growth stimulators was caused by the fertilizer in these growth stimulators.

Up-Start also increased flowering of petunia from 34 to 40 DAT, while PGR IV and Roots2 increased flowering of petunia during the last week of the experiment (42 to 46 DAT) (Fig. 2). This is similar to the findings of Russo and Berlyn (1992), who found that the biostimulant Roots (similar to Roots2) had no effect on vegetative growth of green bean but increased its pod production. PGR IV was the only growth stimulator that increased flowering of impatiens. The flowering-promoting effect of PGR IV and Roots2 occurred when the plants were flowering vigorously, ≈ 10 d after the optimal marketing period for these crops. Thus, this study indicates that these growth stimulators are not of significant value to commercial growers, although the growth stimulators may improve plant performance after transplanting into the landscape.

Applying Up-Start resulted in larger plants with darker leaves. Since this may have been the result of the fertilizer component of Up-Start, similar results could probably have been achieved with higher fertilizer rates. Based on the results of this study, the use of Up-Start for greenhouse production of bedding plants does not seem beneficial, because higher fertilizer rates are less expensive than Up-Start applications.

All growth stimulators used in this study were developed to promote root growth, which may help minimize transplant shock. However, none of them significantly affected root growth under the conditions of this experiment. Since growing conditions in greenhouse production of bedding plants are designed to limit stress and optimize growth, transplant shock may be minimal. Therefore, these compounds may be more beneficial in the landscape, where environmental conditions are less favorable to the plants and transplant shock generally is more severe.

Literature cited

- Aldworth, S.J. and J. van 'Staden. 1987. The effect of seaweed concentrate on seedling transplants. *S. Afr. J. Bot.* 53:187-189.
- Bonner, J. and P.S. Devirian. 1939. Growth factor requirements of four species of isolated roots. *Amer. J. Bot.* 26:661-665.
- Crouch, I.J. and J. van Staden. 1994. Commercial seaweed products as biostimulants in horticulture. *J. Home Consumer Hort.* 1:19-76.
- Kontaxis, D.G. and D. Cox. 1984. Effect of vitamin B₁ on vegetable transplants. *Calif. Agr.* 38(9):15-16.
- Livingston, S.D., R.D. Parker, and J.A. Landivar. 1993. An evaluation of urea and PGR IV for maintaining yield potential of cotton subjected to water damage or nitrogen uptake deficiencies. In: *Proc. 1993 Beltwide Cotton Conferences*, vol. 3. Natl. Cotton Council Amer., Memphis, p. 1342-1343.
- Oertli, J.J. 1987. Exogenous application of vitamins as regulators for growth and development of plants—A review. *Z. Pflanzenernähr. Bodenk.* 150:375-391.
- Oosterhuis, D.M., A. Steger, and D. Zhao. 1996. Cotton seed treatment with plant growth regulators to enhance growth and yield. In: *Proc. Plant Growth Regulat. Soc. America. 23rd Annu. Meeting*, p. 282. (Abstr.)
- Russo, R.O. and G.P. Berlyn. 1990. The use of organic biostimulants to help low input sustainable agriculture. *J. Sustainable Agr.* 1:19-42.
- Russo, R.O. and G.P. Berlyn. 1992. Vitamin-humic-algal root biostimulant increases yield of green bean. *HortScience* 27:847.
- Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. *Ann. Bot.* 78:661-669.
- Styer, R.C. and D.S. Koranski. 1997. Plug and transplant production. A grower's guide. Ball Publishing, Batavia, Ill.

