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Chemical Control of Excessive Vegetative Growth of Fruit Trees with Emphasis on Stone Fruit Species

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Abstract

The following are the main achievements of this project.

1. It was demonstrated that paclobutrazol has growth promoting characteristics at low concentration on apple callus tissue. Part of this effect results from increase in cell division.
2. Paclobutrazol was shown to change root morphology in peaches grown in hydroponic system. Increase in root diameter was found to be a very rapid and sensitive response to paclobutrazol. This effect was shown in both root and top application. Paclobutrazol at low concentration reduced top/root ratio, increased root branching and improved water use.
3. From ^{14}C -paclobutrazol trials and from other experiments it was demonstrated that paclobutrazol moves predominantly acropetally but a basipetal movement was also demonstrated via the phloem to the roots.
4. A slow radial movement of paclobutrazol in the peach plant was shown in a split root system.
5. Dose and timing of the effect of a few triazole growth retardants on various peach cultivars was examined and defined. Biennial use of paclobutrazol was suggested to be integrated in the meadow orchard system.
6. Trials with labeled precursors of gibberellins did not prove that paclobutrazol inhibits or interferes 'in vivo' with GA biosynthesis at the oxidation steps from ent-kaurene to ent-kaurenoic acid.
7. A comparative study of a few triazole growth retardants specified their effect in apple and peach in soil spray strip, subsoil injection, drip irrigation and trunk paint. The latter treatment was especially effective with nectarines and sweet cherries.
8. Application of triazole retardants in an organic solvent to trunks of stone fruits was calibrated with respect to time and dose to obtain the required response.
9. It was shown that with apple, fruit sugar content was increased by triazole retardants.
10. A new analytical procedure was developed to test translocation and metabolism of triazoles. The method is rapid and sensitive and uses internal standard that adds to its accuracy.
11. In a comparative work with a few triazole retardants it was shown that BAS111 which has a shorter period of effect in retarding vegetative growth is metabolised by the apple tissues to produce 2 metabolites. In the solution the chemical is stable.

Objectives of the project:

1. To determine the dose-response relationship from soil application of paclobutrazol to various fruit tree species mainly stone fruits.
2. To examine the uptake translocation and metabolism of paclobutrazol using ^{14}C labeled compound, from foliar and root applications.
3. To study the mutual relationships between vegetative growth, root growth and reproductive development, as affected by paclobutrazol in a few fruit species.
4. To study the effect of paclobutrazol on the endogenous hormonal situation of the plant with special reference to gibberellins.

All the objectives were met with extension of the work to other new triazoles and improved analytical procedures for triazoles.

1.The effect of paclobutrazol on apple callus growth.

Materials and methods

Callus tissue was obtained from 'Golden Delicious' apple fruit and kept in culture on Miller's medium including 2 mg/l of NAA and 1mg/l of Kinetin (8). The callus tissue was transferred to new medium every 30 days. The tissues grew on solid agar medium containing 10% agar and 3% sucrose . The tissue requires continuous supply of cytokinin for its growth.

For the experiments with paclobutrazol, a liquid medium containing the same components as in the Agar medium but for the Agar was used. 10 ml of the solution was poured into a 125 ml ehrlenmeyer, the GBI added at various concentrations, autoclaved and put on a continuously rotating shaker at a frequency of 100 rpm at 27⁰C in the dark. A piece of tissue of ca 10 mg in fresh weight was transferred aseptically to the solution. Fresh and dry weight were checked after 30 days. For evaluation cell size, tissues were treated according to Jensen (6), fixed in FAA , embedded in paraffin and sections 10 μ thick were observed under a light microscope at a magnification of x400. All the cells were parenchyma type non differentiated ones. Using a calibrated grid, length and width of the spheric cells at their center were measured and volume calculated.

Results

The effect of paclobutrazol on growth of apple callus tissue was checked in a few experiments. Not in all did we get the same effect on growth (Fresh and dry weight bases), but the general response was an enhancing effect at a narrow range of concentrations between 0.5-1.0 μ g paclobutrazol /ml. In Fig 1.1a the effect on fresh weight and cell volume is compared. A slight increase in cell volume was found but clearly this increase in cell volume can not explain the increase in total tissue weight. Maximal effect was obtained at 0.5 μ g paclobutrazol per ml when added to a kinetin containing medium. A steep reduction is observed above this level. Comparison on a relative basis show a slight effect to increase in cell volume (up to 22%) as against over 300% in tissue fresh weight (fig 1.1b). Therefore, the main effect of paclobutrazol is on cell multiplication. In another experiment, concentrations of 0.25 and 0.5 μ g of paclobutrazol per ml were compared to no paclobutrazol . Similar pattern of effect was found (Fig 1.2a). Again, only slight cell volume increase due to paclobutrazol was noted compared to a strong effect of total increase in fresh weight of the tissue.

Fig 1.1

Fig 1.2

Fig 1.1: The effect of fresh weight and cell volume of apple tissue culture grown for 30 days in presence of various concentrations of paclobutrazol. Cell volume is reported in $10 \times 10^3 \mu^3 (cv \times 10)$.

A: actual values; B: relative values. Fig 1.2: as in Fig 1.1 but for a different experiment.

Fig 1.4

Fig 1.3: Interaction of paclobutrazol and kinetin in their effect on fresh (A) or dry (B) apple tissue growth in 30 days. Fig. 1.4: as in Fig 1.3 but for a different experiment.

Fig 1.5

Fig 1.5: The effect of paciobutrazol (paclo) and uniconazol (unico) on fresh weight growth of apple tissue in 30 days.

Fig 1.6

Fig. 1.6: The effect of low concentration of ABA on growth of apple tissue in 30 days (A).
A comparison of the effect of ABA on growth of apple tissue in presence (fwt-p) or absence (F wt) of paciobutrazol 0.5 $\mu\text{g/ml}$ (B).

The following is a summary of 5 separate experiments with various levels of paclobutrazol and in addition to other added growth regulators.

Table 1.1: The effect of added paclobutrazol to the medium on callus growth.

