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Uniconazole Inhibits Stress-Induced Ethylene in Wheat and Soybean Seedlings

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Abstract. Previous studies have shown that uniconazole inhibits ethylene synthesis and protects plants from various stresses. The present research was conducted to delineate the mechanism of ethylene inhibition by uniconazole [(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol]. Following heat stress of 48°C for 3 h, the shoots of the control wheat seedlings became desiccated, and the seedlings lost 23% of their fresh mass 8 h after stress. The control soybean seedlings had epinastic unifoliate leaves 5 h after foliar application (4.4 g.a.i./ha) of the herbicide triclopyr [(3,5,6-trichloro-2-pyridinyl)oxyacetic acid]. Soil drench applications of uniconazole, a potent member of the triazole family, reduced these symptoms associated with heat and herbicide stress in wheat (5.0 mg/L) and soybean (0.4 mg/L) seedlings, respectively.

Basal ethylene production was inhibited 32 and 48% by uniconazole in the wheat and acotyledonous soybean seedlings, respectively. Following a 48°C heat stress, 1-aminocyclopropane-1-carboxylic acid (ACC) levels increased 40% in both the control and uniconazole-treated wheat seedlings. After triclopyr application, ACC levels increased 400% in both the control and uniconazole-treated soybean seedlings. The increased ACC levels, following stress, were accompanied by increased ethylene production from the control, but not from the uniconazole-treated wheat and acotyledonous soybean seedlings. Uniconazole treatment did not significantly change the basal or stress-induced N-malonyl-1-aminocyclopropane-1-carboxylic acid (MACC) levels compared to controls. These results suggest that uniconazole inhibits ethylene synthesis by interfering with the conversion of ACC to ethylene in wheat and acotyledonous soybean seedlings. Ethylene production and ACC conversion were not inhibited by uniconazole in excised soybean cotyledons. These results indicate that different ethylene-forming enzyme (EFE) systems operate in the soybean acotyledonous seedling and cotyledon, and the system in the former is inhibited by uniconazole.

It has been suggested (Rademacher et al. 1987) that the triazole group of plant growth regulators reduce shoot elongation through inhibition of cytochrome P-450-dependent oxidations during gibberellin biosynthesis. In addition to inhibiting plant growth, the triazoles mediate a number of biochemical, hormonal, and morphological changes in plants, including stimulated rooting and protection from various environmental and chemical stresses (reviewed by Fletcher and Hofstra 1985, 1988; Davis et al. 1988).

Ethylene is a gaseous plant hormone, which is synthesized by the oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang 1979). The tendency of stress to promote ethylene biosynthesis has led to the term "stress ethylene." The conversion of ACC to ethylene is mediated by the ethylene-forming enzyme (EFE) which has not been characterized, although several systems with apparent EFE activity have been described (Yang and Hoffman 1984). It has been suggested that lipoxygenase (EC 1.13.11.12)-generated hydroperoxides could oxidize ACC to ethylene (Bousquet and Thimann 1984; Kacperska and Kubacka-Zebalska 1989), while other reports dismiss this hypothesis (Lynch et al. 1985; Wang and Yang 1987). Recently, it has been suggested that a cytochrome P-450-mediated monooxygenase system may be responsible for the conversion of ACC to ethylene (Grossman et al. 1989). In addition to ethylene formation, ACC can be conjugated to form N-malonyl-1-aminocyclopropane-1-carboxylic acid (MACC) (Hoffman et al. 1982). The conjugation of ACC to

MACC is essentially irreversible and has been suggested to have a role in controlling ethylene biosynthesis (Yang et al. 1985). The phenomena of stress to promote ethylene biosynthesis, and the ability of the triazoles to protect plants from stress, suggests an interesting relationship between ethylene and triazoles.

