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Gibberellin Biosynthesis Inhibitors: Comparing Growth-Retarding Effectiveness on Apple

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Abstract. The relative growth inhibitory activities of paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol]; XE-1019 [(E)-(1-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol]; flurprimidol [α-(1-methylethyl)-α-[4-(trifluoromethyloxy)-phenyl]-5-pyrimidine-methanol]; and triadimefon (a fungicide) [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] were evaluated and compared by treating the root zone of young greenhouse-grown tissue-culture-propagated 'Gala' (*Malus domestica* Borkh.) trees. At 0.25 mg/plant, only XE-1019 significantly reduced new stem length and number, area, and dry weight of leaves after 115 days. Paclobutrazol and flurprimidol both significantly reduced growth compared to controls when applied at 0.5 mg/plant, but XE-1019 was more effective. All three gibberellin (GA) biosynthesis inhibitors effectively retarded growth at a dosage of 1 mg/plant. Triadimefon applied at 10 mg/plant had essentially no effect on growth, but at 50 and 100 mg/plant it caused significant but less dramatic growth retardation when compared with the GA inhibitors. Major differences in effectiveness among the triazole GA biosynthesis inhibitors may be due to longevity of effect as well as to extent of inhibition.

A number of the commercially important plant growth regulators are retardants or inhibitors (Nickell 1982, Steffens 1980). Several are classed as gibberellin (GA) biosynthesis inhibitors and have uses or potential uses in commercial agriculture (Dalziel and Lawrence 1984, Jaggard et al. 1982, Jung 1984, Williams 1983). Examples include triazole compounds (Davis et al. 1987) rep-

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resented by paclobutrazol (Fig. 1, Cultar, Bonzi) (Sugavanam 1984), and the closely related XE-1019 (Fig. 1, Prunit, Sumagic) (Izumi et al. 1984). Other new triazole compounds also have plant growth-regulating activity (Rademacher and Jung 1986). Flurprimidol (Fig. 1, Cutless), a pyrimidine, has been shown to be a potent inhibitor of plant growth via the GA biosynthesis inhibition route (Hare 1984). Other potent GA biosynthesis inhibitors include ancymidol (A-Rest) [α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidinemethanol] (Jung 1984) and tetcyclacis (Kenbyo) [5-(4-chlorophenyl)-3,4,5,9,10-pentoaza-tetra-cyclo [5,4,1,$0^{2,6}$,$0^{8,11}$]dodeca-3,9-diene (Graebe 1982, Jung 1984). All have the ability to inhibit the oxidation of *ent*-kaurene to *ent*-kaurenoic acid in cell free systems (Coolbaugh and Hamilton 1976, Graebe 1982, Izumi et al. 1985, Sauter 1984).

Severl triazoles, including triadimefon (Fig. 1, Bayleton), inhibit ergosterol biosynthesis in fungi and have become commercially important fungicides (Sisler and Ragsdale 1985). They primarily inhibit the cytochrome P-450-dependent sterol C-14 dimethylation reaction in the conversion of lanosterol to ergosterol. When used as fungicides, they also may retard growth of the host plant.

The objective of this study was to compare relative growth-regulating activities of the structurally similar compounds (Fig. 1) paclobutrazol, XE-1019, flurprimidol, and triadimefon, especially with respect to residual activity.

Materials and Methods

Apple plants (cv. Gala) recently propagated via tissue culture techniques (Zimmerman and Fordham 1985) were greenhouse grown in 15-cm pots filled with equal portions of soil (sandy loam), peat, and perlite. When ~50 cm in height, individual plants were treated at the soil–stem interface with 10 ml of aqueous emulsions containing 0.2% Regulaid surfactant and the designated growth-regulating chemical (wettable powders) at dosages determined from preliminary experiments. Control plants received only the 0.2% surfactant solution. Each of 13 treatments was replicated 5 times to individual plants using a completely random block design. The plants were fertilized with a water-soluble 20:20:20 ($N:P_2O_5:K_2O$) formulation at weekly intervals. To monitor shoot elongation, the uppermost one-third fully expanded leaf was marked and new shoot growth measured at approximately weekly intervals. Leaves, stems, and terminal buds that formed above this leaf were designated "new shoots." Radiation sources in the greenhouse consisted of natural daylight and 400-W high-pressure sodium lamps that provided a PAR level of ~400–500 mol · s^{-1} m^{-2} for 12 h/day (07:00–19:00 hours). Temperatures were ~25/20°C (day/night). Plants were harvested 115 days after treatment and divided into various portions (Table 2). Leaf areas were determined with a Li-Cor 3000 area meter and dry weights were obtained after drying for 48 h at 68°C. Differences in growth of specified plant parts and slopes of growth curves were statistically analyzed by analysis of variance procedures and Duncan's multiple range test. Preliminary experiments showed that triadimefon was considerably less active