Abscisic Acid as a Potential Deciduous Fruit Tree Nursery Stock Defoliant

Fenton E. Larsen and
Stewart S. Higgins¹

ADDITIONAL INDEX WORDS. ABA, apple, European pear, defoliation, *Malus × domestica*, nursery practices, *Pyrus communis*

SUMMARY. Abscisic acid (ABA) was tested as a defoliant for nursery trees of 'Bartlett' pear (*Pyrus communis* L.) and the apple (*Malus × domestica* Borkh.) cultivars Imperial Gala, Gibson Golden Delicious, Scarlet Spur Delicious, Law Red Rome, Granny Smith, Braeburn, and Red Fuji. ABA was sprayed once or twice, with 1 intervening week, at 500, 1000, or 2000 ppm. Percentage defoliation was assessed at 1-week intervals for 4 weeks. For all cultivars, two applications of 2000 ppm ABA ranked among the most effective treatments for rapid defoliation; this treatment led to at least 95% defoliation for all cultivars. For many cultivars, however, other treatments caused similar defoliation percentages by digging time. All tested cultivars were effectively defoliated (>80%) by two 1000-ppm applications ABA or one 2000-ppm application. One or two 500-ppm applications effectively defoliated 'Bartlett', 'Gibson Golden Delicious', and 'Law Red Rome'. Nursery managers, therefore, need to consider a range of ABA concentrations and alternative application protocols to obtain optimum benefit from ABA. Although ABA shows promise as a defoliant, it lacks government approval for commercial use.

¹Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414.

Trees and labor were provided by Willow Drive Nursery and Van Well Nursery in Washington. Partial financial support was provided by funds from the Washington State nursery inspection programs, Washington State Dept. of Agriculture. ABA was provided by Toray, Inc., Tokyo. Statistical assistance was provided by R. Bendel, statistician, Center for Environmental Health, Univ. of Connecticut, Storrs. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Soon after the identification of abscisic acid (ABA) (Addicott et al., 1968; Cornforth et al., 1965; Ohkuma et al., 1965), its use as a defoliant for deciduous nursery stock was investigated (Larsen, 1969). Although ABA showed promise as a defoliant, the high cost precluded extensive experimental use and any possible commercial use. Current availability from a commercial source of agricultural chemicals (Toray Industries, Tokyo) rekindled interest in the defoliation potential of ABA. This work was undertaken to repeat, on a larger scale with currently available ABA, some of the early ABA defoliation work (early work used partial branch units only rather than complete trees) and to examine additional treatment protocol. Some newer, later-maturing, more difficult to defoliate apple cultivars were included in this work.

Materials and methods

The effect of ABA as a defoliant was studied at commercial nurseries during Fall 1994 and 1995. In 1994, one European pear and eight apple cultivars were treated. During 1995, one European pear and seven apple cultivars were used. Since the results were similar for both years, this report is confined to the second year's results. The cultivar/rootstocks combinations were 'Bartlett'/Old Home x Farmingdale 97, 'Imperial Gala'/O.3, 'Gibson Golden Delicious'/MM.111 EMLA, 'Scarlet Spur Delicious'/seedling, 'Law Red Rome'/MM.111 EMLA, 'Granny Smith'/M.26 EMLA, 'Braeburn'/M.7 EMLA, and 'Red Fuji' (BC#2)/O.3. Trees were treated at the end of their first growing season after budding.

The experimental design is presented in Fig. 1. Each cultivar occurred in a single nursery row. Contiguous three-tree plots were designated within the row, and the row was divided into two blocks that served as replications of each treatment. ABA [supplied from Toray as a 15% solution of s-(+)-abscisic acid] was applied at concentrations of 500, 1000, and 2000 ppm (a.i.) with 1250 ppm X-77 (Valent U.S.A. Corp., Walnut Creek, Calif.) added as a spreader. Within a cultivar and within a block, on 4 Oct. 1995, two adjacent plots were sprayed to runoff (spray volume per plot consequently varied with tree size and foli-

age density) with a trombone sprayer using a given concentration of ABA. For example, all six trees in Plots 6 and 7 of Block 1 received 1000 ppm ABA (Fig. 1). One week later, on 11 Oct. 1995, one of these two plots, i.e., the three trees of Plot 7, Block 1 (Fig. 1), was sprayed a second time with the same concentration of ABA. Within each cultivar, then, there were three concentrations of ABA, each concentration having been applied once or twice, for a total of six treatments, with two replications of each treatment, plus one untreated control plot in each block.

