial Garden supervisor. Having as single evaluator is essential for consistency of data collection. Data is entered into Excel on a tablet in the garden and is uploaded to our Trial Garden web site (http://ugatrial.hort.uga.edu/). Plants are rated on a scale of 1-5 (5 being best) on overall vigor, appearance, flower production, and disease and insect resistance. All this data can be found on the web site and is graphically tracked for each plant being evaluated. Graphing is important as it allows for viewing of performance over time and shows how the plant performed from early-summer until fall. Data is also shared with the National Trials database (www.planttrials.org).

During the 3rd week of June each year breeders and growers are invited to attend our Industry Wide Open House. Two weeks later we host a public open house. These events

allow industry professionals as well as the gardening public to see a variety plants all growing at the same location. During each event participants are asked to select five outstanding plants in the garden. This data is collected and shared on the web site and through email communications.

Every week 10-12 plants are selected as "Plants of Distinction" for their extraordinary performance in the garden. These plants are posted on the web site and are also emailed out to all interested parties. At the end of the season the "Classic City Awards" are given to the best 10 plants that had excellent performance all summer. The best cultivars for each genus are also listed under the "Best of the Best" link on the web site.

CLASSIS CITY AWARD WINNERS FOR 2013

***Acalypha* 'Inferno' – Peace Tree Farms**
Acalyphas have provided an incredible range of colors and textures to our garden for several years. Originally from Australia, this year's top eye-popping *Acalypha* has been 'Inferno,' sent to us by Lloyd Traven of Peace Tree Farms. Since receiving these plants in early April, the foliage of 'Inferno' has been a bright fiery red. It has continued to intensify in color throughout the heat and rain of our summer, becoming even more beautiful every day. Our plants were about 3' tall and never flowered.

***Angelonia* 'Balangsparkl', AngelMist™ Angelonia, Spreading Dark Purple – Ball FloraPlant**
Angelonia has become a favorite in our trial garden over the years, with many performing beautifully and some performing less well. AngelMist Spreading Dark Purple was sent by Ball FloraPlant as was AngelMist™ Spreading White. Both are worthy of high merit, but the purple cultivar edged out its white sister slightly. Both cultivars were planted in hanging baskets as well as in the ground and thrived in each location. AngelMist™ Spreading Dark Purple has been a profuse bloomer for the entire summer. It never had any downtime, remaining a tight mound of dark purple beauty all summer.

***Caladium* 'Summer Breeze' – Classic Caladiums**
Caladiums have saved the shady areas of our garden where few plants grow, much less thrive. Practically every visitor to our garden went wild for our caladiums and most of the cultivars earned our "Plants of Distinction" honors throughout the summer. 'Summer Breeze' outshone its siblings as they produced more and more leaves that were a beautiful clean white with rosy red veins. These plants thrived in early sun as well as in deep shade.

***Calibrachoa* Lindura®, 'Light Blue' – Oro**
Out of the over 80 cultivars of calibrachoas in our trial garden this summer, Lindura® 'Light Blue' has been the best cultivar of all. It remained covered in lovely truly light blue flowers for the entire summer. We have had this cultivar for a few years, during which time, it has always performed superbly. This award is a bit over do for Lindura® 'Light Blue', but at last it is receiving its well-deserved respect.

***Euphorbia* 'Star Dust Super Flash' – Red Fox**
The euphorbias we have trialed in the past few years have all been completely maintenance free, beautiful, profuse bloomers. *Euphorbia* 'Star Dust Super Flash' is no exception. After establishing itself in a container, this euphorbia produced endless flowers. Its habit is carefree as it spills its flowers over the edges of containers. As temperature dropped in the fall this cultivar went into overdrive producing more flowers than leaves that resulted in a spectacular floral display.

***Gomphrena* 'Las Vegas Purple' – Benary**
The Las Vegas series of gomphrena has impressed us at UGA for several years. In particular, 'Las Vegas Purple' has really made a bold statement in our trials this summer.

Its large gumball sized flowers dot the plants similar to holiday decorative lights. The dark purple flowers contrast pleasantly with the clean green leaves. As summer warmed, these purple balls multiplied and enlarged. 'Las Vegas Purple' resisted any potential diseases and remained upright throughout our frequent summer rains. It flowered well into the fall.

***Hibiscus* 'Panama Red' PP20,121 – UGA**
Hibiscus 'Panama Red' is an ornamental cultivar developed at The University of Georgia by Dr. John Ruter. The plants display an intense red color in high light, deeply cut foliage, stable foliage color, very large purple flowers, thrives in hot and humid conditions and flowers heavily during short days (November to April) in Zone 10. 'Panama Red' can be grown in the garden or in a container. Plants of 'Panama Red' tend to stay more upright and bushy compared to other cultivars. Some trimming may be necessary if this plant is grown in a crowded location.

Impatiens New Guinea Group (Sun): SunPatiens®, 'Compact Hot Coral' PPAF – Sakata
Early in their development, New Guinea Impatiens bred to be grown in full sun was an anomaly. Now, these beauties growing in the bright Georgia sunshine thrive beyond imagination as the summer's heat attempts to bake them. Month after month, 'Compact Hot Coral' from Sakata has exploded in large brilliant deep coral blooms. These plants were indeed compact and never lodged after any rain shower.

Impatiens New Guinea Group (Sun): Sun Harmony®, 'Salmon' – Danziger
Sun Harmony® 'Salmon' from Danziger has thrived all summer in our unusually wet and not quite blistering hot weather of 2013. These plants were sturdy and covered in large salmon New Guinea Impatiens flowers. Look out SunPatiens®! You now have a viable competitor. Congratulations to Danziger for broadening as well as brightening the field of New Guineas for the sun.

***Petunia Surfinia*® Summer Double™ petunia Rose – Suntory**
This petunia was practically perfect for the entire summer. Elegant, double rose colored clusters of blooms billowed from its container continuously for months. The foliage of 'Summer Double Rose' remained attractive even through our rainy summer, resisting disease and thriving in the summer heat. This cultivar far out lived other petunias and remained a winner into the fall.

***Solenostemon scutellarioides* Stained Glassworks™, 'Luminesce' – Ecke**
Coleus has become a mainstay in many sun gardens, including our trial garden. So many cultivars have been phenomenal especially the hotter the weather becomes. 'Luminesce' thrived, becoming more colorful as the sunshine intensified. Its compact habit enabled us to skip cutting the plants back mid-season as all others were and 'Luminesce' produced very few flowers. This cultivar was more maintenance free than all other coleus in our trials.

Breeding *Ruellia* and Trialing for Sterility at the University of Florida©

Rosanna Freyre
University of Florida/Institute of Food and Agricultural Sciences, Department of Environmental Horticulture, PO Box 110670, Gainesville, Florida 32611, USA
Email: rfreyre@ufl.edu

Sandra B. Wilson
University of Florida/Institute of Food and Agricultural Sciences, Environmental Horticulture Department, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, Florida 34945, USA

Gary W. Knox
University of Florida/Institute of Food and Agricultural Sciences, Environmental Horticulture Department, North Florida Research and Education Center, 155 Research Road, Quincy, Florida 32351, USA

INTRODUCTION

Ruellia is one of the largest genera in the Acanthaceae, consisting of approximately 250 species of perennial herbs, subshrubs, and shrubs, which are found mostly in tropical and subtropical areas. There are many accepted synonyms for *Ruellia simplex* (*R. brittoniana*, *R. coerulea*, and *R. tweediana*) with the name *R. simplex* being the first documented, therefore having taxonomic priority. *Ruellia simplex* ("Mexican petunia") is found in sunny areas on periodically inundated soils in Mexico, the Antilles, and southeastern South America (Ezcurra and Daniel, 2007). It was introduced to Florida sometime before 1940 (Hupp et al., 2009), and since then has become a very popular landscape plant in southern USA due to its high and continuous flowering and low maintenance requirements (Gilman, 1999). However, this introduced plant has escaped cultivation and become invasive in natural areas. For several years, 'Purple Showers' with tall habit and purple flowers was the only sterile commercial cultivar. Since 2007, the breeding objective at University of Florida (UF) has been to develop sterile cultivars with different flower colors such as pink, white, white with a purple corolla tube, and potentially different growth habits, such as, tall, semi-dwarf, and dwarf. Breeding approaches are ploidy manipulations and interspecific hybridizations.

MATERIALS AND METHODS

Breeding

Polyploidization experiments were performed at UF in Gainesville in 2008 using oryzalin on the apical meristem of seedlings as described by Jones et al., 2008. Seedlings were treated at either of two doses (25 or 50 μM) and three application frequencies (1, 2, or 3 times every 12 h) of oryzalin. Ploidy levels were determined on mature plants using flow cytometry as described (Czarnecki and Deng, 2009). The treatments of three applications of 25 or 50 μM oryzalin every 12 h were most successful in inducing polyploidy. A total of 15 tetraploid plants with different flower colors were obtained. Hybridizations were performed with plants of different ploidy levels, such as $4x \times 2x$ and $2x \times 4x$, aiming to obtain sterile triploid plants. $4x \times 4x$ crosses were also performed. A total of 495 *Ruellia* plants were obtained in 2010 and initially evaluated in the greenhouse for growth habit, flowering, and lack of fruit formation. Fifteen breeding lines and five controls were selected for the trials and propagated vegetatively.

Multi-Site Replicated Field Trials 2011

Plants were trialed in three simultaneous field experiments conducted at the North Florida Research and Education Center in Quincy, Florida (FL); at the Plant Science Research and Education Unit in Citra, Florida; and the Indian River Research and Education Center

in Ft. Pierce, FL (northwestern, north central, and southeastern Florida, respectively), as described by Freyre et al. (2012a).

The experimental design used was a randomized complete block with three blocks. Each plot consisted of three plants for each cultivar or breeding line, spaced 50 cm apart. Wild *R. simplex* (2*x*) and 'Purple Showers' (4*x*) were included as purple-flowered comparison lines, 'Chi Chi' (2*x*) as pink-flowered, 'Snow White' (4*x*) as white-flowered, and 'McKee' (2*x*) as white with purple corolla tube. Field rows were covered with black woven nursery ground cover and irrigation was supplied as needed with drip tapes at each site depending on the soil type and weather conditions.

Each plant was evaluated every 4 weeks, from May to October (24 weeks), for landscape performance with a scale from 1–5 where:

- 1 = very poor quality, not acceptable, severe leaf necrosis or chlorosis, poor form;
- 2 = poor quality, not acceptable, large areas of necrosis or chlorosis, poor form;
- 3 = acceptable quality, somewhat desirable form and color;
- 4 = very good quality, very acceptable and desirable color and form;
- 5 = excellent quality, perfect condition, premium color and form, peak landscape performance.

Flowering was rated on a 1-5 scale where:

- 1 = no flowers or buds;
- 2 = buds but no open flowers;
- 3 = 1-10 open flowers;
- 4 = 11-20 open flowers;
- 5 = more than 20 open flowers.

Fruiting was rated on a 1–5 scale where:

- 1 = more than 50 fruits;
- 2 = 21-50 fruits;
- 3 = 11-20 fruits;
- 4 = 1-10 fruits;
- 5 = no fruits.

Field Trials 2012

Fruits were collected at the three field locations in 2011 from open pollination of a selected pink-flowered plant, R10-105. Seed was germinated obtaining 148 progeny, which were then trialed in Citra in similar conditions as previously described.

Multi-Site Replicated Trials 2013

A total of 19 pink-flowered progeny were trialed in Citra using a randomized complete block design with three blocks and one plant per replication. Six plants of 'Chi Chi' or wild *R. simplex* were also included in each replication to ensure having fertile pollen donors for fruit formation. Field rows were covered with silver plastic and irrigation was supplied as needed with drip tape. Evaluations were conducted weekly using the rating system as previously described. This trial was also conducted in Fort Pierce using 14 progeny and one plant of 'Chi Chi' per replication, and evaluations were performed every 4 weeks. In addition, plants were also trialed in 11.4-L pots in a polyhouse in Gainesville, FL, which provided 25% shade. Four plants of 'Chi Chi' were included in each of the three blocks, and 16 plants of wild *R. simplex* were distributed evenly around the trial as pollen donors. Plants were fertigated manually and evaluations were performed weekly.

RESULTS AND DISCUSSION

Field Trials 2011

Average landscape performance for all lines was 3.6 for Fort Pierce, 3.5 for Citra, and 3.3 at Quincy. Three 4*x* plants with different flower colors were outstanding and better than their respective controls at all locations. White-flowered 10-108 and purple-flowered R10-102 had the best performance (4.4 and 4.3, respectively), and for pink-flowered

plants the semi-dwarf R10-105 was the best (4.0). Average flower ratings for all lines were 3.5 at Fort Pierce, 3.3 at Quincy, and 3.2 at Citra. R10-108 and R10-102 had high flowering (4.0 and 3.9, respectively), while R10-105 was medium (3.1). Average fruiting was highest in Fort Pierce and Quincy (4.0, respectively) and lower in Citra (4.2). Both R10-102 and R10-108 had no fruits, so their ratings were on average 5. R10-105 produced some fruits and its rating was on average 4.4.

The three selected breeding lines: purple-flowered R10-102, semi-dwarf pink R10-105, and white R10-108 were evaluated for female fertility by harvesting and germinating open pollinated fruits from the field, and by germinating seeds obtained from manual cross pollinations and self-pollinations in a greenhouse. Additionally, male fertility for each plant was determined by staining pollen grains with lactophenol cotton blue. It was estimated that R10-105 had 5% viable seeds per plant as compared to the invasive wild *R. simplex* and 6% as compared to female and male fertility than the existing commercial pink cultivar 'Chi Chi', and it was not approved for cultivar release by the UF/IFAS Invasive Plants Working Group. However, it was demonstrated that R10-102 and R10-108 are both female and male sterile. These lines were released as new cultivars 'Mayan Purple' and 'Mayan White', respectively, and were commercialized in 2013 (Freyre et al., 2012b).

Field Trials 2012

A total of 29 pink-flowered open pollinated progeny from R10-105 were selected for further trials based on performance and apparent low or no fruiting. These plants were propagated vegetatively and grown in a greenhouse in Gainesville. Nineteen plants were selected for 2013 field trials.

Field and Potted Plant Trials 2013

Data was averaged across dates and analyzed separately for each location. For Citra there were no significant differences between plants for plant quality or flower rating, however for fruiting there were significant differences between pink-flowered plants (ratings between 5 and 3.7) and 'Chi Chi' (2.7). In Fort Pierce the plant numbered R10-105-Q54 had the highest plant quality rating (4.6), while the other pink-flowered plants were significantly different (between 4.3 and 3.6) as was 'Chi Chi' (3.3). 'Chi Chi' had the highest flowering (4.0) while R10-105-Q54 was significantly different (3.0). R10-105-Q54 had no fruiting (5.0), while 'Chi Chi' was significantly different (3.1). Numerous pollinators were observed in the field so it is assumed that fruit formation was mostly due to open pollination. In the potted trials R10-105-Q54 had excellent quality and flowering (4.5 and 2.8) and 'Chi Chi' was not significantly different (4.1 and 3.9). In this trial no pollinators were observed so fruiting was due to selfing. 'Chi Chi' produced abundant fruits (3.4) while the other pink-flowered plants had no fruiting (5.0), with the exception of one plant that produced one fruit.

Overall, R10-105-Q54 was selected as the best performing pink-flowered plant in the trials that had the lowest fruiting. In Citra it was observed that this plant produced some fruits from open pollination (rating of 4.7) but they all seemed to abort prior to maturation. To confirm female fertility, 10 self-pollinations were performed in a greenhouse as well as 20 cross pollinations using either wild *R. simplex* or 'Chi Chi' as males. A few fruits were produced but they all aborted before maturation, with exception of one fruit which matured and dehisced naturally. This fruit contained 14 seeds but they did not germinate. Additionally, it was determined that R10-105-Q54 had only 10% pollen staining compared to wild *R. simplex* with 69%. Since it was demonstrated that R10-105-Q54 had extremely low to null fertility, it was approved for release as a new cultivar by the UF/IFAS Cultivar Release Committee and the Invasive Plants Working Group in October 2013. This line will be commercialized under the name 'Mayan Pink'.

ACKNOWLEDGEMENTS
The development and evaluation of *Ruellia* breeding lines was funded in part, by the USDA/Tropical and Subtropical Agriculture Research (TSTAR) program and the Florida Nursery, Growers and Landscape Association. We thank Adam Moseley, Madeline Bottenhorn, Kelly Ellison, Pat Frey, Keona Nolan, James H. Aldrich, and Brian Owens for their technical assistance; Dr. Zhanao Deng for performing spring field pre-trials in 2011; Terri A. Mellich for performing the flow cytometry ploidy determinations; and Jingsheng Huang for help with the statistical analyses.

Literature Cited

Czarnecki II, D.M. and Deng, Z. 2009. Occurrence of unreduced female gametes leads to sexual polyploidization in lantana. J. Am. Soc. Hort. Sci. 134:560-566.

Ezcurra, C. and Daniel, T.F. 2007. *Ruellia simplex*, an older and overlooked name for *Ruellia tweedinana* and *Ruellia coerulea* (Acanthaceae). Darwiniana 45:201-203.

Freyre, R., Moseley, A., Wilson, S.B. and Knox, G.W. 2012a. Breeding and evaluating for landscape performance and fruitlessness in Mexican petunia. HortScience 47:1245-1251.

Freyre, R., Moseley, A., Wilson, S.B. and Knox, G.W. 2012b. Fruitless *Ruellia simplex* R10-102 ('Mayan Purple') and R10-108 ('Mayan White'). HortScience 47:1808-1814.

Gilman, E.F. 1999. *Ruellia brittoniana*, Fact Sheet FPS-513. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, <http://edis.ifas.ufl.edu>.

Hupp, K.V.S., Fox, A.M., Wilson, S.B., Barnett, E.L. and Stocker, R.K. 2009. Natural area weeds: Mexican petunia (*Ruellia tweediana*). ENH1155, Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Jones, J.R., Ranney, T.G. and Eaker, T.A. 2008. A novel method for inducing polyploidy in *Rhododendron* seedlings. J. Amer. Rhododendron Soc. Summer 2008:130-135.

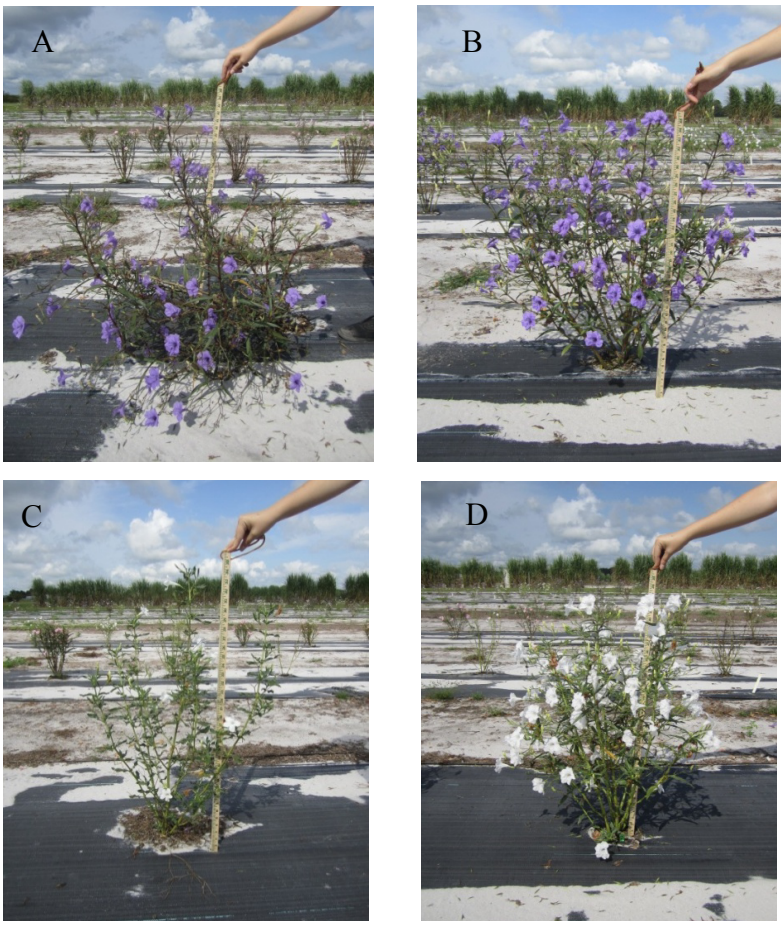

Fig. 1. 16-week old *Ruellia* plants in the field in north central Florida (Citra, FL) in 2011. *Ruellia* 'Purple Showers' (A), 'Mayan Purple' (B), 'Snow White' (C) and 'Mayan White' (D).

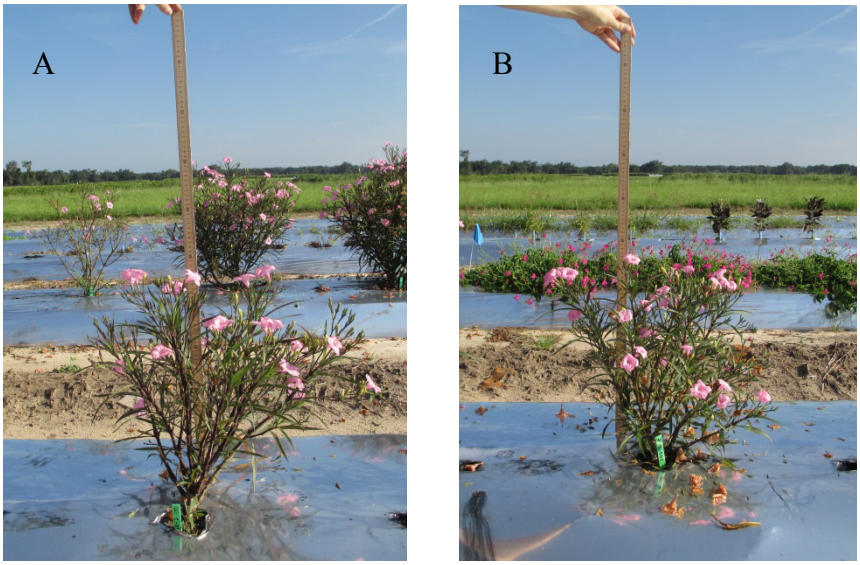

Fig. 2. Sixteen-week old *Ruellia* plants in the field in north central Florida (Citra, FL) in 2013. *Ruellia* 'Chi Chi' (A) and 'Mayan Pink' (B).

Plant Trials and Evaluations: Communicating Results to Consumers©

Allen Owings
Louisiana State University, AgCenter Hammond Research Station, 21549 Old Covington Hwy, Hammond, Louisiana 70403, USA
Email: aowings@agcenter.lsu.edu

INTRODUCTION

Home gardening consumers want new and improved ornamental plants for their landscape. One of the focus areas for ornamental horticulture over the years has been the development of new plant cultivars. New plants spark interest among retailers, landscapers, and especially consumers and provide diversity in the landscape. One of the challenges of trial gardens, plant companies introducing plants, retailers, nursery growers, and others, is developing innovative ways of providing a way to effectively communicate results showing "real world" production and landscape performance of these new cultivars to their customers and the gardening public.

UNIVERSITY TRIAL GARDEN PROGRAMS IN THE SOUTHEASTERN UNITED STATES

The geographical area of the Southern Region — International Plant Propagator's Society has a number of land grant universities conducting trial evaluations of new plant cultivars. At the same time, many also undertake the tasks of evaluation already existing genera, species, and cultivars. Typically, the evaluation of new herbaceous plants is ahead of the evaluation of new woody ornamental plants.

