J Plant Growth Regul (1988) 7:19–26

Journal of
Plant
Growth
Regulation

XE-1019: Plant Response, Translocation, and Metabolism

J. P. Sterrett

U.S. Department of Agriculture, Agricultural Research Service, Bldg. 1301, Fort Detrick, Frederick, Maryland, USA

Received June 2, 1987; accepted September 9, 1987

Abstract. XE-1019 [(E)-1-(4-chlorophenyl)-4,4,-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] was injected into bean plants (*Phaseolus vulgaris* L. 'Black Valentine') at doses of 0.1–1000 μg/plant and caused reduced height growth, fresh weight, and leaf area 7 days after treatment. The sprout growth of California privet (*Ligustrium ovalifolium* Hassk.) was inhibited 52% by 10 μg and growth was further suppressed as the dose was increased to 100 μg, without injury. The shoot growth of American sycamore (*Platanus occidentalis* L.) and yellow-poplar (*Liriodendron tulipifera* L.) was progressively inhibited after 3 months as the injected dose of XE-1019 was increased from 2.5 to 240 mg/tree. Neither species was injured. Growth of 1-year-old trees of 'Golden Delicious' apple (*Malus domestica* Borkh) was inhibited 28 days after injecting the stem with 2 mg of ^{14}C-labeled XE-1019. At this time, 2% of ^{14}C activity has been translocated into the new shoots and 3% was present in the xylem and phloem of the scion. From 96 to 99% of ^{14}C-activity found in the xylem and phloem and 92% in the new shoot tissue chromatographed with XE-1019. This indicates that little degradation of XE-1019 occurred during the initial inhibition period.

The control of vegetative tree growth without causing injury is a major problem for utility company foresters and fruit growers (Williams 1984, Sachs et al. 1986). Treatment with growth retardants by injection into the tree trunk is a technique used to prevent chemical contact with nontarget plants and reduce environmental residues. This approach has had limited success in a variety of woody species, necessitating a search for more effective growth regulators (Brown et al. 1977, Miller 1982, Sterrett 1985, Sterrett et al. 1983). XE-1019 [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] is a new plant growth regulator that inhibits gibberellin biosynthesis as do ancy-

midol [α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine-methanol], paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol], and flurprimidol [α-(1-methylethyl)-α-(4-trifluoromethoxy)phenyl)-5-pyrimidinemethanol]. The reduced gibberellin level results in suppression of shoot internodal growth (Coolbaugh and Hamilton 1976, Dalziel and Lawrence 1984, Izumi et al. 1985, Sterrett and Tworkoski 1987). It is suggested that XE-1019 be applied to the roots, stem, or foliage (Chevron 1984). This study was designed to determine whether stem-injected XE-1019 would inhibit the growth of bean and several woody plants, and to determine the extent to which XE-1019 is translocated and metabolized in a woody species.

Materials and Methods

A randomized complete block design, replicated 5 times, was used in each of the following experiments, which were repeated unless otherwise noted.

Growth Inhibition

Bean seeds were sown in 10-cm-diameter pots filled with a mixture of loam:sand:peat moss:perlite (3:1:1:1 by volume) in a controlled environment chamber at 25 ± 1°C, 60 ± 10% relative humidity, and 158 μmol $s^{-1}m^{-2}$ PAR (400–700 nm) (16-h photoperiod). Ten days after emergence, beans were injected (Sterrett 1979) in the hypocotyl cavity with 100 μl of XE-1019 emulsifiable concentrate (EC) in water at doses ranging from 0 to 1000 μg/plant. Stem height was measured immediately before treatment and 7 days later. Leaf area at harvest was determined with a photoelectric leaf-area meter (Lambda Instruments Corp.).

Two-year-old California privet seedlings, with single 1-cm-diameter stems, were grown in the greenhouse in 1-liter pots filled with loam:sand:peat moss (1:1:1 by volume). Stems were injected (Sterrett 1979) with 10–10,000 μg of XE-1019 EC in 0.5 ml methanol 3 cm above the soil line. Immediately following injection, part of the main shoot was excised, leaving a 10-cm stump. The number and length of new stump sprouts were determined at intervals over a 3-month period after injection. A previous study (Sterrett 1979) with azosulfamide dye indicated that injected solutions were forced throughout the entire stump both above and below the injection site.