One week after the second spray was applied, and for three additional 1-week intervals, each tree in a plot was shaken vigorously to detach any weakly attached leaves. Then, a visual estimate was recorded of the percentage defoliation. All defoliation data were collected by the same investigator.

For statistical analysis, the arcsine transformation (Ott, 1984) was applied to raw defoliation percentages. Within each cultivar, the transformed defoliation data were analyzed as a repeated-measures experiment in a split-plot design (Neter et al., 1990), wherein time was treated as a subplot. Treatment effects were tested using the treatment $\times$ block interaction mean square as the error term. The mean of the transformed data for each treatment at each observation time was obtained using the LSMEANS statement in PROC GLM of SAS (Littell et al., 1991). For each cultivar, the LSD was calculated for a split-plot design (Little and Hills, 1978). After the analysis was completed, means were transformed back to defoliation percentages for presentation (Steel and Torrie, 1980).

Treatments were not assigned at random to the three-tree plots for three reasons. First, by always having the second application of a given ABA concentration adjacent to a single application of the same concentration, the number of borders between adjacent plots to which different concen-

trations were applied was minimized, thereby minimizing the potential for contamination due to drift. Second, plots were always arranged in order of increasing concentration to minimize the likelihood that sprays of 2000 ppm would drift onto adjacent control or 500-ppm plots (since 2000-ppm plots were never adjacent to control or 500-ppm plots). Our approach here is analogous to the well-known dilution series in plant nutrient experiments. Third, since this was one of a larger series of tests (Larsen and Higgins, 1997), there were many more treated plots than those reported here. Due to the necessity to replicate a large number of plots, it was logistically impractical to apply the treatments in random order; treatments occurred in the same order in both blocks. Considerable care was taken, however, to avoid drift. Before spraying, each plot was isolated from neighboring plots and from the adjacent row with a moisture-impermeable cloth shield that was 1.8 m tall.

Results and discussion

When discussing a preference for specific treatments for potential commercial use, the lowest rate of treatment that produced 80% to 90% defoliation over the observation period would normally be acceptable. While

Fig. 1. Experimental design used for a given cultivar. Individual trees within the nursery row are represented by Xs at the bottom of the figure; ellipses represent parts of the nursery row that were dedicated to complementary experiments or treatments that were not included in this report; three-tree plots are designated, P1, . . . , P9; 1x and 2x indicate whether a given concentration of abscisic acid (ABA) was applied once or twice, respectively; ABA concentration and block designations are self-explanatory. So, for example, Plot 8 in Block 2 contained three trees that were sprayed once with 2000 ppm ABA; Plot 5 in Block 1 contained three trees that were sprayed twice with 500 ppm ABA.

100% defoliation in a short time might be sought after, it is not a requirement by nurserymen, and, in fact, may not be desirable, e.g., a rapid response might be associated with greater damage or have a negative impact on carbohydrate accumulation.

For all cultivars except 'Red Fuji', the rate of defoliation during the observation period depended on chemical treatment. For 'Red Fuji', there was no significant interaction between chemical treatment and week of observation. There were, however, differences among chemical treatments, and the trees became increasingly defoliated during the observation period.

'BARTLETT'. By the second week of observation, 2000 ppm ABA applied twice caused >90% defoliation, and by the third week plots with 1000 ppm applied twice also exceeded 90% defoliation (Fig. 2). By the third week of observation, all treatments had caused more defoliation than observed among untreated trees. By the fourth week, only control trees retained many leaves.

While two applications of 1000 or 2000 ppm provided rapid defoliation, even the much less expensive treatment of 500 ppm applied once caused nearly 100% defoliation in time to allow digging before the usual onset of bad weather in the area.

'IMPERIAL GALA'. Defoliation of 90% was achieved by the first week of observation when 2000 ppm ABA was sprayed twice. No other treatment exceeded this level of defoliation until the fourth week of observation, when trees sprayed once with 2000 ppm or twice with 1000 ppm were >90% defoliated (Fig. 2). The latter treatment (1000 ppm twice), while having a slower response time, produced adequate commercial results.