Previous research suggested that triazoles inhibit basal ethylene production through reduced ACC synthesis (Abbas et al. 1989; Hofstra et al. 1989). However, it has been demonstrated that plant growth retardants of the norbornanodiazetine and triazole types inhibit ethylene production due to impaired conversion of ACC to ethylene (Grossmann et al. 1989; Sauerbrey et al. 1988). In order to determine the mechanism of ethylene inhibition by uniconazole, the present study examines ACC, MACC, and ethylene production before (basal) and following two different stresses, heat and an auxin-like herbicide, in a monocotyledonous and dicotyledonous species.

Materials and Methods

Plant Material and Chemical Treatment

Wheat (*Triticum aestivum* cv. Frederick) and soybean (*Glycine max.* cv. Bicentennial), 20 and 6 seeds, respectively, were planted in vermiculite using a double styrofoam cup system (Asare-Boamah et al. 1986). They were drenched with 250 ml of uniconazole solution (50% wettable powder in water) at a rate of 5.0 and 0.4 mg/L for wheat and soybeans, respectively. The controls were drenched with water. Plants were grown in a controlled environment room with day/night lighting and temperatures of 16/8 h and 25/20°C, respectively, with watering as required. Light from Sylvania metarc lamps, reached 200 $\mu E\ m^{-2}\ s^{-1}$ irradiance at plant level. When the plants were 8 days old, the wheat seedlings were heat stressed in a controlled environment chamber at 48°C with 50% relative humidity for 3 h and then returned to the growth room until sampled. Soybean seedlings, with their unifoliate leaves unfolding, were foliar sprayed with an auxin-like herbicide, triclopyr, equivalent to 4.4 g.a.i./ha. Triclopyr [(3,5,6-trichloro-2-pyridinyl)oxyacetic acid] is a pyridine analog of the phenoxy herbicides. In order to minimize the interference of "wound ethylene" in acotyledonous seedlings, ethylene measurements were made 3 h after excision of the cotyledons.

Ethylene Quantification

Two seedlings were weighed and placed in 50-ml test tubes containing 3 ml of distilled water. Four excised cotyledons were incubated in 50-ml Erlenmeyer flasks with 3 ml of distilled water. For ACC-dependent ethylene production, 0.5 μmol of ACC were added to the incubation tube or flask. The tubes were sealed with a rubber serum stopper and incubated in the dark until a detectable quantity of ethylene was present (1–4 h). A 3-ml gas sample was collected and the ethylene content of the sample was determined using a Hewlett-Packard gas chromatograph fitted with an automatic sampling valve, 1.8 m × 3 mm Poropak Q column (60/80 mesh), flame ionization detector, and Hewlett Packard integrator. The injector, column, and detector temperatures were 60°, 80°, and 250°C, respectively, and the helium carrier gas flow was 24 ml/min.

ACC and MACC Quantification

At the required time intervals two whole plants were weighed and homogenized together in 70% ethanol for ACC and MACC analysis. Free ACC content was determined by chemical oxidation of ACC to ethylene, which was measured according to the method of Lizada and Yang (1979). MACC levels were determined by acid hydrolysis (2.0 M HCl at 90°C for 2 h) of MACC to ACC and then the ACC was quantified as before. Subtraction of the free ACC from the ACC levels after acid hydrolysis yielded the quantity of MACC in the tissue. Efficiency of the conversion of the extracted ACC was determined and accounted for using internal ACC standards for each sample.

All experiments and statistical analysis (ANOVA, Duncan's new multiple range test) were based on a completely randomized design.

Results and Discussion

Anatomical Changes Induced by Uniconazole and Stress

Eight-day-old wheat and soybean seedlings treated with 5.0 and 0.4 mg L^{-1} uniconazole showed the typical visual symptoms of retarded shoot elongation and stimulated root growth [reviewed by Fletcher and Hofstra (1988)]. Eight hours after the heat stress period, the shoots of the control wheat seedlings were desiccated and flaccid, while the uniconazole-treated seedlings were turgid; seedlings lost 23 and 2.4% of their fresh mass, respectively (Fig. 1). The dry mass of the control and uniconazole-treated wheat seedlings were not significantly different (data not shown) and for this reason all parameters quantified for the wheat are expressed on a dry weight basis. These results are in agreement with previous findings (Booker et al. 1991) that uniconazole protects wheat seedlings from a 3-h period of 48°C heat stress. They hypothesized that uniconazole-treated plants had a higher root to shoot ratio and were able to sustain higher transpiration rates to maintain lower leaf temperatures. The shoots of the control soybean seedlings