The trunks of 7-year-old yellow-poplar and 5-year-old American sycamore field saplings (Frederick, MD) were injected in late May with 6 and 4 ml, respectively, of XE-1019 in 60% methanol. The dose ranged from 2.5 to 240 mg/tree. Growth data were taken in August from 2-year-old wood on 3 branches pruned in late April.

Translocation

Apple trees (1 year old) were grown in half-strength aerated nutrient solution (Hoagland and Arnon 1950) in 7.3-liter containers and maintained in a con-

trolled environment at 25 ± 1°C, 65 ± 10% relative humidity, and 158 μmol $s^{-1}m^{-2}$ PAR (400–700 nm) (16-h photoperiod). Dormant trees were transferred from cold storage to nutrient solution and injected immediately with 2.0 mg of ^{14}C-XE-1019 (phenyl-ring labeled) (0.32 μCi/tree, specific activity 74.6 mCi/mmol) in 0.5 ml of methanol. The stem was injected 5 cm above the root/stem transition zone and the top pruned to 80 cm above the injection site. All buds on the scion were removed except those located in the top 20-cm section. One group of 4 trees was harvested and assayed immediately after injection. At 28 days after injection, when growth inhibition of the shoots was obvious, a second group of 4 trees was harvested and assayed. Harvesting consisted of separating each tree into 2 parts: (1) 25-cm-long rootstock containing the roots and the injection site, and (2) 60-cm-long scion containing buds or new shoot growth located in the top 20 cm. The wood (xylem), bark (phloem), buds, and new shoots were separated and assayed individually for activity. No buds or shoots were obtained from the rootstock. Fibrous and tap roots were assayed together. Harvested plant parts were immediately freeze dried, ground to 0.5 mm in a Wiley mill, and oxidized (Peterson 1969). Ground tissue samples from 3 trees were retained for extraction and chromatography. The amount of $^{14}CO_2$ evolved was quantitated by liquid scintillation spectrometry using Reich solution. In the repeat experiment, 500 μl of ^{14}C-flurprimidol in methanol (2.5 mg; 0.3 μCi/tree) was also injected into apple and assayed after 28 days.

Metabolism

Samples of the dried, ground xylem (1.0 g) and phloem (1.5 g) from the rootstock and complete shoots (2.5 g) from the scion of 1-year-old apple trees 28 days after injection with ^{14}C-XE-1019 were extracted with 80% methanol. Water was added and the extract was evaporated to an aqueous solution (pH 7.0), followed by partitioning with methylene chloride. Also, fortified extracts of xylem, phloem, and shoots obtained from untreated plants were chromatographed. Extracts were then applied to TLC plates (Redi-Plate Silica Gel GF, 250 μM; Fisher Scientific Co.) and the chromatograms were developed for 15 cm in chloroform:ethyl acetate:acetic acid (3:6:1 by volume). Developed chromatograms were quantified by an imaging scanner (Bioscan Inc.). The results of chromatography of extracts from 3 trees were pooled for data analyses.

Results and Discussion

Growth Inhibition

Posttreatment height growth and final fresh weight and trifoliolate leaf area of bean plants were reduced as the dose of XE-1019 increased (Fig. 1). Higher doses of XE-1019 (10–1000 μg/plant) caused epinasty of trifoliolate leaves and increased chlorophyll pigmentation in the leaf. Beans treated with 0.1 or 1 μg XE-1019 per plant appeared normal except that growth was inhibited.

