J Plant Growth Regul (1989) 8:45–51

Journal of
Plant
Growth
Regulation

Uniconazole Reduces Ethylene and 1-Aminocyclopropane-1-Carboxylic Acid and Increases Spermine Levels in Mung Bean Seedlings

G. Hofstra, L. C. Krieg, and R. A. Fletcher

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Received May 3, 1988; accepted August 8, 1988

Abstract. Uniconazole reduced growth of etiolated mung bean seedlings and increased lateral root formation. Ethylene production for whole seedlings was reduced by 80% within 24 h after treatment and 1-aminocyclopropane-1-carboxylic acid concentrations were reduced by approximately 40% in 12 h. Uniconazole treatment increased spermine levels by 100% by day 4, whereas spermidine and putrescine levels were not affected. Uniconazole, by inhibiting ethylene synthesis, may be increasing spermine levels, which in turn stimulate formation of root primordia.

The triazoles, a group of potent fungitoxic and plant growth regulatory compounds, increase hardening against several stresses (Fletcher and Hofstra 1985), and it has been established that they inhibit ergosterol and gibberellin biosynthesis in fungi and higher plants, respectively (Fletcher and Hofstra 1988, Graebe 1987). Contradictory results have been reported with respect to triazole-induced changes in ABA levels; results range from continued increases (Lürssen 1987), to a transient rise in ABA (Asare-Boamah et al. 1986), to no change (Izumi et al. 1988), and to decreases in ABA concentrations (Norman et al. 1986, Grossmann et al. 1987, Wang et al. 1987). The inconsistencies may reflect differences in plant species or time of measurement after triazole treatment. It has been suggested that ABA is involved in the cross-adaptation of plants to various stresses (Boussiba et al. 1975). Furthermore, increases in cytokininlike activity have also been reported (Fletcher and Arnold 1986, Grossmann et al. 1987, Izumi et al. 1988). Subsequently, it has been proposed that the triazoles appear to affect plant responses, at least in part, through changes in the growth regulators produced by the isoprenoid pathway (Fletcher and Hofstra 1985, 1988).

Compounds produced by other metabolic pathways have also been impli-

cated in the stress response. A plant's ability to withstand conditions of environmental stress has been related to changing polyamine concentrations (Shen and Galston 1985, Ormrod and Beckerson 1986). The levels of ethylene, produced from the same substrate as polyamines, are also drastically affected by environmental stresses such as drought and chilling (Yang and Hoffman 1984). The triazole, paclobutrazol, prevented stress-ethylene production, the accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-malonylaminocyclopropane-1-carboxylic acid (MACC), and the rise of putrescine and spermidine in water-stressed apple leaves (Wang and Steffens 1985).

The triazoles also stimulate rooting in both cuttings and intact plants (Sankla et al. 1985, Fletcher et al. 1988). Polyamines have also been shown to stimulate rooting (Fletcher et al. 1988, Jarvis et al. 1983) and to increase with the initiation of root primordia (Schwartz et al. 1986) and active cell division (Walker et al. 1985).

Since the triazoles affect the levels of both ethylene and polyamines, two biochemically linked growth regulators, as well as associated physiological processes, this study was undertaken to determine the effects of a potent triazole, uniconazole (S-3307), on the levels of polyamines, ACC, MACC, and ethylene in mung bean seedlings, and to determine the relationship of these changes to the stimulation of rooting.

Materials and Methods

Plant Material and Uniconazole Treatment

Mung bean seedlings were grown in sectioned plastic flats in vermiculite. On the day of planting, vermiculite was moistened with distilled water and flats were placed in an unlit growth chamber at 21°C. After 24 h, seedlings were treated with 0.5 μg/ml uniconazole. Treatment solution was applied as a soil drench. It was not required that plants be rewatered past this point.

Ethylene Quantification

At various time intervals seedlings were weighed, placed in 20 ml vials with 2–3 drops of distilled water, and immediately sealed with rubber serum stoppers. Whole seedlings were used to eliminate the production of stress ethylene from cutting of the tissue. After 3 h a 3 ml gas sample was injected for ethylene quantification into a gas chromatograph fitted with an automatic sampling valve, 1.8 m × 3 mm Porapak Q column (60/80 mesh), and a flame ionization detector.

ACC and MACC Quantification

At specific time intervals 1-2 g of whole seedlings were collected for ACC and MACC analysis according to the procedures described by Lizada and Yang

(1979) and Mikitzel (1983). Free ACC content was determined by chemical degradation of ACC to ethylene followed by quantification of the ethylene produced. The MACC content was determined by acid hydrolysis of MACC to ACC and conversion of the total ACC to ethylene. Subtraction of ethylene produced from the free ACC, from that produced from the total ACC, yielded ethylene produced solely from the converted MACC. Efficiency of chemical conversion of the extracted ACC, and the hydrolyzed MACC, to ethylene was determined, and accounted for, using internal ACC standards.