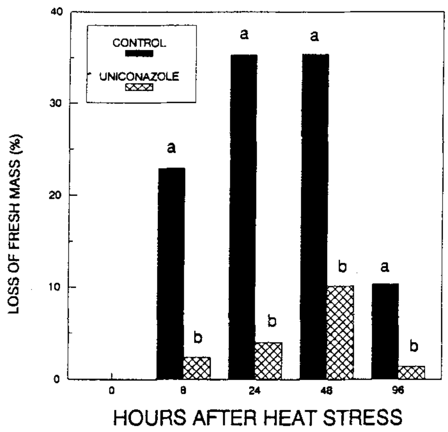

Fig. 1. Effect of uniconazole treatment (5.0 mg/L) on the loss of fresh mass in 8-day-old wheat seedlings following a heat stress of 48°C for 3 h. After the heat stress the plants were returned to the growth room and watered. Within each time period, treatment means marked by different letters are significantly different (p = 0.05; N = 3).

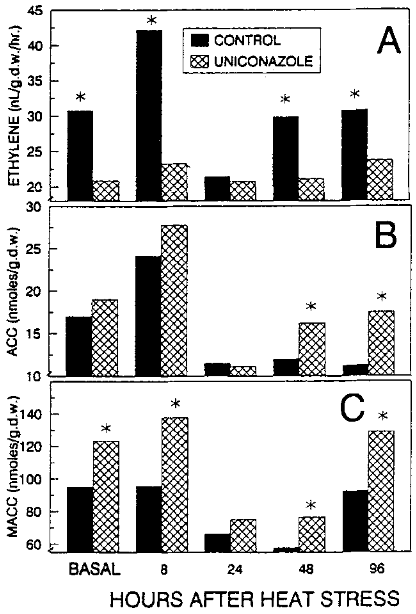

Fig. 2. Influence of uniconazole (5.0 mg/L) on the basal and stress-induced (**A**) rate of ethylene production, (**B**) ACC, and (**C**) MACC levels from 8-day-old wheat seedlings following a heat stress of 48°C for 3 h. Within each time period, treatment means marked by an asterisk (*) are significantly different (p = 0.050; N = 3).

had epinastic unifoliate leaves 5 h after triclopyr application, while the uniconazole-treated seedlings did not. Epinasty is a classic symptom of elevated ethylene production (Crocker et al. 1932).

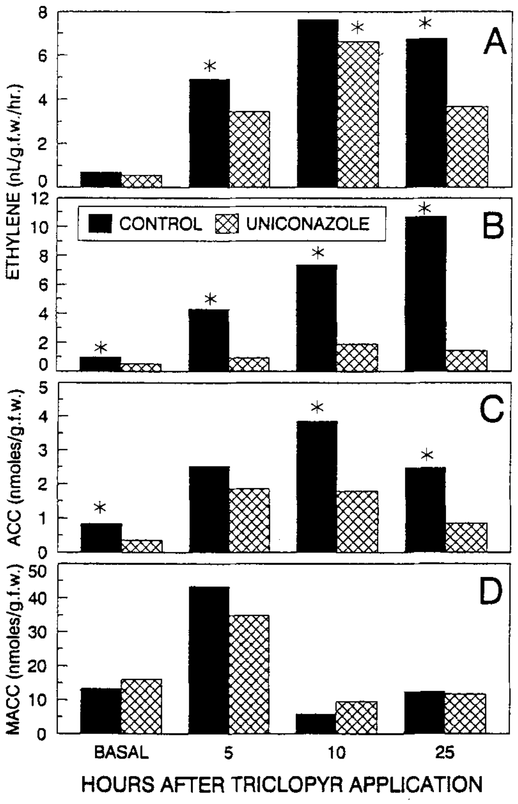

Fig. 3. Influence of uniconazole (0.4 mg/L) on the basal and triclopyr-induced (4.4 g.a.i./ha) (**A**) rate of ethylene production from intact soybean seedlings, (**B**) rate of ethylene production from acotyledonous soybean seedlings, (**C**) ACC, and (**D**) MACC levels from 8-day-old intact soybean seedlings. Within each time period, treatment means marked by an asterisk (*) are significantly different (p = 0.05; N = 3).

