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Morphological Changes in *Citrus* Associated with Relatively High Concentrations of Paclobutrazol

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Abstract. Progressively higher concentrations of Paclobutrazol markedly reduced germination of Valencia sweet orange (*Citrus sinensis* (L.) Osbeck) seed and induced significant changes in the morphology, growth, and development of roots of Valencia seedlings and rough lemon (*C. limon* (L.) Burm. f.) leaves. Threshold concentrations for significant visible effect to radical change ranged from 10^3 to 10^5 ppm (2.84×10^{-4} to 2.84×10^{-2} M) (ai). Initial change was readily evident in reduced lateral and fibrous root development at the lower concentrations (10^3 ppm). Higher concentrations (10^5 ppm) resulted in no secondary root formation and progressive basal enlargement terminating in a bulbouslike apex of the primary root. Lack of secondary roots suggests disruption in the pericycle or severe inhibition of meristematic initial cells. Root system changes were not visibly indicated in shoot growth other than strong inhibition of extensions.

Paclobutrazol (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol; ICI Americas, Inc.) is a relatively new plant growth regulator, which influences the growth of fruit trees (Williams and Edgerton 1983), ornamentals (McDaniel 1983), and other plants (Barrett and Bartuska 1982). Apparently, Paclobutrazol inhibits the biosynthesis of gibberellins (Graebe 1982), which in turn affects tree size (Quinlan 1981).

Much of the initial work on controlling tree size and increasing fruit production has been focused on apple trees (Curry and Williams 1983). Miller (1982) found Paclobutrazol the most effective growth inhibitor when pressure-in-

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jected into apple (*Malus domestica* Borkh.) seedlings. Paclobutrazol has also been used with some success on pear trees (*Pyrus communis* L.) (Raese and Burts 1983) and, more recently, *Citrus* (Aron et al. 1985). One of the most effective ways to achieve maximum tree response is to apply Paclobutrazol to the soil (Williams and Edgerton 1983).

Paclobutrazol is persistent in soils (Williams and Edgerton 1983; Couture, personal communication). In an earlier report we observed morphological changes occurring in the topmost roots exposed to a soil drench of Paclobutrazol (Bausher and Yelenosky 1986). Similar observations were made on apples by Atkinson and Crisp (1983). Paclobutrazol apparently inhibits the biosynthesis of GA at the same enzymatic sites as ancymidol, with similar specificity and efficiency (Hedden and Graebe 1985, Rademacher et al. 1984). The influence of Paclobutrazol on root systems is of interest since the levels of compound necessary for citrus growth inhibition are typically much higher than those reported for other plant systems (Bausher and Yelenosky 1986, Aron et al. 1985). The work presented here further studies the influence of high concentrations of Paclobutrazol on the growth and morphology of citrus root tissues.

Materials and Methods

Paclobutrazol

Paclobutrazol coded as GFU029 was obtained from ICI Americas Inc., Goldsboro, NC 27530, as a 50% (ai) wettable powder with a water solubility of 35 ppm. Aqueous-solution suspensions and 95% ethanol solutions ranged from 0 to 10^5 ppm Paclobutrazol in factors of 10. These concentrations were chosen because of earlier experiments showing the relative insensitivity of *Citrus* to Paclobutrazol. All trials were conducted under greenhouse conditions where maximum natural daylight exceeded 10^3 μmol · m^2 · s^{-1} (PAR); maximum day temperature reached 32°C, and minimum night reached 20°C; relative humidity ranged from 30 to 60% during the day and from 93 to 98% during the night.

Seed Germination

Valencia sweet orange and rough lemon seed were extracted from fruit of an open-pollinated tree in a citrus planting. Seeds were treated with 8-hydroxyquinoline sulfate and stored for 3 months at 4°C. Twenty-five seeds each were soaked for 30 min in five different aqueous solution–suspension concentrations of Paclobutrazol in Petri dishes. Treated seeds were planted in Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) and watered daily. Germination counts were based on visible emergence of seedlings during a 119-day period. Nongerminated seeds were considered viable if no discoloration or necrosis of the embryo was apparent at the termination of the experiment.