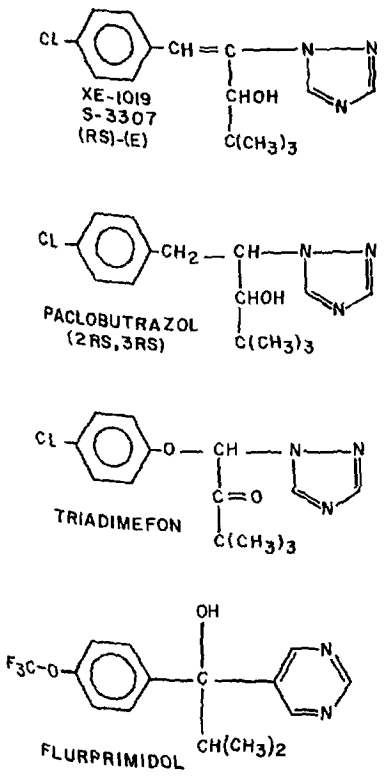

Fig. 1. Chemical structures of XE-1019, paclobutrazol, triadimefon, and flurprimidol.

for inhibiting growth of apple than the triazole GA inhibitors, so triadimefon dosages chosen were 10, 50, and 100 mg/plant verus 0.25, 0.50, and 1.0 mg/plant for the other three chemicals.

Results

Growth during the first 51 days following treatment with 0.25 mg (low dosage) of paclobutrazol, flurprimidol, and XE-1019 differed significantly from the control, but differences among the three chemicals were not significant (Table 1). However, between days 56 and 115, rate of growth of plants treated with XE-1019 remained unchanged whereas growth rates of plants treated with paclobutrazol or flurprimidol were more rapid and differed significantly from that of XE-1019. This low dosage of paclobutrazol and flurprimidol had no significant effects by the end of the 115-day treatment period on any of the measured growth parameters except new stem dry weight, which was reduced (Table 2). However, XE-1019 significantly reduced all parameters except dry weight of roots and specific leaf weight.

At the medium dosage (0.5 mg), growth patterns of plants treated with pa-

Table 1. Effect of root-applied paclobutrazol, flurprimidol, XE-1019, and triadimefon on growth rates (slope) of young tissue-culture-propagated apple trees, cv. 'Gala'.

Growth inhibitor	Dosage (mg tree^{-1})	Rate of shoot elongation (cm day^{-1})	
	Low	*Days 7–51*	*Days 56–115*
Control	0	1.44 a[a]	0.82 ab
Paclobutrazol	0.25	0.52 b	1.12 a
Flurprimidol	0.25	0.76 b	1.13 a
XE-1019	0.25	0.48 b	0.51 b
Triadimefon	10.00	1.44 a	0.75 ab
	Medium	*Days 7–70*	*Days 78–115*
Control	0	1.31 a	0.77 a
Paclobutrazol	0.50	0.20 c	1.03 a
Flurprimidol	0.50	0.20 c	0.75 a
XE-1019	0.50	0.10 c	0.02 b
Triadimefon	50.00	0.76 b	0.73 a

[a] Separation of means within each column at a given chemical dosage by Duncan's multiple range test (0.05 level).

clobutrazol and flurprimidol were similar (Fig. 2B). Compared with the low dosage (Fig. 2A), the inflection point for the initiation of rapid new growth for plants treated with paclobutrazol and flurprimidol was at about 70 days rather than 51 days. Plants treated at the medium dosage with XE-1019 did not elongate between days 78 and 115 (Table 1). However, all three chemicals at the medium dosage significantly reduced stem length and weight, and new leaf area and dry weight, but had no effect on leaf weight per unit area or root dry weight (Table 2). The number of new leaves was also reduced by flurprimidol and XE-1019. At this dosage, paclobutrazol and flurprimidol affected the growth parameters to about the same extent, but XE-1019 had a more marked effect on shoot length and leaf number, area, dry weight, and specific weight.