Experiment	Paclo. µg/ml	GA ₃ µg/ml	added fresh weight (%)	added dry weight (%)
1. (Oct.86)	0.25		40.2	18.5
	0.5		104.6	43.0
	-	1	21.2	5.9
	0.5	1	103.3	33.8
2. (Jun.87)	0.1		8.5	-0.4
	0.25		18.8	9.6
	0.5		16.9	-2.8
3. (Jul.87)	0.25		6.2	2.1
	0.5		19.7	5.4
	1.0		9.4	3.5
4. (Aug.87)	0.5		10.8	-2.5

In general, increase in added fresh weight was noted in all the experiments although to varying degrees. The effect on added dry weight was not as clear. In all cases, increase in dry weight was lower than with fresh weight and in the last experiment there was no increase at all. The reason for this variability is not known.

The interaction of the effects on callus growth of paclobutrazol and kinetin were tested on agar medium. It was found that the enhancing effect was repeated in the presence of 1 mg/l of kinetin, to a lesser degree with 10 mg/l of kinetin while with 0.1mg/l a retarding effect increasing with paclobutrazol concentration was noted (Fig 1.3a and b). Repeating this experiment with a liquid medium revealed an enhancing effect on callus growth only with 0.5mg/l of kinetin at 1.0 µg/ml of paclobutrazol. (Fig 1.4a and b)

In a few experiments a comparison was done between the effects on apple callus growth of paclobutrazol and uniconazol. In one such experiment (Fig 1.4) a comparison of their effects point to a similar pattern of activity of increase in weight of the tissue but at different concentrations. Uniconazol was found to affect tissue

growth by a similar manner to paclobutrazol but at a much lower concentration. It seems to be more active by about 1 order of magnitude than paclobutrazol.

It can thus be concluded that under a strict and narrow concentration, a strong stimulus of cell multiplication occurred when gibberellin biosynthesis inhibitors (GBI) were added to the tissue. It is interesting to note that when GA was added, increase in tissue weight was also found. Added 0.5 µg/ml to the tissue increased its growth further.

Examination of the effect of ABA on growth of apple tissue in presence of kinetin show (Fig 1.6a) a promotive effect at concentration of 0.5 mg/l. When paclobutrazol was added to this system no change of effect was found (Fig 6b).

Discussion

The enhancing effect of paclobutrazol on growth of callus tissues in presence of a cytokinin seems inconsistent with its general strong inhibiting effects on growth. Other growth retardants like ABA were also found to exert a growth promoting effect at low concentrations. It should be noted that the data reported deal exclusively with non differentiated tissue. The response of this tissue to added gibberellin is relatively small as was shown. The only other promotive effect of paclobutrazol on growth of a tissue was shown here too is the increase in root radial growth. In this case, the increase is quite strong as root diameter increases a few folds. We do not know the cause for the response but it seems it is connected with high levels of the cytokinins. With low levels this effect could not be demonstrated. With no added cytokinins, no positive effect to paclobutrazol on growth was found. The range where the enhancing effect was shown is quite narrow: between 0.25 and 1.0 µg paclobutrazol/ml which correspond to between 0.85 and 3.41 µM. Uniconazol revealed this effect at about 1/10 of the paclobutrazol concentration.

2. paclobutrazol applied to hydroponically grown peach plants

Most of the reports on the effect of paclobutrazol on fruit trees are concerned with the effects on vegetative and reproductive development of plant top. Very little is known on the effects of GBI on the root system. In order to study the response of the root system to GBI we followed the response of small peach plants to these growth retardants applied in hydroponic systems.

Materials and methods.

Peach plants were raised from cuttings as described before (5). The cutting were left to root in either sand medium or in a peat moss volcanic tuff medium 1:4 ratio.

The rooted plants were transferred into the hydroponic units. Generally young rooted cuttings were used, but for one case where one-year-old plants bearing fruit were used. Hydroponic culture was performed in 'Ein-Gedi' round hydroponic units of 35 cm diameter and a height of 25 cm containing 10 l of solution and a central suction unit operating continuously that sucks solution from the bottom and sprays it on the upper part of the container. This continuous circulation and spray of solution on the root system, produced a combination of aeroponic (above the level of the solution) and hydroponic conditions. The solutions were topped every second day with tap water and replaced every fortnight. Since aeration was excellent, growth of the root system was undisturbed. Four plants could be grown in each unit. The plants introduced in special adaptors that support the plant and allowed free development of its root system. A half Hoagland nutrient solution was used throughout. Occasionally, Fe-sequestrene at a level of 30 mg/l was added. The hydroponic units were put in a greenhouse with temperature fluctuations between 10 and 30°C in March-April, Between 15 and 30°C in May and between 20 to 35°C in June and July. On one occasion, the hydroponic units were put in an air-conditioned greenhouse in mid-summer allowing temperature fluctuations between 20 and 25°C.

GBI were applied to the root system or to the top. In the first case, the inhibitor was added to the medium only after a strong root system was established and for different periods. On terminating the exposure to the GBI, the root systems and the containers were rinsed a few times with water and filled with a GBI free solution. In the second case, the top of the plants was dipped in the GBI solution verifying no contact with the root system.

The following measurements were carried out: Increase in top growth, measured twice a week; increase in root growth by measuring premarked roots; change in thickness of the growing roots; root branching. In one experiment determination of the root weight was carried out by weighing the plant with the roots submerged in water using the system of Young and Werner (11) the total plant was weighed in the air as well, and the root and top weights were calculated separately. On ending an experiment, fresh and dry weight of the different plant parts was determined.

Results

Experiment 1:

One-year-old 'Earligrande' peach plants grown in hydroponic system, 8 replicates per treatment, were exposed on March 6 to 4 levels of paclobutrazol: 0,

0.1, 0.8 and 2.4 mg/l in the nutrient solution. After exposure for 14 days, half of the plants were transferred to paclobutrazol-free solutions (short exposure). The remaining plants were left another 30 days in paclobutrazol. On April 12 when the fruits matured, the plants were harvested and fresh and dry weight of the various organs determined in both the short and the long exposure periods to paclobutrazol. Total vegetative weights were found to be similar at harvest between the short exposure treatments (Fig 2.1.1b) while fruit weight was higher in the 2.4 and 0.8 mg/l paclobutrazol than in the control. On dry weight basis, increase in both fruit and root weight was noted in the 0.8 mg/l treatment. (Fig 2.1.2b). Analysis of the vegetative growth shows reduced foliage and reduced new shoot formation especially in 0.1 and 2.4 mg/l treatments. (Fig 2.1.1a). This difference in leaves disappeared on dry weight basis (Fig 2.1.2a) indicating a higher specific leaf weight to the paclobutrazol treatment. The 0.8 mg/l treatment, which had the heaviest fruit weight, was also the higher producer of dry leaf weight per plant. The difference in old shoots' weight only in the highest paclobutrazol treatment, was due to lower secondary thickening of these branches (Fig 2.1.2a).