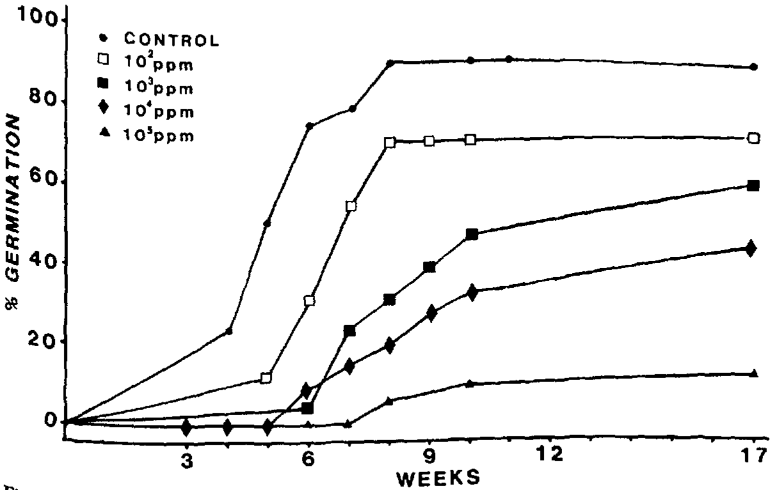

Fig. 1. Germination of *C. limon* seeds with the addition of Paclobutrazol. Experimental conditions listed in Materials and Methods.

Root Growth and Development

A 200-ml soil drench of equivalent concentrations of Paclobutrazol was used in seed germination trials and growth and development of citrus roots. The growing media was sterilized Astatula fine sand in 2.4-l pots containing one seed or seedling with 10 replicates per treatment. Soil-drench concentrations ranged from 0 to 10^5 ppm of Paclobutrazol (ai) per pot. Differences were based on visible observation of root extension, secondary root formation, root thickening, and root stimulation.

To further determine if Paclobutrazol influenced root formation, rough lemon (*C. limon* Burm. f.) leaves were planted in Astatula fine sand with three leaves per pot and five pots per treatment. Leaves were cut at the end of the petrioles, dipped in 0–10^5 ppm Paclobutrazol, and planted to a depth of about 5 cm. Continuous mist was applied 12 h/day for 6 months.

The anatomy of the root systems of treated and nontreated plants was examined 1 cm from the distal end of the seedling. Roots were fixed in a solution of 10% Formalin in 95% ethanol, dehydrated with a t-butyl alcohol series, and infiltrated with paraffin. Sections (12 cm) were stained with safranine and fast green and examined by light microscope.

Results

Paclobutrazol delayed the start, reduced the rate, and decreased the percentage of germination of Valencia orange seed during 4 months of observations (Fig. 1). Maximum delay was about 2 weeks at 10^3 ppm and higher concentrations. Without Paclobutrazol, total germination was essentially completed after 8 weeks, whereas seed soaked in the higher concentrations were

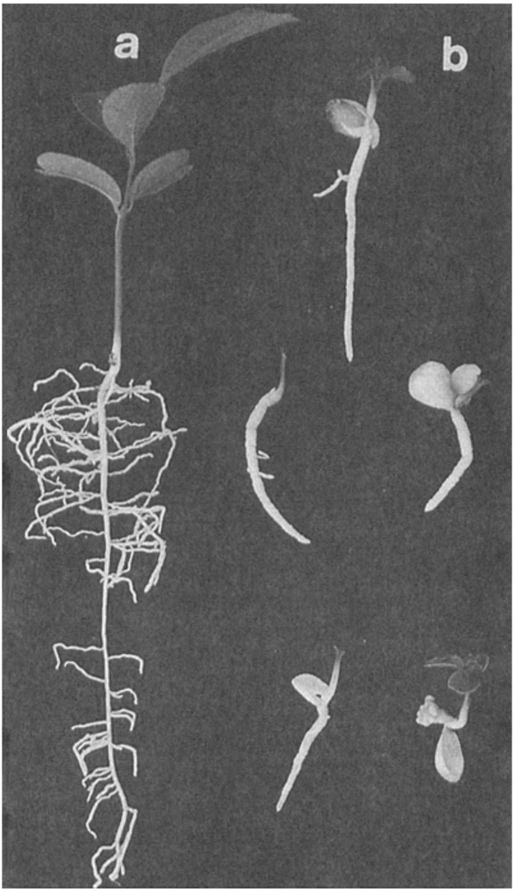

Fig. 2. Paclobutrazol-induced changes in the growth and development of Valencia sweet orange seedlings from seed soaked for 30 min immediately before planting in Astatula fine sand under greenhouse conditions for 17 weeks. (a) 0 ppm; (b) 10^5 ppm.

