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Effect of Plant Growth Regulators on Ethylene Production, 1-Aminocyclopropane-1-carboxylic Acid Oxidase Activity, and Initiation of Inflorescence Development of Pineapple

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Abstract. With the development of pineapple [*Ananas comosus* (L.) Merr.] as a fresh fruit crop, it became common to force inflorescence development with ethephon [(2-chloroethyl)phosphonic acid] or ethylene throughout the year. Environmental induction (EI) of inflorescence development disrupts scheduling of fruit harvest and may cause significant losses if small plants are induced, resulting in fruits that are too small to be marketable. Our objective was to identify plant growth regulators (PGRs) that could inhibit EI. Because circumstantial evidence indicates that EI occurs in response to naturally produced ethylene or changes in plant sensitivity to it, most work was done with PGRs that inhibit ethylene biosynthesis or block ethylene action. The synthetic auxin 2-(3-chlorophenoxy)propionic acid (CPA) was included because in one study it reduced the percentage of EI. GA_3, aminooxyacetic acid (AOA), aminoethoxyvinylglycine (AVG), daminozide [butanedioic acid mono-(2,2-dimethylhydrazide)], and silver thiosulfate (STS) had no effect on EI. CPA, paclobutrazol [(2*RS*,3*RS*)-1-(4-chlorophenyl)methyl-4,4-dimethyl-2(1h-1,2,4-triazol-1-yl)penten-3-ol], and uniconazole [(*E*)-(*p*-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] delayed or inhibited EI of pot-grown pineapple plants. Uniconazole and paclobutrazol inhibited growth and ethylene production by leaf basal-white tissue, and either or both effects could account for the inhibition of EI. Production of 1-aminocyclopropane-1-carboxylic acid (ACC) was unaffected by these compounds, but the activity of ACC oxidase, which converts ACC to ethylene, was inhibited and probably accounts for the reduced ethylene production by leaf basal-white tissue. CPA stimulated ethylene production by stem apical tissue approximately fourfold relative to the control. ACC oxidase activity and the malonyl-ACC (MACC) content in stem apical tissue were also greater than in the control, indicating that CPA greatly stimulated the production of ACC and its sequestration into MACC. The mechanism by which CPA delayed or inhibited EI is not known. CPA, paclobutrazol, and uniconazole appear to have some potential for inhibiting EI of pineapple. Their effect on yield needs to be determined.

Key Words. Pineapple flower induction—Paclobutrazol—Uniconazole—Ethylene production

Abbreviations: ACC oxidase, 1-aminocyclopropane-1-carboxylic acid oxidase; CPA, 2-(3-chlorophenoxy)propionic acid; AOA, aminooxyacetic acid; AVG, aminoethoxyvinylglycine; daminozide, butanedioic acid mono-(2,2-dimethylhydrazide); DM, dry mass; ethephon, [(2-chloroethyl)phosphonic acid]; FM, fresh mass; GA, gibberellin; EI, environmental induction of inflorescence development; IA, inflorescence appearance; LSD, Fisher's protected least significant difference; MACC, malonyl-ACC; NAA, naphthaleneacetic acid; PGR, plant growth regulator; paclobutrazol, (2*RS*,3*RS*)-1-(4-chlorophenyl)methyl-4,4-dimethyl-2-(1h-1,2,4-triazol-1-yl)penten-3-ol]; uniconazole, (*E*)-(*p*-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol; STS, silver thiosulfate; M-leaf, fourth leaf; M1-L, first leaf younger than M-leaf.

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Pineapple [*Ananas comosus* (L.) Merr.] is a crop of the tropics and subtropics, and fruits can be harvested in most months of the year. Environmental induction of inflorescence development (EI) of pineapple in Hawaii occurs primarily in response to short daylengths and cool temperatures (Bartholomew and Malézieux 1994); once induced, development to fruit maturity continues without interruption (Bartholomew 1985). Even in the December–January time period when EI normally occurs in

Hawaii, the percentage of plants initiating an inflorescence is unpredictable and is not relied upon in commercial practice. Thus, on commercial scale farms, inflorescence development is forced at all times of the year with ethylene, ethephon, or NAA (Bartholomew and Criley 1983). Forcing synchronizes inflorescence initiation, narrows the harvesting peak, and makes it possible to harvest fruit throughout the year. EI that occurs prior to the scheduled forcing is a sporadic but widespread and important problem in pineapple-producing regions because it disrupts crop scheduling and results in fruits that either are too small to be marketable or too few in number, which makes harvesting unprofitable (Bartholomew and Malézieux 1994, Scott 1993).