Growth was inhibited throughout the experimental period by 1-mg dosages of paclobutrazol, flurprimidol, and XE-1019 (Fig. 2C). Note that the growth curve for plants treated with 0.5 mg of XE-1019 (Fig. 2B) followed the same pattern as for XE-1019 applied at 1.0 mg (Fig. 2C). At this high dosage, all 3 GA inhibitors significantly reduced new stem length and dry weight as well as new leaf number, area, and dry weight compared to the control. In addition, XE-1019 significantly increased specific leaf weight as well as root dry weight.

As shown in Fig. 2A, triadimefon at 10 mg had no effect on shoot elongation. At the medium dosage (50 mg), plants grew more slowly than controls, but the growth rate from day 7 through day 115 remained rather constant (Fig. 2B). Triadimefon applied at 100 mg (Fig. 2C) was no more inhibitory to shoot elongation than 50 mg/plant. At the end of the 115-day treatment period, triadimefon applied at 10 mg had no significant effect on any of the growth parameters measured compared to the control (Table 2). At 50 and 100 mg, it reduced new stem length and dry weight and had minor effects on leaf weight, but markedly reduced root dry wight (by nearly 60% compared to controls).

Table 2. Effect of root applied paclobutrazol, flurprimidol, XE-1019, and triadimefon on selected growth characteristics of young tissue-culture-propagated apple trees, cv. 'Gala'—heights at treatment were ca. 50 cm; harvested 115 days after treatment.

Growth inhibitor	Chemical dosage (mg tree^{-1})	New shoot length (cm)	New stem dry wt (g)	Root dry wt (g)	New leaves			
					No.	Total area (cm^2)	Dry wt (g)	Dry wt (mg cm^{-2})
Low								
Control[a]	0	128 a[b]	35.4 a	16.1 a	56.6 a	2833 a	25.7 a	9.0 a
Paclobutrazol	0.25	109 a	21.2 b	11.6 a	55.4 a	2977 a	26.7 a	9.0 a
Flurprimidol	0.25	116 a	23.4 b	13.0 a	56.6 a	2854 a	26.1 a	9.1 a
XE-1019	0.25	64 b	8.7 c	17.0 a	47.8 a	1938 b	18.0 a	9.2 a
Triadimefon	10.00	126 a	33.3 a	12.7 a	56.4 a	2922 a	26.0 a	8.9 a
Medium								
Control[a]	0	128 a	35.4 a	16.1 ab	56.6 a	2833 a	25.7 a	9.0 ab
Paclobutrazol	0.50	70 b	8.9 c	9.8 c	53.6 a	2236 b	18.7 bc	8.4 b
Flurprimidol	0.50	57 c	6.1 c	10.9 bc	47.0 a	2022 b	16.0 c	8.1 b
XE-1019	0.50	21 d	1.7 c	17.5 a	16.2 b	580 c	5.9 d	10.1 a
Triadimefon	50.00	90 b	16.8 b	6.6 c	53.4 a	2277 b	20.9 b	9.2 ab
High								
Control[a]	0	128 a	35.4 a	16.1 b	56.6 a	2833 a	25.7 a	9.0 b
Paclobutrazol	1.00	30 c	3.1 c	13.3 b	41.2 ab	1425 b	12.3 b	8.8 b
Flurprimidol	1.00	13 d	1.4 c	17.8 b	23.2 bc	674 c	6.5 c	9.8 ab
XE-1019	1.00	20 cd	2.0 c	26.6 a	16.0 c	537 c	5.8 c	10.7 a
Triadimefon	100.00	95 b	22.8 b	6.9 c	57.2 a	2568 a	23.7 a	9.2 b

[a] Only one control treatment was included; the data are repeated for ease of making comparisons.

[b] Separation of means (n = 5) within each column at a given chemical dosage by Duncan's multiple range test (0.05 level).