Test gardens in the southeastern United States include Texas A&M University in Overton; Stephen F. Austin State University in Nacogdoches, Texas; three locations at the University of Arkansas; Louisiana State University (LSU), AgCenter's Botanical Gardens at Burden in Baton Rouge and Hammond Research Station in Hammond; Mississippi State University efforts in Poplarville, Verona, Crystal Springs, and Starkville; University of Tennessee garden trials in Knoxville and Jackson; University of Georgia in Athens; University of Florida in Quincy; J.C. Raulston Arboretum at North Carolina State University in Raleigh; and more. Also, public gardens are now joining in plant trials and evaluations with the Dallas Arboretum being at the forefront of this effort.

LOUISIANA SUPER PLANT – PROMOTION AND MARKETING PROGRAM

Universities have plant promotion and marketing programs that attempt to provide plant trial results to the consumer horticulture audience. In addition, there are national programs, such as All-America Selections, that conduct similar efforts.

In Louisiana, the LSU AgCenter in cooperation with the Louisiana Department of Agriculture and Forestry re-initiated an ornamental plant marketing and promotion program in fall 2010. This new program is called Louisiana Super Plants. Funded has been provided from the Specialty Crop Competiveness State Block Grant. A previous program, called Louisiana Select, ran in Louisiana from 1996-2000.

The Louisiana Super Plants program has three parts. The first identifies outstanding plants. The second makes sure the plants are available at retail nurseries and garden centers. The third promotes the plants to Louisiana gardening consumer.

Each Super Plant must have at least two years of rigorous evaluations and have a proven track record under north and south Louisiana growing conditions. Louisiana Super Plants must prove hardy across the state. Louisiana Super Plants must be easily produced and available for all nursery and landscape industry wholesalers and retailers to market and sell.

Louisiana Super Plants are selected two years in advance of release. The Louisiana Super Plants selection committee composed of LSU AgCenter personnel selects plants based upon observations made in replicated plots and demonstration trials across the state.

The Louisiana Super Plants advisory committee, which is composed of nursery and

landscape industry personnel from across the state, meets with the plant selection committee for further scrutiny of the plant's landscape ability and marketability. This selection process gives each Super Plant the combined rating of university-tested and industry-approved.

To ensure that Louisiana Super Plant selections are available at retail nurseries and garden centers, the Louisiana Super Plants selection committee works closely with Louisiana wholesale growers so they produce plenty of the selected plants. At the same time, retail sellers are kept informed of the selections and are encouraged to carry them in their garden centers and nurseries. In addition, display signs containing plant photos and growing information are provided to nurseries and garden centers to help customers find and choose Louisiana Super Plants.

The first Louisiana Super Plants were promoted in the fall of 2010. Through 2013, twenty-two plants have been identified as Louisiana Super Plants with an additional five selected for 2014 promotion. More than 200 retail and grower operations have signed up to participate in the program. Surveys of participants were conducted to determine the impact the Louisiana Super Plants program had on sales. In the first season (fall 2010) of Louisiana Super Plants, one wholesale grower reported a 145% increase in sales of Amazon dianthus over the previous year. A retail garden center had a 1,920 percent increase in Camelot foxglove sales. Sales of the woody ornamental Shishi Gashira camellia were up by 45% at one wholesale grower.

A larger survey was conducted during the summer of 2012 after four marketing seasons. Retail and wholesale businesses participating in the Louisiana Super Plants program were contacted by email, and 15% responded. Of the participants who responded, 40% described their business as retail, 40% as wholesale, none as landscape design, and 20% as landscape installation and maintenance. Eighty percent of the respondents said the program had a positive effect on their business. Fifty percent of the respondents said sales or use of Super Plants in their business increased from 21% to 40% after the promotion began; the other 50% indicated increased sales of 20% or less.

The survey indicated that not only did the program increase sales of Louisiana Super Plants, but overall sales at a business also increased. More than 60% said the Super Plants program increased traffic flow or interest in their business. All the respondents indicated that the program increased overall sales in their business from 10% to 60%. Eighty-five percent of the respondents said the Louisiana Super Plants program had been beneficial to the nursery and landscape industry.

When asked to name the Super Plant that had the greatest impact on sales, one respondent wrote "no one plant, but an increase in general plant knowledge and interest."

In other states, programs that are similar to the Louisiana Super Plant program include the Texas Superstar program coordinated at Texas A&M University and the Mississippi Medallion program co-sponsored by the Mississippi State University Cooperative Extension Service, Mississippi Department of Agriculture, and Forestry and Mississippi Nursery and Landscape Association. Other states such as Florida (Plants of the Year, now Florida Garden Select), Georgia (Gold Medal Plant Program), and Virginia (Beautiful Gardens) participate in program efforts to marketing, promote and/or introduce new plants to consumers.

LOUISIANA STATE UNIVERSITY AGCENTER MASS COMMUNICATION AND SOCIAL MEDIA EFFORTS

How are universities conducting more timely communication of research results and improving industry, consumer outreach? Communication efforts have been initiated by the LSU AgCenter via Facebook, electronic newsletter updates, and trial garden reports.

Faculty at the LSU AgCenter's Hammond Research Station initiated a Facebook social media page in May 2011. The page is updated 3-5 times weekly with ornamental plant of the week postings, interactive discussion questions and posts pertaining to research and extension programs at the station and elsewhere at the LSU AgCenter. Nine hundred twenty people currently like the page and monthly active users number approximately

500. The page results in 2,000-3,000 contacts weekly. A separate Facebook page created by LSU AgCenter communications faculty in April 2010 for the LSU AgCenter has 5,800 friends (likes) with 500-1000 monthly active users and 5,000-7,500 contacts weekly.

The LSU AgCenter Hammond Research Station's ornamental horticulture electronic e-mail newsletter was initiated in July 2007 and distributes information weekly to 950 recipients through June 2013. The current format is twice monthly. Updates are formatted as PDFs. The e-news consist of 8-10 pages with 3-4 photos, weekly ornamental plant of the week, event calendars, links to landscape horticulture news articles and 2-3 ornamental horticulture timely topics.

The LSU AgCenter trial garden report from the Hammond Research Station is sent twice monthly via e-mail to 950 recipients. This media communication was initiated in Sept. 2011. Issues are formatted as one-page, three-columned, letter size PDFs and dated the first and fifteenth of each month. Two to three photos are included in each issue and along with 3-4 short plant trial related items. The original formatted page was 8.5 × 11 in. A new expanded format started recently (8.5×14 in.). The LSU AgCenter ornamental horticulture e-news updates and trial garden reports are archived on the Louisiana Nursery and Landscape Association website (www.lnla.org).These mass media efforts resulted in 436,000 primary clientele contacts in 2012.

NATIONAL PROGRAMS FOR PLANT PROMOTION

Several independent, non-profit groups are involved in plant promotions. These include All-America Selections, National Garden Bureau, Perennial Plant Association, Herb Society of America, and the American Garden Rose Selections.

The All-America Selections mission is "to promote new garden varieties with superior garden performance judged in impartial trials in North America." The group desires to test new, unsold plants of flowers, bedding plants, and vegetables. Other goals are to inform gardens of AAS winners and to earn gardener's trust in AAS winners. A tagline is "tested nationally and proven locally". There are almost 200 gardens in 55 locations across the country testing potential AAS winners. All-America Selections actively participates in the American Garden Award (AGA) program with the National Garden Bureau.

The National Garden Bureau (NGB) started in 1920. Their mission is "to improve the quality of life and the environment through increased use of seeds and plants". Their purpose is "to educate, to inspire and motivate people, to increase their use of plants in homes, gardens, and workplaces by being the marketing arm of the gardening industry". Efforts of the NGB include the previously mentioned AGA program, "year of the" annually for an edible, annual and perennial, and new variety announcement listings each year.

Roses have been traditionally been promoted to gardening consumer via the All-America Rose Selections (AARS) program. This program started in 1938 and was dedicated to the introduction and promotion of exceptional roses. Sadly, AARS is being dissolved. A new program for evaluating and promoting roses has launched and is called the American Garden Rose Selections (AGRS). Twelve gardens initiated trials in 2013 with the first AGRS winner(s) being announced in 2017. Regional winners will be named in this program in addition to fragrant roses. A program motto is "bringing you great roses you can grow where you live".

The Perennial Plant Association has a plant program called "Perennial Plant of the Year" and the Herb Society of America has a program called "Herb of the Year". The All-America Daylily program has named 20 winning cultivars in previous years. Another program is the "Rhododendron of the Year" from the American Rhododendron Society.

A new initiative with potential is the National Plant Trials Database (NPTD). This program stated in 2011 and is an on-line resource to serve as a central repository for trial data of the plants from the breeders who choose to participate. The trials grounds that choose to participate agree to adopt a standardized trialing protocol and a minimum set of standardized scoring procedures. At the end of the trialing season, each trial manager

updates this database with their scores and other trial data. Currently, only participating companies and cooperators have access to the data but this may be a public way to disseminate trial garden data in the future. There are 10 breeding company supporting sponsors thus far with 30 trial gardens participating.

And, another new program launched over the past 2 years is the Plant Something campaign. So far, there are 11 states with Arkansas, Virginia, and North Carolina in the IPPS-Southern Region area participating. The program has a commercial that "imagines live without plants" and also promotes the "perks of plants". The group also promotes "growth investments" (boost resale value of home with landscaping), "shady deal" (reduction of energy consumption using plants and trees), local color (neighborhood satisfaction is tied to landscaping) and greener is cleaner (trees removing carbon dioxide).

USEFUL WEBSITES FOR MORE INFORMATION

- Louisiana Super Plants - <www.lsuagcenter.com/superplants>
- Texas Superstars - <www.texassuperstar.com>
- All-America Selections - <www.all-americaselections.org>
- American Garden Rose Selections - <www.americangardenroseselections.com>
- National Garden Bureau - <www.ngb.org>
- National Plant Trials Database - <www.planttrials.org>
- Plant Something - <www.plant-something.org>

Growing Native Azaleas from Seed©

J.P. Jackson and Lindy Johnson
Appalachian Native Plants Inc, PO Box 736 Mountain City, Tennessee 37683, USA
Email: appalnativeplants@gmail.com

INTRODUCTION

Appalachian Native Plants Inc. is a 501(c)(3) organization dedicated to preserving and propagating native azaleas and rhododendron from seed. We are located near Mountain City in the Blue Ridge Mountains of north east Tennessee. The U.S.D.A. Plant Hardiness Zone is 6A.

In practice there are many different native azalea seed propagation methods that yield relatively successful results. One of our goals is to produce healthy, fully rooted 50 cell plugs from seed in 6 to 8 months. Integrated Pest Management (IPM) methods are used through out our plant production.

Growing azaleas and rhododendron from seed is an old topic which has been presented several times to International Plant Propagators Society meetings. Our plant mentor, Zophar Warner, gave a presentation titled "Azaleas from Seed" at the Forth Annual Plant Propagators Society meeting on 4 Dec. 1954 in Cleveland, Ohio.

The following is a quote from Mr. Warner's presentation: "Now, if I seem to be going a little bit too much into detail, the people who know how to do this aren't going to change their method anyhow and I am sure the people who don't know can have success by using this method."

PROPAGATION OF NATIVE AZALEAS AND RHODODENDRON FROM SEED

It is very important to know your seed source. The seed parents are chosen based on characteristics of flower, foliage, structure, and health. We predominately grow open pollinated seed. Open pollinated seed appeals to us because there is variability and sometimes an exceptional plant comes through a seedling population. We believe that genetic variability is good for plant populations.

In our mountain region seed collecting begins in October but many friends send us seeds that are collected earlier in the year from plant populations further south. The seed pods are dried in open pans. They should not be crushed, cutting or breaking them in half yields cleaner seed and fewer problems.

Cleaned seed is sown from December through January. Some species require longer growing times in order to finish a plug in one season. No pretreatment stratification is required prior to sowing. The seed is sown by hand directly onto 25×50×5 cm (10×20×2 in.) community trays filled to a depth of 4.4 cm (1.8 in.) with "Growers" grade milled sphagnum peat moss. The medium should be moist but not be too wet (soggy). After sowing we spray the seed and media surface with fungicide to prevent "damping off" problems. Germination generally takes place in 9 to 14 days after sowing. Clear plastic domes are used to cover the trays, creating a high humidity environment. The trays are placed on tables covered with propagation mats set at 21°C (70°F). For illumination, 2.4 m (8 ft) long cool white shop lights are hung six inches above the media surface and are illuminated continuously. After the seedlings have formed a second set of leaves, a liquid fertilizer [21-7-7 (21N-3.0P-5.8K) with minors] is mixed at 75 ppm and applied every 10 days as a foliar spray.

In early to mid-March the seedling trays are moved to the greenhouse propagation tables and hardened off for 2 weeks prior to transplanting into 50-cell propagation trays. These propagation tables have bottom heat provided by hot water circulated through ½-in. pex pipe embedded in perlite. The water is heated with three solar hot water panels and a 40-gal electric hot-water heater. The greenhouse has an air based geo-thermal system which prevents freezing temperatures and cools the greenhouse during the day. This allows for significant savings in fuel costs. There has been no propane consumed during

the last four growing seasons. The geo-thermal system buffers greenhouse temperatures. In mid-April the greenhouse is covered with 30 or 40% shade cloth.

This group of plants is classified "woody ornamentals" although the first 6 to 8 months of growth they are herbaceous. It was suggested that we treat the seedlings as herbaceous plants while they are in the greenhouse. A more "preventive" growing protocol for the greenhouse phase of production was adapted and has yielded significant improvements.

A commercially prepared medium consisting of peat, pine bark, and perlite is used to fill the plug trays. The peat-based medium is a vector for pests and pathogens. Prior to transplanting the media is drenched with fungicides designed to prevent *Phytophthora* spp. and *Rhyzoctonia solani*. The trays are then allowed to rest for 2 or 3 days prior to transplanting. It is very important that the seedlings are planted in the plug tray at the same depth as they were growing in the community tray. Planting too deep causes a slow growth response and from our experience can kill the seedling in a few weeks. At the time of transplanting the seedlings are sprayed with an adjuvant mixed with a broad spectrum foliar fungicide. This treatment reduces transplant shock and disease pressures. This is one of the preventative measures adopted after talking with our friend that grows bedding plants. All transplanting from the community trays to plug trays should be completed by May 1st. After the transplanting is complete the plug trays are drenched with a product containing *Streptomyces lydicus* (strain WYEC 108) and a neonicotinoid class insecticide. Other insecticides are applied as needed. We apply alternating fungicide drenches every 2 weeks for the first 6 weeks. In the greenhouse liquid fertilizers are used as "constant feed" at a rate of 75 ppm. The following fertilizers are alternated every 10 days:

- 21-7-7 (21N-3.0P-5.8K) acid special with minors 21-7-7 (21N-3.0P-5.8K)
- 20-20-20 (20N-8.7P-16.7K) with minors
- 4-18-38 (4N-7.7P-31.9K) hydroponic tomato fertilizer

Beginning in July the plug trays are moved from the greenhouse to cold frames covered with 30% shade cloth. Fertilization and IPM continues in the shade houses. In late November the cold frames are covered with 3-mil white poly for over wintering. No supplemental heat is used through the winter.

The following April and May plugs that are not sold are transplanted into 11-cm (4.5-in.) square containers. The volume of these containers is ten times that of the plugs root mass. We found that an overly large container slows plant growth.

The containers are filled with composted pine bark fines and drenched with *Streptomyces lydicus* (strain WYEC 108). Slow release fertilizer with minors is applied at the low rate as a top dressing. These quarts finish by fall.

Also during April and May unsold finished quarts are transplanted into 3-gal squat pots filled with composted pine bark fines and top dressed with the same slow release fertilizer. On average it takes 18 months to finish a 3-gal azalea from a quart liner.

SUMMARY

These growing methods have improved plant growth and have reduced average production times.

Our opinion is that this success has been accomplished by using prophylactic measures in the herbaceous stage of growth, providing constant feed fertilization, Integrated Pest Management and proper timing of transplanting.

Methods of Propagation of New Selections at Nurseries Caroliniana©

Ted Stephens
Nurseries Caroliniana, Inc., 143 Mims Grove Church Rd., North Augusta, South Carolina 29860, USA
Email: nurcar1@nurcar.com

INTRODUCTION

Since Nurseries Caroliniana specializes in many new and unusual plant selections that are not typically on the market, many plants are acquired where little to no propagation information is available. One must determine whether methods used should be similar to other plants in the same genus or family or whether completely new procedures should be explored. Methods which are investigated are seed, cuttings, grafting, budding or layering.

ARDISIA CRENATA 'BENI KAJAKU'

Pink peacock coralberry is a striking cultivar of this species which was acquired in Japan that has brilliant burgundy and pink new growth with an added attraction of pink flowers and dark red berries in the fall. An attempt was made to root this cultivar by typical semi-hardwood to hardwood cutting methods under mist with 0% success. The original plants of this selection were allowed to flower and fruit. Berries were harvested and planted in January in 3-gal community pots with approximately 50 to 75 seed per pot using a pine-bark-based nursery growing medium. The containers were placed outside under light pine shade. In early April the seed began to germinate at close to 100%, and surprisingly all came true to type with deep burgundy leaves with not a single green leaf among them. The seedlings were then pricked out in the fall, planted in 4-in. plastic pots and placed in a cold frame.

ARDISIA CRISPA 'KOKKOU DARUMA'

This species is a much more cold-hardy species than the previous *A. crenata* and is hardy into U.S.D.A. Zone 7b. This particular cultivar has white borders to its leaves with extremely "wavy" margins and brilliant red berries in the fall follow small white flowers in spring. It was purchased in Japan as a grafted plant at a nursery specializing in *Ardisia* species and cultivars. As with the previous selection, an attempt was also made to root this selection with no success. It was thought that the unusual variegated and morphological characteristics of this cultivar would not come true from seed, but upon producing fruit, they were allowed to ripen and removed from the plants and planted in community pots similar to the previous selection. The seed germinated at a very high percentage but showed only green leaves, but late in the growing season, they began to display characteristics similar to its parent with white and wavy leaf margins. It has not been proven that second generation seedlings will produce seedlings as true to form.

LIRIOPE PLATYPHYLLA 'KOREAN GIANT'

This species of *Liriope* has extremely tall flower spikes to over 1.3 m (50 in) tall, but it is an exceedingly slow divider. Now that the demand is growing, and more product is needed, planting in raised beds gives a faster rate of division. It is a good seed producer and seed have been collected and planted with approximately 50% germination when the testa is removed and planted on the soil surface so that the seed will be exposed to light. The seedlings grow quite slowly initially and may not be economically feasible since they may be crossed with other nearby species. This is not clear since they have not matured enough. Putting this selection in tissue culture might be a more feasible way to increase production and keep it true to form.

ROHDEA JAPONICA
Nippon lily is an exceptional dry shade landscape subject, which much like the previous selection, divides quite slowly. With many new variegated forms available, there is increased demand for these selections. Frequent division seems the most viable means of increase when done from early summer to late winter. The only time to avoid would be late winter to late spring when new foliage is being produced. Tissue culture has proven feasible for green-leaf forms and some vigorous variegated forms, but there is the danger of some reversions in tissue culture, so rigorous culling must be practiced.

***EUCOMIS COMOSA* 'SPARKLING BURGUNDY'**
Sparkling burgundy pineapple lily is in the lily family, *Liliaceae*, and is a bulbous perennial hardy from U.S.D.A. Zone 6-10. It will divide slowly on its own, but far too slowly for commercial production. Initially efforts to tissue culture this selection proved futile. It was found that leaf cuttings could be taken in June and the leaves could be sectioned into 5-7 cm (2-2.8 in.) sections cut horizontally across the fleshy leaf. Being careful to maintain the polarity of the leaf cuttings, they are stuck approximately 1.5 cm (0.6 in.) into un-amended nursery potting soil in a vertical to slightly angled position in plastic trays. These are left un-watered for 2 days to allow the cut portions to callous and then they are watered in and covered with a clear polymer tray-cover to maintain the humidity. The trays are then placed under high pine shade. They are checked every few days for moisture and if necessary the covers are removed and watered with a sprinkling can. Roots begin to emerge from the basal end of the cuttings in about 3 weeks, after which small bulbs will begin to form. The covers are removed when plantlets are about 2 cm (0.8 in.) in height. Trays are then moved to a cold frame which is kept just above freezing for the duration of the winter. Each leaf section will produce from 1 to 8 offsets. The following spring, when the plantlets are approximately 7-8 cm (2.8-3.1 in.) in height, they are divided and potted in a nursery growing medium in 7.5-cm (3-in.) pots and when these become well established they are planted into trade gallons. Thereafter, the plants are grown outside with no protection. *Heloniopsis orientalis* is a closely related species native to Japan which can be produced by this same method, but its leaves are bent into an inverted "U" with both the basal end as well as the apical end inserted into the rooting medium. Propagules will then form at both ends of the leaf cuttings.

***PONCIRUS TRIFOLIATA* 'SNOW DRAGON'**
This variegated contorted form of hardy citrus is most attractive particularly in spring and early summer when it is producing new growth. It is also extremely cold hardy even when grown in U.S.D.A. Zone 6. When most new growth appears, it is practically devoid of chlorophyll in its leaves and stems, but as the growth matures, the leaves will tend to get slightly greener and its stems will slowly turn green also. This plant will root under mist from summer cuttings, but the growth rate is quite slow on its own roots. But when grafted with a simple cleft graft or side veneer graft in February and March with a scion of 2-3 buds, a saleable plant can be produced in one growing season. *Poncirus trifoliata* 'Flying Dragon' seedlings planted in 1 quart pots are used as under-stocks. Grafts are covered with clear plastic cups until new growth is observed.

***WISTERIA FLORIBUNDA* 'NISHIKI'**
Japanese wisterias are usually sold for their extremely long and elegant flowering racemes with colors ranging from purple to lavender to pink and one double flowering cultivar. There are also several variegated foliage types. One is 'Mon Nishiki' with a heavily speckled gold variegation, and another is 'Nishiki' which actually appears to have green speckling over a white background. The former cultivar roots well with semi-hardwood cuttings under mist. But 'Nishiki' has so little chlorophyll that it is almost impossible to root from cuttings, but it is easily grafted with a side veneer graft using seedling *W. sinensis* as the under-stock in February or early March. By this method, one can produce a 3 gallon saleable plant in one growing season.

DIOSPYROS RHOMBIFOLIA
This species of Chinese persimmon is quite popular in Japan as a bonsai subject because its fruit is in scale for a bonsai specimen. There is some dispute as to whether it is *D. rhombifolia* or *D. cathayensis*, with the latter being more evergreen. Some authorities say that they are one and the same. This selection has bright red fruit. Even though this species is dioecious, it was assured by Japanese nurserymen that this cultivar produces fruit without pollination, possibly by means of parthenocarpy. Most of the larger edible types of persimmon are grafted, but initially cuttings of this selection were taken in late spring using semi-hardwood cuttings, wounding and treating with a 10-sec dip of a 10:1 dilution of Dip'N Grow liquid rooting hormone. Initially there was little activity, but after about 8 weeks, 90% rooting occurred with some initiating new growth upon rooting.

***ERIOBOTRYA JAPONICA* 'DOKA'**
This variegated loquat is quite popular in Japan with its white margins, and most of the plants found there are grafted using a side veneer graft. Since green leaf cultivars of this species root quite well with firm wood on bottom heat in late fall and winter, initially this was tried with 'Doka' with poor results. Hence, an attempt to air layer this plant using sphagnum moss as a substrate, and a commercially available Press'n Seal™ food wrap was used to hold the sphagnum in place. The transparent wrap gives good visibility to observe when roots are emerging. The stems were girdled using a pair of bird nail clippers for the space of approximately 2 cm. This was initiated in a cold frame in February and the rooted stems were removed in August. A problem was had with the cambium layer bridging the girdled portion on a number of the layers. Another method which produced good results was a whip in tongue approach graft using seedling loquats in 1-qt containers, attaching them to concrete re-enforcing wire encircling the parent plant. This method produced almost 100% success but it is very labor intensive. But when only small numbers are needed, it is adequate. Both the layers and the grafts were potted with excellent survival.

