BIOLOGIA PLANTARUM (PRAHA)

31 (5) : 370–375, 1989

Anatomy of *Zea mays* and *Glycine max* Seedlings Treated with Triazole Plant Growth Regulators

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Abstract. Soil drenches containing 250 μg of paclobutrazol or uniconazol (50 ml of a 17 μM solution) reduced the height of both corn (*Zea mays* L. cv. How Sweet It Is) and soybean (*Glycine max* (L.) MERR. cv. A2) seedlings. With corn, uniconazol was considerably more active than paclobutrazol in reducing height whereas with soybean both compounds had similar dwarfing effects. The compounds increased foliar chlorophyll content and leaf thickness in soybean but had no effect on these parameters in corn. The increase in leaf thickness with soybean was due primarily to an increase in the thickness of the palisade cell layer. Chloroplast size and ultrastructure of both species were unaffected by the compounds. The growth regulators increased root diameter in both corn and soybean because of increased size of cortical parenchyma cells and particularly in soybean because of radial rather than longitudinal growth of the first few layers of the cortical parenchyma.

The triazole compounds, paclobutrazol (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol) and uniconazol ((E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol) are new synthetic gibberellin biosynthesis inhibitors which are active at extremely low dosages. These compounds retard shoot growth in a wide range of species and have a variety of potentially important applications in agriculture (DAVIS *et al.* 1986, 1988). In addition to reducing shoot growth, the triazoles reduce leaf expansion (JAGGARD *et al.* 1982, WOOD 1984, SANKHLA *et al.* 1985, 1986, STEFFENS *et al.* 1985) and generally increase chlorophyll content (JAGGARD *et al.* 1982, DALZIEL and LAWRENCE 1984, WOOD 1984, SANKHLA *et al.* 1985, STEFFENS *et al.* 1985, FLETCHER *et al.* 1986). It is not clear, however, whether increased chlorophyll content is due to enhanced chlorophyll biosynthesis or is simply a "concentrating effect" due to reduced leaf expansion or increased leaf thickness.

In addition to influencing above-ground plant organs, the triazoles alter root morphology. Roots of treated plants are generally shortened and thickened com-

Received November 21, 1988; accepted February 16, 1989

pared to untreated plants (STEFFENS *et al.* 1985, SANKHLA *et al.* 1986, WANG and FAUST 1986). Treated plants may also form a number of thickened adventitious roots just above the soil level (SANKHLA *et al.* 1985). The increased diameter of paclobutrazol-treated *Prunus persica* roots has been attributed to increased size of the cortical parenchyma cells (WILLIAMSON *et al.* 1986).

With the exception of some preliminary work on a few woody species (WOOD 1984, WILLIAMSON *et al.* 1986, BAUSHER and YELENOSKY 1987) little is known regarding the anatomical and ultrastructural changes that accompany the growth inhibition caused by soil-applied triazole growth regulators. The objective of this research, therefore, was to describe anatomical changes in leaves and roots of sweet corn and soybean seedlings treated with triazole compounds.

MATERIALS AND METHODS

In one experiment, corn (*Zea mays* L. cv. How Sweet It Is) and soybean (*Glycine max* (L.) MERR. cv. A2) were grown in a peat-perlite medium (1 :1 v/v) in a greenhouse with day/night temperatures of approximately 23/18 °C. Twenty-one days after sowing of the corn and 10 days after sowing of the soybean, plants were treated with soil applied paclobutrazol or uniconazol at a rate of 250 μg per 10 cm pot (50 ml of a 17 μM solution). Controls received water only. Additional plants were sprayed to runoff with a 6741 mg l^{-1} (8 mM) solution of the triazole herbicide, amitrol-t (3-amino-1,2,4-triazole), which is known to damage developing chloroplasts. The latter treatment was used to provide damaged chloroplasts as an additional comparison against the uncertain variation of paclobutrazol- and uniconazol-treated chloroplasts.