With the long exposure to paclobutrazol till harvest time, weight of both root and top vegetative parts were negatively correlated with paclobutrazol concentration on both fresh (Fig 2.1.1d) and dry (Fig 2.1.2d) bases. The fruit on the other hand had a higher fresh and dry weight in 0.1 and 0.8 mg/l of paclobutrazol than in the control. Analyzing the top, reveals a similar effects of all paclobutrazol concentration in reducing total leaf and new shoot fresh weight (Fig 2.1.1c). On dry weight basis (Fig 2.1.2c), similar leaf weight and reduced shoot weight were found. Again this finding testifies to the increase in specific leaf weight by paclobutrazol.

Analysis of the growth recovery of the shoots after suspending the exposure to paclobutrazol, shows a connection between paclobutrazol concentration and the time for initial growth recovery. All paclobutrazol treatments showed a strong growth inhibition. With 0.1mg/l and to a lesser degree with 0.8 mg/l growth was evident 13 days after suspension of paclobutrazol (Fig 2.2a). 2.4 mg/l did not recovered by that time. With the long exposure when the plants were in continuous contact with the chemical, only the 0.1 mg/l did show a slight growth recovery on the 44th day of exposure (Fig 2.2b). On March 17, 11 days after application, evaluation of the root quantity of all plants was done in a scale of 1(low) to 4 (high). Clearly paclobutrazol inhibited also root growth (Fig 2.2c). A distinct characteristic of the treated plant was the appearance of thick root tips that when not

Fig 2.1.1: The effect of exposure of 1-year-old 'Earligrande' peach plants to paclobutrazol for 14 (A and B) and 44 (C and D) days on growth of the various plant organs on fresh weight basis. Exposure on March 6, experiment terminated on April 19 1987.

FIG. 2.1.1

SHORT (14 days) APPLICATION

LONG (44 days) APPLICATION

Fig 2.1.2: The effect of exposure of 1-year-old 'Earligrande' peach plants to paclobutrazol for 14 (A and B) and 44 (C and D) days on growth of the various plant organs on dry weight basis. Exposure on March 6, experiment terminated on April 19 1987.

Fig 2.2: The effect of exposure of 1-year-old 'Earligrande' peach plants to paclobutrazol for 14 (A and B) and 44 (C and D) days on growth of the various plant organs on fresh and dry weight bases as measured on termination of the experiment on April 19 1987.

FIG. 2.2

11 DAYS AFTER APPLICATION

FIG. 2.3

Fig 2.3: The effect of exposure of 1-year-old 'Earligrande' peach plants to paclobutrazol for 14 (B and D) and 44 (A, C and E) days on growth of the various plant organs and on top/root ratio.

Exposure on March 6, experiment terminated on June 30 1987.

inhibited in growth produced thick roots. This appeared in all paclobutrazol levels but the length of these thick roots was negatively correlated with the paclobutrazol concentration (Fig 2.2d). From this it may be concluded that root thickness is more sensitive to paclobutrazol than root extension growth.

Part of the plants that were not harvested at fruit maturation time, were left to grow till June 30 when the plants were harvested. Total weight, top weight and root weight in the short (Fig 2.3b,d,f) and the long (Fig 2.3 a,c,e) periods of exposure to paclobutrazol revealed strong effects of the inhibitor. In the long exposure (44 days), reduction in total and top weight on both fresh (Fig 2.3a) and dry (Fig 2.3c) bases was noted. Regarding root weight, 0.8 mg/l treatment induced after suspension of paclobutrazol, a heavier root system than the untreated control. The few replicate number (only 2) was not enough to analyze statistically this result. With the short exposure of only 14 days to paclobutrazol, a clear enhancing effect on total top and root systems was found to 0.1 mg/l paclobutrazol on fresh (Fig 2.3b) and dry (Fig 2.3d) weight bases. A comparison of the effect of short and long exposures to paclobutrazol (Fig 2.3.1) stresses the enhancing effect on root growth in the short exposure treatment. A gradual reduction of growth especially of the top with paclobutrazol concentration was noted (Fig 2.3b and d). The root system showed also a reduction trend with increase in paclobutrazol concentration but in comparison with the control, the root systems of all paclobutrazol treatment was either heavier or equal to that of the untreated control. As a result, a reduction in top/root ratio was found with all treatments in the long (Fig 2.3e) and in the short (Fig 2.3f) exposure treatments.

Experiment 2:

To repeat this treatment, rooted cuttings of the peach 'Maravilha' were allowed to develop in the hydroponic units. 0, 0.1 and 0.8 mg/l of paclobutrazol were introduced on June 22 to the root system of 4 replicates per treatment. After exposure for 34 days the paclobutrazol treatment was suspended. On October 27 the plants were harvested. The strongest inhibition on the top (Fig 2.4a,b) was on the extension growth of the shoots followed by the weight of the leaves. Smaller effect was noted with the trunk that its Increase in weight was in negative correlation with paclobutrazol level. In this experiment too, the inhibition of the top by paclobutrazol was much stronger than the inhibition of the roots (Fig 2.4c,d) resulting in a reduced top root ratio (Fig 2.4e,f).

FIG. 2.4

Fig 2.4: The effect of exposure of 1-year-old 'Maravilha' peach plants to 0.1 or 0.8 mg/l of paclobutrazol for 34 and 38 days respectively on fresh (A and C) and dry (B and D) matter accumulated and on top/root ratio on fresh (E) and dry (F) bases. Treatment started on June 22 and on June 28 1987 in the 0.1 and 0.8 mg/l of paclobutrazol respectively and terminated on October 27 1987.

FIG. 2.5

Fig 2.5: The effect of exposure of 'Maravilha' peach plants to 0.1 mg/ml of paclobutrazol for 34 days in comparison to untreated control, on water consumption in the course of 9 weeks.