Polyamine Quantification

At various time intervals seedlings were collected, homogenized, and analyzed for polyamine levels according to the method of Roberts et al. (1984). A varian 5000 liquid chromatograph with a 250 mm Whatman Partisil 10 silica gel reverse phase column was used for separation. A methanol: 10% acetonitrile in water gradient eluted the benzoyl-derivatized polyamines within 20 min running from 45 to 80% methanol at 1ml/min. A 254 nm Varian selectable wavelength UV-5 detector was used for peak measurement. Respective retention times and quantifications were determined using authentic benzoylated standards.

Results and Discussion

Growth Parameters

Within 24 h after treatment, uniconazole applied as a soil drench to 1-day-old etiolated mung beans significantly reduced root and hypocotyl lengths by 15% and 35%, respectively (Table 1). Within 48 h plant epicotyl lengths were also reduced by approximately 20%, and by 30% in 96 h. Growth of the treated seedlings remained reduced throughout the duration of the experiment, and by 96 h hypocotyl length was the most severely retarded (reduced by 45%). Thus, growth of etiolated mung bean seedlings treated with uniconazole followed the general pattern of triazole-induced growth retardation observed in other species (Fletcher and Hofstra 1988). The number of lateral roots was also significantly increased in the treated seedlings, with the roots being thicker and whiter. Similar observations have been made for other species (Sankla et al. 1985). No adventitious roots developed during the duration of the experiment.

Fresh weight results were more variable: no differences were found with respect to cotyledon weights or the root weights; treated epicotyls weighed significantly ($p = 0.01$) more than control epicotyls only between 48 and 72 h after treatment; hypocotyl weights were, however, consistently reduced ($p = 0.05$) compared to control values for the duration of the experiment. Etiolation thus appears to dampen the previously observed shifts in, and distribution of, plant fresh weight as affected by the triazole compounds (Fletcher and Hofstra 1988).

Table 1. Effect of 0.5 μg/ml uniconazole on etiolated mung bean root, hypocotyl and epicotyl lengths (cm ± SE), and number of secondary roots

		Length (cm)				
		Hours after uniconazole application				
Tissue		0	24	48	72	96
Root	Control	2.2	5.1	8.2	9.1	10.8
	Uniconazole	—	4.3*	6.3*	7.7*	8.8*
Hypocotyl	Control	NA	3.4	8.6	13.4	16.5
	Uniconazole	—	2.3*	5.2*	6.0*	8.9*
Epicotyl	Control	NA	0.2	0.5	0.8	3.8
	Uniconazole	—	0.2	0.4*	0.5*	2.6*
Number of secondary roots	Control	NA	NA	2.9	11.4	16.5
	Uniconazole	—	—	3.8	15.4*	23.1*

* Significantly different from respective control values at $p = 0.05$.

Ethylene

Applications of uniconazole significantly reduced ethylene production from whole etiolated mung bean seedlings. Ethylene production (Table 2) was reduced by 17% within 6 h after treatment and reached a maximum reduction of 80% 24 h after treatment. Ethylene synthesis by the uniconazole-treated seedlings remained significantly below that of control seedlings for the duration of the experiment (96 h).

Ethylene production was high in all seedlings on the day of treatment. Beyer (1985) reported similar trends: a peak in ethylene production by 2 days after imbibition of pea seeds. This peak was subsequently followed by a gradual decline. Thus, the observed production pattern may be a normal trend for germinating seeds and young seedlings.

Uniconazole significantly reduced ACC levels by 12 h after treatment (Table 2), thereby chronologically preceding the observed decrease in ethylene production. This would be expected since ACC is the immediate precursor for ethylene biosynthesis. Maximum reductions in ACC concentrations were reached at 12 h after treatment (40% of controls) and then, again following the pattern of ethylene inhibition, declined with time. These trends imply that the observed inhibition of ethylene production by uniconazole is, at least in part, induced via a reduction in ACC synthesis.

However, the maximum reduction in ethylene of 80% was twice that of ACC and occurred by 24 h. Therefore, it may be that the reduction in ACC synthesis accounts for only a fraction of the observed reduction in ethylene production. A reduced conversion of ACC to ethylene was also observed by Abbas et al. (1988), who found that the reduction in ethylene synthesis in the roots could not be overcome by the addition of ACC. Increased conjugation of ACC to MACC does not appear to play a role since conjugation of ACC to MACC was delayed in the uniconazole-treated seedlings (Table 2).