***YUCCA ALOIFOLIA* 'TRICOLOR'**
This is a rare form of variegation with this cold hardy species which has a broad central yellow band leaf with margins of green on either side. Since division is so slow, an attempt was made to root stem cuttings. The trunk of the plant was cut in sections of approximately 12.5 cm (5 in.) and allowed to dry for 2 days. Except for the terminal, the stems were stripped of all leaves and potted in a pine-bark-based nursery mix with approximately half of the stem cutting below the soil. The rooting medium was watered every 5 days. Roots began to emerge in approximately 3 weeks and then numerous buds began to emerge from around the stems. Upon growing these sprouts off, they are removed and rooted in a similar way and then grown off. If there would be a great demand for this cultivar, tissue culture would be an excellent method to produce larger numbers.

***OSMANTHUS FRAGRANS* CULTIVARS**
The cultivars of this species are our number one selling product at Nurseries Caroliniana. We are trying to acquire new cultivars of this on a regular basis, particularly deeper orange flowering forms and variegated leaf types. We have found that when acquiring new cultivars from Asia during fall and winter, we can grow these under extended day length to induce growth through periods of shorter days by using 100 W light bulbs spaced 2.5 m (8.2 ft) apart in the greenhouse. The ambient temperature will range from 2°C (35°F) to 30°C (86°F) during the winter months and this will result in almost continuous flushes of new growth with a bottom heat of 20°C (68°F). During this period cuttings can be taken when the stems are still green and just firm enough not to "flop." Dip'N Grow rooting hormone is used at a 10:1 dilution rate with a 10-s dip and stuck in 6.5-cm (2.5-in.) diameter plastic pots with mist and bottom heat set at 20°C (68°F). Under these conditions, root initiation can be observed in 15 days.

RHODOLEIA HENRYI

This is a relatively unknown species in the West in the witchhazel family, *Hamamelidaceae*; whereas, the species *R. championii* has been an infrequent occupant of gardens for years, mostly along the gulf coast and down into Florida, mainly in U.S.D.A. Zones 9-10. But *Flora of China* reports *R. henryi* is found at elevations up to 2450 m (8038 ft), which would make this a likely Zone 7 subject if not Zone 6b. The provenance of our selections are not known, but they have survived outside in U.S.D.A. Zone 8 in containers with not protection down to -11°C (12°F) with no damage. It is a large shrub to small tree with red to pink flowers of 5-6 cm (2-3 in.) in diameter from mid-March to mid-April in Zone 8. Even temperatures as low as 7°C (20°F) did not damage its flowers when they were open. We experimented with rooting cuttings from fairly green wood in June and more mature wood in October, using a 10:1 dilution of Dip'N Grow rooting hormone and a 10-s dip placed under mist. Fall cuttings had bottom heat of 20°C (68°F) with extended day length of 18 h using 100 W bulbs spaced 2.5 m on center in the propagation house which was covered with white polyethylene. The younger cuttings rooted at 90%; whereas, the older wood cuttings rooted at 85%. The rooted cuttings were potted the following June in 2-qt pots and grew off quite well with some producing as much as 1 m of growth with little branching. The older wood liners grew off more vigorously than the softer wood cuttings. There were sparse flower buds set approximately 12 months after cuttings were taken, but when shifted to 2-gal containers, there was fairly heavy flower bud set 24 months from the time cuttings were taken.

Laser Tag: Intelligent Sprayers Change the Pest Management Game©

Amy Fulcher and Diana Cochran
University of Tennessee, Department of Plant Sciences, 2431 Joe Johnson Drive, Ellington Plant Science Building Room 252, Knoxville, Tennessee 37996, USA
Email: afulcher@utk.edu

Robin Rosetta
Oregon State University, North Willamette Research and Extension Center, 15210 NE Miley Rd., Aurora, Oregon 97002, USA

Randall Zondag
The Ohio State University/OARDC, 1680 Madison Ave., Wooster, Ohio 44691, USA

Heping Zhu
USDA-ARS Application Technology Research Unit, 1680 Madison Avenue, Wooster, Ohio 44691, USA

INTRODUCTION

Pests pose a substantial threat to the sale of nursery crops (LeBude et al., 2012) and increase the cost of producing ornamental crops. For example, losses due to plant disease in Georgia nurseries were estimated at $43.4 million in 2007 (Martinez, 2008). Application of pesticides, as part of an Integrated Pest Management (IPM) program, can serve an important role in decreasing plant mortality, maintaining plant quality to a market acceptable level, and complying with plant trade requirements (Cloyd, 2008). However, pesticide use by its very nature can pose a threat to human and ecosystem health. By refining pesticide applications, environmental and human risk can be reduced.

Air-assisted sprayers are conventionally used to apply pesticides to nursery crops. However, less than 30% of pesticide applications are intercepted by the intended nursery canopy (Zhu et al., 2006). Increasing spray application efficiency could improve worker safety by reducing active ingredient residue on plant surfaces and air contamination. Additionally, because of the increased efficiency, the tank would be refilled less frequently, reducing opportunities for the spray applicator to come into contact with concentrated pesticides during mixing. Increasing efficiency would not only reduce the total amount of active ingredients applied but also decrease the water footprint of each pesticide application, improving environmental quality.

To increase spray application efficiency, two variable-rate output spray systems that integrate plant characteristics in real time were developed for nursery applications: an air-assisted sprayer for wide species of nursery and fruit tree crops (Chen et al., 2012) and a hydraulic boom sprayer for young, narrow trees such as liners (Jeon and Zhu, 2012). Both sprayers are sensor-guided, employing a high-speed laser scanning sensor and ultrasonic sensor for the air-assisted and boom sprayers, respectively. The sensors detect the presence or absence of a plant, plant architecture, canopy volume, and tractor speed, while controllers manipulate the solenoids to produce variable-rate spray outputs based on plant characteristics and plant occurrence in real time. Sprayers were developed at the USDA-ARS Application Technology Research Unit in Wooster, Ohio.

SPRAYERS

Variable-Rate Air-Assisted Sprayer Performance

Spray consumptions between the intelligent sprayer, non-intelligent sprayer, and a conventional air-assisted sprayer in an orchard were compared at three different growth stages (Beginning to leaf, half foliage, and full foliage). Application rate for the conventional sprayer was 50 gpa, determined by a tree-row volume method.

Variable-Rate Hydraulic Boom Sprayer Performance
Tests were conducted to verify deposition uniformity inside tree canopies at different travel speeds. The test plot consisted of two rows of six tree species (*Acer rubrum* 'Franksred'; *Carpinus betulus*; *Malus sargentii*; *Prunus ×cistena*; *Acer ×freemanii* 'Jeffersred'; *Acer palmatum*). Tree species ranged in height from 0.8 to 2.5 m, and in caliper from 0.5 to 5.4 cm. The travel speeds for the test were 2, 3, 4, and 5 m/h. Spray deposition and coverage by the hydraulic boom sprayer were compared with 100 gpa constant-rate application and tree row volume estimated rate applications. Water sensitive papers were mounted inside canopies to measure the spray coverage, and a fluorescent tracer was mixed with water to quantify spray deposits.

Variable-Rate Hydraulic Boom Sprayer Pest Control
In Oregon, *Quercus rubra* liners were rated from 16 June to 30 Sept. 2011 to monitor aphid levels and compare control of aphids by variable-rate and constant rate applications with a modified vertical boom sprayer. One side of the sprayer contained the intelligent system and produced variable-rate output, while the other side of the sprayer remained a conventional boom sprayer to produce constant-rate output. *Acer platanoides* liners were also rated from 6 June to 30 Sept. 2011 to monitor powdery mildew and to compare control achieved by the two applications. For both experiments, five of the newest, fully expanded leaves were examined for each of the 20 trees per treatment.

Variable-Rate, Air-Assisted Sprayer Pest Control
Cornus florida trees grown in a nine-row block were used to compare powdery mildew control by a conventional air-assisted sprayer with the variable-rate air-assisted sprayer and to determine if tree position within the block affected powdery mildew control. Trees were sprayed with Daconil on 20 June and 19 July 2013. Powdery mildew infection level was evaluated on the date of fungicide application and weekly thereafter for three weeks.

RESULTS AND DISCUSSION

Variable-Rate Air-Assisted Sprayer Performance
Pesticide consumption was dramatically reduced with the variable-rate intelligent sprayer. Spray application rate and percent spray volume reduction by the intelligent air-assisted sprayer at three growth stages are shown in Figure 1. The intelligent sprayer had 70, 66, and 52% spray mixture reduction at the beginning to leaf, half foliage and full foliage growth stages, respectively. Intelligent sprayer coverage and deposition were more stable over different growth stages at approximately 40% coverage compared to approximately 45-90% saturated coverage for the non-intelligent and a conventional air-assisted sprayer (data not shown).

Variable-Rate Hydraulic Boom Sprayer Performance
Spray deposit and coverage were relatively uniform regardless of changes in the canopy size, plant morphology, and travel speed (data not shown). Conventional spray application rates estimated with the tree-row volume method were 131, 60, 40, 36, and 28 gpa, compared with variable-rates of 38, 32, 25, 16, and 16, respectively. The variable-rate sprayer reduced spray volume up to 86.4 and 70.8% compared to a constant 100 gpa and tree-row volume estimated rate applications, respectively.

Variable-Rate Hydraulic Boom Sprayer Pest Control
Following the insecticide application, aphid populations decreased with no significant difference due to sprayer type until 30 Sept. 2011 when the plants sprayed with the intelligent sprayer had a lower aphid population (Table 1). Once fungicide applications commenced, powdery mildew ratings were not different or infection was lower for plants sprayed with the intelligent sprayer on all dates but one (Table 2).

Variable-Rate Air-Assisted Sprayer Pest Control

Powdery mildew infection was not different at the beginning of the experiment (Week 1) and was not affected by sprayer type on six out of seven dates thereafter (Table 3). Trees in rows within the interior of the block did not have higher levels of infection than one or the other exterior row of trees (data not shown).

Field laboratory and nursery tests demonstrated that both variable-rate intelligent sprayers controlled spray outputs by continually matching canopy characteristics and consequently reduced off-target losses. In the pest control evaluation of the variable-rate, intelligent- sprayers with the conventional sprayers, insect and disease control was generally not affected by sprayer type. Thus, both intelligent sprayers have the potential to effectively control pests while drastically decreasing pesticide use and associated economic inputs, and potentially increase environmental quality and enhance worker safety.

ACKNOWLEDGMENTS

USDA SCRI award "Intelligent Spray Systems for Floral and Ornamental Nursery Crops"; Walker Nursery, Morrison, Tennessee; Willoway Nurseries, Inc., Avon, Ohio; Sunleaf Nursery, LLP, Madison, Ohio; Herman Losely & Son, Inc., Perry, Ohio; Klyn Nurseries, Inc., Perry, Ohio; Possum Run Greenhouse, Bellville, Ohio; Wearren & Son Nursery, Taylorsville, Kentucky; Green Ridge Tree Farm, Elizabethtown, Kentucky; J. Frank Schmidt & Son Co., Boring, Oregon; Hans Nelson & Sons Nursery, Inc., Boring, Oregon; Bailey Nurseries, Yamhill, Oregon.

Literature Cited

Chen, Y., Zhu, H. and Ozkan, H.E. 2012. Development of variable-rate sprayer with laser scanning sensor to synchronize spray outputs to tree structures. Transactions of the ASABE 55(3):773-781.

Cloyd, R. 2009. Pesticide use in ornamental plants: what are the benefits? Pest Mgt Sci. 65:345-350.

Jeon, H.Y. and Zhu, H. 2012. Development of variable-rate sprayer for nursery liner applications. Transactions of the ASABE 55(1):303-312.

LeBude, A., White, S., Fulcher, A., Frank, S., Klingeman, W., Chong, J.-H., Chappell, M., Windham, A., Braman, K., Hale, F., Dunwell, W., Williams-Woodward, J., Ivors, K., Adkins, C. and Neal, J. 2012. Assessing the integrated pest management practices of southeastern U.S. ornamental nursery operations. Pest Mgt. Sci. 68:1278-1288. DOI 10.1002/ps.3295.

Martinez, A. 2008. 2007 Georgia plant disease loss estimates. Univ. Georgia Coop. Ext. Serv. Pub. SB 41-20. 6 Feb. 2009.

Zhu, H., Derksen, R.C., Guler, H., Krause, C.R., Zondag, R.H. and Ozkan, H.E. 2006. Foliar deposition and off-target loss with different spray techniques in nursery applications. Transactions of the ASABE 49(2):325-334.

Table 1. Comparison of aphids on red oak trees sprayed with the variable-rate or conventional boom sprayer in a commercial nursery.

Date	Average number of aphids	
	Variable-rate	Conventional
6/16	0 a[y]	0 a
8/4	2.3 a	1.8 a
8/18	11.6 a	9.1 a
8/30	46.1 a	39.5 a
9/8[z]	0.6 a	0.4 a
9/15	0.4 a	0.1 a
9/30	0.3 b	3.4 a

[z]8 days following Diazinon insecticide application.
[y]Values in a row followed by the same letter are not significantly different at the 0.05 level.

Table 2. Comparison of powdery mildew infection on Norway maple trees sprayed with the variable-rate or conventional boom sprayer in a commercial nursery.

Date	Average disease rating	
	Variable-rate	Conventional
6/16	0.06 a[wv]	0.05 a
6/30	0.52 b	0.67 a
7/6[z]	0.79 a	0.84 a
7/14	0.99 a	1.00 a
7/26	1.01 a	1.08 a
8/1[y]	0.68 b	0.84 a
8/11	0.12 a	0.17 a
8/18[x]	0.56 a	0.47 a
8/25	0.83 a	0.61 b
9/30	1.10 b	1.70 a

[z], [y], [x]Five, six, and six days following Chlorothalonil 720 SFT (July 1, 2011), Eagle 20 EW (July 26, 2011), and 3336F (August 12, 2011) treatments, respectively.
[w]Values in the same row followed by the same letter are not significantly different at the 0.05 level.
[v]The following rating system was used: 0=no sign of powdery mildew, 1=1 to 25% powdery mildew, 2=26 to 50%, and 3=51 to 100%.

Table 3. Dogwood powdery mildew infection level following fungicide application with conventional and intelligent, air-assisted sprayers.

Sprayer type	Date							
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
Intelligent sprayer	1.8	2.4	3.2a[z]	4.4	4.9	3.9	6.3	6.3
Conventional sprayer	1.7	2.4	2.9b	3.7	3.7	3.1	5.6	5.4

Abbreviation: Wk = week.

[z]Values in the same column followed by different letters are different at the 0.05 level.

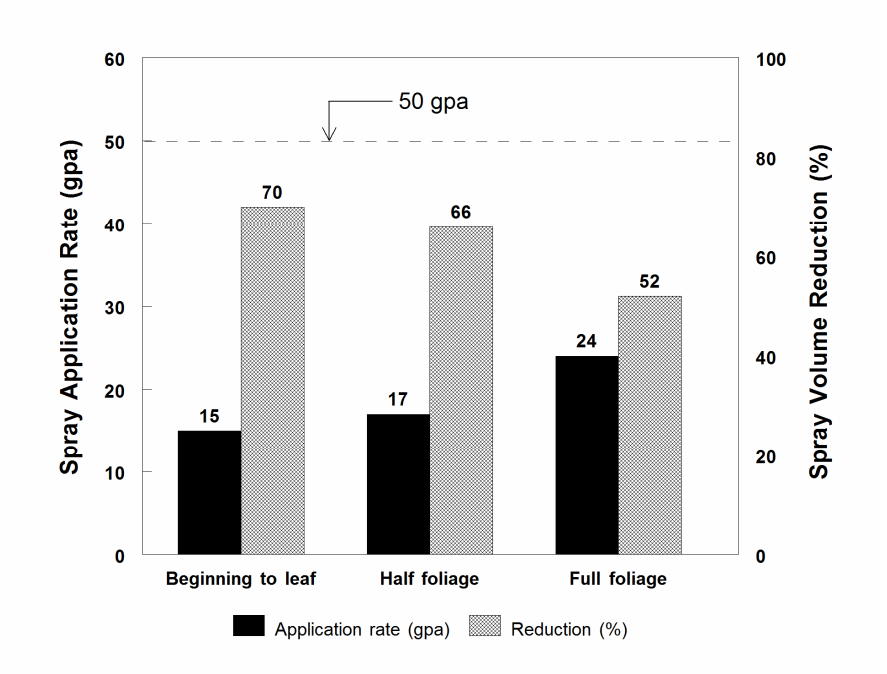

Fig. 1. Spray application rate and percent spray volume reduction from intelligent sprayer, compared with the conventional 50 gpa spray application rate at beginning to leaf, half foliage and full foliage stages.

Fungicide Resistance in *Pythium* and *Phytophthora* from Ornamentals in Georgia©

Jean L. Williams-Woodward and Max E. DeMott
Department of Plant Pathology, University of Georgia, Athens, Georgia 30602, USA
Email: jwoodwar@uga.edu

INTRODUCTION

The majority of root and crown diseases on ornamental crops are caused by oomycete pathogens, including species of *Pythium* and *Phytophthora*. Both Pythium and *Phytophthora* cause root, crown, stem, and foliage blights. Symptoms often include root softening, sloughing, darkening of roots, crowns and stems, wilting, foliage chlorosis, leaf drop, stem dieback, and leaf and petiole blighting.

Oomycete pathogens or "water molds" as they are commonly called, which also includes downy mildew causing pathogens, are unique and are not true fungi. They are more closely related to brown algae than fungi. One of the major differences between oomycetes and true fungi is in their cell wall components. Oomycete cell walls are composed of β-1,3 and β-1,6 glucans, whereas true fungi cell walls are composed of chitin. This is an important distinction because the mode of action of many fungicides is to act on and inhibit chitin cell wall biosynthesis. Since Oomycete cell walls do not contain chitin, these products have no activity on these pathogens. This has resulted in a limited number of commercially available fungicides with activity against *Pythium*, *Phytophthora*, and downy mildew diseases.

The predominant fungicide used against *Pythium* and *Phytophthora* diseases in ornamentals has been the phenylamide systemic fungicide, metalaxyl, which was replaced by mefenoxam (the R-enantiomer of metalaxyl), and marketed under the trade names of Subdue 2E and Subdue Maxx (Syngenta Crop Protection, Inc., Greensboro, North Carolina), respectively. Metalaxyl was registered for use in the United States of America in 1980 and within 4 years fungicide resistance in *Pythium* causing turf blight was identified (Sanders, 1984).

Mefenoxam fungicide resistance, or rather insensitivity, has been noted in several states in *Pythium* and *Phytophthora* species causing root and crown rots of ornamental plants. In Pennsylvania, 32.5% of the 120 *Pythium* isolates recovered from infected plants were insensitive to mefenoxam (Moorman et al., 2002). Eleven species of *Pythium* were identified from the 120 isolates. The most common species were *P. irregulare* and *P. aphanidermatum* of which 36.8 and 37.5% of these species, respectively, were insensitive to mefenoxam. In North Carolina, three species of *Phytophthora* (*P. nicotianae*, *P. cryptogea*, and *P. palmivora*) were recovered as the predominant species infecting floriculture crops (Hwang and Benson, 2005). Although, all isolates of *P. palmivora* were still sensitive to mefenoxam, 100% of the *P. cryptogea* and 21% of *P. nicotianae* isolates were insensitive. In a more recent North Carolina study, *P. nicotianae*, *P. drechsleri*, *P. cryptogea*, and *P. tropicalis* were the most commonly recovered *Phytophthora* species from floriculture corps, of which 66% of these *Phytophthora* isolates were insensitive or intermediate in resistance to mefenoxam (Olson and Benson, 2011).

These studies would suggest that mefenoxam insensitivity is widespread within floriculture production. However, another study involving multiple states in the southeastern USA concluded that across six states and 488 isolates that only 6% of the *Phytophthora* isolates were insensitive to mefenoxam (Olson et al., 2013). The viability of mefenoxam as a valuable tool in managing *Pythium* and *Phytophthora* root diseases is of great concern. The objectives of this study was (1) to identify species of *Pythium* and *Phytophthora* from symptomatic plants within both floriculture and woody ornamental crops in Georgia, and (2) to evaluate the recovered isolates for mefenoxam sensitivity.

MATERIALS AND METHODS

Isolate Collection

From 2010-2011, plant samples exhibiting symptoms of root or crown rot were collected from 17 wholesale ornamental production facilities (nine specializing in container-grown woody shrubs and eight specializing in floriculture or herbaceous crops). Discolored root and/or crown tissue were washed with tap water to remove rooting substrate, blotted dry, and placed on filter paper for selection and direct isolation. Symptomatic tissue sections were plated onto V8-PARP medium [15 g Bacto agar (Becton, Dickerson and Co., Sparks, Maryland); 50 ml clarified V-8 juice (Campbells, Camden, New Jersey); 67 mg 75% PCNB (Terraclor; Chemtura, Middlebury, Connecticut); 400 µl pimaracin (Sigma-Aldrich, St. Louis, Missouri); 250 mg ampicillin (Sigma-Aldrich, St. Louis, Missouri); 10 mg rifampicin (Sigma-Aldrich, St. Louis, Missouri) in 950 ml deionized water] (Jeffers and Martin, 1986). Plates were incubated in the dark at 22°C for up to 10 days. Putative *Pythium* and/or *Phytophthora* colonies were transferred onto new V8-PARP plates. After 24 to 72 h, actively growing colonies were then transferred by hyphal tip to new V8-PARP or non-amended V8-agar (15 g of Bacto agar; 100 ml of clarified V8 juice; 900 ml of deionized water) plates to obtain an axenic culture.

Morphological Identification

Isolates were grown on V8-agar for 72 h. Two agar plugs from the leading edge of the suspected *Pythium* and *Phytophthora* colony were transferred to a 35-mm plastic petri dish and flooded with non-sterile soil extract solution (NSES). Non-sterile soil extract solution was prepared by stirring 15 g of loamy field soil in 1 L of distilled water using a magnetic stirrer for at least 4 h and allowing the solution to settle overnight. The supernatant was decanted and centrifuged for 10 min at 8,000 rpm. If needed, the solution was vacuumed filtered to remove any debris left in the solution. Plates were then grown at 22-24°C for 24-72 h and examined for sporangia and morphological characteristics. Isolates resembling either *Pythium* or *Phytophthora* were then prepared for internal transcribed spacer (ITS) sequencing.

Identification with Internal Transcribed Spacer Sequencing

The ITS region (ITS1, 5.8S, and ITS2) of the rDNA of each isolate was sequenced for DNA-based identification. Suspected *Pythium* and *Phytophthora* isolates were grown on V8-agar at 22-24°C for 72 h. Hyphae was scraped and/or lightly touched with a 200-µl pipette tip. The tip was then placed into a 0.5-ml PCR tube containing a PuReTaq Ready-To-Go™ PCR Bead (GE Healthcare), 1 µl of 10 µM ITS-1 primer (5'-TCCGTAGGTG AACCTGCGG-3'), 1 µl of 10 µM ITS-2 primer (5'-GCTGCGTTCTTCATCGATGC-3'), and 23 µl of sterile nuclease-free water and mixed by gently pipetting up and down several times. Total PCR reaction volume was 25 µl. Thermal cycling conditions consisted of an initial denaturation at 94°C for 5 min; followed by 34 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and a final extension step of 72°C for 5 min, followed by a 4°C hold (Moorman et al., 2002). To improve sequencing results and for confirmatory testing, ITS-2 primer was replaced with ITS-4 (5'-TCCTCCGCTTA TTGATATGC-3') in the PCR reaction. Amplification products were confirmed with gel electrophoresis (1% molecular grade agarose; 100V for 55-60 min). PCR products were purified using QIAquick Purification Kit (Qiagen, Inc., Valencia, California) and submitted to the Georgia Genomics Facility (Athens, Georgia). Isolate DNA was stored and maintained at -20°C. DNA sequences were aligned and manually edited using Geneious software (Biomatters Ltd., Auckland, New Zealand). Internal transcribed spacer sequences were BLAST analyzed in GenBank (National Center for Biotechnology Information, Bethesda, Maryland) and the *Phytophthora* Database (http://www.phytophthoradb.org/).