Water consumption by the plants under 0 and 0.1 mg/l paclobutrazol were followed from the 2nd through the 11th week following application. Due to its reduced foliage area per plant, water consumption was much lower in the paclobutrazol treated plant. Although leaf weight was reduced to about one half in the 0.1 mg/l paclobutrazol compared to the control, water use was about one third than that of the control during the examination period (Fig 2.5). In this experiment, weekly or biweekly determination of top and roots in a nondestructive manner was carried out. Growth of the top in the control followed a curved line indicating a gradual slowing down of growth. (Fig 2.6a). The paclobutrazol treated plants showed an extremely restricted top growth that continued linearly. The roots in the control and the paclobutrazol treatment showed a linear growth pattern with a little smaller angle for the later treatment. (Fig 2.6b). As a result, top/root ratio was reducing with time, steeper in the control than in the paclobutrazol treatment but even in the last measurement the ratio was lower for the treatment than for the control (Fig 2.6c).

Experiment 3.

On April 6 1988 4 single tree replicates of 'Earligrande' peach plants were exposed to 0 or 0.1 mg/l paclobutrazol in hydroponic system for a period of 13 days. Starting from the 5th day from application, frequent measurements of top and root growth were carried out. A very quick response was noted in vegetative growth in the treated plants already in the 13th day of treatment (Fig 2.7a). This inhibiting effect persisted for another 24 days although the retardant was not present in the solution. The roots did not show any inhibition at this level of paclobutrazol (Fig 2.7b), on the contrary, there was a slight trend for a stronger growth of the roots. The growing roots were showing the typical increase in diameter already 13 days from application and continued to show it another 17 days when abruptly they returned to a normal diameter (Fig 2.7c).

Experiment 4.

On April 27 1988 4 single tree replicates of 'Earligrande' peach plants were exposed to 0 or 0.1 mg/l paclobutrazol and to 0.1 and 0.025 mg/l of uniconazol in the nutrient solution. The retardants were removed after 14 days on May 10 and the plants were harvested on July 25. The weight of the shoots the foliage and the trunks was reduced in all treatments compared to the control on both fresh (Fig 2.8a) and dry (Fig 2.8b) weight bases. The reduction by uniconazol was much

FIG. 2.6

Fig 2.6: The dynamics of change in fresh matter accumulation in top (A) root (B) and top/root ratio (C) in 0.1 mg/ml of as compared to untreated control.paclobutrazol treated plants.

FIG. 2.7

Fig 2.7: The effect of exposure of 'Earligrande' peach plants to 0.1 mg/ml of paclobutrazol for 13 days on shoot (A) and root(B) growth rates and on the thickness of the growing roots (C). The arrow indicates the end of exposure to paclobutrazol.

FIG. 2.8

Fig 2.8: The effect of exposure of 'Earligrande' peach plants to 0,0.1 mg/ml of paclobutrazol and to 0.025 and 0.1 mg/l of uniconazole for 14 days on growth of various plant organs on fresh (A) and dry (B) bases, on top/root ratio (C) and on root diameter (D) and length (E). The arrow indicates the end of exposure to GBI.

stronger than that by paclobutrazol in the same concentration (almost identical molecular weights to both chemicals). Even the lower concentration of 0.025mg/l uniconazol was more retarding than 0.1 mg/l of paclobutrazol. Roots on the other hand showed no reduction in weight with an indication of increased growth with the 0.1 mg/l uniconazol. Top/root ratios reflected the level of inhibition of the tops by the GBI with a lower ratio the stronger the inhibition (Fig 2.8c). On harvesting, the trees determination of trunk cross section area and root branching were determined (Table 2.1) TCA follows closely the trunk fresh weight (Fig 2.8a) with lowest value for the 2 uniconazol treatments and an intermediate value for paclobutrazol. Root branching was found to be enhanced dramatically by all the retardants with a correlation between the level of the top inhibition and branching. Over 4 folds of increased branching was noted with 0.1 mg/l uniconazol (Table 2.1). Root diameter was followed during the period of exposure to the GBI and afterwards (Fig 2.8d). The stronger effective uniconazol at 1mg/l induced a thicker root in all 3 measurement periods. Furthermore, on the 25th day from application this was the only treatment that showed thicker roots than the control. Observing the development of the roots till day 25 from application revealed a quick recovery of the roots from partial inhibition by 0.025 mg/l of uniconazol (fig 2.8e) while the top remained inhibited for another 70 days.

Table 2.1: The effect of GBI on root branching and trunk cross-section area

Treatment	Lateral roots ⁽²⁾ (no±SE)	TCA ⁽¹⁾ (cm ² ±SE)	Top F.wt/TCA (g/cm ²)	Top D.wt/TCA (g/cm ²)
Control	3.2±0.5	80.6±18.6	1.96	0.72
Paclo 0.1 mg/l	5.6±0.6	64.3±19.2	1.83	0.68
Unico 0.025mg/l	7.9±1.0	44.2±5.0	1.39	0.56
Unico 0.1 mg/l	14.6±0.9	43.2±5.2	1.73	0.67

(1) Trunk Cross-section Area

(2)Number of lateral roots per 5 cm length of main root.

Fig 2.3.1: the effect of paclobutrazol on roots of hydroponically grown peach plants. **0.1 ppm** = 42 days after end of exposure for 30 days to 0.1 mg/l. **0.1 ppm=>0** = 58 days after end of exposure for 14 days to 0.1 mg/l.

Experiment 5.

One-year-old 'Rhodes' peach plants were grown in the hydroponic units since June 8. . On August 15 the units with the plants moved into an airconditioned green house. On August 25 1988, when no extension growth was evident, 4 plants were exposed to each of the following 3 treatments for 14 days: Untreated control; 0.1mg /l of paclobutrazol in the solution and dip of the top in 0.5% of paclobutrazol. Swelling of the root tips was evident first in the solution treatment (after 3 days from treatment but also the treated tops did show this effect starting from the 7th day after application. All the root growth returned to normal appearance on the 21st day from starting the treatments. In order to examine the possible way of translocation of the stimulus to the roots, the treated plants that recovered their normal root growth (no visible vegetative growth of the top was evident in any of the plants) were exposed again to paclobutrazol on October 4 for another 14 days to the same treatment. Half of the plants were girdled immediately after treatment. It was found on termination the experiment on November 9 that girdling the treated plants caused a rapid deterioration of the root system with no new root development. The nongirdled plants responded as before with the production of thick roots in both types of treatment.