The lowest dose of XE-1019 (10 μg/plant) inhibited sprout growth of Cali-

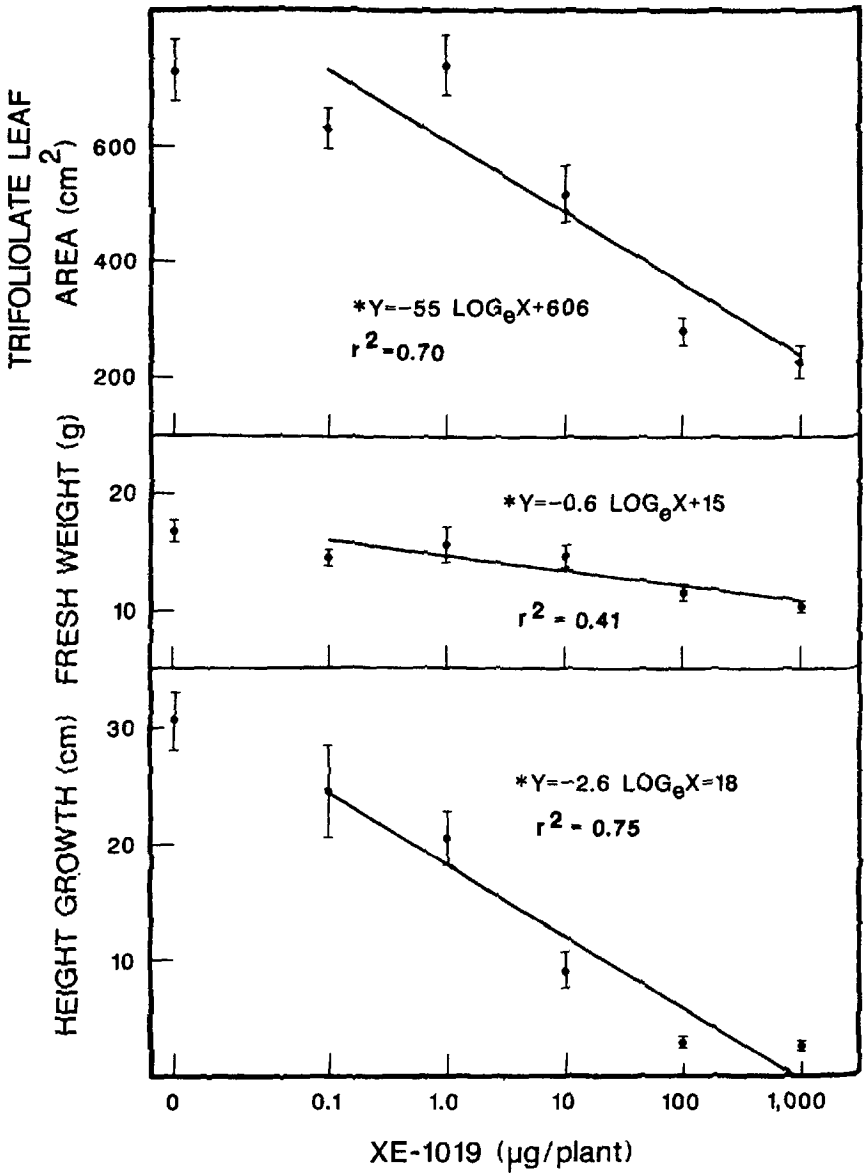

Fig. 1. Response of 10-day-old bean plants to stem injection of dilutions of XE-1019 of 100 µl in water 7 days after injection as derived from regression analysis. Vertical lines indicate standard error of the mean. Asterisk indicates significant linear regression coefficient, 5% level.

fornia privet 52% and growth was further suppressed as dose was increased (Fig. 2). Foliage of treated privet seedlings was smaller and darker green than that of untreated seedlings. These effects were also observed when California privet was injected with paclobutrazol or flurprimidol (Sterrett 1985, Sterrett

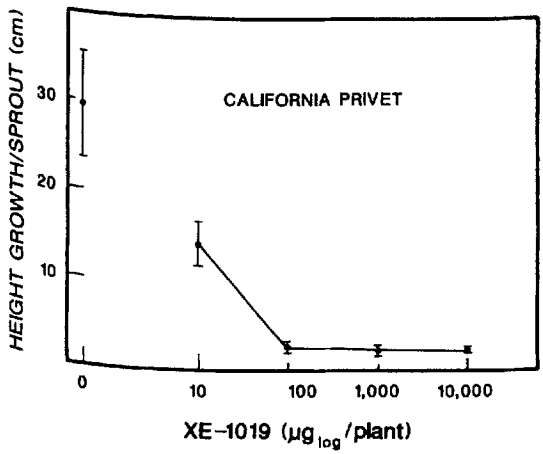

Fig. 2. Sprout growth from California privet stumps 77 days after injection of dilutions of XE-1019 EC in 0.5 ml methanol. Vertical lines indicate standard error of the mean.