'GIBSON GOLDEN DELICIOUS'. ABA at 2000 ppm caused rapid defoliation, but by the end of the 4-week observation period the only treatments that produced <90% defoliation were single applications of 500 and 1000 ppm (Fig. 2). A double application of 500 ppm appeared to give adequate commercial defoliation.

'SCARLET SPUR DELICIOUS'. Although several treatments caused significant defoliation relative to the untreated trees early in the observation period, no treatment exceeded 90% defoliation until the third week of observation (Fig. 2). By the fourth week,

plots with one or two applications of 2000 ppm and two applications of 1000 ppm had reached at least 90% defoliation. Two applications of 1000 ppm produced adequate commercial defoliation.

'LAW RED ROME'. Almost complete defoliation was achieved by the first observation date by 2000 ppm applied once or twice (Fig. 3; the line for 2000 ppm applied once is hidden in the figure). All chemical treatments were equally effective by the end of the observation period. Consistent with the thought that the lowest treatment to produce ≈90% defoliation during the observation period would be acceptable, one or two applications of 500 ppm produced adequate results.

'GRANNY SMITH'. The earliest high level of defoliation for 'Granny Smith' was achieved with two applications of 2000 ppm by the second week of observation. By the end of the observation period, one application of 2000 ppm and two applications of

1000 ppm caused defoliation similar to 2000 ppm applied twice (Fig. 3). Consequently, two applications of 1000 ppm produced adequate results. 'Granny Smith' was one of only two cultivars studied in which untreated trees retained all of their leaves at the end of the observation period.

'BRAEBURN'. Only two treatments, two applications of 1000 ppm or 2000 ppm, caused >90% defoliation by the third week of observation, although by the end of the observation period defoliation by one application

Fig. 2. Defoliation percentage for nursery trees of four tree fruit cultivars over a 4-week observation period in response to sprays of abscisic acid (ABA). ABA concentrations ranged from 0 (untreated) to 2000 ppm, and were applied either once (1x) or twice (2x). LSD, within or among observation dates, was calculated at $P = 0.05$, and is presented for each of the respective cultivars.

of 2000 ppm produced similar results (Fig. 3). Two applications of 1000 ppm produced adequate commercial results. As with 'Granny Smith', defoliation of untreated trees had not begun by the end of the observation period.

'Red Fuji'. Although the interaction between chemical treatment and time of observation was not significant ($P = 0.0961$), we present the results as for all other cultivars. Pooling the results for each chemical treatment over

all observation times would obscure individual treatments that exceeded 90% defoliation by the end of the observation period. Ninety percent defoliation was not noted until the third week of observation, and then only with two applications of 2000 ppm. No other treatment exceeded 90% defoliation even at the end of the observation period (Fig. 3). However, 1000 ppm applied twice was $\approx 90\%$ and would be an acceptable commercial treatment.

Substantial differences among cultivars were expected. Rootstock differences may have had some influence that could indirectly affect the rate of defoliation via plant nutrition. In 'Bartlett', however, there is scant evidence that rootstock substantially influences leaf mineral nutrition (Chaplin and Westwood, 1980; Woodbridge, 1973). Rootstock effects on apple leaf mineral status vary among orchards (Tukey et al., 1962) and between years

(Fallahi et al., 1984). Our past experience indicated that rootstock probably has a small influence on defoliation compared with the scion cultivar. We have also noted that some pears can be expected to defoliate faster (but are more subject to damage) than most apples and that apples that cease vegetative growth early, e.g., 'Golden Delicious', defoliate more rapidly than those that mature later, e.g., 'Granny Smith' or 'Braeburn'. Both observations were repeated here. Results may have been more uniform across cultivars if treatment applications had been timed to coincide with the physiological stage of the trees rather than with the calendar, since we have noted that chemically induced defoliation is more rapid and the tendency for damage is less once terminal buds have set. This stage is different for each cultivar and varies with weather, moisture, and nutritional status. The practical objective, however, was to force nursery stock to defoliate early enough to avoid bad weather. In central Washington (and similar areas), the useful period to accomplish chemical defoliation is typically from ≈ 15 Oct. to 15 Nov. Depending on weather and management, some cultivars continue to produce shoot growth during this period. However, inclement weather is typical after 15 Nov. Hand stripping is the traditional approach to remove leaves of cultivars that do not defoliate naturally before this time (i.e., most apples).