In order to further clarify the effect of paclobutrazol on the roots, another trial was set-up on October 28 with 'Flavortop' nectarines that were still in full foliage with an active root system. The treatments included:

1. 0.1 mg/l paclobutrazol.
2. 0.1 mg/l paclobutrazol; plants defoliated completely.
3. 0.5% paclobutrazol dip of one branch out of 2 per plant.
4. 0.5% paclobutrazol dip of one branch out of 2 per plant and girdling the treated branch.
5. control.

While 5 showed a normal growth of roots, 1 showed typical root thickening 4 had no thickening, 3 showed a sectorial effect of thickening while 2 exhibited arrested root growth. It could thus be suggested that in order to have the typical effect of thickening in the roots, the chemical has to be absorbed into the plant, and probably transported to the leaves. The cause for the effect moves by the phloem and exhibits its effect in the root tip.

Discussion

From observation of the effects of GBI on the whole peach plant it seems that apart from the dramatic effects on vegetative growth of the top, the effects of these group of chemicals on the root system has a special significance. The change in root morphology that was reported here agrees with previous reports on apple (1) and on peach (10). We have shown an extremely early response in the roots diameter to paclobutrazol. We have shown that with low concentration of the GBI, root growth may continue while the top will be fully inhibited. With such concentration, a drastic reduction of the top/root weight ratio is obtained. This change is due to the following reasons:

1. Resumption of growth of the roots while the top is still under inhibition.
2. Growth of thick heavier roots under the effect of paclobutrazol.
3. Increase in root branching and as a result an increase in the number of growing points.

This may lead to a change in the hormonal balance of the plant regarding root produced ones like cytokinins and gibberellins compared to top produced auxins. Therefore, a rapid recovery from the inhibition is expected once the effect of the inhibitor disappears.

The transport of GBI in the plant was reported to be acropetal. If this was the case, one would expect a large difference in appearance of a treated plant if top or root treated. This was not the case as both roots and tops responded similarly in both treatment methods. Furthermore, the thickening of the roots in treated plants testifies to a basipetal movement of the effect. We have not checked the cause for the effect but we have shown that it very probably moves in the phloem. It could well be a chemical different from the GBI but induced by them probably in the leaves.

It should be noted that the effect on water consumption may have special implication if these findings could be repeated under field conditions.

3.Radial movement of paclobutrazol in peach plants.

Control over the effect of paclobutrazol on fruit trees when the chemical applied to the root system is one of the major problem in the practical use of this retardant. One of the main questions is the overall effect of paclobutrazol when applied to or when reaching only part of the root system. This may occur when application or movement of the retardant is not uniform in the soil, or when the roots develop into

soil zones where no inhibitor is found. From observation in the field, it is noted frequently that a sectorial response is observed when the chemical was applied to one side of the tree. In this set-up of experiments, we wanted to test the radial movement of paclobutrazol in small peach plants.

Materials and methods:

Young one-year-old 'Maravilha' peach plants grown in were transferred to a split root system by dividing in spring the roots into 2 similar parts and planting them separately in two adjacent plastic containers. Another group of the same plants were grafted on April to each other by cutting their main stem longitudinally about 2 cm long at about 20 cm height and tying them together. The trees were irrigated by drip system with a diluted nutrient solution one emitter per container. The plants were exposed in the next spring to a differential treatment to their root system. Only to one out of two containers that supported the plants 1 mg of paclobutrazol was applied. All growing branches were marked to their size and the added growth till the end of the observation was measured. The shoots on every tree with split-root system were separated according to their position on the plant to above treated container and to above the non-treated one. With the grafted trees the branches of every individual tree of the twins were measured separately on July 17.

Results

Comparison of added growth on these trees shows (Fig 3.1a) a strong sectorial effect on the grafted trees and a weaker but still sectorial response also to the split-roots trees. In the first comparison the growth ratio of non-treated side vs treated was 1:2.25 and in the second 1:1.5. It is clearer to relate the branches to their corresponding roots in the grafted plants as against the root-split ones. This may be one reason for the less sectorial response observed. Checking the bud density and the internode length (Fig 3.1b) revealed similar response ie. shorter internodes and higher bud density in the treated trees with a stronger sectorial response with grafted than with divided roots. When to these trees water was applied to one container out of the two, no signs of water stress could be seen on shoots connected to the non-irrigated part during a period of 1 month. It was concluded that by comparison to water, that moves easily radially in the stem, paclobutrazol has a limited radial movement. Our data support the field observation that in order to get the full retarding effect of paclobutrazol the chemical has to reach all or most of the root system.

Fig 3.1

Fig 3.1: The response of grafted or divided root system peach trees to one-side application of paclobutrazol (p) $\pm$ SE.

Fig 5.1

Fig. 5.1: The effect of spring applied paclobutrazol (440g/ha) in a drip irrigation system on growth of three peach cultivars. Seasonal growth was determined in autumn after end of vegetative growth.

4. Trials with ^{14}C paclobutrazol on small peach plants.

With the purpose of following the movement of paclobutrazol in peach plants from the location of application a set of experiments was conducted with small plants grown in sand or in hydroponic systems.

4.1 Application to plant top

^{14}C paclobutrazol was applied to young Maravilha peach plants grown in sand and irrigated with a diluted nutrient solution. Conviron E7H growth chambers at 25 : 15°C day : night temperature with a 16 hr photoperiod and with light intensity of $100 \mu\text{M s}^{-1}\text{m}^{-2}$ at plant height. On July 10 1986 150 μl of methanol containing $3.27 \times 10^6 \text{dpm}$ equivalent to $1.49 \mu\text{Ci}$ was applied to 4 plants

a: lower side of a fully developed leaf in 2 replicates of single plant each.

b: to the growing apex including the very young expanding leaves in 2 replicates of single plant each. On July 16 1986 a dose of 500 μg of cold paclobutrazol was applied to a leaf above the treated one in a or to the same apex in b. As the treated leaf showed damage symptom probably due to the methanol, a second treatment on the same plants with the same dose was repeated on July 17 on an adjacent leaf but on its upper surface. On August 17 and on September 26 two plants one leaf treated and another apex treated were separated to leaves, apex, stem (the stems were cut longitudinally) and roots and were dried and autoradiographed.

The autoradiograms revealed:

Exposure July 10 - August 17 (39 days)

a: Leaf treated

A strong activity in the treated leaf.

Activity observed in leaves proximal to the treated ones.

Activity observed along the stem on phloem side.

Activity found in root system (Obvious but not strong).

b: Apex treated

A strong activity in apex

Activity observed along the stem on phloem side

Activity clearly observed in the root system.