Anatomical evidence for the reduced ethylene production was seen in the

Table 2. Effect of 0.1 μg/ml uniconazole on evolution of ethylene (per h) and the content of ACC and MACC in mung bean seedlings

Tissue		Concentration (n moles/g FW)						
		Hours after uniconazole application						
		0	6	12	24	48	72	96
Ethylene	Control	0.50	0.29	0.18	0.19	0.09	0.13	0.09
	Uniconazole	—	0.24*	0.10*	0.05*	0.04*	0.07*	0.06*
ACC content	Control	—	1.60	1.90	1.30	2.19	1.71	1.85
	Uniconazole	—	1.46	1.13*	0.91*	1.71*	1.38*	1.39*
MACC content	Control	—	1.03	1.79	1.70	4.30	3.33	3.66
	Uniconazole	—	1.02	1.34	1.71	2.58*	3.57	3.95

* Significantly different at $p = 0.05$.

chronologically promoted unhooking of the treated mung bean hypocotyls (data not reported) and the enhanced development of lateral roots (Table 1).

Uniconazole and Polyamines

Whole mung bean seedlings initially contained relatively high concentrations of both spermidine and spermine, each of which declined with age of the seedling (Table 3). Conversely, between days 1 and 3 putrescine levels increased by more than two fold (Table 3). This increase in putrescine could be associated with the falling demand for both spermidine and spermine synthesis as the seedlings aged. Shen and Galston (1985) and Smith et al. (1985) discussed progressively declining spermine and spermidine levels with increasing age of pea roots and pea internodes; the highest concentrations were found in young, meristematic, predominantly unelongated tissue. Dumortier et al. (1983) have specifically correlated elevated spermine levels to discrete zones of mitotic activity in corn seedlings, with polyamine concentrations declining as the zones matured, whereas Schwartz et al. (1986) found a rise in spermidine levels and a decrease in putrescine levels in the apices of corn roots. Shen and Galston (1985) have suggested that increasing putrescine levels may be correlated with a block in the conversion of putrescine to spermidine and a subsequent switch from cell division to elongation.

Consequently, observed trends between age and spermine or spermidine levels may be explained via the use of whole germinating mung beans with concentrated regions of mitotic activity, which decrease over time in importance with respect to their contribution to total plant polyamine levels.

Uniconazole significantly increased, over control levels, the spermine content of the seedlings by day 2 after treatment. Spermine concentrations in treated mung bean increased consecutively from 68% greater than control values on day 2 after treatment to 100% greater by day 4. Uniconazole did not affect either spermidine or putrescine concentrations (Table 3).

Uniconazole also increased the number of secondary roots differentiating

Table 3. Effect of 0.1 μg/ml uniconazole on the levels of putrescine, spermidine and spermine in mung bean seedlings.

		Polyamines n moles/g FW)					
		Hours after uniconazole application					
Tissue		0	12	24	48	72	96
Putrescine	Control	66.3	61.4	60.1	72.7	144.7	84.3
	Uniconazole	—	64.7	69.6	77.9	114.6	68.7
Spermidine	Control	322	326	297	146	150	108
	Uniconazole	—	307	326	197*	148	122
Spermine	Control	126.8	62.4	49.8	22.9	12.4	6.2
	Uniconazole	—	61.1	63.2	38.4*	23.8*	12.0*

* Significantly different at $p = 0.05$

from the treated mung beans (Table 1). Increases in root number and polyamine levels by triazoles have also been shown in apple seedlings (Wang and Faust 1986). Considering the times at which ethylene synthesis was inhibited (6 h after treatment) and elevated spermine concentrations were detected (24 h after treatment), and lateral roots were first observed (48 h after treatment), it is possible that uniconazole, by inhibiting ethylene production, stimulates spermine synthesis (Ickeson et al. 1985), which in turn regulates RNA and DNA levels (Cohen et al. 1984, Smith 1985) and the cellular division required for root development and unhooking of the hypocotyls and epicotyls.

Acknowledgments. This research was supported by the Natural Science and Engineering Research Council of Canada. We thank V. Kallidumbil for technical assistance and Chevron Chemical Company for a generous supply of uniconazole (S-3307).