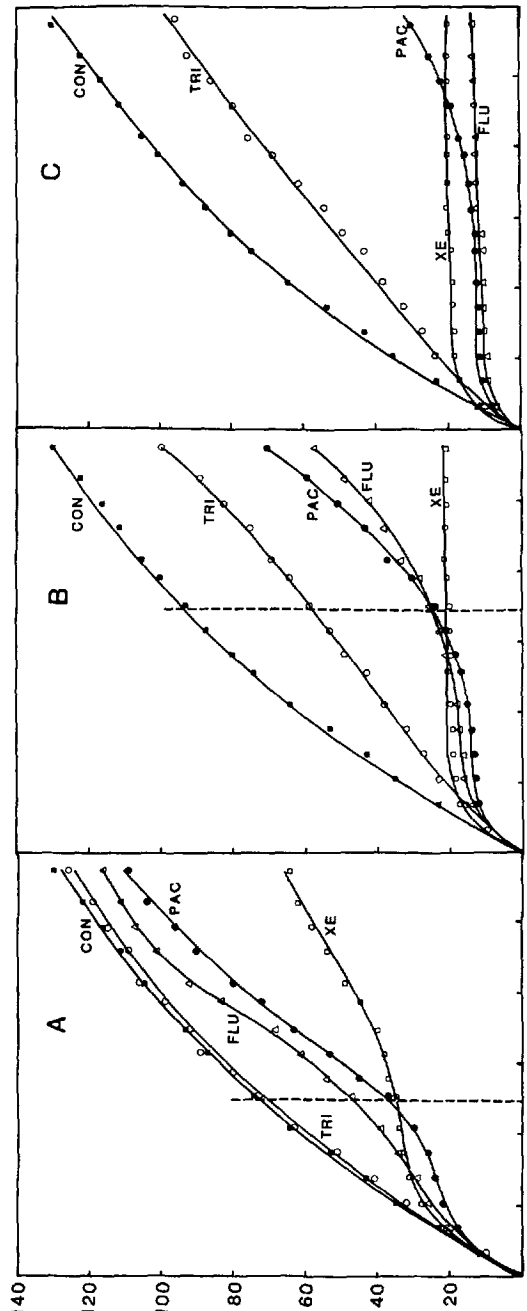

Fig. 2. Increase in length of shoots of greenhouse-grown 'Gala' apple trees: ■, control (CON); ●, paclobutrazol (PAC); △, flurprimidol (FLU); □, XE-1019 (XE); and ○, triadimefon (TRI). (A) Paclobutrazol, flurprimidol, and XE-1019 at 0.25 mg and triadimefon at 10 mg/plant. (B) Paclobutrazol, flurprimidol, and XE-1019 at 0.50 mg and triadimefon at 50 mg/plant. (C) Paclobutrazol, flurprimidol, and XE-1019 at 1.00 mg and triadimefon at 100 mg/plant.

Discussion

These results indicate that paclobutrazol, flurprimidol, and XE-1019, when taken up by roots, are all relatively active growth inhibitors for apple and, for a period of time, inhibit growth to about the same degree. The difference, especially between XE-1019 versus paclobutrazol or flurprimidol, is in residual activity rather than potency. This is most readily seen at the medium dosage (0.50 mg), where degree of inhibition of stem elongation for all 3 compounds was essentially the same for the first 70 days after treatment (Fig. 2B). XE-1019 inhibited growth over a longer period of time than did paclobutrazol or flurprimidol and thus may be considered to have more effectively inhibited growth. Comparison of the growth curve for XE-1019 at 0.25 mg (Fig. 2A) versus the growth curves for paclobutrazol and flurprimidol at 0.50 mg (Fig. 2B) suggests that paclobutrazol and flurprimidol were about 50% as effective as XE-1019 in these studies.

When inhibitor dosage was high enough, internode elongation nearly ceased for 6–8 weeks (see Fig. 2B), but leaf development progressed so the terminal portion of the plants bore rosettes of leaves. After internode elongation started, it was rather rapid for the low dosage of both paclobutrazol and flurprimidol compared to the control for the day-56 through day-115 period (Fig. 2A). Rapid growth rate after release from inhibition may be related to a buildup of GA precursors (Izumi et al. 1985) that may become available for rapid GA biosynthesis when the inhibitors are no longer present or have become diluted or inactivated. In addition, we have previously shown (Steffens et al. 1983, Wang et al. 1985) an accumulation of nonstructural carbohydrates in all parts of triazole-inhibited plants, which also would be readily available to support renewed growth.