Plant heights in each treatment were measured just prior to microscopy fixation. Leaf samples from the first fully expanded leaf below the apex for soybean and the third leaf down from the apex for corn were taken for microscopic observation 21 d after treatment with paclobutrazol and uniconazol (when growth inhibition was clearly evident) and 5 days after treatment with amitrol-t (when visible damage began to occur). The tissue was fixed in gluteraldehyde acrolein in cacodylate buffer (0.2 M, pH 7.2–7.4) for 2–3 h with a post fixation in 1% osmium tetroxide in the same buffer for 1–2 h and stained with 0.05 % aqueous uranyl acetate. The tissue was then dehydrated in a graded ethanol series and, following changes in absolute acetone, was embedded in Spurr's epoxy resin (SPURR 1969). Thick sections were cut for light microscopy using a Fisher J134 microtome and were stained with a solution of 1% methylene blue, 1% Azure II, and 1% sodium carbonate. Ultrathin sections were placed on carbon coated grids, stained with lead citrate, and viewed in a transmission electron microscope (Hitachi HU11E).

Chlorophyll measurements were made using DMSO extraction (HISCOX and ISRAELSTAM 1979) on leaf tissue from the same area of the plant as that used for microscopy. Colorimetric measurements of extracts were made using an uv-visible spectrophotometer. Leaf thickness measurements were made by viewing leaf sections with a toolmaker's microscope.

In a second experiment, soybean and corn seeds were germinated in Petri dishes on filter paper soaked daily with either 5 mg l^{-1} paclobutrazol (17 µM), 5 mg l^{-1} uniconazol (17 µM), or water (control). The dishes were set in a growth chamber at 22 °C with a 9 h photoperiod. After approximately 60 h, when root length was between 10 and 20 mm, root sections approximately 2.5 mm from the seed were prepared for microscopy in the same manner as discussed above for leaf tissue. Root diameters were measured by viewing light microscope slides with a toolmaker's microscope.

Both experiments were conducted using a completely randomized design with a minimum of four replications per treatment.

RESULTS AND DISCUSSION

Plant heights of both species were reduced by the growth regulator treatments (Table 1). Both compounds had similar dwarfing effects with soybean, but with corn uniconazol was considerably more active than paclobutrazol in reducing plant height. The results with corn are in accordance with preliminary studies by BASSI *et al.* (1986) using *Poa pratensis* and *Gossypium hirsutum* which indicate that uniconazol is generally more active than paclobutrazol in retarding shoot growth.

TABLE 1

Plant height, foliar chlorophyll content, and leaf thickness for corn and soybean plants 21 days after treatment with 0 (control) or 250 µg (50 ml of a 17 µM solution) of paclobutrazol (Paclo) or uniconazol (Uni)

Parameter/ Species	Treatment		
	Control	Paclo	Uni
Plant height [cm]			
Corn	66 2a*	55 4b	43.1c
Soybean	22.8a	17.4b	18.0b
Chlorophyll [mg g^{-1} F.M.]			
Corn	2.26a	2.24a	2.06a
Soybean	2.05a	2.66b	2.41b
Leaf thickness [mm]			
Corn	0.148a	0.152a	0.154a
Soybean	0.136a	0.274b	0.268b

* Values within a row with a common lower case letter are not significantly different at the 5 % level of probability.

Soybean, however, may be an exception to this generality. We have observed that even at relatively low dosages (50 μg per 15 cm pot), paclobutrazol and uniconazol have similar dwarfing effects on soybean (DAVIS and SANKHLA 1987).

Foliar chlorophyll contents were significantly increased by paclobutrazol and uniconazol in soybean, but not in corn (Table 1). Leaf thickness was nearly doubled by the triazoles in soybean whereas corn leaf thickness was unaffected by the treatments. Light microscope photographs of paclobutrazol- and uniconazol-treated soybean leaf cross sections revealed that the increase in leaf thickness resulted mainly from an increase in the number of rows and elongation of palisade cells (Fig. 1). The anatomy of the mesophyll layer was relatively unaffected by the triazoles. With corn, no differences in leaf cross section anatomy were observed between treatments.