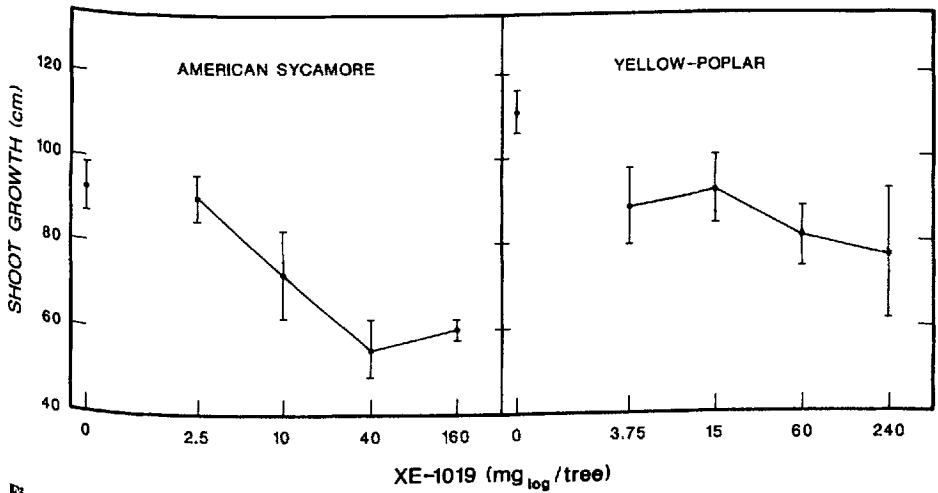

Fig. 3. Growth per shoot of woody saplings in August (3 months) after injections of dilutions of XE-1019 EC in 60% methanol in May (4 ml American sycamore and 6 ml yellow-poplar). Vertical lines indicate standard error of the mean.

and Tworkoski 1987). There was no apparent injury to California privet from XE-1019 and, unlike bean, no leaf epinasty was evident.

Shoot growth of American sycamore decreased as the dose of XE-1019 increased and all doses injected into yellow-poplar inhibited shoot growth (Fig. 3). Neither species displayed symptoms of injury or malformation other than shortened shoots and smaller, darker green leaves. Shoot growth was not inhibited during the second growing season after treatment 12 months later (data not shown). Apparently, either the dose of XE-1019 was not high enough to provide inhibition beyond one growing season or XE-1019 had degraded. A similar lack of carryover activity was observed by Sterrett (1985) and Sterrett and Tworkoski (1987) on woody saplings treated with paclobutrazol or flurprimidol.

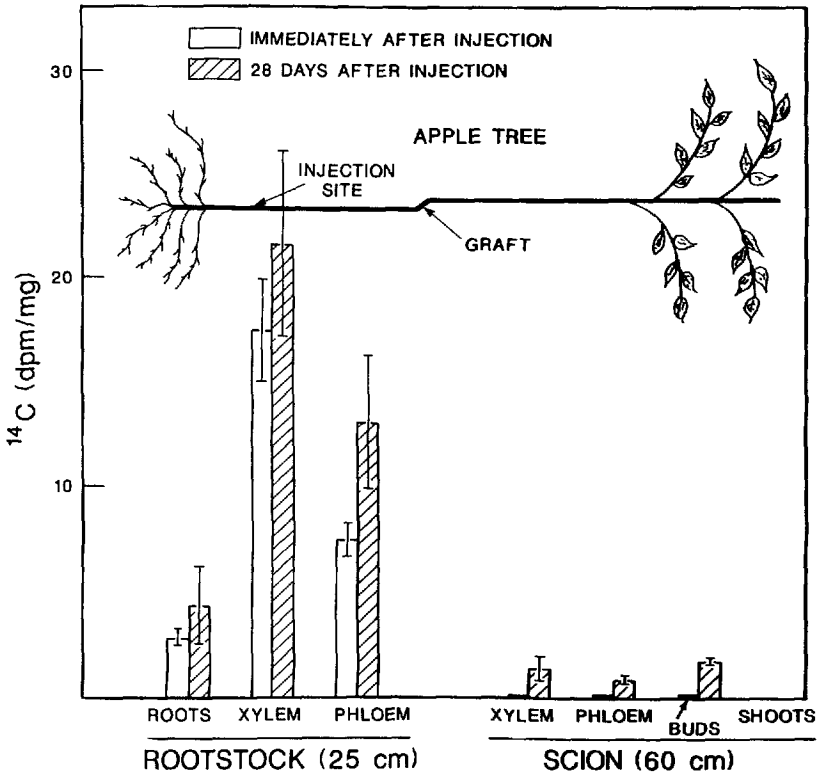

Fig. 4. Location of ^{14}C activity in young 'Golden Delicious' apple trees immediately and 28 days after injection with 2 mg of ^{14}C-XE-1019. Vertical lines indicate standard error of the mean.