Mefenoxam Sensitivity Assays

All isolates were screened for sensitivity to mefenoxam in vitro by amending V8-agar (50 ml clarified V8 juice; 15 g Bacto agar; 950 ml deionized water) with 100 μg a.i./ml of mefenoxam by suspending Subdue Maxx (Syngenta Crop Protection, Greensboro, North Carolina) in water and distributing it in molten agar prior to pouring into 35-mm plastic petri plates and evaluating mycelia growth compared to the growth of the same isolate on non-amended medium. Agar plugs (7 mm in diameter) were cut from the leading edge of a 3-4 day old isolate culture and inverted onto the center of mefenoxam-amended and non-amended plates. Two non-amended and two mefenoxam-amended plates for each isolate was incubated at 22°C in the dark for 24-48 h depending upon isolate growth rate. Plates were evaluated macroscopically. Mycelial growth was measured from the inoculated plug edge to the edge of the colony along two radii per plate. Isolates that grew ≥50% of the non-fungicide amended control plates (EC_{50} > 100 ug a.i./ml) were considered insensitive. Isolates that grew <50% as compared to the control were considered to be sensitive.

Isolates were further tested depending on their results from the initial screening. If the isolate was determined to be sensitive (growth <50%), it was further evaluated at tested at a concentration of 10 μg a.i./ml. If isolates were considered to be insensitive (growth ≥50%), then they were further evaluated at 500 and 1000 μg a.i./ml.

RESULTS AND DISCUSSION

Out of the 152 symptomatic samples collected, oomycete root pathogens were recovered from 80% of them. Either no pathogen or a non-oomycete pathogen was recovered from the remaining samples. Of the 121 oomycete isolates recovered, 39 were identified as *Phytophthora* spp., 77 as *Pythium* spp., and five as *Phytopythium* spp. The *Phytophthora* species identified included *P. nicotianae*, *P. pini*, *P. undulata*, *P. cinnamomi*, *P. citrophthora*, *P. palmivora*, *P. dreschsleri*, and *P. cryptogea*, with *P. nicotianae* being the most prevalent (30% of the *Phytophthora* isolates). Approximately, 21% of the *Phytophthora* isolates could not be identified to species based upon morphology or DNA sequencing. This is not uncommon and has been seen in previous studies (Hwang and Benson, 2005; Olson and Benson, 2011; Olsen et al., 2013).

Pythium species recovered included *Pythium irregulare*, *P. myriotylum*, *P. aphanidermatum*, *P. monospermum*, *P. chamaehyphon*, *P. vexans*, *P. diclinum*, *P. cucurbitacearum*, *P. zingiberis*, and *P. acanthophoron*. *Pythium irregulare* was the most prevalent and accounted for 12.5% of the identifiable species. The majority of the *Pythium* isolates recovered (approximately 50%) could not be identified to the species level.

Of note in this study is the identification of five *Phytopythium* isolates recovered from diverse symptomatic plants including *Coreopsis lanceolata*, *Hydrangea arborescens*, *Rosmarinus officinalis*, *Tagetes patula*, and *Thymus praecox* from three production facilities. *Phytopythium* is a relatively new taxonomic genus whose members were classified as clade K species of *Pythium*, and have more characteristics similar to *Phytophthora* than other *Pythium* species. *Pythium litorale* and *Pythium heliocoides* are now classified as *Phytopythium* species (Robideau et al., 2011) and both were recovered in this study. In recent studies, *Phytopythium heliocoides* was found to be pathogenic to begonia in Virgina (Yang, et al., 2013) and *P. litorale* was pathogenic to squash in Georgia (Parkunan and Ji, 2013).

Across all oomycete isolates in this study, 45.5% were mefenoxam insensitive. Insensitivity was identified in 27.2% of the *Phytophthora* isolates. All isolates *of P. undulata* and *P. palmivora* recovered from three production facilities and 57% of the unidentifiable *Phytophthora* spp. were insensitive to mefenoxam. However, all *P. nicotianae* and *P. pini* isolates, which accounted for over 50% of the total number of *Phytophthora* isolates recovered, were sensitive to mefenoxam.

Mefenoxam insensitivity was found in 58.4% of the *Pythium* isolates recovered. Approximately 28% of all *Pythium* isolates identified were *P. irregulare*, *P. myriotylum*,

and *P. aphanidermatum*. Of these species, only one of the 21 isolates (<5%) was insensitive to mefenoxam. Most of the insensitive isolates were uncommon or unidentifiable *Pythium* species. In addition, all of the *Pythopythium* isolates recovered were mefenoxam insensitive.

The seemingly high occurrence (45.5%) of mefenoxam-insensitive *Phytophthora*, *Pythium*, and *Phytopythium* isolates recovered in this study would suggest that the usefulness of mefenoxam to manage oomycete root diseases is questionable. However, many *Pythium* species are known to be saprobic. It is plausible that many of the unidentifiable *Pythium* isolates recovered, of which the majority were mefenoxam insensitive, are saprobic and not plant pathogenic. Until pathogenicity is proven, the high occurrence of mefenoxam insensitivity, particularly within *Pythium* and *Phytopythium* isolates, may be misleading.

Literature Cited

Hwang, J. and Benson, D.M. 2005. Identification, mefenoxam sensitivity, and compatibility type of *Phytophthora* spp. attacking floriculture crops in North Carolina. Plant Dis. 89:185-190.

Jeffers, S.N. and Martin, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.

Moorman, G.W., Kang, S., Geiser, D.M. and Kim, S.H. 2002. Identification and Characterization of *Pythium* Species Associated with Greenhouse Floral Crops in Pennsylvania. Plant Dis. 86:1227-1231.

Olson, H.A. and Benson, D.M. 2011. Characterization of *Phytophthora* spp. on floriculture crops in North Carolina. Plant Dis. 95:1013-1020.

Olson, H.A., Jeffers, S.N., Ivors, K., Steddom, K.C., Williams-Woodward, J.L., Mmbaga, M.T., Benson, D.M. and Hong, C.X. 2013. Diversity and mefenoxam sensitivity of *Phytophthora* spp. associated with the ornamental horticulture industry in the southeastern United States. Plant Dis. 97:86-92.

Parkunan, V. and Ji, P. 2013. Isolation of *Pythium litorale* from irrigation ponds used for vegetable production and its pathogenicity on squash. Can. J. Plant Pathol. 35(3):415-423.

Robideau, G.P., De Cock, A.W., Coffey, M.D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D.W., Désaulniers, N., Eggertson, Q.A., Gachon, C.M., Hu, C.H., Küpper, F.C., Rintoul, T.L., Sarhan, E., Verstappen, E.C., Zhang, Y., Bonants, P.J., Ristaino, J.B. and Lévesque, C.A. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol Ecol Resour. 11(6):1002-1011.

Sanders, P.L. 1984. Failure of metalaxyl to control Pythium blight on turfgrass in Pennsylvania. Plant Dis. 68:776-777.

Yang, X., Richardson, P.A., Olson, H.A. and Hong, C.X. 2013. Root and stem rot of begonia caused by *Phytopythium helicoides* in Virginia. Plant Dis. 97(10):1385.

Shrub Evaluation at Stephen F. Austin Gardens©

David Creech
SFA Gardens, Arthur Temple College of Forestry and Agriculture, PO Box 13000, Stephen F. Austin State University, Nacogdoches, Texas 75962, USA
Email: dcreech@sfasu.edu

INTRODUCTION

Stephen F. Austin (SFA) Gardens is a collector's garden, one that adds hundreds of new taxa each year to the plantings. Those that survive, perform well, and impress visitors make their way into propagation, promotion, and distribution. This program has introduced and promoted numerous plants through a wide range of print and electronic media, many of which have made an impact in the nursery industry, well been documented in past IPPS Proceedings.

STEPHEN F. AUSTIN GARDENS

Stephen F. Austin Gardens comprises 128 acre (58 ha) of on-campus property at Stephen F. Austin State University (SFA), Nacogdoches, Texas. Stephen F. Austin Gardens is the umbrella organization responsible for the activities, growth, and development of five gardens. Representing the oldest plantings, the 10-acre (4.5 ha) SFA Mast Arboretum was initiated in 1985 and includes the horticulture facility of the Agriculture Department. The Ruby M. Mize Azalea garden is an 8-acre (3.2 ha) garden of primarily azaleas, camellias, and Japanese maples that was dedicated in April, 2000. The 42-acre (19 ha) Pineywoods Native Plant Center (PNPC) was dedicated by Lady Bird Johnson in April 2000. The newest land resource, SFA's Recreational Trail and Gardens was dedicated in March 2010 and comprises 68-acre (31 ha) acres of mostly undisturbed forest. As the result of a donor with a vision, SFA Gardens is now home to the Gayla Mize Garden, a 8-acre (3.2 ha) spot in the SW portion of SFA's Recreational Trails and Gardens, which is directly across University Drive from the Ruby M. Mize Azalea Garden. This newest garden has allowed SFA Gardens to revisit shrub and small flowering tree evaluation in a big way. Stephen F. Austin Gardens enjoys four full time employees and two half-time employees, all funded by a combination of state and external grant funding.

Shrub evaluation at SFA Gardens is scattered across gardens and landscapes. In most cases, shrubs have been placed in what we hope is an appropriate environment. For the most part, the azaleas are under high pine canopy shade. Dry loving shrubs and small trees are placed in full sun, often on a mild berm to improve drainage. Not everything is perfect. In too many cases, shrubs brilliantly placed 20 years ago are now swamped by their neighbors. Many never reveal their potential, something we deal with by pruning, moving the plants to a better location, or just letting them languish. While the list of shrubs in the collection is huge, this paper focuses on those that have performed well and are already well known in the market place.

AZALEAS

Azaleas are major nursery and landscape shrubs in the South. Since the first plantings in Fall 1985, SFA Gardens has grown the collection to include over 8,000 azalea plants, which is comprised of more than 550 species and/or cultivars. That collection is documented (see website) and mapped. In the last decade, nothing has stirred the azalea industry more than the phenomenon of repeat blooming. While the major spring azalea bloom show at SFA Gardens is mid-March to mid-April, there have long been cultivars that bloom at other times of the year. Since the late 1990s, reblooming azaleas have grown from only six to over 76 cultivars that are part of various branding programs. First to impact the market and now with 28 cultivars, Encore™ is the oldest brand, perhaps one of the best known brands in all of Horticulture. All begin with the word 'Autumn', which also imprints the plant into the buying public's mind. More recent participants include (1) the Proven Winners® brand, the BLOOM-A-THON® group (six cultivars); (2) Garden

Debut's® REBLOOM™ group (nine cultivars); (3) the Gardener's Confidence Collection, BLOOM-N-AGAIN®, which features 28 cultivars; (4) HGTV's Always Azaleas™ (five cultivars); and, finally, (5) JBerry Nursery's Dejavu Bloom™ series (five or six cultivars) which will be released in 2014. Our count tells us there are over 80 patented or trademarked azaleas, all touting reblooming, flower color, and habit qualities.

Deciduous azaleas are now a major focus at SFA Gardens and our goal is simple: to have the best collection of deciduous azaleas in the South. This is a more coherent group to work with and our collection now includes over 162 taxa on trial. They are characteristically truly fragrant, lose their leaves during the winter, and feature blooms before the leaves emerge. We have long promoted deciduous azaleas as worthy of greater use. Once fully established after several years, we find them to be very drought and heat tolerant and rarely devastated by the impact of lacebugs, a common pest in southern USA landscapes.

HYDRANGEAS

In 1997, SFA Gardens began an effort to collect a wide range of *Hydrangea* taxa and place them into a side-by-side trial. By 2005, we had accumulated over 250 taxa. With our usual enthusiasm, we measured plant height and width, number of blooms, size of blooms, and date of the bloom show. We used groups of three visitors to rank their top picks, came up with a "top ten", and thought we had the situation nailed. The best cultivars included 'Ami Pasquier', 'Mariesii Perfecta' (syn. 'Blue Wave'), 'Nikko Blue', 'All Summer Beauty', 'Frillibet', 'Europa', 'Goliath', Bläuling' (syn. 'Bluebird'), 'Preziosa', 'Beauté Vendômoise', 'Souvenier du President Paul Doumer', 'Bailmer' Endless Summer™ hydrangea, 'Blushing Bride', and 'David Ramsey'. Then in 2007, we listed those cultivars producing blooms later in the season: 'All Summer Beauty', 'Decatur Blue', 'Endless Summer', 'Penny Mac', 'David Ramsey', 'Blaumeise', 'Fuji Waterfall', and 'Twist n Shout'.

In 2006, a virtual flood of new cultivars entered the market picture, most patented and trademarked to one brand or another. In 2012, Michael Dirr provided a fine treatment of hydrangea breeding and advancement (Dirr, 2012a) and noted the increased pace of cultivar releases. At SFA Gardens, it became almost impossible to keep up and we decided to abandon the idea of evaluating new hydrangeas. We took a break. That is, until 2013, when Allen Owings of Louisiana State University, Hammond, Louisiana, and I decided to take a look at only the new cultivars. The plant evaluation program at LSU, Hammond, is a young one, and this garden is growing fast and smart. Allen and I knew we could secure plants from nurseries and plant an even-aged set of three plants at each location. That project is under way and it hasn't taken long to realize that the market is packed with new cultivars.

There are over 95 new cultivars of lace cap and mop head hydrangeas since 2006 that tout reblooming as a key attribute, and most fall under the umbrella of a major brand. Brands include Endless Summer® (Bailey), Forever & Ever™, Cityline™, Edgy™, Everlasting ™ (Plants Nouveau), Mystical™, Hovaria® (Kaleidoscope®), Japanese Lady Series (Halo™, Frau™, and Angel™), Let's Dance™ (Spring Meadow), Next Generation™ (Ball Ornamentals), and Showstopper Hydrangeas™, a series promoted by HGTV which includes eight varieties. While it's hard to imagine the improvements, future breeding projects might include better flower shedding, more reblooming, and burgundy foliage color.

CRAPEMYRTLES

Another standard shrub and small tree that remains a major commodity in the southern USA is crape myrtle, purported to exceed 50 million dollars at the wholesale level. I remember when the Texas market only included red, pink, and white, period. The "red" was usually a cultivar called 'Watermelon Red' which was not red, but nearly so. Everything changed in the 1950s with the introduction of *Lagerstroemia fauriei* by John Creech. Many of those early seedlings are still with us. One patriarch, now named 'Bayou

View', rests on a Timberline Avenue in Shreveport and is a magnificent single trunk specimen with a 2.5 m (8.3 ft) circumference at breast height. This specimen is derived from the first seed distribution from the U.S. National Arboretum. 'Townhouse' and 'Fantasy' originated at the JCR Arboretum in Raleigh, North Carolina.

Many Texas horticulturists contend that Lynn Lowrey introduced the first hybrid, 'Basham's Party Pink', which was found as a seedling near a *L. fauriei*. This superseded the introductions by Donald Egolf of the U.S. National Arboretum's breeding program. That program resulted in 25 excellent introductions, the Indian Tribe Series, with 'Natchez' and 'Muskogee' the first introductions that are still fine trees in the landscape. Later, 'Chickasaw' and 'Pocomoke' were introduced as the first true genetic dwarfs. In Oklahoma, Dr. Carl Whitcomb, Stillwater, bred 'Whit II', Dynamite® crape myrtle (true red) and 'Whit IV', Red Rocket® crape myrtle (true red), which are both 3-4.6 m (10-15 ft) upright large shrubs, or small multi-stem trees. The last decade has seen a proliferation of cultivars under the ever increasing presence of branding. The compact Dazzle® series, a Filligree™ Series from Fleming's, an Early Bird™ series, a Barnyard Collection™ introduced through McCorkle Nurseries, and Plant Introductions, Inc. has introduced the Magic series, Coral, Plum, Purple, and Red. Purple Magic may be the best purple flower on the market. There are others. In 2012, Dirr provided a thorough and comprehensive treatment of the history of *Lagerstroemia* breeding to 2012 (Dirr, 2012b).

Since 2012, nothing has excited the crapemyrtle industry more than dark burgundy foliage cultivars. Since the introduction of *Lagerstroemia* Delta Jazz™ ('Chocolate Mocha') (PP 21,540) there have been 10 new cultivars enter the market place with burgundy foliage that lasts throughout the season. 'Chocolate Mocha' features small flower heads of bubblegum pink, an upright stature, and leaves best described as often cupped and less than attractive. In full sun, the cultivar is relatively free of disease, but in part shade conditions we have observed significant powdery mildew. The genes of this cultivar led to five Black Diamond™ cultivars (JBerry Nursery) with flower colors ranging from red to white to blush. To confuse things a bit, Ebony Crapemyrtles and Black Diamond Crapemyrtles are the same clones under different names. Black Diamond 'Pure White' is 'Ebony & Ivory', 'Best Red' is 'Ebony Flame', 'Blush' is 'Ebony Glow', 'Crimson Red' is 'Ebony Fire', and 'Red Hot' is 'Ebony Embers'. That was followed by the release of four dark-foliaged Delta™ cultivars by Plant Development Services, Inc. (PDSI), Loxley, Alabama. Plant Introductions, Inc. has introduced two patented dark-foliaged cultivars 'Midnight Madness' and 'Moonlight Madness'. These are in the first year of trials at SFA Gardens and at LSU, Hammond, Louisiana.

Crapemyrtles are a major commodity in the South. From very few selections in the market place, there are now hundreds to choose from. Cultivars vary in ultimate size, from small shrub to large tree, flower season and color, form, and, in recent years, foliage color. Nothing has boosted the crapemyrtle world more than burgundy foliage color. Future improvements might include dark foliage color on superior dwarf forms like 'Cherry Dazzle'. The recent advent of *Lagerstroemia* scale, beginning in McKinney, Texas, a few years ago, and now found in east Texas, Louisiana, and recently in Memphis, Tennessee, suggests a research focus on cultivars resistance and pest control. In November 2013, as part of a U.S.D.A. scientific exchange team, we viewed the impact of *Lagerstroemia* scale in Beijing, Nanjing, and Kunming, China. In some cases, plants were reduced to pitiful specimens. Scientists at one location remarked that they had long had the scale, but the impact had gotten worse in the last 2 or 3 years. Some even speculated that this was a new form brought in on USA hybrids cultivars.

OTHER SHRUBS WITH OPPORTUNITY

Most species of beautyberry are drought resistant and extremely durable deciduous shrubs. *Callicarpa americana* berries, a key fall and early winter feature, are available in various shades of dark purple, lavender, pink, and white. There are numerous purple-berry forms of *C. americana* and I suspect most are derived from local provenances with little to no selection work. However, there are several white-berry forms that are in the

trade (we have three) and there are differences in sun tolerance of the berries. For pink berries, there are two genotypes. One is the original 'Matt's Pink', a clone found in the Davy Crockett National Forest by Matt Welch, one of my former students. The other is a form found by this author along a roadside near Stonewall, Louisiana. They seem identical. When seedlings are allowed to fruit, we do find progeny with a good mix of purple and pink berried forms. We have a small trial of several hundred seedlings in full sun and hope to make an advanced selection in the next few years. *Callicarpa acuminata*, the Mexican beautyberry, features a larger more pubescent leaf and dark purple to almost-black berries. *Callicarpa dichotoma* 'Duet' is a superior variegated beauty berry released by the National Arboretum which has proven to be extremely stable and features very clean foliage. *Callicarpa dichotoma* 'Shiji Murasaki', Wine Spritzer™ beautyberry is a new variegated form. Several relatively unknown beautyberries are in our testing program. While I have long admired *C. kwantungensis*, it has not made a big mark in our garden. It features dark foliage with attractive white blooms and berries but has proven to be quite drought-sensitive in our east Texas climate. In the right spot, moist and part shade, it is a truly special plant. *Callicarpa longissima* is a rarely encountered drought-tolerant Asian species that has performed well at SFA Gardens. It is robust and becomes a large plant to 3 m (10 ft) in a few years featuring showy lavender blooms and white berries. We have just acquired *C. salicifolia*, another Asian species, featuring pink flowers, dark green glabrous foliage, and a small shrub that appears to have excellent habit. Breeding goals would be more showy blooms, denser branching, and better habit and, perhaps, fall color.

Vitex agnus-castus, the chaste tree, is a large shrub or small tree that has been much maligned across the southern USA for many years. The main feature is a summer bloom of bright blue, pink, or white blooms. The species is woody in the southern USA and an herbaceous perennial in more northern regions. We have six blue-flowered cultivars in our collection and find all of them to be good performers. 'Montrose Purple' and 'LeCompte' are most attractive. The first light pink-flowered variety, 'Salina Pink', was introduced several years ago by Greg Grant, the Research Associate at SFA Gardens. That cultivar was used as the foundation for finding the first true pink flowered form, 'Flora Ann', which is becoming more common in the trade. L.E. Cooke has introduced four new chaste tree cultivars, and one, 'Cooke's Pink'™, features pink flowers. At this stage, the plant looks very similar to 'Flora Ann'. Breeding goals would include dwarf forms, dense branching, and more flowers.

Ilex vomitoria 'Scarlet's Peak' is destined to become a standard across the South. Introduced by Dan Batson of Green Forest Nursery, this yaupon is classically columnar and reliably produces a crop of clean bright red berries. It is a great improvement over 'Will Fleming Upright', which is a male with no berry show and plagued with a rather unkempt habit as it ages. Since first encountering this plant several years ago, we have concluded that 'Scarlet's Peak' is destined to be a leader in the yaupon market. It's unique, offering a much needed columnar red berried shrub to the market, something that has not existed before.

Distylium, the isu tree, is perhaps best referred to as the evergreen witch hazel. Stephen F. Austin Gardens is home to several large specimens of both *D. racemosum* and *D. myricoides*. We have promoted and distributed for years an interesting variegated form, 'Mr. Ishi's variegated'. 'Vintage Jade', 'Blue Cascade', and 'Emerald Heights' are three recent introductions to SFA Gardens. They have similar habits, but vary in height from a few feet to 5 to 6 ft tall and wide. These cultivars are the result of Mike Dirr's breeding program in Georgia, crosses of *D. racemosum* and *D. myricoides*. They are adapted to the heat and dry times of SFA Gardens and provide a neat and clean non-invasive shrub useful as a screen or grouping where its foliage and form can be appreciated up close.

Dirr lists about 50 cultivars of *Loropetalum chinense*, Chinese fringe flower (Dirr, 2009). The burgundy foliaged cultivars 'Blush' and 'Burgundy' were the first burgundy foliaged cultivars introduced in the early 1990s. We have a fine old hedge trimmed and trained into quite striking large multi-stem shrubs. We have many specimens in the

gardens that are over a decade old and performing beautifully. When in bloom, few flowering shrubs can compare. The foliage is always attractive year round. They were remarkably resilient during the heat and drought of 2010 and 2011. We have lost a few large plants over the years due to a sudden death. In most cases, we've concluded that a drainage issue was the likely cause. In other cases, we did not know. Many of the older touted as dwarf or modestly dwarf are proving to be quite large with time. I have strolled under large old *Loropetalum* trees in China. Given a few hundred years, this plant can be a giant. *Loropetalum chinensis* 'Sparkling Sangria' is our most recent acquisition.