A number of attempts have been made to find chemicals that will delay or inhibit EI of pineapple. It has been known for many years that large amounts of NAA inhibit EI (Gowing 1956, Leeper 1965, Millar-Watt 1981), but auxins are not a practical solution because they force plants when applied in smaller amounts. The mechanism by which higher concentrations of NAA inhibit EI of pineapple is not known. That ethephon and ethylene force pineapple (Bartholomew and Criley 1983) and NAA apparently forces pineapple by stimulating the production of ethylene (Burg and Burg 1966) lead to EI inhibition studies using compounds that inhibit ethylene production or action. Silver ion, which inhibits ethylene action (Abeles et al. 1992), as silver nitrate or STS, partially (Sanford and Bartholomew 1981) or completely (Min and Bartholomew 1993) blocked floral induction of pineapple by ethephon. Millar-Watt (1981) found that three applications of silver nitrate at 1-month intervals between March and May reduced the percentage of EI from 57 to 27. Mekers et al. (1983) found that 10 ppm of AVG, an inhibitor of ethylene biosynthesis (Yang and Hoffman 1984), prevented flowering of ornamental bromeliads. Recently, Scott (1993) found that treating pineapple with CPA reduced the percentage of precocious fruit from 48.5 to 8.2. Scott (1993) attributed the effect to reduced shoot growth.

Based on the hypothesis that EI of pineapple occurs in response to naturally produced ethylene or increased plant sensitivity to ethylene, or both (Min and Bartholomew 1993), in this study we evaluated compounds that inhibit ethylene biosynthesis (AOA, AVG) or ethylene action (STS). We also evaluated PGRs that decrease plant tissue ethylene production but do not directly inhibit ACC synthase or ACC oxidase, such as uniconazole (Krause et al. 1991), paclobutrazol (Wang and Steffens, 1985), and daminozide (Gussman et al. 1993). GA_3 and CPA were also included in different experiments to evaluate their effects on EI. To explore possible mechanisms of action of some of the PGRs on EI of pineapple, we measured ethylene production, ACC oxidase activity, ACC, the precursor of ethylene, and MACC contents of tissues from treated plants. MACC is evidence of excess ACC production and effectively sequesters and removes it from the metabolic pool (Hoffman et al. 1982).

Materials and Methods

Plant Materials and Treatments

The Smooth Cayenne pineapple clone Champaka F-153 was used in all experiments. Three trials installed in commercial fields in two different years to explore the effects of PGRs on EI failed because no EI occurred. The incidence of EI in Hawaii is highest when fertilizer nitrogen is withheld and temperatures are unusually cool (Bartholomew and Criley 1983). We had no control over how plants were managed in the field and attribute the absence of EI to conditions unfavorable for its occurrence. Thus, all studies reported here were conducted using plants grown in pots because previous experience had shown that EI occurred readily with such plants. Plants were grown in 8.5 liter (23.2-cm diameter by 20.0-cm height) pots in a 1:1 (by volume) mixture of Sunshine 4 potting mix (Sun Gro Horticulture Inc., 110 110th Ave. N.E., Bellevue, WA 98004) and Perlite (Supreme Perlite Co., 4600 N. Suttle Rd., Portland, OR 97217). Stem shoots (suckers) weighing an average of 325 g fresh mass (FM) were used in Experiment 1. In all other experiments, fruit tops (crowns) with an average FM of 175 g were used. Plants were watered weekly, and at 1 month after planting each plant was fertilized biweekly with 0.25 liters of a solution containing 0.04 g of $CaCl_2$, 0.3 g of urea, and 1.0 g of Gaviota Foliar 62 (Brewer Environmental Industries, Honolulu), a soluble fertilizer containing in mg, 120 nitrogen; 105 phosphorus, 200 potassium, 0.4 magnesium, 1.0 iron, 0.13 copper, 0.1 boron, 0.2 molybdenum, and 1.42 zinc. Pots were flushed with tap water approximately monthly to prevent the accumulation of nutrients. Extra plants were grown, and those used in the experiments were selected for uniformity of size; plants with a visible inflorescence were discarded. All treatments were applied by pouring the specified quantity of water (control) or PGR solution into the leaf whorl of each plant. The amounts of active ingredients used in all experiments were based on a previous study (Min and Bartholomew 1993) and other studies with pineapple (Scott 1993) or ornamental bromeliads (Mekers et al. 1983). The effects of treatments on forcing or EI were assessed in all experiments by noting the date when the inflorescence was visible (inflorescence appearance, IA) in the leaf whorl and about 1.0 cm in diameter, usually 60 days or more in Hawaii (Bartholomew 1977).