Translocation and Metabolism

A high concentration of ^{14}C activity was found in the rootstock within 20 cm of the injection site immediately after injection (Fig. 4). The radioactivity in this region represented about 99% of that recovered. Most of the ^{14}C-XE-1019 was recovered from the rootstock xylem. The significant increase of ^{14}C activity in the rootstock after 28 days cannot be explained entirely on the basis of translocation because at the time of injection it was impossible to prevent some ^{14}C-XE-1019 solution from contacting the outer bark adjacent to the injection site. This makes it difficult to separate quantitatively the ^{14}C activity in the phloem tissue from other bark tissue (Fig. 4). After 28 days, when obvious growth inhibition of the injected trees had occurred, only about 2% of the ^{14}C activity was detected in the new shoots and 3% in the xylem and phloem of the scion. Standard error of the mean analyses indicated that there was no significant movement of ^{14}C into the roots. The slight increase in ^{14}C activity in the roots of trees harvested 28 days after injection versus trees harvested immediately after injection should not be interpreted as basipetal transport of XE-1019 because, in addition to the lack of significant movement (overlapping standard error lines), there was some variation in tree size. Although the trees appeared

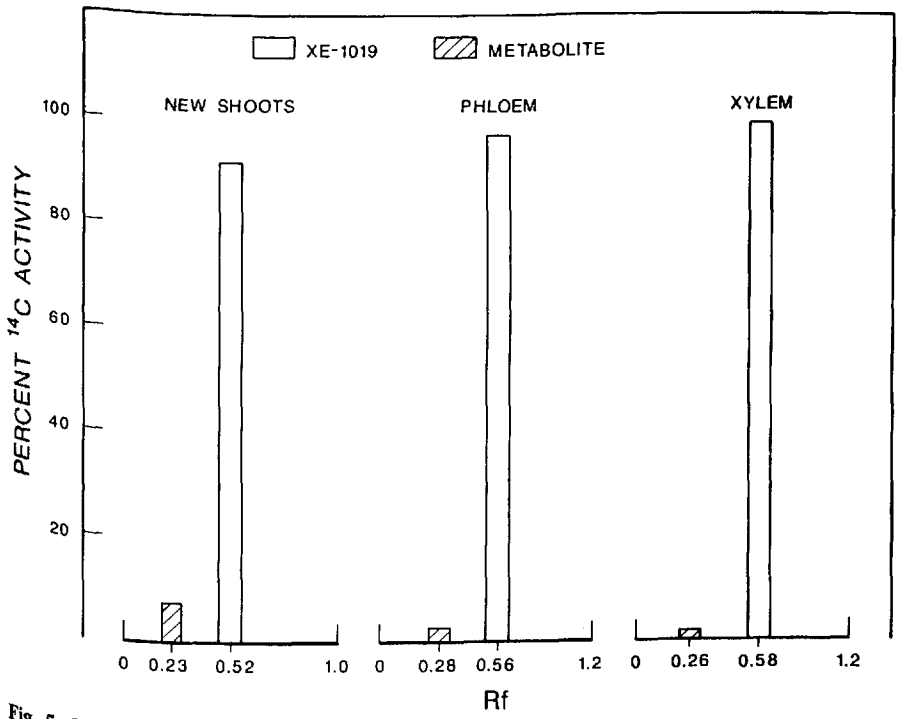

Fig. 5. Distribution of ^{14}C in extracts of xylem and phloem from rootstock and new shoot tissue from scion of apple trees 28 days after injection of XE-1019 (ring labeled). Methanolic extracts were thin-layer chromatographed for 15 cm in chloroform:ethyl acetate:acetic acid (3:6:1 by volume).

similar at treatment time, the rootstock dry weight of the trees harvested immediately after injection was slightly higher (by chance) than that of the trees harvested after 28 days, thus lowering the dpms/mg. Similarly, paclobutrazol and flurprimidol were not translocated basipetally (Sterrett 1985, Sterrett and Tworkoski 1987). The ^{14}C activity found in the roots was forced there by the pressure injector. The overall recovery of ^{14}C was 88%.