still germinating after 17 weeks. Rate of germination from planting to maximum number averaged about 12% per week for nontreated seed to as low as 3% for seed soaked in 10^5 ppm Paclobutrazol. Over the entire range of concentrations, total germination was decreased more than 50% with about a 10% decrease per 10-fold change in ppm. Neither the coefficient of absorption through the seed coat nor the total amount of Paclobutrazol absorbed into the seed during the soaking period is known. Also, adhesion of Paclobutrazol to the outside of the seed coat may be significant, especially at high concentrations, where gross changes occurred in growth and morphology of seedlings (Fig. 2). Thus these results are limited to surface exposure of seeds to soil application of Paclobutrazol.

These gross changes included cotyledons which were not necessarily hypogean (remaining in the soil during germination as usually occurs in citrus seed germination) (Frost and Soost 1968). The earlier extension of the plumule relative to the development of the primary root exemplified marked delays in the developmental stages of emerging seedlings. Seedlings from nontreated seed

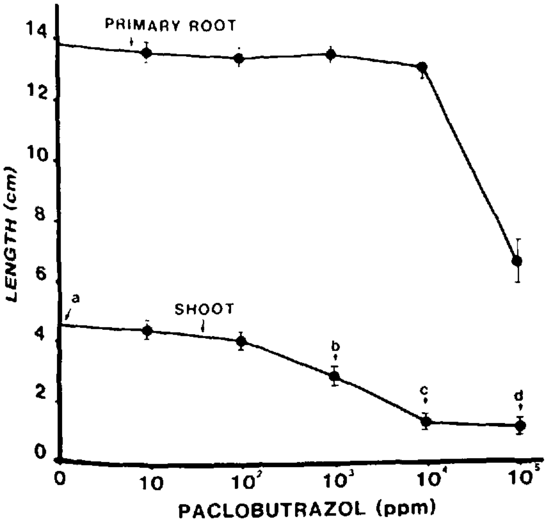

Fig. 3. Primary root and shoot extension of Valencia orange seedlings during 1 month in Astatula fine sand in 2.4-l pots; soil drenched with 200 ml of Paclobutrazol under greenhouse conditions.

were well into secondary root formation and three leaves beyond the first two true leaves; seedlings from treated seed (10^5 ppm Paclobutrazol) were still in the initial stages of plumule and primary root development. The primary root showed abnormal curvature not attributed to a tougher testa or a position difference of the embryo relative to gravity. Occasionally, rudimentary secondary roots were present but extremely low in number. Primary root extension was severely inhibited with abnormal thickening of the primary root (including the hypocotyl). In some instances, primary root extension did not occur beyond the hypocotyl region, although the plumule developed into the first two leaf stages. Overall, shoot development was severely inhibited and often did not reach a well-developed, two-leaf stage.

Paclobutrazol-induced inhibition of secondary roots was also found in tests where Valencia seed were planted in Astatula fine sand containing different amounts of Paclobutrazol applied as a soil drench (Fig. 3). Significant loss of secondary roots was first noted at 10^3 ppm soil drench of Paclobutrazol per pot and progressively worsened to virtually no secondary roots at the highest concentrations used (10^5 ppm). Inhibition of shoot extension (evident at 10^2 ppm) apparently preceded inhibition of primary root extension, which did not occur until 10^4 ppm. Highest concentrations used did not affect the downward orientation but did induce copious thickening of the primary root, which terminated in a bulbous rather than a cone-shaped root tip.