Exposure July 10 - September 26 (80 days)

a: Leaf treated: Total detected label low, Detected label in the leaves proximal to the treated leaf, trace label can be seen along the stem and in the root system.

b: Apex treated: Strong label in application zone, trace label can be seen along the stem and in the root system.

In these trials we followed the movement of paclobutrazol applied to a leaf or the apex in the plants. Although we did not expect to get a label in the parts of the plants proximal to site of application we got in both treated organs a clear indication for label movement from the top to the root system. The label was detected after 39 days of application but almost none after 80 days from application. The cause for this may be a loss through the root system or by metabolism of the chemical and loss as CO₂. We do not have a quantitative determination but the presence of the label in the root system together with appearance of label in the conducting tissues point to a certain basipetal movement. We do not have any proof that the labeled chemical was still paclobutrazol but considering its limited and slow metabolism and the fact that the product of its metabolism in the plant is also biologically active, we find it critical to note that contrary to the reports in the literature (7,9,) we do show this movement to a certain extent.

4.2 Application to plant roots

12 plants of Flordagold peach were grown in 'Ein-Gedy' hydroponic units (See section 2) 4 in each. The volume of the liquid phase in the units was reduced by introducing filled and closed plastic bags leaving room for just 3 l. On April 6 1986 cold paclobutrazol was introduced at 1 mg/ unit in two units. On April 18 when the average growth reached about 30 cm and at fruitlet stage 2.3 μ Ci was added to 3 l of nutrient solution to each of two units. Three plants were collected, on May 5 after 17 days of exposure to the inhibitor. Another 3 plants were taken on May 30, after exposure for 42 days. Two plants was used for autoradiography. The plants taken for analysis, were divided individually into: Thick roots, white thin roots, leaves, Stem and shoots and fruits. The plant material was dried, weighed, milled with a Wiley mill and passed a 40 mesh sieve. Fifty mg of the dried material of every organ were extracted with 5 ml of 80% methanol under continuous shaking overnight. The extract was left in a refrigerator at 4⁰C for another 30 days. An aliquote of the supernatant was added to 25% Lumax and read in a scintillation counter equipped with an external standard. Readings of similar parts of the control plant material were carried out simultaneously and the control readings were deducted from that of the readings of the ¹⁴ C paclobutrazol treated plants. The data obtained represented by DPM/mg Dry weight of plant organ and as % of the total radioactivity found are presented in Table 4.1

Table 4.1: The distribution of ^{14}C from labeled paclobutrazol applied to the roots of Flordagold peach plants in the various organs

Plant part	<u>DPM/mg D. Wt</u>		<u>Radioactivity (% of total)</u>	
	17 days	42 days	17 days	42 days
Main roots	22.6	5.2	42.0	18.2
White roots	23.6	5.8	5.6	2.7
Leaves	46.7	66.6	25.2	73.2
Stem & shoots	121.0	4.5	18.3	1.5
Fruit pulp	9.0	0	2.2	0
Fruit stone	12.5	0	4.0	0

Out of the total label introduced per hydroponic unit we could account in an average for only 17.0% in the 17 day-treatment and 15.2% in the 42 day-treatment. Per unit dry weight, the highest value was obtained in the stem and shoots in the short exposure and in the leaves in the long exposure treatment. We could detect low levels of activity in the pulp and in the stone in the unripe fruit. When observing the values per organ, the roots were leading in the short exposure treatment with highest values in the woody roots indicating probably a possible storage function. The longer exposure means a more advanced situation of development of the tree and the fruit. The leaves were dominant in accumulating paclobutrazol over all other organs. The increase in paclobutrazol in the leaves occurred on the expense of the "stored" inhibitor in the stem and roots. No paclobutrazol was detected at this stage in either the fruit or the stone.

For autoradiography, two plants exposed to paclobutrazol since April 18 were harvested on June 10 (53 days of exposure). The plants were dried and then exposed to x-ray sensitive film for 40 days. It was found that the roots were heavily labelled, while all the transpiring leaves were also labelled especially along the veins indicating a xylem mobility of the inhibitor.

4.3. Application to split-root system

Two small Redhaven peach trees were prepared with a split root system (see section 3). On June 21 1985 $1.1 \mu\text{Ci}$ of ^{14}C paclobutrazol was applied together with 2.5 mg of cold paclobutrazol to one of the 2 containers. After 40 days the

plants were collected and treated as described above and analyzed by reading of the supernatant with a scintillation counter. The data (Table 4.1) point to the presence of the label also in the root system that was not treated. A similar level of the label was found in the two parts of the trunk above the treated and the non-treated roots. The same experiment was repeated with 3 small 'Babcock' plants raised from cuttings in winter 1984, were grown since Summer 1985 with their roots split between two containers filled with sand. On May 28 1986, 2 μCi of ^{14}C paclobutrazol + 1 mg of cold paclobutrazol were applied to one container out of the two that supported the plant in each of three trees. 37 days later the plants were harvested, and the label evaluated as indicated above.

It was found (Table 4.2) that the majority of the chemical is found in the shoots and leaves. But in 2 out of the three replicates, label was detected again in the non-treated root testifying to a basipetal transport of paclobutrazol or its metabolite.

This supports our previous evidence for some basipetal movement of paclobutrazol, or its metabolite.

Table 4.2: The distribution of radioactivity in the various plant organs of split-root peaches that were treated with ^{14}C paclobutrazol in a sectorial manner to the root system.

<u>Cultivar</u>	<u>Organ</u>	<u>% total activity</u>		<u>DPM/mg dw</u>	
		Treated side	Non-tr. side	Treated side	Non-tr. side
Redhaven	Roots	26.8	10.2		
	Trunk	15.7	14.9		
	Top	----32.4-----			
Babcock	Roots	25.6	5.8	36	11
	Trunk	0.8	2.0	22	24
	Top	---- 65.8-----		----26----	

Discussion

In this work we confirmed that the main transport of paclobutrazol in peach plants is by the xylem acropetally. But we obtained also evidence that there is a basipetal transport of paclobutrazol or its metabolite. The evidence in this direction is as follows:

1. We noticed repeatedly a thickening of the roots when paclobutrazol was applied to the top. We noticed also a retardation of root growth from top application of paclobutrazol.
2. Autoradiograms of paclobutrazol treated leaves or apex show label in the root system after a period of 39 days.
3. Labelling one part of the root system reveals the label in another non treated root. This could only happen if the chemical moved first acropetally and later basipetally to the roots.
4. Girdling seems to interfere with thickening of the roots when paclobutrazol is applied to the top of the plant distal to the girdle indicating movement of the cause by the phloem.
5. ^{14}C treated leaf did show label distributed to other plant parts which testifies also for a basipetal mobility.