A high percentage of ^{14}C activity detected was XE-1019; 92% of the ^{14}C activity in new shoot tissue, 96% in the phloem, and 99% in the xylem chromatographed with XE-1019 (Fig. 5). One apparent metabolite of XE-1019 (R_f 0.23–0.28) was evident in all tissues. These results indicate that XE-1019 was translocated into the xylem and phloem of the scion and into the new shoots.

These results indicate that XE-1019 is stable during the initial 28-day period and not highly mobile. The translocation that did occur was acropetal similar to gibberellin biosynthesis inhibitors paclobutrazol and flurprimidol and was probably apoplastic. The presence of ^{14}C-XE-1019 in the phloem of the scion means that XE-1019 either was translocated via the phloem tissue directly from the rootstock or was translocated via the xylem to the upper stem from whence it was moved laterally into the phloem. Since there was no significant basipetal movement, the latter is the most plausible explanation. XE-1019 ap-

pears to be a more active growth retardant than flurprimidol per unit of active ingredients. Based on previous research with flurprimidol (Sterrett and Tworkoski 1987), and on the comparison of both growth regulators in this study, 4 times as much flurprimidol (20 μg/g) was found in inhibited apple shoot tissue as XE-1019 (5 μg/g).

XE-1019 should be an effective injectable growth inhibitor for woody plants. It is not phytotoxic. The only visible symptoms of XE-1019 presence in woody plants were smaller, dark green foliage and shortened shoots. Since there was no detectable basipetal movement of XE-1019, it appears to be transported primarily via the xylem similar to paclobutrazol and flurprimidol. Research is under way to determine the fate and metabolism of XE-1019 over a longer period since little degradation of XE-1019 occurred during the initial growth inhibition period.

Acknowledgments. The author thanks C. R. Baker, N. Monath, and L. Iype for technical assistance and the Chevron Chemical Company for furnishing samples of XE-1019 (uniconazole, Prunit, Sumagic, S-3304). The author also thanks J. G. Phillips for statistical assistance.

References

Brown GK, Kwolek WF, Wuertz DE, Jumper GA, Wilson CL, Carr SR (1977) Regrowth reduction in American elm and sycamore by growth regulator injection. J Am Soc Hortic Sci 102:748–751

Chevron Chemical Company (1984) Technical information bulletin. Ortho XE-1019 (S-3307), 3 pp

Coolbaugh RC, Hamilton R (1976) Inhibition of *ent*-Kaurene oxidation and growth by α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidine methyl alcohol. Plant Physiol 57:245–248

Dalziel J, Lawrence DK (1984) Biochemical and biological effects of kaurene oxidase inhibitors, such as paclobutrazol. Br Plant Growth Regul Group Monogr 11:43–57

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif Agr Exp Stn Circ 347 (rev edn)

Izumi K, Kamiya Y, Sakurai A, Oshio H, Takahashi N (1985) Studies of sites of action of a new plant growth retardant (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazole-1-yl)-1-penten-3-ol (S-3307) and comparative effects of its stereoisomers in a cell-free system from *Curcurbita maxima*. Plant Cell Physiol 26:821–827

Miller SS (1982) Growth and branching of apple seedlings as influenced by pressure-injected plant growth regulators. HortScience 17:775–776

Peterson JI (1969) A carbon dioxide collection assessory for the rapid combustion apparatus for preparation of biological samples for liquid scintillation analysis. Anal Biochem 31:189–201

Sachs RM, Campidonia M, Steffen J, Hodel D, Jauniaux M-P (1986) Chemical control of tree growth by bark painting. J Arbor 12:284–291

Sterrett JP (1979) Injection methodology for evaluating plant growth retardants. Weed Sci 27:688–690

Sterrett JP (1985) Paclobutrazol: a promising growth inhibitor for injection into woody plants. J Am Soc Hortic Sci 110:4–8

Sterrett JP, Tworkoski TJ (1987) Flurprimidol: plant response, translocation, and metabolism. J Am Soc Hortic Sci 112:341–345

Sterrett JP, Hodgson RH, Snyder RH (1983) Growth retardant response of bean (*Phaseolus vulgaris*) and woody plants to injections of MBR 18337. Weed Sci 31:431–435

Williams MW (1984) Use of bioregulators to control vegetative growth of fruit trees and improve fruiting efficiency. Am Chem Soc Book Ser no. 257, ch 9:93–99