Electron micrographs of chloroplasts from each treatment revealed that there were no major differences in ultrastructure between paclobutrazol-treated, uniconazol-treated, and control chloroplasts (Fig. 2). It was also observed that triazoles did not influence individual chloroplast size. In contrast to paclobutrazol and uniconazol, amitrol-t caused serious disruption of chloroplasts within five days after treatment. The micrographs showed loss of membrane structure as well as a drastic reduction of grana and stroma thylakoids in amitrol-treated plants (Fig. 2). Hence, the ultrastructure of the chloroplasts from the paclobutrazol and uniconazol-treated plants was much more similar to that of the untreated controls than that of the amitrol-t treated plants.

Triazoles have generally increased chlorophyll content of treated plants, although exceptions have been reported (WIELAND and WAMPLE 1985). Based upon our observations, it seems likely that the increase in chlorophyll content in triazole-treated soybeans observed in the present study as well as others (SANKHLA *et al.* 1985, 1986) is a result of increased leaf thickness, and in particular because of the increased size of the palisade layer which contains a large number of chloroplasts. Chloroplast ultrastructure and size were not dramatically changed and were not likely causes of the dark green color observed in paclobutrazol- and uniconazol-treated soybean plants. It is interesting to note that with corn, the triazoles strongly inhibited shoot growth but did not increase chlorophyll content and did not affect leaf thickness.

The triazoles did not affect the germination rate or percentage of the corn or soybean seed. Likewise, root length was unaffected by the triazoles. Root diameters were increased by the triazoles, particularly in soybean (Table 2). It was also observed that the xylem tissue of soybean roots appeared to be somewhat disorganized in the triazole-treated roots as compared with the controls. The number of rows of cortical parenchyma cells in soybean was not increased by the triazoles, but rather individual cells were larger in treated roots compared to controls (Fig. 3). Cortical parenchyma cells of corn roots showed a similar effect, although not as pronounced. Another reason for the increased root diameter in triazole-treated

TABLE 2

Root lengths and cross section diameters for corn and soybean fixed approximately 60 h after treatment with either water (control) or 5 mg l^{-1} (17 μM) paclobutrazol (Paclo) or uniconazol (Uni)

Parameter/Species	Treatment		
	Control	Paclo	Uni
Root length [mm]			
Corn	11.0	10.7	12.5
Soybean	17.5	17.5	16.9
Root diameter [mm]			
Corn	0.897	1.225*	1.035
Soybean	1.215	2.065*	2.094*

* Significantly different than the control at 5 % probability level based on *f* test.

seedlings was that the inner rows of cortical parenchyma cells grew radially rather than longitudinally. This effect was most pronounced in the paclobutrazol-treated soybean seedlings. Similar morphological changes have been observed in paclobutrazol-treated *Citrus sinensis* seedling roots (BAUSHER and YELENOSKY 1987). The paclobutrazol concentration required to induce significant morphological changes in *C. sinensis* roots (1000 mg l^{-1} or 3.4 mM), however, was much greater than that used in the present study with soybean and corn (5 mg l^{-1} or 17 μM).

The results of the present study indicate that both paclobutrazol and uniconazol are effective inhibitors of shoot growth in corn and soybean. Although anatomical changes such as increased palisade layer thickness can accompany triazole-induced growth inhibition, such changes do not always occur as was observed with triazole-treated corn leaves which exhibited normal anatomy. Furthermore, we find no evidence that the triazoles alter chloroplast ultrastructure in these species. It is clear that the triazoles alter root anatomy, but it is not known how these changes affect root functions such as mineral and water uptake.

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Figures at the end of the issue.