All this evidence support our conviction that such a movement within the plant does occur. This should have a bearing on both application of these chemicals as well as on understanding their effects in plants. In their work with labeled paclobutrazol Quinlan and Richardson (9) checked only the plant top and therefore could not detect movement to the root system. Early and Martin (2) followed the labelled paclobutrazol after feeding the root system. They could not observe a relatively small basipetal translocation of the retardant.

It was demonstrated that GBI at low concentrations have a growth promoting effect on callus growth in the presence of a cytokinin and an auxin. This effect is at least in part due to enhanced cell multiplication.

It was also shown that paclobutrazol has only a limited level of radial transport and there is a high degree of sectorial movement of the chemical in peach plants.

5. Orchard trials with soil-applied paclobutrazol.

Soil applied paclobutrazol was found to be the most efficient method of application in arid areas (4,7). Commercial use of the GBI must deal with the long lasting effect of these type of triazol inhibitors. For practical purposes, it is ideal if a dose applied will last up to 1 year so that a repeated application in the following

year will not have a stronger effect than in the previous year. The orchard trials tried meant to

1. Determine the effect of dose and time of application
2. Compare the effect of a few GBI's.
3. Examine the potential utilization of the chemical inhibitor in the orchard

management.

5.1. Determine the effect of dose and time of application

Materials and methods.

Peach plants grown in a meadow orchard system (3) were used for this experiment. For evaluating the relationship of dosage and time of application of paclobutrazol on growth control in a peach meadow orchard, two levels (50 and 100 mg/tree) and three periods (autumn, winter and spring) of application were compared in 1984/1985 season in Bet-Dagan. Six replicates of 3 trees each per treatment were used.

Table 5.1: The effect of dosage and timing of paclobutrazol treatment on vegetative growth of "Earligrande" peach trees grown in a meadow orchard system.

Treatment ¹ (mg/tree)	Total vegetative growth(April 30) (cm)	Added growth (April 16-30) (cm)	Vegetative devel- opment (May 3) (1-5) ²
Control	32.9 a	10.2 a	5.0 a
25(e)+25(l)	20.4 b	5.5 b	3.1 c
50(e)	7.9 c	2.5 cd	2.8 cd
50(m)	8.7 c	1.6 d	2.7 cde
50(l)	20.3 b	4.7 bc	3.0 cd
100(e)	12.2 bc	3.1 bcd	2.0 f
100(m)	9.4 c	1.4 d	2.2 ef
100(l)	16.7 bc	2.1 cd	2.6 de

¹e = Autumn, October 1984; m = winter, January 1985; l = spring , March 1985

²Evaluation on 5 grades: 1=severely restricted; 2=insufficient growth; 3=optimal growth; 4=excessive growth; 5=extreme growth.

Measurements of growth of preselected branches and evaluation of the overall vegetative state of the trees, showed that both dosage and timing of paclobutrazol treatment play an important role in the level of vegetative growth inhibition (Table 5.1). Total vegetative growth from bud break (early February) to the end of April was affected most by autumn and winter treatments. A much weaker effect was noted with the spring treatment, due to the delay in activity of the chemical resulting from the late application. No difference in total vegetative growth was noted in the premarked branches between doses of 50 and 100 mg paclobutrazol per tree applied in the same period. On the other hand, evaluation of total vegetative development on May 3, 1985, showed a stronger effect to 100 mg/tree. The double 25 mg treatment was less effective than any single 50 mg treatment. Optimal vigor was obtained with 50 mg applied in spring and in the double 25 mg treatment. Additional growth during 14 days in April, as observed on preselected branches, showed the strongest growth inhibition in the winter treatment by 50 mg and 100 mg. A significant difference was noted only between treatments in autumn or winter and those in spring with 50 mg. Yield in the various treatments did not differ significantly and there were only minor differences in mean fruit weight. The largest fruits were obtained in the 100 mg/tree autumn treatment. In the following year some inhibition in spring was noted with the 100mg treatments but none with the 50 mg one. By the end of the first year, measurements of annual shoot length in the paclobutrazol treated trees and in the control in the 3 cultivars revealed a connection between the vigor of the cv and the response to paclobutrazol treatment. Thus Maravilha was inhibited very severely, Rhodes to a lesser degree and Earligrande almost none (Fig 5.1).

5.2.1. Effect of various growth retardants on Maravilha peach

On February 10 1987 40 mg of the growth retardants paclobutrazol uniconazol and Triapenthenol were applied under each dripper to Maravilha trees planted at 0.75 x 1.80 m in a meadow orchard. Drippers were distributed every 50 cm along the lines. This amounted to 444 g ai /ha. Every treatment was replicated three times with 5 to 8 trees per replicate. The effect on vegetative growth was different among the various chemicals. Triapenthenol showed the strongest inhibition and a complete stop of growth while the other two had but a reduced growth rate. On the other hand, growth resumption was the quickest with triapenthenol already after two months from treatment. The yield obtained (Table 5.2) was higher in paclobutrazol and uniconazol than the control in the year of treatment. In the next

year uniconazol caused a severe reduction in yield due to the restricted growth and the heavy autumn bloom (see Table 5.3), while paclobutrazol and triapenthenol did not have any effect. Fruit weight was higher in the paclobutrazol and triapenthenol treatments than in the control in the year of treatment. In the next year paclobutrazol and uniconazol still produced heavier fruit. Fruit total soluble solids and fruit color did not differ from the control. During late summer, loss of a part of the tree foliage occurred and in October autumn bloom appeared on part of the trees. Evaluation of the density of bloom (Table 5.3) showed a small effect to paclobutrazol and a marked bloom only with uniconazol. This phenomenon testifies to a lighter stage of dormancy in the uniconazol treated trees than in the other treatments or in the control.

Table 5.2: The effect of GBI on yield and fruit weight in the year of treatment and the following year.