References

Abbas S, Fletcher RA, Murr DP (1988) Alteration of ethylene synthesis in cucumber seedlings by triadimefon. Can J Botany, In Press

Asare-Boamah NK, Hofstra G, Fletcher RA, Dumbroff EB (1986) Triadimefon protects bean plants from water stress through its effects on abscisic acid. Plant Cell Physiol 27(3):383–390

Beyer EM (1985) Ethylene metabolism. In: Roberts JA and Tucker GA (eds) Ethylene and plant development. Butterworths, Toronto, pp 125–138

Boussiba S, Rikin A, Richmond AE (1975) The role of abscisic acid in cross adaptation of tobacco plants. Plant Physiol 56:337–339

Cohen E, Arad S, Heimer YH, Mizrahi Y (1984) Polyamine biosynthetic enzymes in the cell cycle of *Chlorella:* correlation between ornithine decarboxylase and DNA synthesis at different light intensities. Plant Physiol 74:385–388

Dumortier FM, Flores HE, Shekhawat NS, Galston AW (1983) Gradients of polyamines and their biosynthetic enzymes in coleoptiles and roots of corn. Plant Physiol 72:915–918

Fletcher RA, Arnold V (1986) Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol Plant 66:197–201

Fletcher RA, Asare-Boamah NK, Krieg LC, Hofstra G, Dumbroff EB (1988) Triadimefon stimulates rooting in bean hypocotyls. Physiol Plantarum, 73:401–405

Fletcher RA, Hofstra G (1988) Triazoles as potential plant protectants. In: Berg K, Plempel M (eds) Sterol biosynthesis inhibitors—Pharmaceutical and Agrochemical Aspects. Ellis Harwood Ltd., UK

Fletcher RA, Hofstra G (1985) Triadimefon: a plant multi-protectant. Plant Cell Physiol 26: 775–778
Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465
Grossmann K, Kwiatkowski J, Siebecker H, Jung J (1987) Regulation of plant morphology by growth retardants. Plant Physiol 84:1018–1021
Ickeson I, Goldlust A, Apelbaum A (1985) Influence of ethylene on S-adenosylmethionine decarboxylase activity in etiolated pea seedlings. J Plant Physiol 119:335–345
Izumi K, Nakagawa S, Kobayashi M, Oshio H, Sakurai A, Takahashi N (1988) Levels of IAA, cytokinins, ABA and ethylene in rice plants as affected by a gibberellin biosynthesis inhibitor, uniconazole-P. Plant Cell Physiol 29:97–104
Jarvis BC, Shannon PRM, Yasmin S (1983) Involvement of polyamines with adventitious root development in stem cuttings of mung bean. Plant Cell Physiol 24:677–683
Lizada MCC, Yang SF (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100:140–145
Lürssen K (1987) Physiological effects of triazole PGRs in relation to their biochemical mode of action. Pesticide Sci 21:310–311
Mikitzel LJ (1983) Bruise-induced stress ethylene of tomato fruits and a possible role for calcium in stress ethylene production. M.Sc. Thesis, Univ. of Guelph
Norman SM, Bennett RD, Poling SM, Maier VP, Nelson MD (1986) Paclobutrazol inhibits abscisic acid biosynthesis in *Cercospora rosicola*. Plant Physiol 80:122–125
Ormrod DP, Beckerson DW (1986) Polyamines as antiozonants for tomato. Hort Sci 24(4):1070–1071
Roberts DR, Walker MA, Thompson JE, Dumbroff EB (1984) The effects of inhibitors of polyamine and ethylene biosynthesis on senescence, ethylene production and polyamine levels in cut carnation flowers. Plant Cell Physiol 25(2):315–322
Sankla N, Davis TD, Upadhyaya A, Sankla D, Walser RH, Smith BN (1985) Growth and metabolism of soybean as affected by paclobutrazol. Plant Cell Physiol 26:913–921
Schwartz M, Altman A, Cohen Y, Arzee T (1986) Localization of ornithine decarboxylase and changes in polyamine content in root meristems of *Zea mays*. Physiol Plantarum 67:485–492
Shen H, Galston AW (1985) Correlations between polyamine ratios and growth patterns in seedling roots. J Plant Growth Regul 3:353–363
Smith MA, Davies PJ, Reid JB (1985) Role of polyamines in gibberellin-induced internode growth in peas. Plant Physiol 78:92–99
Smith TA (1985) Polyamines. Annu Rev Plant Physiol 36:117–143
Walker MA, Roberts DR, Shih CY, Dumbroff EB (1985) A requirement for polyamines during the cell division phase of radicle emergence in seeds of *Acer saccharum*. Plant Cell Physiol 26(5):967–997
Wang SY, Steffens GL (1985) Effect of paclobutrazol on water stress-induced ethylene biosynthesis and polyamine accumulation in apple seedling leaves. Phytochemistry 24:2185–2190
Wang SY, Faust M (1986) Effect of growth retardants on root formation and polyamine content in apple seedlings. J Am Soc Hort Sci 111:912–917
Wang SY, Sun T, Ji ZL, Faust M (1987) Effect of paclobutrazol on water stress-induced abscisic acid in apple seedling leaves. Plant Physiol 84:1051–1054
Yang FA, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol 35:155–189