The amount of inhibition in shoot development does not necessarily reflect the growth and morphology of the roots growing in Paclobutrazol soil. This was evident in instances where soil drenches of Paclobutrazol were applied to 1-week-old Valencia seedlings. After the 10th week, shoot development was inhibited similarly for all concentrations used, but root systems were strikingly different (Fig. 4). At 10^3 ppm, absence of secondary roots was pronounced. At 10^4 ppm, secondary roots were severely inhibited until the primary root out-

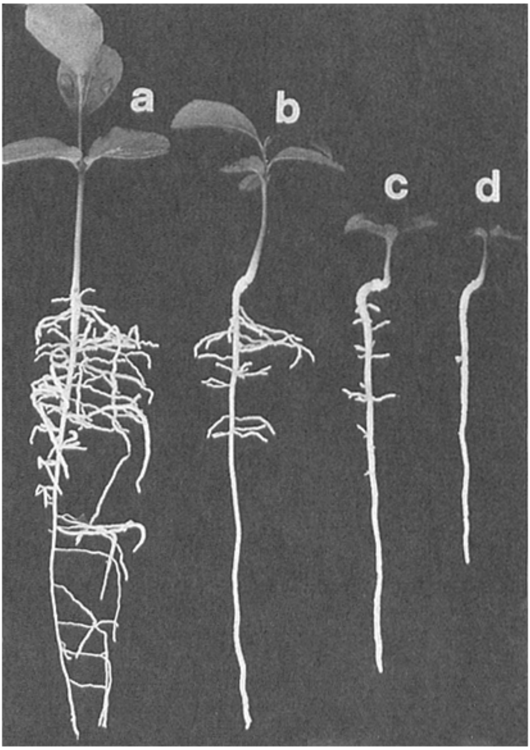

Fig. 4. Differences in root systems of Valencia orange seedlings growing for 10 weeks under greenhouse conditions in field sand in 2.4-l pots without (a) and with 200 ml PP 333 soil drench at 10^3 ppm (b), 10^4 ppm (c), and 10^5 ppm (d) when seedlings were 1 week old.

grew the critical threshold of Paclobutrazol at the bottom of the pot and/or periodic watering diluted Paclobutrazol in the soil. Apparently, once Paclobutrazol enters the primary root, the effects tend to persist in the areas of absorption (Bausher, unpublished). Strong, localized retention and relatively poor mobility are indicated. Paclobutrazol applied to seeds (10^5 ppm) affected the amount of lateral increase in size by the expansion of the cell surfaces in the cortex (Fig. 5). This increase in cortical cell diameter causes the thickening of the roots associated with the Paclobutrazol using the 10^5 ppm treatment. Interior portions of the roots are relatively the same size, showing the secondary phloem most apart in the treated root sections.

Paclobutrazol also induced roots to form from areas not generally regarded as rooting areas in citrus morphology. This was the case in the rooting of rough lemon leaves. Roots formed from the midvein of leaf petioles that had been dipped into 10^5 ppm Paclobutrazol for 2 min before they were in Astatula fine sand (Fig. 6). Although rooting occurs prolifically from basal callus, the midvein of citrus leaf petioles is not usually noted for root formation. In general, the roots arising from callus of leaf petioles dipped into Paclobutrazol were characteristically thicker and less prone to develop secondary roots than untreated leaf petioles. The occurrence of thickened roots arising from the midvein of leaf petioles is another indicator that Paclobutrazol is relatively immo-

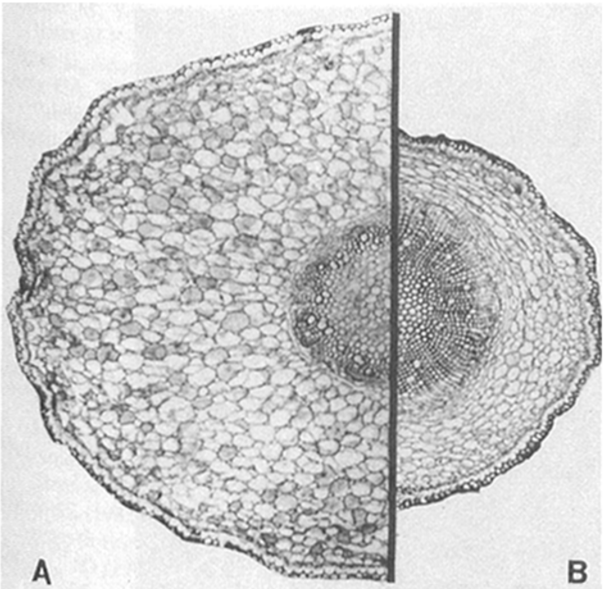

Fig. 5. Cross section of citrus roots taken 1 cm from the distal end at the same magnification (200×). (A) 10^5 ppm Paclobutrazol-treated. (B) Control.

bile in the vascular system of citrus owing to the changes seen in root morphology. The relatively low solubility, 35 ppm, of Paclobutrazol in water may or may not be directly related to the mobility in the plant.