Edgeworthia chyrsantha is a rarely encountered shrub that deserves greater use. This deciduous shrub typically reaches 2.4 m (8 ft) or taller and is usually slightly wider than tall. Large strappy leaves lend a tropical look to the plant. The key feature is winter bloom which occurs after the leaves have fallen. The foliage drops in mid December to reveal attractive bark and the large terminal flower buds. The flower buds open slowly from mid-December to early March and produce a fragrant show of pendent white or yellow flowers. The orange-red form, *Edgeworthia chrysantha* 'Red Dragon' (syn. 'Akebono') on the market is less fragrant. Mike Dirr reports that a hybrid of the large tetraploid form and the shorter diploid form exists and should be entering the market in the future. One obscure fact associated with the plant is a cultural one that I've observed in public gardens in China. The branches of *Edgeworthia* are very pliable and can easily be tied into "love knots" that continue to grow unabated. This is a favorite practice for young people in love and newlyweds.

Photinia serratifolia (syn. *serrulata*) is a bullet-proof large Asian evergreen shrub of various forms. There are only a few cultivars on the market. The species is extremely drought tolerant. We are distributing plants propagated from a clone we named 'Akin', named after Sherwood Akin of Sibley, Louisiana. A long time nurseryman, Sherwood maintained that this shrub had better form. After a decade, our specimen is very clean and dense, football shaped, 3.7 m (12 ft) tall and 2.1 (7 ft) wide.

There are many other shrubs we've come to admire after many years. After many years at SFA Gardens, we admire *Agarista* (syn. *Leucothoe*) *populifolia* as a durable evergreen shrub with its pleasant relaxed branch arching. It is best in masses or as a screen and individuals in the right spot can become quite large. *Agarista* populifolia 'Taylor's Treasure', Leprechaun™ leucothoe PP#13347 is a plant of more subdued stature and should be utilized more in our region. We find *L. axillaris*, a low growing evergreen shrub, a superior performer in part shade if there's a modest irrigation during the summer. In our garden, 'Jenkins Form' is a beautiful plant. We have long admired *Gordonia axillaris*, the fried egg plant. The new hybrids of *Gordonia*, *Schima*, and *Franklinia* via Dr. Tom Ranney, North Carolina State University, Asheville, North Carolina, appear particularly promising. While only with us a few years, they appear surprisingly vigorous and early to flowers in part shade conditions. There are advances in gardenias and SFA Gardens has a fine collection. True dwarfs with clean foliage and good habit are here. There's an ever-increasing list of viburnums in our garden and we've reached the conclusion they are durable in the landscape, charming in flower, and have good foliage interest. However, in our region, while many do well in the landscape, they have yet to gain a big market share in Texas. *Mahonia* 'Soft Caress' and some relatives appear well suited to the garden. Trips to China have led me to admire a huge range of Mahonia species in China, Mexico and Texas. These are durable evergreen shrubs with flower, habit, and foliage quality attributes. While there is good opportunity for bispecific hyrbridization and other breeding strategies, the genus remains relatively unexploited.

CONCLUSIONS

Dr. Charles Hall, Ellison Chair, Texas A & M University, has coined the phrase 'hypercompetition" to describe the acceleration of branding, patenting, and marketing of new plants as nurseries attempt to gain market share. Many psychologists contend that more choices may lead to a poorer decision or a failure to make a decision at all. Hall has referred to this as a kind of analysis paralysis, the process perhaps leading to rational

ignorance (when the cost of educating oneself outweighs any potential benefits). Dilution may not be the solution. As a long ago Horticulture student at Texas A&M University, I was trained with the mantra that new selections should be evaluated in many locations over many years before introduction. The current flood of new plant materials is bewildering. Nomenclature issues are complicated, a topic well covered by Tony Avent in 2012 (Avent, 2012). There's a relatively new trend to rebrand, remarket, and reintroduce cultivars introduced long ago. I am slowly concluding that University and other woody plant trialing programs may not be as relevant to the nursery market place as we used to be. By the time a new cultivar succeeds or fails in trials, the industry has already made a major market push or totally left the scene when a newer, more exciting and more fashionable plant arrives on the scene. For the consumer, it may not really matter one way or the other. To keep excitement high, perhaps we need to offer 96 reblooming azaleas in the market place.

Maybe 10 major brands of reblooming hydrangeas really is a good idea. Perhaps the industry will benefit from presenting customers with 11 different black leaf crapemyrtle cultivars. In the short term, there are certainly profits to be made by nurseryman able to position themselves. In the long term, perhaps we are creating customers confused by the barrage of new plants. I have concluded there's no relief in sight, and, perhaps, the only course is simply to just sit back enjoy the ride. After all, our mission at SFA Gardens remains the same: educate, entertain, evaluate, and enlighten. That we can do.

Old age, believe me, is a good and pleasant thing. It is true you are gently shouldered off the stage, but then you are given such a comfortable front stall as a spectator." — Confucius

Literature Cited

Avent, T. 2013. Name that Plant: The misuse of trademarks in horticulture. <http://www.plantdelights.com/Article-Trademarks-in-Horticulture>.

Dirr, M. 2009. Manual of Woody Landscape Plants. 6th Ed. Stipes Publishing Co., Champaign, Illinois.

Dirr, M. 2012a. Hydrangeas: Breeding, selecting and marketing. <http://www.plantintroductions.com/hydrangeasbreedingselectionandmarketing.html>.

Dirr, M. 2012b. *Lagerstroemia* – Crapemyrtle: Advances in crapemyrtle breeding. <http://www.plantintroductions.com/advancesincrapemyrtlebreeding.html>.

Seed Germination of *Rhododendron calophytum* Planch. in Response to Temperature, Light, and GA_3©

Bing Zhao
Northwest A&F University, Yangling, 712100, China

Jin-Ying Dong and Donglin Zhang
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: donglin@uga.edu

INTRODUCTION

Rhododendron calophytum Planch., commonly named large leaf *Rhododendron* or meili *Rhododendron* is in the *Ericaceae* family, *Rhododendron* genera. It is an endemic evergreen plant with beautiful flowers, found in high mountains at altitudes of 1,300 to 4,000 m in south-west China (Ran et al., 2010). This includes the Qinling Mountains, where the species is beneficial for helping to maintain the stability of the ecosystem. *Rhododendron calophytum* germplasm is endangered because of excessive excavation activities. Additionally, few cultivars are cultivated and utilized in modern city landscape.

In order to protect *R. calophytum* from extinction, help maintain its diversity, and utilization of its multiple-color landscape cultivars, it is necessary to develop propagation systems for *R. calophytum*. Seed propagation can be used to protect germplasm and enrich genetic diversity of the species (Zhang et al., 2010). More importantly, for wild resources, seedlings of diverse populations are more easily adapted to new environments than seedlings collected and transplanted from the mountains.

External factors such as light and temperature are known to affect seed germination. Studies of how environmental factors affect seed germination in *Rhododendron* genus have provided various results. Some species need higher temperatures to germinate (Antonidaki-Giatromanolaki et al., 2008; Sajad et al., 2012; Fan et al., 2011). Conversely, lower temperatures are favorable for germination of other species (Vologdina, 2006; Zhang et al., 2007).

Phytohormones can be used for breaking seed dormancy. Germination requirements of many *Rhododendron* species indicate that the seeds have a non-deep, simple, morphophysiological dormancy, but gibberellic acid (GA_3) application can increase seed germination of selected *Rhododendron* species (Tiwari and Chauhan, 2007; Gao et al., 2010; Su et al., 2011).

There are studies on *R. calophytum* using tissue culture (Luo et al., 2007), chemical analysis (Tian et al., 2010) and cultivation management (Si et al., 2012), but few on seed propagation.

The objective of the present study was to develop seed propagation systems for germinating *R. calophytum* by manipulating photoperiod, temperature, and GA_3 treatments. Establishing standard germination procedures is important for conservation strategies, including providing future breeding material of *R. calophytum*.

MATERIALS AND METHODS

Seed Source

Mature capsules of *R. calophytum* were harvested from Niu Beiliang district of Qinling Mountain at the end of November in 2012. The capsules were air-dried at room temperature until the seed released. Seed was collected, put into paper bags and refrigerated at 4°C (39°F) until experimentation.

Experimental Design

The main treatment effects were: light, photoperiod, and GA_3, each of which was treated as a single factorial. There were three photoperiod treatments were 0, 16 light/8 dark, and 24 h light. The three temperatures treatments were 20, 30, and 20°C (16 h)/30°C (8 h).

The six GA_3 treatments were 0, 200, 400, 600, 800, and 1,000 mg·L. Each treatment was replicated three times with 100 seeds per replicate, n=3.

Seed Germination Tests

Germination tests were performed in incubators (Conviron A1000, Canada) with automatic temperature and light control. Seeds were immersed in GA_3 for 24 h, then rinsed three times with deionized water and dried on filter paper. Finally, 100 seeds were counted out and put inside 12-cm petri dish containing absorbent gauze and filter paper. To control fungal contamination, seeds were periodically transferred to clean petri dishes, and the absorbent gauze and filter paper were changed and moistened with new deionized water every day. The germination dishes were randomly rearranged daily to avoid effects of potential temperature and light differences and other factors. The germinated seeds were recorded everyday for 6 d. Seeds were considered to have germinated as soon as radicle emerged from seed coats. Germination performance was evaluated according to germination percentage and germination vigor.

Statistical Analysis

Germination percentage and germinating vigor were calculated with the Excel software. Germination percentage GP = (n/N) × 100% ,where n is the number of germinated seeds, N is the number of seeds tested; germinating vigor (GV) is the germination percentage on the 11^{th} day, final day of test run.

RESULTS AND DISCUSSION

Seeds Morphology Observation

Seeds were small and flat, with an oval or long oval shape. There were obvious vertical stripes on the surface of seed and developed wings around the seed. The average length and width was around 0.2 and 0.1 cm, respectively. The seed coat was brown and weighed on average around 0.2 g.

Effect of Different Temperature on Germination of *Rhododendron calophytum*

Temperature was an important environmental factor affecting seed germination. Temperature of 30°C for 24 or 16 h depressed germination, while the optimal temperature (for control GA_3 treatment) was 20°C for 24 h (Fig. 1). Initial germination occurred after Day 8, and peaked 5 or 6 days later (Fig. 1). The results showed that there was little difference among the effects of the three temperature on the starting germination time and germination speed. While seed germination of *Cynanchum bungei* (Zhang, 2012) and *Rhododendron delavayi* (Duan et al., 2007) showed that the higher the temperature, the earlier seeds began to germinate (Zhang, 2012). There were significant difference among the effects of the three temperature regimes on germination percentage and vigor (Table 1). Both germination percentage and vigor were significantly highest at 20°C, and lowest at 30°C is the lowest (Table 1). The optimal lower temperature response may be due to this species being adapted to low temperatures, since wild populations of *R. calophytum* are found in mountainous areas with heights of 1,300 to 4,000 m and cooler temperatures. Our results are in agreement with seed germination of *R. molle* (Shi et al., 2010), but contrast to *R. irroratum* (Fan et al., 2011); the later findings reported that germination speed and germination vigor were best at 30°C. In our study, the germination vigor at 20°C was significantly greater than the higher temperature regimes, emphasizing the importance of seed propagation and cultivation of this species under cooler conditions.

Effect of Different Light Time on Germination of *Rhododendron calophytum*

A 24-h light exposure delayed seed germination of all GA_3 treatments until after Day 8, whereas seed exposed to 0 and 16-h light germinated by Day 8 (Fig. 2). At the end of the 13-day experimental runs, the final percent germination and germination vigor was greatest at 0- and 16-h light exposure and lowest at 24-h light exposure (Table 1). While

our results agree with Roberts (1973), they conflict with the benefits of light on germination percentage and germination vigor of four other *Rhododendron* species that require light for germination (Zhang, 2012).

Effect of Different Gibberellic Acid Concentration on Germination of *Rhododendron calophytum*

Compared with the control, seeds treated with GA_3 had greater germination percentage and germination vigor (Table 1, Fig. 1). Seed treated with GA_3 germinated after Day 8. While 200 $mg \cdot L^{-1}$ GA_3 had greater germination percentage and vigor than the control, the greatest benefit occurred with 400-1,000 mg·L GA_3. There were no significant differences among 400-1000 mg·L GA_3. Under the adverse conditions of 30°C for light exposure of 24-h to 16-h, there was a trend in 800 $mg \cdot L^{-1}$ GA_3 having the greatest benefit (Fig. 1). This suggests that a range of 400 to 800 $mg \cdot L^{-1}$ GA_3 is beneficial for seed germination.

The control did not germinate under darkness (0-h light) and also 24 h light; the germination percentage of the control under 16-h light was also lower than that of the seeds treated with GA_3 treatment (Fig. 2). Seeds of *R. jiulongshanense* and *R. annae* treated with 200 $mg \cdot L^{-1}$ GA_3 for 15 min had increased germination percentage and vigor (Gao et al., 2010), whereas in our study 200 $mg \cdot L^{-1}$ GA_3 was only marginally effective, i.e., GA_3 treatments of 400-800 $mg \cdot L^{-1}$ were more effective.

This research demonstrated seed propagation is a valuable method for the reproduction and conservation of *R. calophytum*. The high germination percentage obtained with this protocol will facilitate the conservation and development of new cultivars of *R. calophytum* germplasm.

ACKNOWLEDGEMENTS

This research was conducted in woody ornamental plant lab at the University of Georgia in USA. Funding for this research was provided by National Natural Science Foundation of China (k305021110), Shaanxi Natural Science Foundation (2012JQ3008), and construction project of Forestry Department of Shaanxi Province Shan (2011(70)).

Literature Cited

Antonidaki-Giatromanolaki, A., Dragassaki, M., Papadimitriou, M. and Vlahos, I. 2008. Effects of stratification, temperature and light on seed germination of *Colchicum macrophyllum* B.L. Burtt. Prop. Ornam. Plants 8:105-107.

Duan, Xu, Chen, X. and Zhao, Y.Y. 2007. Study of the seed germination of *Rhododendron delavayi*. J. Anhui. Agri. Sci. 35:9199-9200.

Fan, C.L., Chen, X. and Xing, J.N. 2011. Effects of different treatments on seed germination of *Rhododendron.* Seed 30:106-108.

Gao, G.L., Long, X.Q., Hu, X.J., Lu, X.L. and Chen, X. 2010. Effect of GA on two species of Alpine *Rhododendron* seed germination. Seed 5:22-25.

Luo, P., Zhuang, P. and Bai, J. 2007. Tissue culture of *Rhododendron decorum* Franch， *Rhododendron calophytum* Franch. and *Rhododendron discolor* Franch. Plant Physiol. Commun. 2:326-328.

Roberts, E.H. 1973. Predicting the storage life of seed. Seed Sci. Technol. 1:449-514.

Ran, F., Wu, C.C., Peng, G.Q., Korpelainen, H. and Li, C.Y. 2010. Physiological differences in *Rhododendron calophytum* seedlings regenerated in mineral soil or on fallen dead wood of different decaying stages. Plant Soil 337:205-215.

Sajad, R., Shailesh, P. and Nautiyal, S. 2012. Studies of seed germination in four *Rhododendron* species of Garhwal Himalayas. Indian Forest. 138:284-288.

Shi, D.H. and Chen, X. 2010. Effect of different treatments on *Rhododendron molle* G.D seed germination. Seed 9:91-94.

Su, J.L., Li, C., Chen, L., Liu, X.Q., Chen, S.P. and He, L.S. 2011. Effects of different pretreatment methods on seed germination of *Rhododendron aureum* and *R. parvifolium*. J. Plant Resour. Environ. 20:64-69.

Si, G.C., Zhang, Y.L., Gu, X., Wang, Y.Q. and Zhao, B. 2012. Study on cutting propagation technology of QinLing Wild *Rhododendron calophytum*. North. Hort. 3:77-79.
Tian, P., Fu, X.L., Zhuang, P., Bai, J. and Chen, F. 2010. Analysis on the volatile oils from *Rhododendron calophytum* Franch. by GC-MS. Chinese J. Appl. Environ. Biol. 5:734-737.
Tiwari, O.N. and Chauhan, U.K. 2007. Seed germination studies in *Rhododendron maddenii* Hook.f. and *Rhododendron niveum* Hook.f. Indian J. Plant Physiol. 12:50-56.
Vologdina, O.S. 2006. Biology of *Rhododendron dauricum*, *R. mucronulatum* and *R. sichotense* (*Ericaceae*) seeds germination. Rastitel'nye Resursy. 42:55-60.
Zhang, H.Y. 2012. Seed germination and early seedling growth of *Cynanchum bungei* Decne (*Asclepiadaceae*) in response to photoperiod, temperature, and seed size. HortScience 47:1338-1341.
Zhang, J.L., Wu, Y.W., Wu, H.Z., Zhao, H.Z. and Wang, Y.Y. 2012. Studies on seed germination of four species of Subgen. Hymeanthes (Blume) K. Koch *Ericaceae* in Yunnan. J. Agricul. Univ. 27:875-881.
Zhang, F., Yu, S.L. and Wang, J.H. 2010. Studies on the photosynthetic characteristics and its relationship to yield in Radix *Cynanchum bungei* Decne. J. Nuclear Agr. Sci. 24:176-180.

Table 1. Effect of temperature, light time, and gibberellic acid (GA_3) concentration on germination of *Rhododendron calophytum*.

Factors	Germination percentage (%)	Germination vigor (%)	Factors	Germination (%)	Germination vigor (%)
Light (h)	**	**	GA_3 ($mg \cdot L^{-1}$)	**	**
24	62.3 a	52.0 a	0	14.5 a	9.1a
16	83.4 b	76.5 b	200	64.7 b	58.6 b
0	80.4 b	76.8 b	400	72.9 c	64.2 c
Temperature (°C)	**	**	600	74.5 c	67.7 c
20	87.8 a	77.4 a	800	79.4 cd	74.7 cd
30	5.0 b	4.3 b	1000	76.7 cd	70.0 cde
20/30	62.3 c	52.0 c			

** Different letter has significant difference at 0.01 level.

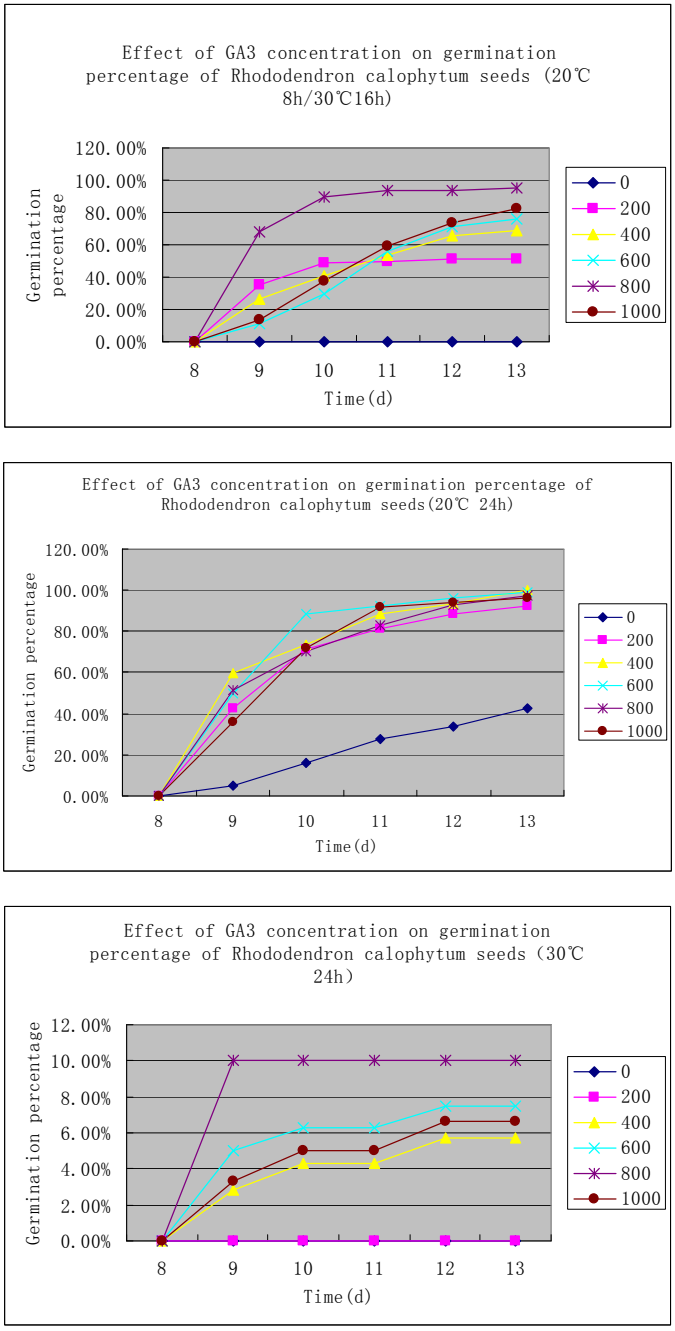

Fig. 1. Effect of six gibberellic acid (GA_3) concentrations under three temperature regimes on germination percentage of *Rhododendron calophytum*.

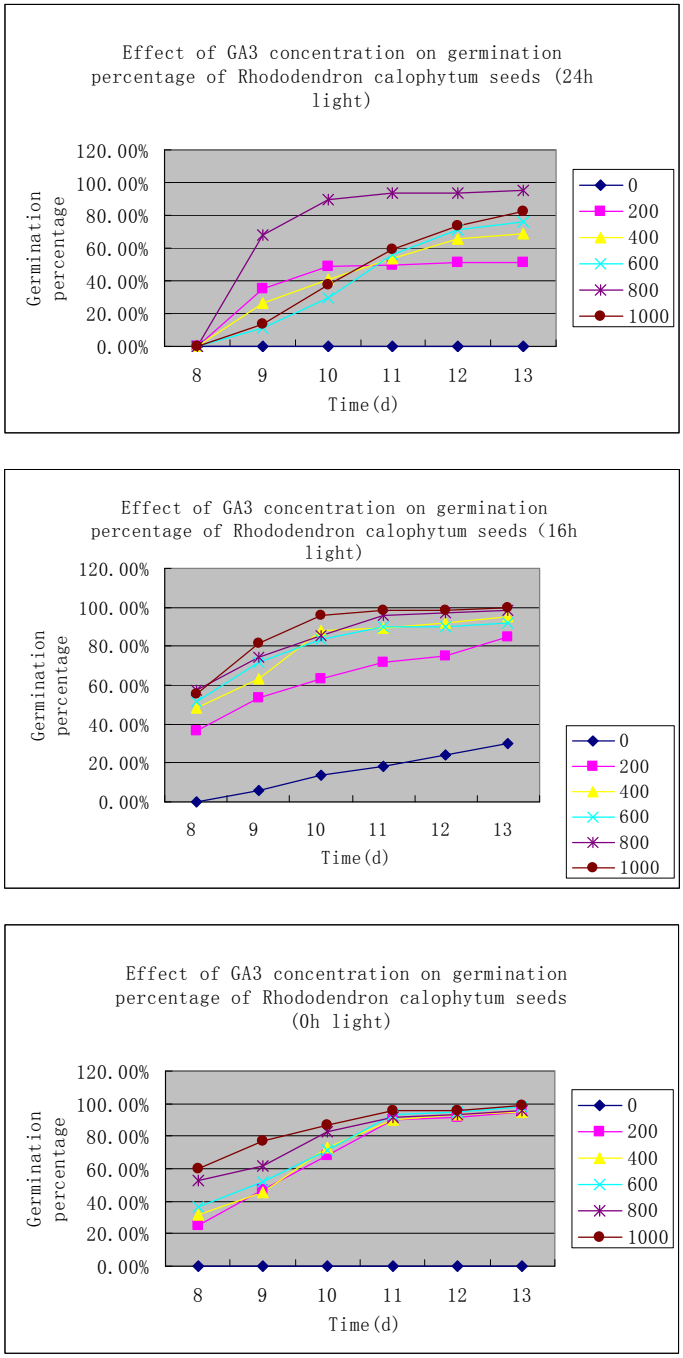

Fig. 2. Effect of six gibberellic acid (GA_3) concentrations and three photoperiod regimes on germination percentage of *Rhododendron calophytum*.