Experiment 1

Suckers were planted as described above on March 5, 1992. On August 13, 1992, each of 12 plants was treated with 10 mL of water or a water solution containing either 20 mg of GA_3 or 10 mg of uniconazole. Six plants in each treatment were retained to observe the effects of the PGRs on days to IA. At 93 days after the PGRs had been applied, 10 mg of ethephon was applied in 10 mL of a 2% urea:water solution to six plants in each treatment to observe their response to forcing. A second application of ethephon was made to all unforced plants 73 days later.

Experiment 2

Crowns were planted as described above on February 17, 1993. On November 10, 1993, six plants were treated with 10 mL of water each or a water solution containing 1.0 mg of uniconazole, and five plants

were treated with 1.0 mg of AOA or 19 mg of STS each (1:4 molar ratio of silver nitrate and sodium thiosulfate).

Experiment 3

Crowns were planted as described above on June 5, 1993. On November 20, 1993, four plants were treated with 10 mL of water each or a water solution containing in mg 1.0 AOA, 20 daminozide, 10 paclobutrazol, 9.5 STS, or 1.0 uniconazole. The AOA and STS treatments were reapplied on January 12, 1994. Dates and number of plants sampled for measurement of ethylene metabolism and growth are provided in Results section.

Experiment 4

Crowns were planted as described above on January 7, 1994. On October 7, 1994, each of eight plants was treated with 25 mL of a water solution containing in mg 0.5 CPA, 5 paclobutrazol, or 0.5 uniconazole; 17 plants were each treated with water, 2.5 CPA, 25 paclobutrazol, or 2.5 uniconazole. The effect of PGRs on leaf appearance rate was obtained by counting newly emerged leaves bimonthly from October 7, 1994 to February 8, 1995. The effect of the PGRs on the length of the longest leaf to emerge after treatment was recorded on March 9, 1995.

Experiment 5

Crowns were planted as described above on April 4, 1994. The treatments in mg per plant applied in 20 mL of water were 1.0 AVG applied once (AVG-1), 1.0 AVG applied twice (AVG-2), 10 STS applied three times, 1.0 uniconazole, or water alone. The treatments were applied on December 2, 1994; a second application of AVG was made on December 30; and second and third applications of STS were made on December 23, and again on January 13, 1995. There were 13 plants in each treatment. Three plants from each treatment were sampled on February 1, 1995 to measure ethylene production and ACC oxidase activity of leaf basal-white and stem apical tissues.

Leaf basal-white tissue and the upper 1.0 cm of stem apical tissue were used for the measurement of ethylene because they were the most responsive to PGR treatments (Min and Bartholomew 1993). Approximately 1.0 g of leaf or stem tissue was incubated at room temperature (25 ± 0.5°C) for about 2 h (exceptions are noted) in a 17.5-mL test tube capped with a serum stopper. After incubation, a 1.0-mL gas sample was withdrawn from the headspace and injected into a gas chromatograph equipped with a flame ionization detector. Previous results (Min and Bartholomew 1993) showed that ethylene production by leaf basal-white tissue varied with leaf age. In Experiment 4, tissue of uniform age for the measurement of the effect of PGRs on ethylene production and ACC oxidase activity was obtained by marking the fourth leaf (M-leaf) older than the youngest visible leaf at the time plants were treated.

Measurement of ACC Oxidase Activity, ACC, and MACC

ACC oxidase activity was estimated by measuring the conversion of ACC to ethylene in vitro following a procedure similar to that of Starrett and Laties (1991). About 1.0 g of fresh tissue was dipped into a 1.0 mM ACC solution for 2 min; stem apical tissue was cut into slices approximately 0.2 cm thick to promote absorption of ACC by the tissue. The tissue was blotted dry, placed in a 17.5-mL test tube, which was then sealed with a serum stopper, and incubated for approximately 2 h. Ethylene production was measured as described above.