Discussion

The results of this study suggest that the modifying effect of Paclobutrazol on root formation and morphology is new and additional (Atkinson and Crisp 1983, Steffens et al. 1983) to root elongation effects found for other fruit trees, but for lower concentrations of material (39 ppm) (Steffens et al. 1985). However, soil distribution and method of point of application of Paclobutrazol can markedly influence root growth of plants. Root growth may be inhibited or stimulated or may undergo morphological changes as emulated in this study. Citrus differs from deciduous crops where the cortex breaks down after 1–4 weeks (Atkinson 1980) in that the cortex of the root remains intact throughout its life span (Schneider 1968) if secondary growth does not occur. Plant species and cultivars apparently differ in this sensitivity to the plant growth regulator, and citrus roots are seemingly not sensitive to Paclobutrazol at levels found effective in other systems, and relatively high concentrations of Paclobutrazol in the soil show no signs of phytotoxicity in foliage. This is not the case with other antigibberellin compounds such as AMO-1618 or ancymidol, which can cause phytotoxicity in *Citrus* at concentrations far less than used in these experiments (Yelenosky 1979). This compound is interesting in that it has the

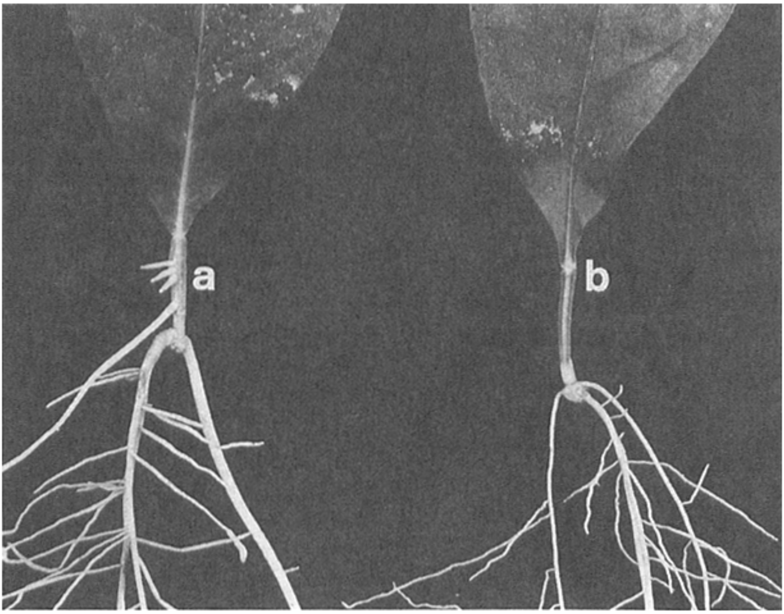

Fig. 6. (a) Paclobutrazol-induced root formation from midvein of a rough lemon leaf petiole and thicker roots from basal callus caused by dipping petiole into 10^5 ppm Paclobutrazol for 2 min before planting leaves 5 cm deep in Astatula fine sand in pots under a 12-h/day continuous mist for 6 months in a greenhouse. (b) Untreated leaf.

same mode of action as ancymidol (Dalzeil and Lawrence 1984), the inhibition of kaurene oxidase; however, the morphological changes have never been reported. Other compounds, such as homovanillic acid, induce the shortening of roots in citrus (Burger and Small 1983). The presence of this compound caused the swelling of the root tips of the treated plants at 180 μg/l. Treatment with 2,4-dichlorophenoxyacetic acid will produce hypocotyl and root apex swelling with adventitious root formation in hydroponic systems using kidney beans (*Phaseolus vulgaris*) (Taylor 1945). There exists the possibility that some contaminant exists in the technical material that may be responsible for the results found in this study. Further experimentation should investigate this possibility.