Ethylene Production

Compared to the controls, uniconazole significantly inhibited the basal rate of ethylene production by 32% in wheat (Fig. 2A) but not in the intact soybean seedlings (Fig. 3A). However, in the acotyledonous soybean seedlings, uniconazole treatment significantly reduced basal ethylene evolution by 48% (Fig. 3B). This supports previous findings (Abbas et al. 1989; Fletcher et al. 1988) that the triazoles, uniconazole and triadimefon, inhibit basal ethylene production in wheat and cucumber seedlings, respectively.

Eight hours after heat stress, the rate of ethylene production from the control wheat seedlings increased 12 nL/h, while the uniconazole-treated seedlings only increased 2 nL/h (Fig. 2A). Twenty-four hours after the heat stress, the rate of ethylene production from the control wheat seedlings rapidly declined to that of the uniconazole-treated seed-

Table 1. Ethylene production and exogenous ACC (0.5 μM) conversion rates (nl/g fresh wt/h) in excised soybean cotyledons.[a]

	C	U	C + T	U + T
Ethylene	0.438 b	0.447 b	1.079 a	1.030 a
ACC conversion	9.545 a	8.357 a	8.079 a	9.439 a

[a] The cotyledons were excised from control (C), uniconazole-treated (U), control sprayed with triclopyr (C + T), and uniconazole-treated seedlings sprayed with triclopyr (U + T) 5 h prior to excision. Ethylene production and ACC conversion rates followed by different letters are significantly different ($p = 0.05$, $N = 4$).

lings. It is reported (Apelbaum and Yang 1981; Wright 1977) that ethylene production decreases when tissue loses more than 10% of its fresh mass. Likewise, the control wheat seedlings lost 36% of their fresh weight (Fig. 1) and this could account for the reduction. Subsequently, 96 h after heat stress, the control wheat seedlings were only 10% dehydrated and the rate of ethylene production returned to the basal level (Fig. 2A).

Five hours after triclopyr application the rate of ethylene production in the control and uniconazole-treated soybean seedlings increased 7.4- and 8.0-fold, respectively. This increase continued for 10 h after triclopyr application and then declined by 25 h (Fig. 3A). However, when the cotyledons were excised 3 h prior to ethylene quantification, ethylene production in the acotyledonous soybean plants increased progressively (Fig. 3B). This increase was prevented and the absolute levels of ethylene were significantly lower than the controls in the uniconazole-treated seedlings (Fig. 3B).

These results demonstrate that uniconazole virtually eliminates the increased rates of ethylene production following stress (stress ethylene) in wheat and acotyledonous soybean seedlings. The fact that uniconazole is a less efficacious inhibitor of ethylene production from intact soybean seedlings indicates that there may be an ethylene-forming system in the cotyledons which is not inhibited by uniconazole.

Ethylene Production in Excised Soybean Cotyledons

Since uniconazole did not effectively inhibit ethylene production from soybeans when the cotyledons were attached to the plant, the characteristics of ethylene production from excised cotyledons were examined. Uniconazole did not inhibit the basal or triclopyr-induced rate of ethylene production from excised cotyledons (Table 1). Triclopyr treatment resulted in a twofold increase in ethylene from the cotyledons but the same treatment in the intact plants resulted in up to an eightfold increase (Fig. 3A). Hence, the conversion of exogenous ACC was examined in the excised cotyledons and it was demonstrated that uniconazole and triclopyr have no influence on the rate of ACC conversion in excised cotyledons (Table 1). Matheussen (1989) demonstrated that *Phaseolus vulgaris* cotyledons are sinks for ACC in young seedlings. In our laboratory, it was observed that ethylene production from excised soybean cotyledons approached undetectable levels 5 h after excision (data not shown). It is possible that excised cotyledons have a limited capacity to produce ACC but have an EFE system which is very active. The high capacity of the cotyledons to covert ACC to ethylene (Table 1) coupled with the high lipoxygenase activity in the cotyledons (Axelrod et al. 1981) warrant further investigation to characterize their relationship.