Propagation and Cross Compatibility of *Abutilon*©

Fanghua Niu, Donglin Zhang and John Ruter
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: niuniu@uga.edu

Zhihui Li
College of Forestry, Central South University of Forestry and Technology, Changsha, Hunan 410004, China

INTRODUCTION

Abutilon, flowering maple, is a large genus in the mallow family. The genus comprises of 100-150 species and is distributed in the tropics and subtropics (Servin et al., 2013). Leaves are lobed, maple-like, and light green. Flowers come in red, pink, yellow, white, and pastel shades (Kim and Suh, 2013). The diversified and long-lasting flowers are very attractive and have brought a lot of attention from all over the world (Matlawska and Sikorska, 2005), especially in the southeastern United State of America.

Flowering maple should be placed in areas of full sun to light shade in well-draining moist soil. Light shade will prevent wilting during the hottest parts of the day. A fast grower in warm climates, *Abutilon* is generally hardy in U.S.D.A. Zones 8 and 9 and thrives in the cooler temperatures of spring and fall. As for problems, flowering maple is sensitive to temperature fluctuations, which can cause leaves to drop. Higher temperatures experienced in some parts of southeastern USA can be detrimental to growth and development of flowering maple. In Georgia, temperatures can range from 27-38°C (80-100°F) in the summer. Consequently, wilting can happen to plants directly grown in the sun. This problem can be made worse if plants are grown in containers that hold small volumes of water and substrate (Yeager et al., 2010). Flower color, size, form, and longevity can also be compromised by extreme summer heat.

Due to the significance of *Abutilon* and increasing market demand, better cultivars that can survive summer heat without extra care and more efficient propagation techniques are required. *Abutilon* is mainly propagated from seeds and cuttings. Buckstrup (2005) reported that it was easier to develop new cultivars from mutations rather than hybridization. He noted that some cultivars appear to be self-sterile and did not form seeds easily.

In this research, 10 *Abutilon* clones were selected and propagated by cuttings with varying hormone treatments. Growth and development of rooted cuttings were also recorded after transplanting. We also investigated the cross-compatibility for breeding among the 10 clones.

MATERIALS AND METHODS

Plant Materials

Semi-hardwood terminal stem cuttings of 10 clones of *Abutilon* were collected from the Trial Garden at the University of Georgia on 18 Sept. 2012. They are A08-0401, A08-1603, A08-1607, A08-2110, A08-2112, A08-2114, A08-2121, A08-2125, A08-2127, and A08-2131. The cuttings were placed into black plastic bags and sprayed with water. In the greenhouse, each cutting was trimmed to approximately 10 cm (4 in.) in length and stripped from the bottom to 3-4 top leaves. To reduce respiration and transpiration, two-thirds of each remaining leaf on the cuttings was removed. The cuttings were rooted under different concentration and hormone treatments.

Experimental Treatments

Cuttings were treated with K-IBA (1000, 3000, and 8000 ppm) and Hormodin #1 (1,000 ppm IBA). Cuttings were dipped into the liquid K-IBA for about 10 s followed by at least 15 min of air-drying. For the Hormodin #1 treatments, cuttings were dipped in tap water

first and then dusted with the Hormodin #1 powder. Treated cuttings were inserted into 36-cell flat trays filled with a propagation substrate of milled peat moss and perlite (1:3, v/v). All cuttings were then placed on a mist bench. The mist system was set at an interval of 10 min for 15 s for the first week, then 20 min for 15 s thereafter.

The cuttings initiated rooting in about 2 weeks and were transplanted into 1 trade-gal pots (2.8 L) with soilless substrate (Fafard 1P; Fafard, Agawam, Massachusetts) after 4 weeks. All plants were fertilized using slow-release fertilizer at 15 g per pot after 2 weeks in the greenhouse. Six uniform plants were selected from each clone for observation of growth and development observation as well as cross-hybridization. All transplanted plants reached full bloom in about 2 months and reciprocal crosses were conducted among all 10 clones.

Data Collection

Rooting data were collected after 2 weeks of root initiation. Root quality was classified into six groups based on root length and quantity (Table 1). Plant height was recorded weekly. The total number of crosses, the number of successful crosses, fruit set, and seed set were documented.

Experimental Design and Data Analysis

A randomized complete block design was employed in this experiment. There were three replicates per treatment and six cuttings per replicate, n=3. The data were analyzed using SAS. The cross-compatibility among these ten clones was calculated as a percentage.

RESULTS AND DISCUSSION

Effect of Hormone on Rooting of Cuttings

The K-IBA and hormodin treatments significantly affected rooting quality of the 10 clones. Compared to control, the root quality of the cuttings with the four hormones was significantly higher. However, there were no significant differences among the four treatments (Fig. 1).

Rooting Quality

For the hormone treatments, rooting quality was different among clones (Fig. 2). The A08-2125 clone showed the best rooting quality and followed by A08-1607. However, the A08-0401 and A08-1603 clones had the lowest rooting quality ratings (<3).

Plant Height

After transplanting, plant growth of the ten clones varied. The rooted cuttings grew at the same rate for the first 7 weeks (Fig. 3). From the 8th week, growth rate diverged. A08-2127 showed a faster rate of growth than other clones. At the end of the experiment, A08-0401 and A08-1607 followed closely with a height increase ≥25 cm within 17 weeks. Clone A08-1603, A08-2112, and A08-2114 had the slowest growth rates. Their height increased less than 10 cm during the entire experiment. The growth rate of the four other clones ranged from 12.47 cm to 16.01 cm (Fig. 3).

Similar trends in height in both garden and greenhouse conditions (Fig. 4) suggest that the variability in height was genetically controlled with less influence of the environment. Abutilon being perennial plants grow bigger and faster over time after proper root establishment and this might also explain the variability observed between stock plants and rooted plant.

Hybridization

Successful crosses were only made among three clones: A08-1607, A08-2112, and A08-1603. The crossing percentage differed among different pairs of clones. A08-1607 X A08-2112 had 41.7% fruit set and produced seven seeds per fruit; while A08-2112 X A08-1607 had 1.96% fruit set with three seeds. A08-1603 X A08-2112 had 25.5% fruit set and

produced 14 seeds per fruit; while A08-2112 X A08-1603 had 4.4% fruit set with five seeds. A08-1603 X A08-1607 had 13.7% fruit set and produced six seeds per fruit; while A08-1607 X A08-1603 had no fruit (Fig. 5). A08-2112 performed better as a pollen donor and formed more fruits and seeds than A08-1607, A08-1603. In term of maternity, A08-1603 formed more fruit and seed than the other two clones (Fig. 5). The crossed hybrids were being grown in containers and had survived summer warm temperatures. They were showing mixed morphological features of both parents. Their growth is being monitored and will be reported in the future, as well as their ability to tolerate heat.

Literature Cited

Buckstrup, M. 2005. Modern *Abutilon*. Amer. Nursery 201(9):20.

Kim, J.H. and Suh, J.K. 2013. Evaluation of *Abutilon* hybrids as potted plant. Acta Hort. 1000:319-326.

Matlawska, I. and Sikorska, M. 2005. Flavonoids from *Abutilon theophrasti* flowers. Acta Polon. Pharmaceut. - Drug Res. 62(2):135-139.

Servin, W.P., Devi, C., Moin, S. and Sahaya Shibu, B. 2013. In vitro phytochemical screening, free radical scavenging activity and anticancer activity of *Abutilon hirtum* (Lam.) Sweet (*Malvaceae*). In Vitro 5:155-161.

Yeager, T., Million, J., Larsen, C. and Stamps, B. 2010. Florida nursery best management practices: Past, present, and future. HortTech. 20:82-88.

Table 1. Rooting classification according to length of longest root, average root length, root quantity, rooting height.

Root classification	Length of longest root (cm)	Avg. root length (cm)	Root quantity
0	1	0-1	1-2
1	1-2	1-2	2-5
2	2-3	2-3	5-10
3	3-5	3-4	10-25
4	5-10	4-5	25-40
5	>10	>5	>40

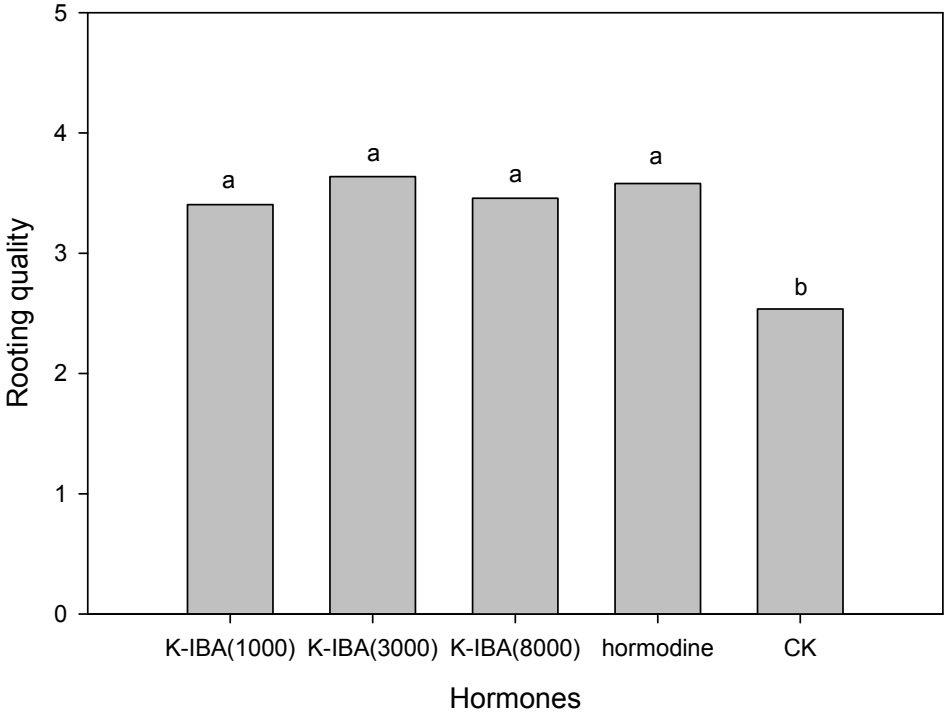

Fig. 1. Effect of hormone treatment on rooting quality. Different letters mean significant differences (α=0.05), (HSD).

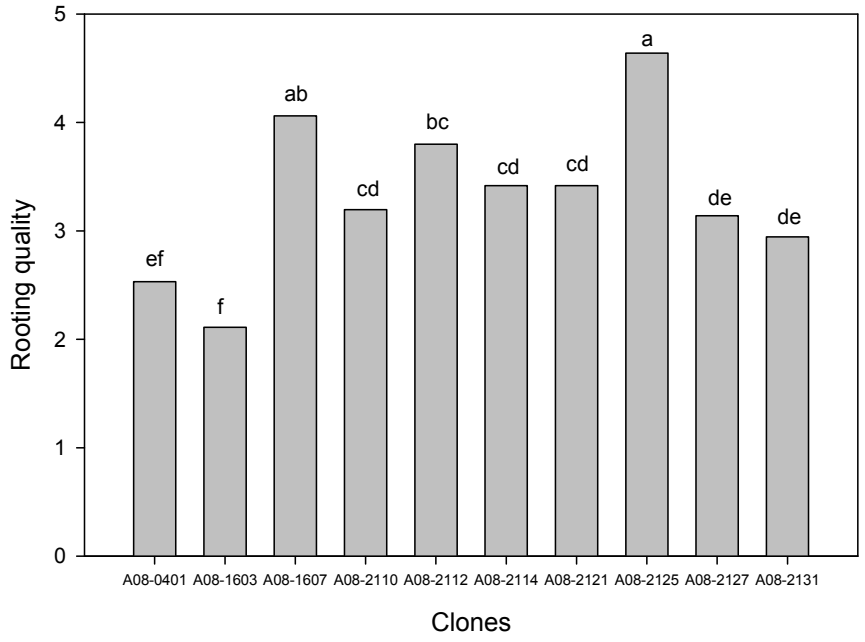

Fig. 2. Rooting quality of different clones. Rooting quality was graded in a scale of 0 to 5, 0 being the poorest root quality. Bars with different letters mean significant differences according to Turkey test (α=0.05).

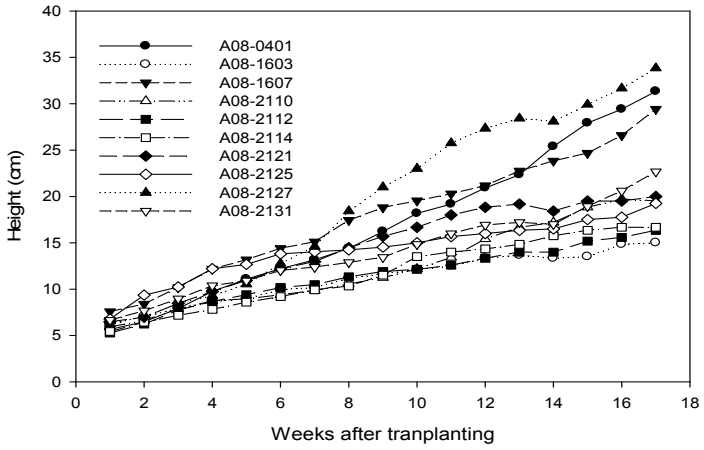

Fig. 3. The plant height growth (cm) after transplanting.

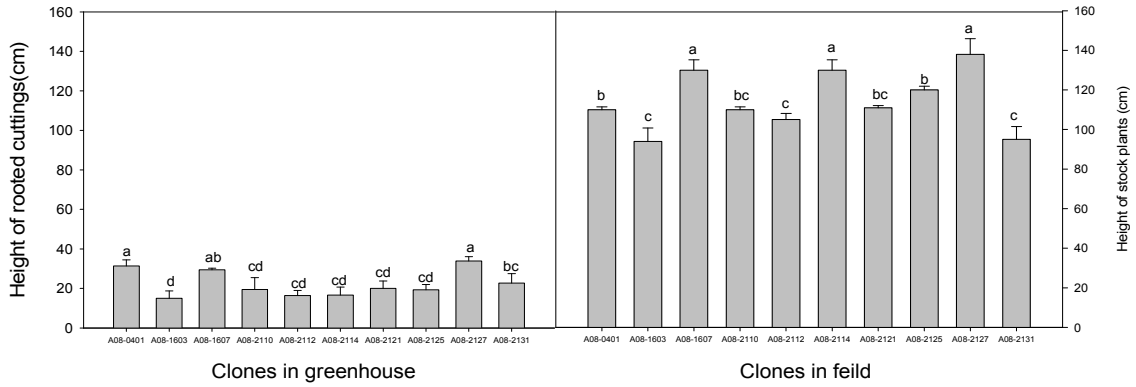

Fig. 4. Height of the ten clones both in greenhouse and garden. Different letters mean significant differences (α=0.05), (HSD).

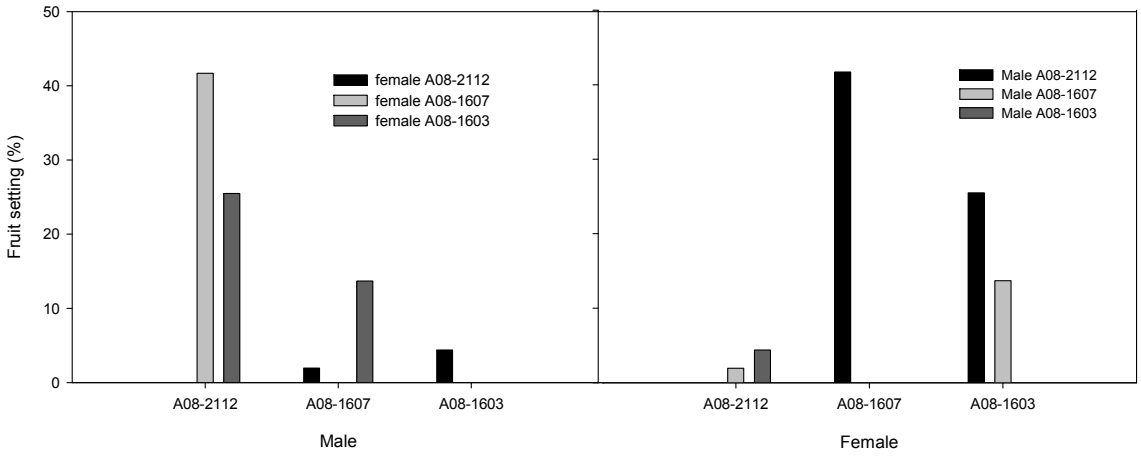

Fig. 5. Fruit setting percentage among the three clones: A08-1607, A08-2112, A08-1603.

Sanitation Can Be a Foundation Disease Management Tool: Potential of Spreading Binucleate *Rhizoctonia* from Nursery Propagation Floors to Trays Containing Azalea Stem Cuttings©

Warren E. Copes
U.S. Department of Agriculture, Agricultural Research Service, Thad Cochran Southern Horticultural Laboratory, Poplarville, Mississippi 39470, USA
Email: warren.copes@ars.usda.gov

INTRODUCTION

Many people see sanitation as simple control techniques with limited application. However, a technical definition of sanitation is any control action that lowers the initial pathogen level so that the amount of final crop loss is reduced or a damaging threshold of disease is delayed. The resulting reduction in disease, whether from a single control action or integrated disease management, can be dramatic and desirable (Daughtrey and Benson, 2005; Jones et al., 2001; Williams-Woodward and Jones, 2001). Ultimately, pathogen reduction is a desirable goal that reduces the need for other controls or magnifies their effectiveness. The potential for pathogen reduction (sanitation) to be beneficial is greater in ornamental plant production than any other commodity system, yet this potential has not been imaginatively explored using current research concepts.

Binucleate *Rhizoctonia* species (BNR), the cause of web blight, are present all year on stems, in dead leaves below the canopy, and in the pine bark media of many container-grown azalea cultivars in the southern USA (Copes et al., 2011). Azalea shoots collected for stem cutting propagation can harbor the pathogen, thus allowing the pathogen to be carried into the propagation house. Temperature and moisture conditions in propagation houses are favorable for plant root development and pathogen growth, which allows *Rhizoctonia* to infest next year's crop. Copes and Blythe (2009) showed binucleate *Rhizoctonia* can be eliminated prior to vegetative propagation by submerging stem cuttings in 51°C (123°F) water for 21 min. Root development progressed normally for 12 azalea cultivars ['Conleb' (Autumn Embers), 'Fashion', 'Formosa', 'Gumpo White', 'Hardy Gardenia', 'Hershey Red', 'Macrantha Pink', 'Midnight Flare', 'Red Ruffles', 'Renee Michelle', 'Roblel' (Autumn Debutante), and 'Watchet'] receiving hot water treatment (Copes and Blythe, 2011).

Rooted cuttings are removed from propagation houses in the spring. Propagation managers typically remove organic debris and leave houses empty for 6 to 8 weeks before the next crop of stem cuttings are collected. The objective of the current study was to evaluate (1) the presence of *Rhizoctonia* on bare propagation floors, (2) survival of the pathogen over 6 weeks of exposure to full sun and ambient temperatures, and (3) risk of rooting trays with stem cuttings becoming colonized by *Rhizoctonia* present on polyethylene fabric and gravel floors in propagation houses.

MATERIALS AND METHODS

Experiment 1: Recovery of *Rhizoctonia* from Propagation House Floors

Polypropylene fabric and gravel floors of commercial propagation houses were sampled 1 to 7 days after trays with rooted cuttings were removed in 2011 and 2012. Recovery of binucleate *Rhizoctonia* from polyethylene fabric and gravel floors were sampled by rubbing a sterile damp synthetic sponge on the floor at 96 randomly selected spots from a defined grid layout. The sponge was placed in sterile tubes and plated on Ko and Hora agar in the laboratory. *Rhizoctonia* was identified by morphological traits and nuclei counted using a safarnin red staining procedure.

Experiment 2: Survival of *Rhizoctonia* over Six Weeks on Floors in Empty Propagation Houses
Fabric strips and gravel were inoculated with *Rhizoctonia*. Fabric strips were stapled to the bottoms of wooden frames. Gravel was set in wooden frames that had fabric bottoms. Both floor materials were exposed to factorial treatment combinations of (1) full sun or 70% shade, (2) ambient rain only or irrigation at 2-h intervals plus rain, and (3) free of organic matter or partially covered with peat. Substrates were sampled at 2-week intervals over 6 weeks and plated on Ko and Hora agar in the laboratory. *Rhizoctonia* was identified by morphological traits.

Experiment 3: Potential for *Rhizoctonia* to Grow from Infested Floor Surfaces into Trays Filled with Peat Where Stem Cuttings Are Being Rooted
Fabric strips (1/3 to 2.5 in. long) and gravel (1 to 6 pieces) were inoculated with *Rhizoctonia* and set beside or under rooting trays. *Rhododendron* 'Gumpo White' azalea cuttings were submerged in 51°C (123°F) water for 20 min, inserted in peat media for propagation, and placed under a daily 7-s mist duration at 15-min intervals from 7:00 to 19:00. Cuttings were maintained for 15 weeks, and then three cores of peat per tray were collected for isolation and plated on Ko and Hora agar in the laboratory. *Rhizoctonia* was identified by morphological traits.

RESULTS

Experiment 1: Recovery of *Rhizoctonia* from Propagation House Floors
Binucleate *Rhizoctonia* were recovered from 1 to 9% of 96 swab samples per an area containing a single cultivar on fabric floors and 3 to 9% of 96 swab samples per an area containing a single cultivar on gravel floors.

Experiment 2: Survival of *Rhizoctonia* over Six Weeks on Floors in Empty Propagation Houses
Absence or presence of peat and absence or 2-hour intervals of irrigation in addition to rain did not significantly influence *Rhizoctonia* survival. Binucleate *Rhizoctonia* recovery declined 75% under shade and 86 to 96% under full sun over 6 weeks.

Experiment 3. Potential for *Rhizoctonia* to Grow from Infested Floor Surfaces into Trays Filled with Peat Where Stem Cuttings Are Being Rooted
After 15 weeks, binucleate *Rhizoctonia* was not recovered from any trays regardless of inoculum level or placement. Of the inoculum placed beside and below the trays, *Rhizoctonia* was recovered from 60 and 94% of the inoculated substrates after 15 weeks in 2011 and 2012, respectively.

CONCLUSIONS
Binucleate *Rhizoctonia* does persist on fabric and gravel floors after rooted cuttings are removed from the propagation house. While the pathogen population declines over the 6 weeks houses are empty, some *Rhizoctonia* may still be viable. However, under normal rooting conditions, none of the *Rhizoctonia* grew into rooting trays in this study. Since *Rhizoctonia* is known to readily colonize dead azalea leaves, it is important to clean floor surfaces of organic matter. Once the organic matter is removed, the risk for rooting trays becoming contaminated appears to be low. Disinfestants are being evaluated for sanitizing floor surfaces of binucleate *Rhizoctonia*. Applications of disinfestants would be a precautionary step that likely is not needed, but would further reduce risk of contamination. With the results to date, the prospective looks favorable for producing azaleas free of binculeate *Rhizoctonia*. If this practice is followed, fewer infected plants will be present on the nursery each successive year that clean propagation material is generated. This approach could eliminate the need for fungicides to control web blight, although the fungus likely would never be totally eliminated from the nursery.

