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Effect of Paclobutrazol on Abscisic Acid Levels in Wheat Seedlings

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Abstract. Contradictory results have been reported for the effects of triazoles on abscisic acid (ABA) levels in plants. Paclobutrazol reduced the height, fresh weight, and ABA levels of wheat seedlings. The magnitude of the inhibitory effect of paclobutrazol on ABA levels was dependent on the length of time after application. ABA levels in plants as determined by gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS) were reduced by 50–60% 2 days after soil application. A further 15% reduction occurred by the seventh day after treatment. ABA level analyses began at the time of treatment with 4-day-old seedlings and continued until 21 days after treatment when only a 20% reduction was detected. These determinations using GC-SIM-MS should increase the understanding of triazole effects on ABA levels.

Paclobutrazol (PP 333) is one of a group of triazole fungicides, which have been under intensive investigation as crop plant growth regulators (Jung et al. 1987). The principal plant response reported has been the inhibition of internode growth of plants as seen in cereal grains due to a reduction of gibberellic acid biosynthesis (Dalziel and Laurence 1984, Hedden and Graebe 1985). Recently, however, the levels of *cis*-abscisic acid (ABA), another plant hormone, were found to be affected by triazole applications to plants. Results have been reported that include increases in ABA levels (Lurssen 1987), a transient rise in ABA levels (Asare-Boamah et al. 1986), no change (Izumi et al. 1988), and decreases in ABA levels (Grossman et al. 1987, Wang et al. 1987). The measurements of ABA levels were reported on different species of plants and at different plant growth stages after treatment. Little information has been reported concerning ABA levels in triazole-treated plants over a period of time other than the effect of triadimefon on beans for 8 days (Asare-Boamah et al. 1986).

Since the growth-retarding effects of triazoles on cereal grains are seen within 1 week after treatment, this study was done to determine whether ABA levels in young wheat seedlings might also be affected during the early periods of growth. An examination of the relationship between growth retardation and ABA levels should provide further information on the importance of time of measurement after treatment or plant species studied in understanding the effect of triazole treatment on ABA levels.

Materials and Methods

Wheat seeds (*Triticum aestivum* L.), "Purple Straw" (5 g), were sown in soil, and seedlings were treated 4 days later by soil application with paclobutrazol formulations: R_1 = 0.3 mg/pot and R_2 = 3.0 mg/pot (11 cm diameter). Each treatment was replicated three times. The plants were grown in a greenhouse during the month of May and watered uniformly as needed. Whole plant samples were taken at 0, 2, 5, 7, 9, 14, and 21 days after treatment. Plant heights (tip of the most expanded leaf) were measured for each of the 10 plants randomly selected per replicate at each sampling date. Top fresh weights for 30 plants were obtained after the plants were cut off at soil level. Plant material was frozen at −80°C for subsequent analysis.

For the ABA analysis, plants after partial thawing were extracted with 100% acetone in a Waring Blendor (duplicate samples). The internal standard, 750 ng of d_6-ABA (which was an intermediate quantity with respect to endogenous ABA levels), and BHT were added prior to blending (Rivier et al. 1977). After filtration and solvent evaporation, the aqueous residue was adjusted to pH 8.0 to minimize deuterium exchange and extracted twice with EtOAc. The aqueous residue was then adjusted to pH 2 and partitioned twice against EtOAc. The EtOAc layer, after evaporation, was subjected to high-performance liquid chromatography (HPLC) fractionation on a polystyrene PRP-1 semiprep column (0.8 × 25 cm) with a gradient of 20–100% CH_3CN in 0.006 N HCl over 30 min at 2.0 ml/min. The ABA-containing fractions were collected and treated with diazomethane. The MeABA-containing fractions were collected after reinjection us-

Table 1. Effect of paclobutrazol on height and fresh weight of wheat seedlings.

	Length (cm)[a] Days after paclobutrazol application						
	0	2	5	7	9	14	21
Paclobutrazol							
0.3 mg/pot		11.1	13.6	15.8	16.8	19.4	21.2
3.0 mg/pot		9.9	10.3	11.8	12.5	13.7	14.9
Control	3.0	13.8	19.0	21.2	23.9	27.1	32.1
LSD 0.05		1.6	0.7	0.8	0.8	1.1	1.6
	Weight (g)[b] Days after paclobutrazol application						
	0	2	5	7	9	14	21
Paclobutrazol							
0.3 mg/pot		26.6	54.3	55.8	71.1	94.7	112.7
3.0 mg/pot		24.3	44.5	55.0	59.5	75.8	104.2
Control	4.25	33.7	58.1	74.4	95.1	118.3	131.0

[a] Average length of three replicates of 10 plants.
[b] Weight of 30 plants.

ing the previously mentioned HPLC chromatography conditions. The MeABA samples were analyzed by gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS) using a HP 5992 instrument equipped with a J & W DB-1201 15-m capillary column, 0.25-μm film thickness using helium as the carrier gas at a flow rate of 1 ml/min. Ions m/z 162 and 190 for ABA and 166 and 194 for the deuterated ABA internal standard were monitored during the analysis. Duplicate injections were made for each sample.