Treatment	<u>yield(kg/tree)</u>		<u>Mean fruit wt (g)</u>	
	1987	1988	1987	1988
Control	2.1 b	1.2 a	94±4	106
Pclobutrazol	3.4 a	1.2 a	140±7	140
Uniconazol	3.1 a	0.3 b	105±5	147
Triapenthenol	2.3 b	0.9 a	117±6	114

Table 5.3: Comparison of the effect of GA-biosynthesis inhibitors on autumn bloom

Treatment	Evaluation of bloom * (Nov.9.1987)
Control	0.15
Paclobutrazol	0.25
Uniconazol	1.24
Triapenthenol	0.05

* Evaluation in 4 stages: 0- no bloom; 1- few flowers; 2- medium bloom density; 3- abundant bloom.

Evaluating the stage of residual retardation effect in the next year revealed no effect in both the paclobutrazol and the triapenthenol but a still strong effect with uniconazol (see fig.2 of section 5.3).

5.2.2 Comparing the effect of paclobutrazol on a few peach cultivars.

For evaluating the sensitivity to paclobutrazol, three peach cultivars: Maravilha, Rhodes and Earligrande all grown on their roots in a meadow orchard system were treated under the drippers in Spring 1988 with 40 mg/drippers. The planting spacing was 1.8m*0.75 with drippers spaced every 50 cm along the irrigation lines. The dose thus reached 444 g a.i./ha. The response in growth arresting varied among the 3 cultivars according to their vigor (Fig 5.1) so that Maravilha the weakest cultivar in vigor was reduced most, Rhodes next and Earligrande the least. It can thus be concluded that the vigor of the cultivar has to dictate the dose required to obtain the growth reduction required. The growth obtained by Earligrande was not reduced enough. A higher dose at the time of application was probably needed.

Discussion

Excessive vegetative growth of peach trees is a severe problem especially in warm climates and with vigorous species. The direct effect of excessive vegetation is competition between fruit and vegetative sinks for carbohydrates, minerals water and hormones. The indirect effects are reduction in fruit quality due to shading; reduced fruiting potential in the shaded parts; elevation of the productive tree mantle; an increase in water requirements due to the increase in leaf area and an increase in the amount of wood that should be pruned.

With peaches, especially with vigorous early maturing cultivars, the excessive vegetative growth is particularly problematic since vigorous growth continues uninterrupted throughout the fruit development and maturation period. In the peach meadow orchard, vegetative growth is strongly enhanced by the severe pruning accentuating the imbalance between growth and fruiting even more (3). Use of growth retardants like daminozide have only a mild and insufficient effect, while paclobutrazol was shown to have a dose - response effect that could be tailored to the level of growth inhibition required. The unique characteristics of this chemical: low water solubility, extremely long survival in soil or on the plant, and predominant xylem movement (7) - make root application attractive. The potential leaching of the chemical when sprayed and by rain makes it difficult to control the dose taken up by the tree. On the other hand, soil application may be inefficient if

the material will not reach part or most of the roots due to fixation by organic matter (7), or if the roots are too deep as occurs with clean cultivation techniques.

We have shown the advantage of using paclobutrazol to control excessive vegetative growth of peach trees with application of the chemical under the drippers. The low solubility of paclobutrazol in water will result in the chemical acting for a relatively long period as a slow-release retardant. The relatively low dosages needed under drip irrigation will allow more economical use of the chemical than by other means of application. In this respect growth regulators act similarly to nutrients which have been shown to be taken up more efficiently with drip irrigation than with other irrigation systems.

In order to inhibit excessive growth, use of paclobutrazol should control tree vigor for one season only. This will allow a repeated application of the suitable dosage annually. Autumn or winter applications are more efficient than later soil treatment. To obtain the same level of growth control with late application, higher dosages are required. The reason for this effect is twofold. First, a lag period between treatment and response will result in too late an effect when treated in late spring and second, the dependence of response to soil application on uptake of applied water that serve as a carrier for the retardant. Rainy weather in winter time will allow root proliferation away from the drip-fed zone on one hand and also will delay need for irrigation till late spring. Hence uptake of the chemical will be too late in many instances to allow for strong enough control. On the other hand, there is an advantage in applying paclobutrazol after observing the initial growth in spring for determination of residual paclobutrazol effect. Uncertainty of paclobutrazol residue left in the tree or in the soil from previous treatment may lead to excess effect. Among the GBI tested, uniconazol is by far the most potent. With 440 g/ha, it kept the trees retarded for two full seasons as against paclobutrazol and triapenthenol that at that level, had an effect that lasted only one season. The dosage needed of paclobutrazol depends on the vigor of the cultivars. The more vigorous a cultivar, the higher the dosage needed to obtain the same level of inhibition. About 400 g/ha of paclobutrazol applied under the drippers is all that is required to obtain a full year's growth control in autumn or early spring application in a medium vigorous peach cultivar. One of the conspicuous effects of GBI is their effect on advanced bud break the year after treatment. Earlier bud break is connected to lower depth of rest in the buds. The finding that a higher level of autumn bloom occurred with GBI especially with uniconazol further strengthens this conclusion.

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Cooperation

In the course of this project, a visit of the principal Israeli investigator to Wenatchee WA (1986) and of the principal USA investigator to Bet-Dagan Israel (1987) were carried out. In these visits direct contact with the set of experiments done by the counterparts was possible. These visits ended with discussion of all the researchers involved regarding analysis of the results and further activities in the project. Mutual implementation of techniques especially of the analytical procedures developed in Wenatchee was carried out.

Other connections among the two groups apart from frequent correspondence was available in the International Horticultural Congress in Davis CA in 1986 and in the meeting of the working group on 'Growth regulators in fruit production' in Penticton Canada in 1988.

Benefit to Agriculture

The following are the immediate benefits coming out of this work:

1. Characterizing the timing and dose of paclobutrazol application to control excessive growth in peaches together with evaluation of the expected response according to cultivar vigor, may allow commercial utilization of this product in peach orchards grown in arid areas and irrigated by drip irrigation.
2. Comparison of the effect of a few triazole growth retardants may further improve the use of an array of chemicals varying in their potency and persistence in the plant tissues.
3. A new method of application to tree trunks was found to be an important and effective technique of application in stone fruits. This technique has a minimal soil pollution effect.
4. Development of new analytical methods to detect triazole residues is critical in knowing if and when to apply another dose of the triazole retardants to avoid excessive growth control.

Other benefits lie in our better understanding of the uptake, translocation and effects of paclobutrazol and other similar compounds in peach and other fruit tree species.