Research has not been done that identifies the predominate means by which *Rhizoctonia* is spread between container-grown azaleas on the nursery, thus the following discussion is speculative. Motorized pruning shears are likely a means for spread between blocks of azaleas. Advice would be to separately prune blocks of azalea that are infested with *Rhizoctonia* and those free of *Rhizoctonia*. Clean and sanitize pruners before pruning azaleas that are free of *Rhizoctonia*. Another possible means of spread is the dispersal of infested leaves during storms. A study is being considered to evaluate what wind speeds spread dead leaves colonized by *Rhizoctonia* across a nursery. Wind-blown leaves would probably result in small spread incidences, especially during severe storms. Natural means of spread are likely a key reason that *Rhizoctonia* cannot be totally eliminated from the nursery. Other means of spread could result from plant handling activities. As with pruning shears, the main concern is when working in plants free of *Rhizoctonia*. Hand washing and stiff brushes to remove organic debris from pants and boots may reduce the potential of this type of spread. One technique would be to schedule workers to complete work among plants free of *Rhizoctonia* first thing in the morning before work is done among other azaleas, hollies or any plant species that have had web blight symptoms.

Literature Cited

Copes, W.E. and Blythe, E.K. 2009. Chemical and hot water treatments to control *Rhizoctonia* AG-P infesting stem cuttings of azalea. HortScience 44:1370-1376.

Copes, W.E. and Blythe, E.K. 2011. Rooting response of azalea cultivars to hot water treatment used for pathogen control. HortScience 46:52-56.

Copes, W.E., Garcia-Rodriguez-Carres, M., Toda, T., Rinehart, T.A. and Cubeta, M.A. 2011. Seasonal prevalence of species of binucleate *Rhizoctonia* fungi in growing medium, leaf litter, and stems of container-grown azalea. Plant Dis. 95:705-711.

Daughtrey, M.L. and Benson, D.M. 2005. Principles of plant health management for ornamental plants. Annu. Rev. Phytopathol. 43:141-169.

Jones, R.K., Simone, G.W., von Broembsen, S.L. and Dutky, E. 2001. Integrated disease management. p.376-383. In: R.K. Jones and D.M. Benson (eds.), Diseases of Woody Ornamentals and Trees in Nurseries. APS Press, St. Paul, MN.

Williams-Woodward, J. and Jones, R.K. 2001. Sanitation: Plant health from start to finish. p.384-386. In: R.K. Jones and D.M. Benson (eds.), Diseases of Woody Ornamentals and Trees in Nurseries. APS Press, St. Paul, Minnesota.

Influence of Propagation Environment on Rooting of Sparkleberry (*Vaccinium arboreum*) Stem Cuttings©

Andrew B. Baker, James D. Spiers and Glenn B. Fain
Dept. of Horticulture, Auburn University, 101 Funchess Hall, Auburn, Alabama 36849, USA
Email: jds0017@auburn.edu

Eugene K. Blythe
Mississippi State University, Coastal Research and Extension Center, South Mississippi Branch Experiment Station, P.O. Box 193, Poplarville, Mississippi 39470, USA

Trials were conducted to determine whether propagation environment and/or substrate would encourage adventitious root formation of juvenile sparkleberry (*Vaccinium arboreum*) cuttings. The first experiment was designed in a 3×2 factorial to test the effects of three substrates (100% perlite, 2:1 perlite/ peat, 1:1 perlite/ peat) and two different environments ("mist tent" and "sweat tent") on rooting of softwood cuttings. The second experiment was designed in a 2×2 factorial to test substrate (Faford® 3B mix and a 2:1 peat/perlite mix) and ± wounding on rooting of hardwood cuttings in two separate environments (mist tent and sweat tent). Due to the low number of cuttings that rooted in all experiments, there were no significant effects of treatments on any of the parameters measured. Previous research indicates that the time of year cuttings are collected is a determining factor for successful vegetative propagation of sparkleberry.

INTRODUCTION

Sparkleberry (*Vaccinium arboreum*) has many potential uses in the landscape and fruit industry. Its uses in the commercial industry have been limited thus far by one crucial factor: *V. arboreum* is extremely difficult to vegetatively propagate. Sparkleberry has many unique landscape features including shade tolerance, exfoliating bark, berries for attracting wildlife, and great fall color. Sparkleberry also has many great features as a potential rootstock for cultivated blueberry species including, increased pH tolerance, drought resistance, and a single trunk growth habit for mechanical harvest. A successful method of asexual propagation is essential to future use in either industry.

Thus far, there has been very little research on sparkleberry propagation. Reese (1992) tested a range of auxin concentrations and found no effect on rooting. Stockton (1976) tested four levels of K-IBA on *V. arboreum* softwood cuttings with concentrations up to 20,000 ppm. No differences in rooting percentages were observed between any of the treatments. Bowerman (2012) tested a range of K-IBA concentrations on softwood, hardwood, and semi-hardwood cuttings. K-IBA concentrations up to 7,500 ppm were tested. Source and type of cutting were evaluated. Both factors were found to make a difference in rooting percentage. The highest rooting percentage (43%) occurred with the use of softwood terminal cuttings collected from water sprouts off of mature plants at the Robert Trent Jones Golf Course.

The objectives of this study were to determine the effects of substrate and environment on rooting percentages of *V. arboreum*.

MATERIALS AND METHODS

Both studies were conducted at the Paterson Greenhouse Complex, Auburn University, Auburn, Alabama. Two environments were evaluated in the two studies. The two environments were a mist tent" and a sweat tent". Rooting response (rooted or unrooted) was recorded for all cuttings, with a cutting considered rooted when any sign of adventitious roots were seen emerging from the stem. All cuttings were trimmed to 10-14 cm long.

Both environments tested were comprised of ½-in. PVC frames covered with white polyethylene film. The mist tents sat on top of expanded metal frames that were left uncovered at the base for drainage, while the sweat tents were completely enclosed by white polyethylene film. The mist tent was misted for 4 s every 10 min. The sweat tent was misted for 60 s at 8 AM and again at 1 PM.

Experiment 1

Experiment 1 was initiated on 9 Sept. 2012 using softwood cuttings. Terminal and sub-terminal cuttings were collected from Stone County, Mississippi (Lat. 30°80' N, Long. 89°17' W, U.S.D.A. Hardiness Zone 8b). Cuttings taken were juvenile cuttings arising from latent buds on mature plants that had been cut back to approximately 1 m in height in Feb 2012. This study was designed as a 3×2 complete factorial to test the effects of three substrates [100% perlite, perlite and peat (2:1, v/v), perlite and peat (1:1, v/v)] in two different environments ("mist tent" and "sweat tent"). The experimental design was a split plot design with environment as a main plot factor and substrate as a sub plot factor. There were four replications for each environment, and eight replications for each substrate. Each substrate contained two sub-samples, with six cuttings/ sub-sample. The mean day temperature in the mist tent was 18°C (65°F) ± 2.7°C (5°F). The mean night temperature was 16°C (62°F) ± 2.7°C (5°F). The mean RH was 97%. The mean day temperature in the sweat tent was 22°C (73°F) ± 5°C (9°F). The mean night temperature was 18°C (65°F) ± 2.7°C (5°F). The mean RH was 99%. Experiment 1 was terminated on 20 Dec. 2012. Additional data collected include number of cuttings that formed a callus, callus caliper (mm), number primary roots, and root length (cm).

Experiment 2

The second experiment was initiated on 28 Feb. 28, 2013 using hardwood cuttings arranged in a completely randomized design. Sub-terminal cuttings were collected from Robert Trent Jones Golf Course in Opelika, Alabama (lat. 32°69' N, long. 85°44' W, U.S.D.A. Hardiness Zone 8a). Cuttings were taken from water sprouts on mature plants. The experiment was designed in a 2×2 factorial to test substrate (Faford® 3B mix [Sun Gro Horticulture Ltd., Agawam, Massachusetts] and a perlite and peat (2:1, v/v) mix) and ± wounding in two separate environments (mist tent and sweat tent). There were 20 replications per treatment, with each cutting considered a replication. The mean day temperature in the mist tent was 24°C (76°F) ± 5°C (9°F). The mean night temperature was 22°C (72°F) ± 2.7°C (5°F). The mean RH was 92%. The mean day temperature in the sweat tent was 28°C (83°F) ± 8.3°C (15°F). The mean night temperature was 23°C (74°F) ± 4.4°C (8°F). The mean RH was 99%. Study 2 was terminated on 31 May 2013. Additional data collected include stem caliper, number of cuttings that formed a callus, callus caliper (mm), number primary roots, and root length (cm), number new leaves, number new shoots, and shoot length (cm).

Data was analyzed using generalized linear models with the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, North Carolina). Rooting and callusing was analyzed using the binomial distribution and a log link function, count data was analyzed using the negative binomial distribution and a log link function, and measurement data was analyzed using the normal distribution and the identity function.

RESULTS AND DISCUSSION

Rooting percentages ranged from 2-8.3% in Experiment 1 (Table 1). Due to the low number of cuttings that rooted, there were no significant effects of substrate, environment, or substrate*environment on rooting, number of roots, or total root length. Though callus percentage ranged from 29.1-54.1 among treatments, there were no effects of treatments on callus or callus caliper. Though Bowerman (2012) observed the greatest rooting percentages with softwood cuttings (~40%), the cuttings were collected in May in that study while the cuttings for this experiment were collected in September. Thus the propagation, e.g., air temperature and day length, were much different than those

experienced in this experiment. The time of year cuttings are collected, and/or the propagation environment of those cuttings may be important factors that contributed to the differences observed in rooting percentages in the present study compared to the study conducted on softwood cuttings by Bowerman et al. (2012).

Experiment 2 was designed to test wounding and substrate on rooting percentages of juvenile hardwood sparkleberry cuttings in two different propagation environments (mist tent and sweat tent). Very few cuttings rooted and there were no effects of substrate, wounding, or substrate*wounding on any of the data collected (Tables 2 and 3). Rooting percentages ranged from 0-7.5% in the mist tent environment (Table 2) and the sweat tent environment (Table 3). Though not significant due to the very low number of cuttings that rooted, percent rooting tended to be slightly higher in the perlite and peat (2:1, v/v) mix in both environments, perhaps due to increased water drainage. Previous research has demonstrated that hardwood cuttings of sparkleberry are very difficult to root. Bowerman (2012) observed the lowest rooting percentages when using hardwood cuttings compared to softwood and semi-hardwood. Rooting percentages in that study ranged from 0.7-10.6% for subterminal hardwood cuttings compared to 34.6-38.6% for softwood cuttings (Bowerman, 2012).

Substrates were included in the experiments presented to allow for differences in water-holding capacities in case water availability was an issue for root formation. However, substrate, wounding, and environment did not affect rooting percentages, as very few cuttings rooted in any of the experiments. The percentage of cuttings with callus formation ranged from 29-54% on softwood cuttings in Experiment 1 (Table 1) compared to 0-20% on hardwood cuttings in Experiment 2 (Tables 2 and 3). The higher percent callus formation observed on softwood cuttings may be indicative of rooting potential. Previous research has shown very little success with the vegetative propagation of sparkleberry cuttings to date. Most of the previous research conducted resulted in many treatments that do not affect rooting percentages, with the most significant finding reported by Bowerman (2012) that softwood cuttings collected in May resulted in 43.3% rooting. Thus far, rooting hormone treatments have proven to be ineffective for enhancing adventitious root formation of sparkleberry softwood, semi-hardwood, and hardwood cuttings (Bowerman, 2012; Reese, 1992; Stockton, 1976). Abscorbic acid appears to play a key role in root formation (Tyburski et al., 2006), and pre-treatment of Japanese stewartia (*Stewartia pseudocamellia*) with 0.1 M ascorbic acid prior to dipping cuttings in low IBA concentrations enhanced rooting percentages (Struve and Lagrimini, 1999). Research is currently being conducted to test the effectiveness of abscorbic acid ± K-IBA to enhance rooting percentages of softwood sparkleberry cuttings.

Literature Cited

Bowerman, J.R. 2012. Propagation of *Vaccinium arboreum* by stem cuttings for use as a rootstock for commercial blueberry production. Auburn Univ., Auburn, Alabama. Thesis.

Reese, J.C. 1992. Propagation of sparkleberry (*Vaccinium arboreum*) for use as a blueberry rootstock. Mississippi State University, Starkville, Mississippi. M.S. Thesis.

Stockton, L.A. 1976. Propagation and autoecology of *Vaccinium arboreum* and its graft compatibility with *Vaccinium ashei*. Texas A&M Univ., College Station, Texas. M.S. Thesis.

Struve, D.K. and Lagrimini, L.M. 1999. Survival and growth of *Stewartia pseudocamellia* rooted cuttings and seedlings. J. Environ. Hort. 17:53-56.

Tyburski, J., Jasionowicz, P. and Tretyn, A. 2006. The effects of ascorbate on root regeneration in seedling cuttings of tomato. Plant Growth Regul. 48:157-173.

Table 1. Effect of environment and substrate on rooting of juvenile sparkleberry (*Vaccinium arboreum*) softwood cuttings.[z]

Substrate	Environment	Callus (%)	Callus caliper (mm)	Rooting (%)	Roots (no.)	Root length (cm)
1:1 perlite/peat	Mist[y]	37.5	5.6	4.1	1.5	3.9
2:1 perlite/peat	Mist	33.3	4.3	2.0	1.0	3.5
100% perlite	Mist	29.1	3.8	4.1	1.0	0.4
1:1 perlite/peat	Sweat[x]	54.1	8.2	6.3	2.7	7.8
2:1 perlite/peat	Sweat	47.9	9.0	8.3	1.5	3.2
100% perlite	Sweat	43.7	5.4	6.3	3.3	3.7
Significance[w]		NS	NS	NS	NS	NS

[z] Softwood cuttings taken 20 Sept., 2012 from Stone County, MS. Juvenile cuttings were taken from latent buds on mature plants that had been cut back to approximately 1 m in height in Feb. 2012.

[y] "Mist tents" were covered with white polyethylene plastic and placed into intermittent mist 4 s every 20 min.

[x] "Sweat tents" were constructed using white polyethylene plastic on all sides with mist provided for 60 s at 8 am and 1 pm.

[w] Nonsignificant (NS).

Table 2. Effect of wounding and substrate type on rooting, callus formation, and new growth of sparkleberry (*Vaccinium arboreum*) hardwood cuttings in "mist tent"[z] environment.[y]

Substrate	Wound	Stem caliper (mm)	Rooting (%)	Roots (no.)	Root length (cm)	Callus (%)	Callus caliper (mm)	New shoots (no.)	Shoot length (cm)	New leaves (no.)
Faford® 3B mix	N	4.2	0	*	*	0	*	*	*	*
Faford® 3B mix	Y	4.5	2.5	3	2.0	0	*	8.0	5.0	63
Perlite:peat (2:1, v/v)	N	4.4	7.5	1	1.7	10	2.5	3.6	3.0	25
Perlite:peat (2:1, v/v)	Y	4.6	5.0	2	2.5	5	4.3	5.5	4.8	28
Significance[x]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] "Mist tents" were covered with white polyethylene plastic and placed into intermittent mist 4 s every 20 min.

[y] Hardwood cuttings were taken 28 Feb., 2012 from Robert Trent Jones Golf Course in Opelika, AL, cuttings taken from water sprouts of mature plants.

[x] Nonsignificant (NS).

Table 3. Effect of wounding and substrate type on rooting, callus formation, and new growth of sparkleberry (*Vaccinium arboreum*) hardwood cuttings in "sweat tent"[z] environment.[y]

Substrate	Wound	Stem caliper (mm)	Rooting (%)	Roots (no.)	Root length (cm)	Callus (%)	Callus caliper (mm)	New shoots (no.)	Shoot length (cm)	New leaves (no.)
Faford® 3B mix	N	3.8	0	-	-	0	-	0	-	0
Faford® 3B mix	Y	3.8	0	-	-	0	-	0	-	0
Perlite:peat (2:1, v/v)	N	4.4	7.5	5.0	4.2	5	4.2	3.6	3.6	30
Perlite:peat (2:1, v/v)	Y	4.6	2.5	4.0	5.0	20	4.7	5.0	4.0	30
Significance[x]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] "Sweat tents" were constructed using white polyethylene plastic on all sides with mist provided for 60 s at 8 am and 1 pm.

[y] Hardwood cuttings were taken on 28 Feb. 2012 from Robert Trent Jones Golf Course in Opelika, AL. Cuttings were taken from water sprouts of mature plants.

[x] Nonsignificant (NS).

Physical Properties of Varying Rain Garden Filter Bed Substrates Affect Saturated Hydraulic Conductivity©

Elizabeth D. Riley, Helen T. Kraus and Ted E. Bilderback
Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695-7609, USA
Email: edbridge@ncsu.edu

Both water flow through and retention time (Ksat) in filter bed substrates in combination with plants remediate polluted stormwater runoff in rain gardens. Two commonly used rain garden filter bed substrates were evaluated: sand (80% washed sand, 15% clay and silt fines and 5% pine bark v/v/v) and slate (100% expanded D-tank slate). Regression analyses showed that slate banded with increasing amounts of composted yard waste (CYW) resulted in a linear decrease in Ksat while, increasing amounts of pine bark (PB) banded resulted in a linear increase in Ksat. The amount of organic matter added to sand did not alter Ksat. Particle size distribution regression analyses showed that increasing the amount of CYW incorporated in sand caused coarse (>2.0 mm) size particles to increase linearly while, there was a quadratic effect on medium (0.5-2.0 mm) and fine (<0.5 mm) size particles. Amendment amount of CYW or PB with slate had no impact on particle size distributions in the coarse, medium, or fine particles.

INTRODUCTION

Stormwater control measures (SCMs) such as rain gardens (also referred to as bioretention cells) are designed to capture polluted stormwater runoff from impervious surfaces. Rain gardens are installed into the landscape by excavating the native soil, filling with an engineered filter bed substrate and planting, creating a depression to capture stormwater runoff and allow infiltration (Dietz, 2007). The environment created within the rain garden by the filter bed substrate and plants allows numerous remediation processes like adsorption, filtration, sedimentation, volatilization, ion exchange, plant uptake, and biological decomposition to occur (NCDENR, 2009).

Sand based filter bed substrates are recommended due to their suitable hydraulic conductivity (Ksat) (Davis et al., 2009; Hsieh and Davis, 2005). Pine bark (PB) is often used as an organic matter source in filter bed substrates to increase Ksat and denitrification; however, it does not provide much pollutant binding opportunities due to its low CEC. Drainage through the rain garden filter bed substrate needs to be slow in order to allow time for plant uptake and binding of the pollutants within the filter bed substrate. Little research has examined different sources and methods of adding organic matter to rain garden filter bed substrates. Rain garden filter bed substrates are combinations of soil and non-soil components and should be described by particle size distributions and Ksat (Kraus et al., 2014). Ksat values are preferred to be between 2.5-5.1 cm/h (NCDENR, 2009).

Sand filter bed substrates are heavy, expensive to ship, and may not be the best choice for stormwater remediation in rain gardens. Pledger (2012) found that slate as a rain garden filter bed substrate remediated all pollutants well in comparison to sand which remediated everything well except nitrogen (N). There are potential alternative filter bed substrates, organic matter sources, and methods of adding organic matter that can support plant growth and remediate polluted stormwater runoff similar to or better than the recommended sand filter bed substrates. The main objectives of this research were to: (1) Determine the role of different sources of organic matter and (2) Evaluate different combination methods and amounts of organic matter additions to filter bed substrates for their effect on particle size distribution and water and air filled fractions.

MATERIALS AND METHODS

An experiment was conducted as a randomized complete block design with a factorial treatment arrangement and three replications to address these objectives. Thirty-two substrates resulted from combinations of two filter bed substrates, two organic matter amendments, two combination methods, and four different organic matter amounts. The two filter bed substrates used were sand [80% washed sand, 15% clay and silt fines, and 5% pine bark (by vol)] (Wade Moore Equipment Company, Louisburg, North Carolina) and D-tank 100% expanded slate (Permatill, Carolina Stalite Company, Salisbury, North Carolina). Both sand and slate were amended with two different sources of organic matter: pine bark (PB) and composted yard waste (CYW) (City of Raleigh Yard Waste Recycling Center, Raleigh, North Carolina). Pine bark and CYW were added as either a band in the depths of 2.5, 5.1, 7.6 or 10.2 cm (1, 2, 3, or 4 in.) or by incorporation of 5, 10, 15, and 20% (v/v) (Fig. 1). Banding and incorporation applied approximately equivalent amounts of organic matter. Hydraulic conductivity was determined by packing each substrate into 1029 cm^3 (62.8 in^3) cylindrical polyvinyl chloride (PVC) columns (5.1 cm diameter, 50.8 cm height). For the banded treatments, 4 in. of either sand or slate were added to the bottom of the column, then the 2.5, 5.1, 7.6, or 10.2 cm (1, 2, 3, or 4 in.) band of CYW or PB was added before the column was topped off with either sand or slate. Columns were slowly saturated from the bottom and allowed to remain at saturation for 2 h. After this saturation period, water flow through the columns and out of an elbow fitting on the top of the column was caught for 5 min, measured, and used to calculate Ksat using Darcy's Law. Particle size distribution (three replications) was only determined for the incorporation combination method by placing oven dried samples of 350 g (12.4 oz) in a Ro-tap Shaker (Model B, W.S. Tyler, Mentor, Ohio) fitted with 13-sieve plates; 6.3 mm (0.25 in.), 4.0 mm (0.16 in.), 2.8 mm (0.11 in.), 2.0 mm (0.08 in.), 1.4 mm (0.06 in.), 1.0 mm (0.04 in.), 0.71 mm (0.03 in.), 0.5 mm (0.02 in.), 0.36 mm (0.01 in.), 0.25 mm (0.009 in.), 0.18 mm (0.007 in.), and 0.106 mm (0.004 in.) for 5 min. The sample from each sieve was weighed, and particle size was expressed as a percentage of the total weight of the sample. Percentages of total sample were then grouped into fine (<0.5 mm), medium (0.5-2.0 mm), and coarse (>2.0 mm) fractions as described by Drzal et al. (1999) for statistical analyses. All variables were subjected to regression analysis and were considered significant at $P \leq 0.05$ (SAS, 2001).

RESULTS

Slate amended by incorporation with either source of organic matter had higher Ksat values than sand (Fig. 2). Additionally, slate had larger percentages of coarse size particles and smaller percentages of fine size particles when compare to sand. Regression analyses showed that slate banded with increasing amounts of composted yard waste (CYW), from 2 to 10 cm (1 to 4 in.), resulted in a linear decrease of Ksat, while increasing amount of pine bark (PB) banded with slate resulted in a linear increase (Fig. 2A). Slate incorporated with PB at varying amounts also had a quadratic trend for medium particle sizes where 10% (45%) and 15% (45%) had the lowest percentages (data not shown).

There were no clear trends in Ksat when increasing the amount of organic matter added to sand by either banding or incorporating (Fig. 2B). Particle size distribution regression analyses showed that coarse (>2.0 mm) size particles in sand incorporated with varying amounts of CYW increased linearly (data not shown). Also, increasing the incorporated amount of CYW in sand had a quadratic trend on medium (0.5-2.0 mm) and fine (<0.5 mm) particle sizes. Sand incorporated with CYW at 10% (50%) had a higher percentage of medium sized particles than 5% (44%), 15% (45%), or 20% (42%). Fine size particles had the lowest percentage at 10% (40%) for sand incorporated with CYW than 5% (44.9%), 15% (42.5%), or 20% (45.1%).