In general, the most effective defoliation treatment, regardless of consideration for the rapidity of effect, was two applications of 2000 ppm ABA. The prudent nursery manager, however, will consider not only the effect of treatment on defoliation but the substantial cost of ABA as a defoliant and the cost of two applications versus one. Also to be considered is the potential for damage that is increased with higher concentrations. Since 100% defoliation in a short period is not essential (nurserymen would willingly do some hand stripping since this is already the standard practice) and a lesser amount is potentially safer and less expensive, double treatments of 500 to 1000 ppm will often produce adequate commercial results.

The results of these tests support earlier work with ABA (Larsen, 1969), indicating its potential for nursery stock defoliation. In fact, the results achieved were perhaps superior to any observed

Fig. 3. Defoliation percentage for nursery trees of four tree fruit cultivars over a 4-week observation period in response to sprays of abscisic acid (ABA). ABA concentrations ranged from 0 (untreated) to 2000 ppm, and were applied either once (1x) or twice (2x). LSD, within or among observation dates, was calculated at $P = 0.05$, and is presented for each of the respective cultivars.

in previous work conducted by F.E. Larson. Refinements are still needed in application protocol. Unfortunately, cost and government approval are still major issues to resolve.

Literature cited

- Addicott, F.T., J.L. Lyon, K. Ohkuma, and W.E. Thiessen. 1968. Absciscic acid: A new name for Abscisin II (Dormin). *Science* 159:1493.
- Chaplin, M.H. and M.N. Westwood. 1980. Effects of *Pyrus* species and related genera rootstocks on mineral uptake in 'Bartlett' pear. *J. Plant Nutr.* 2:335-346.
- Cornforth, J.W., B.V. Milborrow, and G. Ryback. 1965. Chemistry and physiology of dormins in sycamore. *Nature* 205:1269-1270.
- Fallahi, E., M.N. Westwood, M.H. Chaplin, and D.G. Richardson. 1984. Influence of rootstocks and K and N fertilizers on leaf mineral composition and yield in a high density orchard. *J. Plant Nutr.* 7:1161-1177.
- Larsen, F.E. 1969. Promotion of leaf abscission of deciduous tree fruit nursery stock with abscisic acid. *HortScience* 4:216-218.
- Larsen, F.E. and S.S. Higgins. 1997. Deciduous fruit tree nursery stock defoliation with combination sprays of Alanap, Depeg and Ethrel. *J. Tree Fruit Prod.* (In press.)
- Littell, R.C., R.J. Freund, and P.C. Spector. 1991. SAS system for linear models. 3rd ed. SAS Institute Inc., Cary, N.C.
- Little, T.M. and F.J. Hills. 1978. Agricultural experimentation design and analysis. Wiley, New York.
- Neter, J., W. Wasserman, and M.H. Kutner. 1990. Applied linear statistical models regression, analysis of variance, and experimental designs. 3rd ed. Irwin, Boston.
- Ohkuma, K., F.T. Addicott, O.E. Smith, and W.E. Thiessen. 1965. The structure of Abscisin II. *Tetrahedron Lett.* 29:2529-2536.
- Ott, L. 1984. An introduction to statistical methods and data analysis. 2nd ed. Duxbury Press, Boston.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill, New York.
- Tukey, R.B., R. Langston, and R.A. Cline. 1962. Influence of rootstock, bodystock and interstock on the nutrient content of apple foliage. *Proc. Amer. Soc. Hort. Sci.* 80:73-78.
- Woodbridge, C.G. 1973. Effect of rootstocks and interstocks on nutrient levels in 'Bartlett' pear leaves, on tree growth, and on fruit. *J. Amer. Soc. Hort. Sci.* 98:200-202.