ACC Levels

The formation of ACC from S-adenosylmethionine (SAM) is generally considered to be the rate-limiting reaction in ethylene biosynthesis (Yang and Hoffman 1984). ACC levels were investigated as inhibition of ACC synthesis by uniconazole could be responsible for the reduced ethylene production observed from the uniconazole-treated seedlings. The basal ACC levels of the control and uniconazole-treated wheat seedlings were not significantly different (Fig. 2B). However, uniconazole treatment significantly reduced the basal ACC level of soybeans (Fig. 3C). Abbas et al. (1989) demonstrated that triadimefon, a triazole derivative, reduced basal ACC levels in cucumber seedlings and suggested that this could be the mechanism of ethylene inhibition.

Eight and five hours after stress in wheat exposed to heat and soybeans treated with triclopyr, the ACC levels increased 1.5- and fourfold, respectively, and there was no significant difference between the control and uniconazole-treated seedlings (Figs. 2B and 3C). These results do not support the hypothesis (Abbas et al. 1989; Hofstra et al. 1989) that uniconazole inhibits ethylene biosynthesis through reduced ACC synthesis. Removal of ACC by conjugation to MACC (Yang and Hoffman 1984) or inhibition of the conversion of ACC to ethylene are other possible explanations for the observed inhibition of ethylene production by uniconazole.

MACC Levels

The basal and heat stress-induced levels of MACC are significantly higher in the uniconazole-treated wheat seedlings (Fig. 2C). Uniconazole may increase the MACC level of the treated seedlings by stimulating the conversion of ACC to MACC, but the higher MACC levels are likely due to the fact that the uniconazole-treated seedlings have more free ACC, due to high ACC synthesis and reduced ethylene production. In the soybean seedlings, there was no significant difference between the control and uniconazole-treated basal MACC levels (Fig. 3D). Triclopyr treatment resulted in a 2.2- and 3.5-fold increase in MACC levels in the uniconazole-treated and control soybean seedlings, respectively (Fig. 3D). These results suggest that increased conjugation of ACC to MACC is not the mechanism of ethylene inhibition by uniconazole.

In summary, it is concluded that uniconazole treatment prevents the visual symptoms associated with a 3-h period of 48°C heat stress and triclopyr application in wheat and soybean seedlings, respectively. Application of stress resulted in increased ACC levels in both the control and uniconazole-treated wheat and soybean seedlings. This demonstrates that even though the uniconazole-treated seedlings were protected, the levels of ACC increased following stress. Uniconazole did not influence the conjugation of ACC to MACC in soybeans, while having a minimal effect in wheat. The rate of ethylene production remained lower in the uniconazole-treated wheat and acotyledonous soybean seedlings before and following the stress, indicating that uniconazole reduces ethylene production by interfering with the conversion of ACC to ethylene as suggested previously (Grossmann et al. 1989; Sauerbrey et al. 1988). These results demonstrate that monocotyledonous and dicotyledonous species exposed to different stresses respond in a very similar manner in regard to ethylene biosynthesis. However, it appears that the soybean contains two distinguishable EFE systems, one in the acotyledonous plant and another in the cotyledon, and uniconazole only inhibits the EFE system in the acotyledonous seedling. The results of this study indicate that soybeans have two distinct EFE systems and that uniconazole may be a unique and powerful tool in delineating their differences.