DISCUSSION

Due to the nesting effect of different particle sizes within the filter bed substrate, particle size distribution impacts saturated hydraulic conductivity (Ksat) of rain garden filter bed substrates. Generally, slate has a larger percentage of coarse particles and less fine particles than sand allowing faster (at times too fast) water movement through the substrate. This effect is shown by the differences in Ksat between slate and sand. However, when slate is amended with a band of either organic matter source (PB or CYW), the Ksat is slowed compared to incorporation. Banding slate with 4 in. of CYW slowed Ksat significantly and to acceptable rates. Overall, slate generally has higher Ksat values than sand regardless of the combination method. However, banding of CYW with slate makes it have a slower, more comparable Ksat to sand banded with either organic matter source at any amount. Generally, Ksat of sand was not impacted by the amount of organic matter added for either banding or incorporation. Kraus et al. (2014) found that when using sand with an initial particle size distribution of 83% fine, 17% medium and 0.25% coarse particles, it needed to be amended to achieve a final particle size distribution of 67% fine, 30% medium, and 2% coarse to achieve adequate Ksat. The sand particle sizes for this study were all higher than these recommendations for incorporation with both organic matter sources and each increasing amount.

Literature Cited

Davis, A.P., Hunt, W.F., Traver, R.G. and Clar, M. 2009. Bioretention Technology: Overview of Current Practice and Future Needs. J. Environ. Eng. 135(3):109.

Dietz, M.E. 2007. Low impact development practices: A review of current research and recommendations for future directions. Water, Air and Soil Pol. 86:351-363.

Drzal, M.S., Fonteno, W.C. and Keith Cassel, D. 1999. Pore fraction analysis: A new tool for substrate testing. Acta Hort. 481:43-54.

Hsieh, C. and Davis, A.P. 2005. Evaluation and optimization of bioretention media for treatment of urban storm water runoff. J. Environ. Eng. 131(11):1521.

Kraus, H., Bilderback, T., Pledger, R., Riley, E., Fonteno, B. and Jackson, B. 2014. Defining rain garden filter bed substrates based on saturated hydraulic conductivity. Acta Hort. 1034:57-64.

North Carolina Division Environment and Natural Resources (NCDENR). 2009. Stormwater best management practice manual. <http://portal.ncdenr.org/web/wq/ws/su/bmp>.

Pledger, R.L. 2012. Remediation of urban stormwater pollution by three different filter bed substrates and plant effectiveness in pollution sequestration. North Carolina State University, M.S. Thesis.

SAS Institute, Inc. 2001. SAS/STAT User's Guide: Release 9.3 Edition, SAS Inst., Inc., Cary, North Carolina.

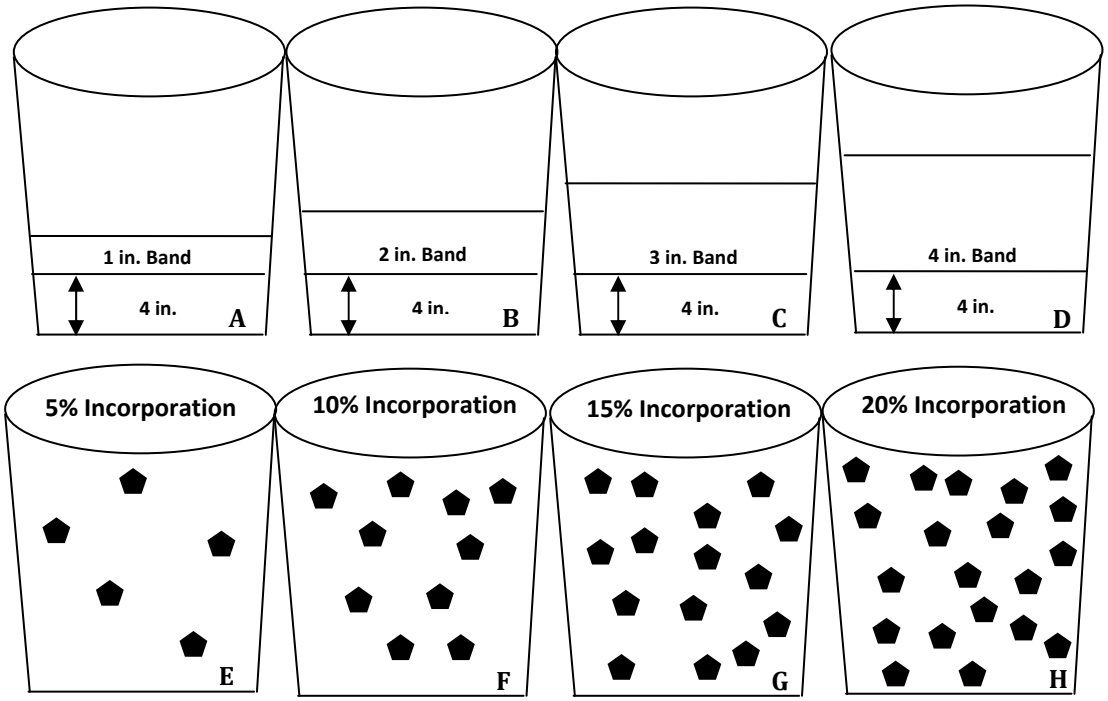

Fig. 1. Schematic of different filter bed substrate combination methods and organic matter amounts. The two organic matter amendments were added as either a band in the depths of 1, 2, 3, or 4 inches or by incorporation using approximately the same amounts of organic matter in the amounts of 5, 10, 15, and 20% (v/v). A: Combination method of banding with combination amount of 1 inch, B: Combination method of banding with combination amount of 2 inches, C: Combination method of banding with combination amount of 3 inches, D: Combination method of banding with combination amount of 4 inches, E: Combination method of incorporation with combination amount of 5%, F: Combination method of incorporation with combination amount of 10%, G: Combination method of incorporation with combination amount of 15%, and H: Combination method of incorporation with combination amount of 20%.

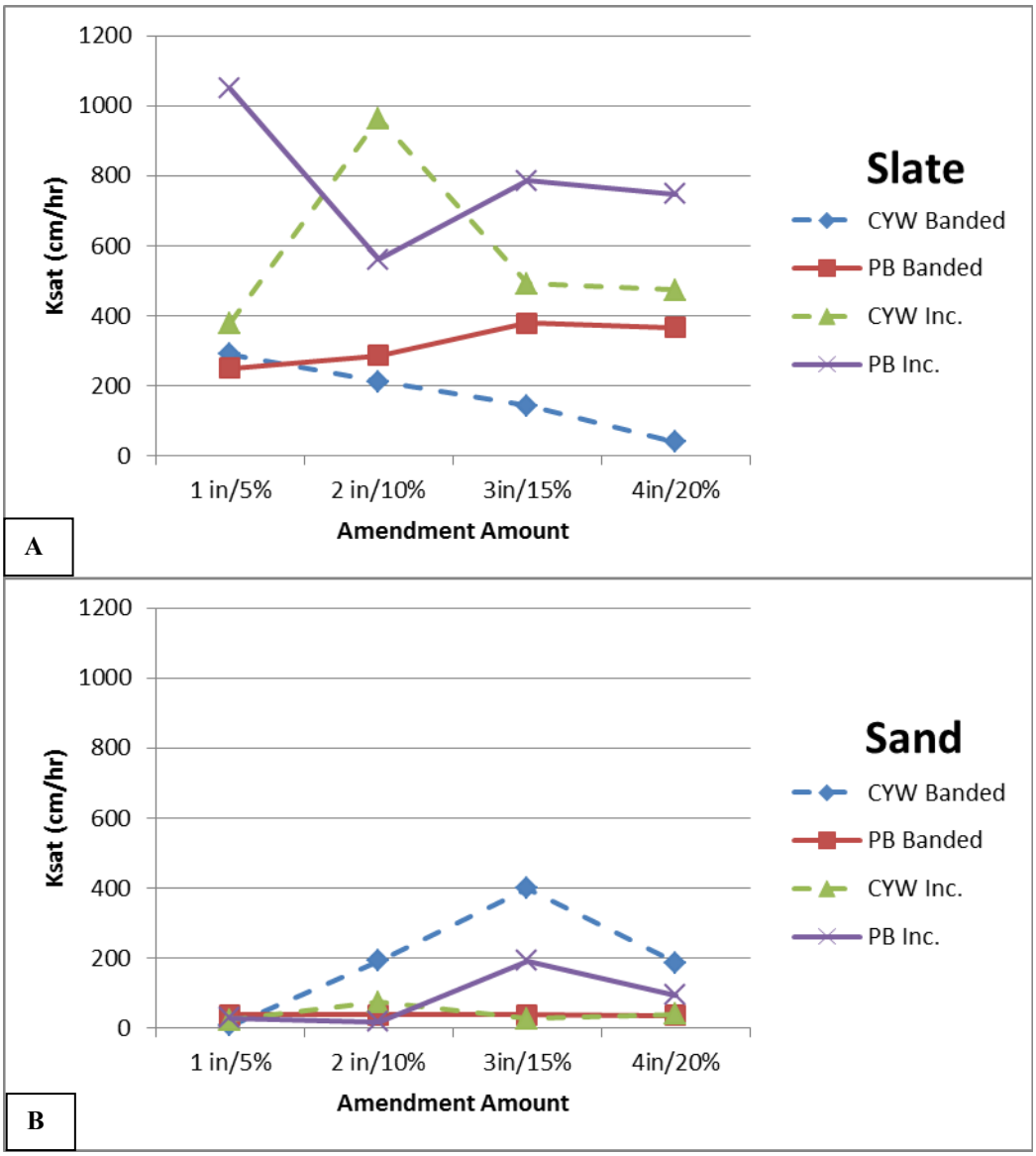

Fig. 2. Effect of amount of organic matter amendment on saturated hydraulic conductivity (Ksat) of a slate (A) and sand (B) rain garden filter bed substrate. The slate base was a 100% expanded D-tank slate. The sand rain garden filter bed substrate was a blend (v/v/v) of 80% washed sand, 15% clay and silt fines, and 5% pine bark. Organic matter amendments included pine bark (PB) and composted yard waste (CYW). Organic matter amendments were added as either a band in the depths of 1, 2, 3, or 4 inches or by incorporation using approximately the same amounts of organic matter in the amounts of 5, 10, 15, and 20% (v/v).

Seed Set and Germination for Interspecific and Intergeneric Hybrids in Two Genera of *Fabaceae*©

Susan M. Hawkins, John M. Ruter and Carol Robacker
Department of Horticulture, University of Georgia, Athens, Georgia 30602, USA
Email: ruter@uga.edu

Interspecific and intergeneric crosses were performed between species in the genera *Baptisia* and *Thermopsis* in an attempt to create hybrids with the best qualities of both parents. Interspecific crosses produced a higher percentage of fertile crosses and number of seeds per fertile cross than intergeneric crosses. Germination rate was not different between interspecific and intergeneric crosses. When comparing species to determine the best female parents, we found no difference between female parents for percentage of fertile crosses or germination rate. However, *Thermopsis* female parents produced a higher number of seed per cross than *Baptisia* female parents. When comparing species to determine the best male parents, crosses with *Thermopsis* male parents produced more seed per cross than those with *Baptisia* male parents, but were not different for percentage of fertile crosses or germination rate. Since seedlings could be obtained from both interspecific and intergeneric crosses, production of a *Baptisia-Thermopsis* hybrid is feasible. Steps to increase the percentage of fertile crosses and number of seedlings include new species as parents, use of bridge parents, embryo rescue, and selection of the male and female parents that produced the most fertile crosses for further breeding efforts.

INTRODUCTION

Interspecific and intergeneric crosses are often used to create new hybrids in ornamental plants. As well as combining good ornamental traits, wide crosses are also used to introgress such traits as disease or pest resistance, heat or cold tolerance, drought tolerance, or greater ease of propagation into a hybrid. *Artemesia*'s resistance to chrysanthemum aphids was conferred on a chrysanthemum (*Dendranthema morifolium*) by *Artemesia vulgaris* hybrid (Deng et al., 2010). A cross between chrysanthemum (*Chrysanthemum grandiflorum* (Ramat.) Kitamura) 'Zhongshanjingui' and *Artemisia vulgaris* produced a hybrid that rooted more easily than its chrysanthemum parent (Deng et al., 2012). Interspecific crosses between *Hibiscus paramutabilis*, *H. sinosyriacus*, and *H. syriacus* were made to incorporate the vigor of the first two species into *H. syriacus* (Van Laere et al., 2007).

In the genus *Baptisia*, interspecific crosses have been used to create many novel cultivars (Avent, 2002; Cullina, 2011). Another genus in the *Fabaceae*, *Thermopsis*, has not been used to create hybrids to our knowledge and is also not much known or used in the ornamental plant industry. Yet several species of *Thermopsis* have good horticultural qualities. Many species of *Thermopsis*, particularly *T. villosa*, are very tolerant of both drought and heat, making *Thermopsis* a good substitute for lupines in the Southeastern United States (Armitage, 1989). *Thermopsis* roots more easily than *Baptisia* (Hawkins et al., 2013) and will bloom 2 years after germination, whereas *Baptisia* requires 3 years to bloom from seed (pers. commun., Heather Alley, State Botanical Garden of Georgia, 2013).

To attempt to introgress these desirable traits into a hybrid between *Thermopsis* and *Baptisia*, intergeneric crosses were made between several species of *Baptisia* and *Thermopsis*. Interspecific crosses were also made, since such a cross might produce a hybrid of good ornamental quality, or one that might be used as a bridge parent for further intergeneric crosses.

MATERIALS AND METHODS

Three species of *Thermopsis* were used in the crosses: *Thermopsis chinensis*, *T. lupinoides*, and *T. villosa* (Table 1). *Baptisia australis* was the only species in its genus to

be used as the female parent. To increase the diversity of male *Baptisia* parents, pollen was collected from *B. alba*, *B. bracteata*, and *B. lanceolata* and used to make crosses (Table 1). *Baptisia australis*, *T. chinensis*, and *T. villosa* plants were obtained from Northcreek Nurseries, Inc., Landenberg, Pennsylvania, as seed-grown liners. *Thermopsis lupinoides* plants were vegetatively produced from a stock plant obtained from Plant Delights Nursery, Raleigh, North Carolina. Reciprocal crosses were made where possible. Since not all species had blooming periods that overlapped or that overlapped for only a brief time, this could not always be accomplished. The crosses were carried out in an enclosed shade house (50% shade) at the University of Georgia Horticulture Farm in Watkinsville, Georgia, from March to May 2013.

Flowers of the female parents were emasculated. Pollen was collected from the male parent onto a small paintbrush and used to pollinate the female parent. Crosses were tagged and seed pods were collected once ripe in May and June 2013. Seeds were extracted from the pods and counted. Seeds were scarified in 0.1 M sulfuric acid for 20 min, rinsed, and soaked for several hours in plain water to imbibe before being sown in a potting substrate containing bark, peat moss, and perlite. Germination data was taken at 4 weeks after sowing.

Statistical analysis on the fertility of the crosses, number of seed per cross, and germination rate was performed using SAS 9.3 (PROC GLM) (SAS Institute, Cary, North Carolina). Percentages were transformed before analysis to normalize data, and retransformed for reporting. Differences were considered to be significant at the level of P=0.05.

RESULTS

Interspecific crosses had a higher percentage of fertile crosses than intergeneric crosses (P=0.005). Number of seed per fertile cross was also higher in interspecific crosses (P=0.008). However, the germination rate was not different between interspecific and intergeneric crosses (P=0.238).

No differences in fertility were found among female parents in the percent of fertile crosses (P=0.760) or in germination rate (P=0.182). The number of seeds per fertile cross was different (P=0.019), with crosses having *Baptisia* as the female parent producing much fewer seed on average than any of the crosses having *Thermopsis* as the female parent. Intergeneric crosses were also compared to determine whether female parents differed in fertility. Average number of seeds per fertile cross was not different among female parents (P=0.084). Neither was germination rate (P=0.155) or the percent of fertile crosses (P=0.231).

Interspecific crosses were also compared to determine which male parents had highest percentage of cross fertility, germination rate, and seed per cross. We found no difference among male parents for percentage of fertile crosses (P=0.370) or for germination rate (P= 0.087). However, we found a difference in the average number of seeds per cross (P=0.008), with all crosses with a male *Thermopsis* parent having higher numbers of seed than crosses with a male *Baptisia* parent.

In intergeneric crosses, the male parent made no difference in percentage of fertile crosses (P=0.661), number of seeds per fertile cross (P=0.573), or germination rate (P=0.656).

DISCUSSION

Pre- or post-zygotic barriers between parents in interspecific and intergeneric crosses will often preclude fertilization of the ovule after pollination, resulting in low seed set. Since percentage of fertile crosses and number of seed per fertile cross was higher in interspecific crosses than in intergeneric crosses, barriers to fertilization seem to be much lower in the interspecific crosses. Though such a result is typical in many species, it is not always the case. Intergeneric crosses in brooms (genera *Genista* and *Cystisus*, family *Fabaceae*) showed greater fertility than interspecific crosses (Bellenot-Kapusta et al., 2006).

The number of seeds obtained per cross when *B. australis* was used as a female parent was less than when *Thermopsis* was used (Table 1). Apparently, this lower seed set is due to factors other than ovule number, as *B. australis* is reported to have numerous seeds per pod, as is *T. villosa* (Cronquist, 1980). Counts of 30 seeds per pod have been reported for *B. australis* (Evans et al., 1989). Southeastern *Thermopsis*, such as *T. villosa*, have been reported to have 12-16 ovules and Asian *Thermopsis*, such as *T. chinesis* and *T. lupinoides*, have been reported to contain 10-14 ovules (Chen et al., 1994).

Since the germination rates for interspecific and intergeneric crosses were not different, mature seeds from intergeneric crosses have a good chance of producing a viable intergeneric hybrid. The challenge for this breeding program is to increase the number of fertile crosses, especially intergeneric crosses. Several techniques could be used to reach this goal. Performing more crosses, especially more intergeneric crosses, would likely result in additional hybrids. Using more diverse genotypes within each species, storing pollen of an early-blooming species to use on a late-blooming species, or using different species in each genus as parents, might also be helpful. Reciprocal crosses should be evaluated for cross fertility, seed set, and germination rate to determine the best cross. For example, the *T. chinensis* × *T. lupinoides* cross had a lower percentage of fertile crosses compared to the reciprocal cross (21.1 vs. 44.0%), but a higher number of seeds per cross (12.1 vs. 8.4) and a higher germination rate (89.4 vs. 81.1%). If these averages held true for the next round of crossing, *T. lupinoides* would prove to be the superior female parent, as 100 crosses of *T. chinensis* × *T. lupinoides* would produce 228 seedlings while the same number of reciprocal crosses would produce 301 seedlings.

Techniques to overcome pre- and post-fertilization barriers could also be used to increase the percentage of fertile crosses. Treatment of the stigma of the female parent with calcium chloride and boron might increase fertilization (Jayavalli et al., 2011). Embryo rescue could also be attempted for intergeneric crosses that had low seed set, since fertilization was proven to be possible for these crosses. In some intergeneric crosses, such as that between *Ascocenda* and *Phalaenopsis* or between *Alstroemeria* and *Bomarea*, embryo rescue has been needed to produce viable progeny (Kashihara et al., 2010; Tsai et al., 2009).

Since putative interspecific *Thermopsis* hybrids were obtained, use of these hybrids as bridge parents should also be investigated. It is possible that cross fertility will be higher with the bridge parents than with the species used in the initial crosses. The blooming period for *T. lupinoides* and *T. chinensis* did not overlap substantially with the blooming period for *B. australis*, while that of *T. villosa* did. A hybrid between *T. villosa* and either *T. lupinoides* or *T. chinensis* might have an intermediate blooming period, making it a more suitable parent in an intergeneric cross with *B. australis*.

Much work remains to be done to create a desirable hybrid between *Baptisia* and *Thermopsis*. However, the initial results indicate that creating such a hybrid is feasible.

Literature Cited

Armitage, A.M. 1989. Herbaceous Perennial Plants: A Treatise on Their Identification, Culture, and Garden Attributes. Varsity Press.

Avent, T. 2002. Revenge of the 'Redneck Lupines'. Horticulture 99:70.

Bellenot-Kapusta, V., Pesteil, C. and Cadic, A. 2006. Diversity study and breeding of brooms (tribe of Genisteae). Acta Hort. 714:29-36.

Chen, C.J., Mendenhall, M.G. and Turner, B.L. 1994. Taxonomy of *Thermopsis* (*Fabaceae*) in North America. Ann. Missouri Bot. Gard. 81:714-742.

Cronquist, A. 1980. Vascular Flora of the Southeastern United States. Chapel Hill: University of North Carolina Press, 116 South Boundary Street, Chapel Hill, North Carolina 27514-3808.

Cullina, W. 2011. Gardener's challenge. Horticulture 108:14-19.

Deng, Y., Chen, S., Lu, A., Chen, F., Tang, F., Guan, Z. and Teng, N. 2010. Production and characterisation of the intergeneric hybrids between *Dendranthema morifolium* and *Artemisia vulgaris* exhibiting enhanced resistance to chrysanthemum aphid

(*Macrosiphoniella sanbourni*). Planta 231:693-703.
Deng, Y.M., Chen, S.M., Chang, Q.S., Wang, H.B. and Chen, F.D. 2012. The chrysanthemum × *Artemisia vulgaris* intergeneric hybrid has better rooting ability and higher resistance to alternaria leaf spot than its chrysanthemum parent. Scient. Hort. 134:185-190.
Evans, E.W., Smith, C.C. and Gendron, R.P. 1989. Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. Oecologia 78:220-230.
Jayavalli, R., Balamohan, T.N., Manivannan, N. and Govindaraj, M. 2011. Breaking the intergeneric hybridization barrier in Carica papaya and Vasconcellea cauliflora. Scient. Hort. 130:787-794.
Kashihara, Y., Hirano, T., Murata, N., Shinoda, K., Araki, H. and Hoshino, Y. 2010. Evaluation of pre-fertilization barriers by observation of pollen tube growth and attempts for overcoming post-fertilization barriers in intergeneric hybridization between Alstroemeria and Bomarea by ovule culture. Acta Hort. 855:159-164.
Tsai, C.C., Chiang, Y.C., Huang, S.C., Liu, W.L. and Chou, C.H. 2009. Intergeneric hybridization, embryo rescue and molecular detection for intergeneric hybrids between Ascocenda and Phalaenopsis. Acta Hort. 829:413-416.
Van Laere, K., Huylenbroeck, J.M. and Van Bockstaele, E. 2007. Interspecific hybridisation between *Hibiscus syriacus*, *Hibiscus sinosyriacus* and *Hibiscus paramutabilis*. Euphyt. 155:271-283.

Table 1. Interspecific and intergeneric crosses of *Baptisia* and *Thermopsis*.

Female parent	Male parent	No. crosses	Crosses with seed (no.)	Crosses with seed (%)	Seeds per cross	Seeds sown (no.)	Seed germinated (no.)	Germination (%)
T. chinensis	*T. lupinoides*	635	134	21.1	12.1	1,617	1,445	89.4
T. chinensis	*T. villosa*	130	25	19.2	9.9	248	158	63.7
T. chinensis	*B. alba*	25	3	12.0	7.3	22	19	86.4
T. lupinoides	*T. chinensis*	600	264	44.0	8.4	2,228	1,808	81.1
T. lupinoides	*T. villosa*	160	21	13.1	10.5	221	171	77.4
T. lupinoides	*B. australis*	10	0	0.0	0.0	0	0	0.0
T. lupinoides	*B. alba*	25	0	0.0	0.0	0	0	0.0
T. villosa	*T. lupinoides*	10	4	40.0	11.5	46	43	93.5
T. villosa	*B. australis*	459	46	10.0	1.4	63	51	80.9
T. villosa	*B. lanceolata*	15	3	20.0	5.7	17	13	76.5
T. villosa	*B. alba*	15	4	26.7	6.5	26	23	88.5
B. australis	*T. chinensis*	5	0	0.0	0.0	0	0	0.0
B. australis	*T. villosa*	340	41	12.1	1.0	42	36	85.7
B. australis	*B. lanceolata*	10	4	40.0	3.00	12	7	58.3
B. australis	*B. bracteata*	5	1	20.0	3.00	3	1	33.3