With respect to relative effectiveness, it should be noted that the paclobutrazol used in these studies consists of a 50:50 mixture of 2 enantiomers, the 2*R*,3*R* and 2*S*,3*S* (Hedden and Graebe 1985, Sugavanam 1984). In cell-free systems of *Cucurbita maxima*, the 2*S*,3*S* enantiomer inhibited *ent*-kaurene oxidation more efficiently than did the 2*R*,3*R* (Hedden and Graebe 1985). The 2*R*,3*R* enantiomer, however, effectively inhibits the C-14 demethylation of fungi sterols (Baldwin and Wiggins 1984). Four stereoisomers are possible with XE-1019 because it has an asymmetric center and a trisubstituted double bond (Izumi et al. 1985). On rice seedlings, the (*RS*)-(*E*) form of XE-1019 (which is the form evaluated here) was only slightly less active than the (*S*)-(*E*) form and both of these forms were considerably more active than the (*R*)-(*E*) and the (*RS*)-(*Z*) forms. It has been shown that the *E* geometric isomer of XE-1019 is the biologically active form, and the *S* enantiomer causes growth-retardant activity whereas the *R* enantiomer is fungicidal (see review by Lenton 1987). Differences in activities of the isomers included as active ingredients in formulations of the paclobutrazol and XE-1019 evaluated may partially account for differences in effectiveness of these 2 tiazoles.

There is evidence that certain triazole isomers (Bladocha and Benveniste 1983, Buchenauer 1977, Burden et al. 1987, Dalziel and Lawrence 1984, Henry 1985, Sisler and Ragsdale 1985) as well as tetcyclacis (Nitsche et al. 1985) can alter or inhibit sterol biosynthesis in plants. Nitsche et al. (1985) and Lenton

(1987) suggest that compounds active as inhibitors of GA biosynthesis restrict cell elongation at low dosages, but at higher dosages may inhibit cell division by inhibiting sterol biosynthesis. Because it has only 1 asymmetric carbon atom, triadimefon can exist as 2 enantiomers and both have nearly the same fungicidal activity (Koller 1987). The carbonyl group on triadimefon, however, can be reduced in both fungi and plants to form the highly active fungicide triadimenol [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanol]. It, like paclobutrazol, can exist in 4 enantiometric forms. Triadimefon and triadimenol were found to have some plant growth-retarding activity, and Buchenauer and Rohner (1981) suggested retardation was the result of both gibberellin and sterol biosynthesis inhibition. Koller (1987) evaluated the 4 triadimenol enantiomers for growth retardation of wheat seedlings and suggested that inhibition of sterol biosynthesis might be the main target for growth retardation rather than gibberellin biosynthesis inhibition. Sterol biosynthesis inhibition results in an accumulation of sterol precursors with a possible consequent membrane integrity loss (Lenton 1987). This may partially account for differences in pattern of growth inhibition of plants treated with triadimefon versus paclobutrazol (see Fig. 2B and note smooth curve for triadimefon).

High dosages of triadimefon (50 and 100 mg) restricted root growth by about 60% and shoot elongation only by about 25%, whereas 1 mg of XE-1019 increased root dry weight by about 65% but decreased shoot elongation by nearly 70%. Buchenauer and Rohner (1981) using barley and wheat showed that triadimefon affected roots more than coleoptiles and primary leaves.

Effectiveness of the highly active GA biosynthesis inhibitors for retarding plant growth may be dependent upon residual activity in addition to the efficiency of the molecular structure of the chemical inhibitor. However, other factors in addition to longevity of activity per se can influence the extent of effect produced by the various triazoles and their isomers. These include rate of uptake, transport, and metabolism within the plant (Lenton 1987) as well as movement in soil and release or binding by soil particles and the vascular system of the plant (Lever 1986). Nevertheless, major differences in residual activity are important when evaluating growth retardants for use on crop plants because long-term or short-term growth inhibition may or may not be desirable.

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