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References

Abbas S, Fletcher RA, Murr DP (1989) Alteration of ethylene synthesis in cucumber seedlings by triademefon. Can J Bot 67:278–280

Adams DO, Yang SF (1979) Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76:170–174

Apelbaum A, Yang SF (1981) Biosynthesis of stress-ethylene induced by water deficit. Plant Physiol 68:594–596

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

Axelrod B, Cheesbrough TM, Zimmer S (1981) Lipoxygenase from soybeans. Meth Enzymol 71:441–451

Booker HM, Gillespie TJ, Hofstra G, Fletcher RA (1991) Uniconazole-induced thermal tolerance in wheat seedlings is mediated by transpirational cooling. Physiol Plant (in press)

Bousquet J-F, Thimann KV (1984) Lipid peroxidation forms ethylene from 1-aminocyclopropane-1-carboxylic acid and may operate in leaf senescence. Proc Natl Acad Sci USA 81:1724–1727

Crocker W, Zimmerman PW, Hitchcock AE (1932) Ethylene-induced epinasty of leaves and the relation of gravity to it. Contrib Boyce Thompsom Instit 4:177

Davis TD, Steffens GL, Sankhla N (1988) Triazole plant growth regulators. Hortic Rev 10:63–105

Fletcher RA, Hofstra G (1985) Triademefon a plant multiprotectant. Plant Cell Physiol 26:775–778

Fletcher RA, Hofstra G (1988) Triazoles as potential plant protectants. In: Berg D, Plempel M (eds) Sterol biosynthesis inhibitors: Pharmaceutical and Agricultural aspects. Ellis Horwood Ltd., England, pp 321–331

Fletcher RA, Santakumari M, Murr DP (1988) Imposition of water stress in wheat improves the efficacy of uniconazole-induced thermal resistance. Physiol Plant 74:360–364

Grossmann K, Hauser C, Sauerbrey E, Fritsch H, Schmidt O, Jung J (1989) Plant growth retardants as inhibitors of ethylene production. J Plant Physiol 134:538–543

Hoffman NE, Yang SF, McKeon T (1982) Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher plants. Biochem Biophys Res Commun 104:765–770

Hofstra G, Krieg LC, Fletcher RA (1989) Uniconazole reduces ethylene and 1-aminocyclopropane-1-carboxylic acid and increases spermine levels in mung bean seedlings. J Plant Growth Regul 8:45–51

Kacperska A, Kubacka-Zebalska M (1989) Formation of stress ethylene depends both on ACC synthesis and on the activity of free radical-generating system. Physiol Plant 77:231–237

Lizada MCC, Yang SF (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100:140–145

Lynch DV Sridhara S, Thompson JE (1985) Lipoxygenase-generated hydroperoxides account for the nonphysiological features of ethylene formation from 1-aminocyclopropane-1-carboxylic acid by microsomal membranes of carnations. Planta 164:121–125

Mathuessen AM (1989) Ethylene, ACC and MACC in the seeds and young seedlings of *Phaseolus vulgaris* L. Diss Abs Intl, Biol Sci Eng 49:12

Rademacher W, Fritsch H, Graebe JE, Sauter H, Jung J (1987) Tetcyclasis and triazole-type plant growth retardants: Their influence on the biosynthesis of gibberellins and other metabolic processes. Pestic Sci 21:241–252

Sauerbrey E, Grossmann K, Jung J (1988) Ethylene production by sunflower cell suspensions: Effects of plant growth retardants. Plant Physiol 87:510–513

Wang T-T, Yang SF (1987) The physiological role of lipoxygenase in ethylene formation from 1-aminocyclopropane-1-carboxylic acid in oat leaves. Planta 170:190–196

Wright STC (1977) The relationship between leaf water potential and the levels of abscisic acid and ethylene in excised wheat leaves. Planta 134:183–189

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189

Yang SF, Liu Y, Su L, Peiser GD, Hoffman NE, McKeon T (1985) Metabolism of 1-aminocyclopropane-1-carboxylic acid. In: Roberts JA, Tucker GA (eds) Ethylene and plant development. Butterworths, London, pp 9–22