Results and Discussion

Inhibition of wheat seedling growth by paclobutrazol was found within 2 days after treatment as seen by leaf length and fresh weight measurements (Table 1). These differences in growth caused by the two levels of paclobutrazol were apparent during the duration of the experiment, although the magnitude of the differences decreased. Plants treated with the larger quantity of paclobutrazol had reduced fresh weights by as much as 23–37% from day 2 to day 14. Even after 21 days, top fresh weights were reduced by 14–20% for both treatments. The effect of paclobutrazol on wheat plant height reduction remained unchanged after 21 days, where plants treated with the larger quantity of paclobutrazol were only 46% of the height of controls. Significant height reduction resulted from both levels of paclobutrazol treatment at each sampling date throughout the experiment.

The ABA levels were determined in 4-day-old wheat seedlings and then at intervals throughout the duration of the study for 21 days (Table 2). Significant decreases of approximately 60% in ABA levels were found in plants 2 days after paclobutrazol treatment (both levels). The ABA levels in the treated wheat plants decreased by 70–80% of levels in untreated plants during the period of 5–7 days after treatment. The magnitude of the decrease in ABA levels due to paclobutrazol was less in plants analyzed 9 and 14 days after treatment. Twenty-one days after treatment, the decreases in ABA levels were only 20% less than levels in untreated controls and were not considered significantly different.

Table 2. Effect of paclobutrazol on ABA levels in wheat seedlings.

Concentration (ng/g fresh wt)	Days after paclobutrazol application 0	2	5	7	9	14	21
Paclobutrazol							
0.3 mg/pot		27.1	16.7	13.5	67.4	23.8	21.9
3.0 mg/pot		34.8	18.2	20.3	51.9	24.3	22.6
Control	47.7	74.8	85.3	65.6	94.7	38.4	28.4
LSD 0.05		11.7	7.2	17.1	10.1	5.4	6.2

Decreases in ABA levels in wheat seedlings resulting from paclobutrazol treatment were found after 2 days and persisted through the duration of the study. The greatest decreases in ABA levels due to paclobutrazol treatment were measured after 5 days, whereas the maximum amount of seedling weight reduction and height inhibition was found after 9 and 21 days, respectively. These decreases in ABA levels in wheat seedlings are similar to those in soybean seedlings where a 50% reduction in immunoreactive ABA was found 21 days after seed treatment with the triazole, LAB 150978, or the norbornenodiazetine, tetcyclacis (Grossman et al. 1987). A 27% decrease in ABA levels in apple leaves was found 28 days after paclobutrazol treatment (Wang et al. 1987). A similar reduction in ABA levels in the fungus *Cercospora rosicola* of 35% due to 0.1 μM paclobutrazol and a 94% reduction after a 100 μM treatment was found after 48 h (Norman et al. 1986). However, ABA levels in rice shoots were not different from controls after treatment with uniconazole-P, another triazole growth regulator, for 17–22 days (Izumi et al. 1988). No difference in ABA levels as compared to controls was found by ABA radioimmunoassay in elephant grass (*Pennisetum purpureum* Schum) treated with paclobutrazol for 5 days (Rajasekaran et al. 1987). The transient rise in ABA levels in bean seedlings found 5 days after triadimefon treatment was of short duration and no difference in ABA levels was found later during the 8-day experiment (Asare-Boamah et al. 1986). The 21 days of measurement of

ABA levels in wheat seedlings reported here gave no clear indication of a transient rise in ABA levels after paclobutrazol treatment. The decrease in ABA levels in wheat reported here that diminished in magnitude with time was also not in agreement with the preliminary report of large increases in ABA levels measured by immunoassay after tripenthenol treatment (Lurssen 1987). The decrease in ABA levels due to paclobutrazol treatment may result from decreased ABA biosynthesis and this biosynthetic pathway is not different in stressed plant tissue (Zeevaart et al. 1989). Measurement of ABA levels in paclobutrazol-treated apple leaves showed no increase after a period of water stress suggesting that the biosynthetic pathway for ABA had been strongly affected by paclobutrazol (Wang et al. 1987).

The reported inconsistencies in triazole-induced changes in ABA levels may reflect differences in plant species or time of measurement after treatment (Hofstra et al. 1989). Additional variability may result from the analytical methods used. Differences in indole-3-acetic acid levels found in several plants were related to the degree of prepurification prior to immunoassay (Cohen et al. 1987). A GC-SIM-MS procedure, similar to the ABA analysis used here, was employed to validate the combination of various immunoassay and bioassay procedures for indole-3-acetic acid analysis. Based on our results, a better understanding of the effects of triazoles on endogenous ABA levels in plants may result if a series of successive measurements of ABA are made.

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