References

Aron Y, Monselise SP, Goren R, Costo J (1985) Chemical control of vegetative growth in citrus trees by Paclobutrazol. HortScience 20(1):96–98

Atkinson D (1980) The distribution and effectiveness of the roots of tree crops. Hort Rev 2:424–490

Atkinson D, Crisp CM (1983) The effect of some plant growth regulators and herbicides on root system morphology and activity. Acta Hort 136:21–28

Barrett JE, Bartuska CH (1982) PP 333 effects on stem elongation dependent on site of application. HortScience 17:737–738

Bausher M, Yelenosky G (1986) Sensitivity of potted-citrus plants to top sprays and soil applications of Paclobutrazol. HortScience 21:140–143
Burger D, Small JGC (1983) Alleopathy in citrus orchards. Sci Hort 20:361–375
Curry EA, Williams MW (1983) Promalin or GA_3 increase pedicle and fruit length and leaf size of "delicious" apples treated with Paclobutrazol. HortScience 18:214–215
Dalzeil J, Lawrence DK (1984) Biochemical and biological effects of kaurene oxidase inhibitors such as paclobutrazol. In: Menhennett R, Lawrence DK (eds) Biochemical aspects of synthetic and naturally occurring plant growth inhibitors. British Plant Growth Regulator Group, Monograph No. 11. Wantage, England, pp 43–57
Frost HB, Soost RK (1968) Seed reproduction: Development of gametes and embryos. In: Reuther W, Batchelor LD, Webber HJ (eds) The citrus industry, University of California Press, Riverside, pp 290–324
Graebe JE (1982) Gibberellin biosynthesis in cell-free systems from higher plants. In: Waring PF (ed) Plant growth substances (1982). Academic Press, London, pp 71–80
Hedden P, Graebe JE (1985) Inhibition of gibberellin biosynthesis by Paclobutrazol in cell-free homogenates of *Cucurbita maxima* and *Malus pumila* embryos. J Plant Growth Regul 4:111–122
McDaniel GL (1983) Growth retardation activity of Paclobutrazol on chrysanthemum. HortScience 18:199–200
Miller SS (1982) Growth and branching of apple seedlings as influenced by pressure-injected plant growth regulators. HortScience 17:775–776
Quinlan JD (1981) New chemical approaches to the control of fruit tree form and size. Acta Hort 120:95–106
Rademacher W, Jung J, Greabe JE, Schwenen L (1984) On the mode of action of tetcylclasis and triazole growth retardants. In: Menhenett R, Lawrence DK (eds) Biochemical aspects of synthetic and naturally occurring plant growth regulators, British Plant Growth Regulator Group, Monograph 11, Wantage, England, pp 1–11
Raese JT, Burts EC (1983) Increased yield and suppression of shoot growth and mite populations of "d'Anjou" pear trees with nitrogen and Paclobutrazol. HortScience 18:212–214
Schneider H (1968) The anatomy of citrus. In: Reuther W, Batchelor LD, Webber HJ (eds) The citrus industry, Vol 2, University of California Press, Riverside, pp 1–85
Steffens GL, Wang SY, Steffens CL, Brennan T (1983) Influence of Paclobutrazol (PP 333) on apple seedling growth and physiology. Proc Plant Growth Regul Soc Am 10:195–206
Steffens GL, Wang SY, Faust M, Byun JK (1985) Growth, carbohydrate, and mineral element status of shoot and spur leaves and fruit of "Spartan" apple trees treated with Paclobutrazol. J Am Soc Hort Sci 110(6):850–855
Taylor DL (1945) Observations on the growth of certain plants in nutrient solutions containing synthetic growth-regulating substances. I. Some effects of 2,4-dichlorophenoxyacetic acid. Bot Gaz 107(4):597–611
Williams MW, Edgerton LJ (1983) Vegetative growth control of apple and pear trees with ICI PP 333 (Paclobutrazol), a chemical analog of Bayleton. Acta Hort 137:111–116
Yelenosky G (1979) Sensitivity of budded young orange trees and grapefruit seedlings to ancymidol in soil mix. HortScience 14(5):600–602