Effects of Soil Amendments and Tillage on Vesicular-Arbuscular Mycorrhizal Fungus Colonization of Citrus

S. Nemec¹

ADDITIONAL INDEX WORDS. symbiosis, *Glomus*, fertilizers, roots

SUMMARY. Three sites (A-C) were prepared for citrus groves in Florida from 1985 to 1986. Nine soil amendments applied at either one or two rates were deep-tilled 3.9 to 4.9 ft (1.2 to 1.5 m) deep and 4.9 ft (1.5 m) wide in the row before planting. Maximum treatment rates were limestone 50,000 lb/acre (56 000 kg·ha⁻¹), phosphoclay at 80,000 lb/acre (89 600 kg·ha⁻¹), humate at 77,612 lb/acre (86 912 kg·ha⁻¹), shrimp waste at 73,052 lb/acre (81 805 kg·ha⁻¹), peat at 250,000 lb/acre (280 000 kg·ha⁻¹), bentonite clay at 73,052 lb/acre (81 805 kg·ha⁻¹), mined gypsum at 2000 lb/acre (2240 kg·ha⁻¹), calcium humate at 2000 lb/acre (2240 kg·ha⁻¹), and phosphogypsum at 10,000 lb/acre (11 200 kg·ha⁻¹). Deep-tilled controls and a no-till control were established at sites A and B and a deep-tilled control at site C. A fourth grove (site D) was planted in 1970 and included the treatments surface tillage (ST), deep tillage (DT), and DT plus lime (DTL) at 45,407 lb/acre (50,848 kg·ha⁻¹). Mycorrhizal fungus infection was found in roots in 1987 at sites A and B and in 1989 in site C. Fungus infection ranged from 6% (no-till control) to 64% at site A, 64% to 81%

at site B, and 15% to 47% at site C. At all sites, amendments did not increase percentage infection and vesicle and hyphae ratings significantly over the deep-tilled control. At site A, percentage infection in the limestone treatment was the highest (64%) and was significantly higher than infection in phosphogypsum, peat, and the no-till control treatments. No treatment had a suppressive effect on infection. Mycorrhizal infection in roots was high (94% to 95%) at site D but did not differ significantly between treatments.

Organic and inorganic natural products as amendments to agricultural soils have affected soil chemistry and structure, plant disease development and plant growth, production, and development (Berry, 1982; Lumsden et al., 1983; Ozores-Hampton and Bryan, 1993; Roe et al., 1993; Sekiguchi, 1977). In view of the increasing interest in the use of organic products to replace fertilizers and pesticides to provide a safer environment, it is essential to learn more about their impact on beneficial soil microflora, which includes mycorrhizal fungi.

Most literature dealing with effects of composts, organic fertilizers, and amendments on vesicular-arbuscular mycorrhizal (VAM) fungi has reported studies done in controlled environments, such as pots and tubs (Guttay, 1982; Maronek et al., 1980; Menge et al., 1982; Nemec, 1992). Such studies are appropriate for commercial production in soils and soilless media in containers, but results are not necessarily applicable to field-plot production of crops. Although limited, studies of field soils treated with manures and composts indicate that organic manures stimulated a more normal development of VAM fungi in roots than inorganic forms of nitrogen and no fertilizer treatments (Azcon et al., 1978; Reed and Frémont, 1935; Sabet, 1945) and increased VAM fungus spore numbers in soil (Kruckelmann, 1975).

It is well established that high levels of mineral fertilizers can often result in a low level of mycorrhizal fungal infection (Hayman, 1982) in roots of plants in pots and the field. However, it has not been established what effect a wide range of soil amendments have on indigenous VAM fungi in crop plant roots in the field. A large selection of soil amendments has been added to soils

¹U.S. Department of Agriculture, Agricultural Research Service, 2199 South Rock Road, Fort Pierce, FL 34945-3138.

Mention of a trademark, warranty, proprietary product or vendor does not constitute a guarantee by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.