The quantity of ACC in tissues was measured by the procedure of Lizada and Yang (1979). The conversion efficiency of ACC to ethylene in each sample was determined by adding 2 nmol of pure ACC in 25 μL water to a replicate sample prior to oxidizing the ACC to ethylene; ethylene was measured as described above. MACC was determined following the procedure of and Sitrit et al. (1988). MACC was hydrolyzed to ACC by adding 1.0 mL of 6 M HCl to one replicate sample, incubating for 3 h at 100°C, neutralizing the sample with 1.0 mL of 6 M NaOH, after which the ACC content was determined as described above. MACC content was calculated by subtraction of the ACC content measured in nonhydrolyzed samples.

Table 1. Effects of CPA, paclobutrazol (Pac), and uniconazole (Uni) on leaf length and leaf appearance rate.

Treatment	Leaf length[a] (cm)	Leaf appearance rate[b] (leaves/day)	
		1	2
Control	79	0.232	0.216
CPA-0.5[c]	75	0.252	0.236
CPA-2.5	59	0.250	0.210
Pac-5	56	0.170	0.308
Pac-25	41	0.143	0.266
Uni-0.5	63	0.221	0.268
Uni-2.5	35	0.195	0.313
LSD (0.05)	7	0.024	0.024

[a] The mean length of the longest leaf that emerged after the growth regulators were applied. Leaf length was measured on March 9, 1995, 153 days after application of the growth regulators (n = 8).
[b] Column 1 is the leaf appearance rate between October 7 (day of treatment) and December 7, 1994; column 2 is the leaf appearance rate between December 8, 1994 and February 8, 1995 (n = 8).
[c] Numbers refer to the amount of active ingredient in mg applied per plant.

Results

Vegetative Growth

Leaf length was unaffected by 0.5 mg of CPA but was reduced significantly by 2.5 mg (Table 1). The 0.5-mg CPA treatment caused a slight twisting of the young leaves, whereas the 2.5-mg treatment killed the basal tissue of some of the younger leaves in the whorl in some plants, caused twisting of others, and killed the stem apical meristems of 6 of 17 treated plants. Both uniconazole and paclobutrazol treatments reduced leaf length significantly, the inhibition increased as the amount of growth regulator increased, and paclobutrazol was approximately one-tenth as active as uniconazole (Table 1). Scott (1993) found that the mass of the youngest physiologically mature leaf and plant canopy height of pineapple were reduced significantly when plants were treated with CPA or paclobutrazol.

Table 2. Effects of 25 mg of paclobutrazol (Pac-25) or 2.5 mg of uniconazole (Uni-2.5) per plant on leaf area and the partitioning of FM and DM of pineapple. Plants were harvested on January 20, 1995, 105 days after treatment (n = 3).

	Treatment			
	Control	Pac-25	Uni-2.5	LSD (0.05)
Young leaf area[a] (cm^2)	5,012	2,092	2,861	849
Other leaf area (cm^2)	12,144	13,050	10,305	N.S.[b]
Total leaf area (cm^2)	17,156	15,142	13,167	2,477
Young leaf FM (g)	864	410	539	149
Young leaf DM (g)	120	65	47	21
Other leaf FM (g)	1,791	1,947	1,429	N.S.
Other leaf DM (g)	280	286	214	N.S.
Total leaf FM (g)	2,654	2,357	1,968	432
Total leaf DM (g)	433	359	311	75
Dead Leaf DM (g)	33	26	32	N.S.
Stem FM (g)	383	506	394	101
Stem DM (g)	101	128	106	N.S.

[a] *Young leaf* refers to green leaves expanded after treatment, which were identified by marking the fourth youngest visible leaf at the time of treatment. *Other leaf* consists of leaves older than the marked leaf.
[b] N.S., not significant at the 0.05 level of probability by F-test.

The leaves of plants treated with GA_3 (Experiment 1) were observed to be longer and thinner than those of control plants, but no data on length were collected. The 19-mg STS treatment (Experiment 2) caused tip burn of some young leaves, but the lesser amounts used in other experiments caused no visible damage. The amounts of AOA, AVG, and daminozide used had no visible effect on vegetative growth and caused no obvious injury to pineapple.

Leaf appearance rate in the first 2 months after treatment was unaffected by CPA but decreased in both the uniconazole and paclobutrazol treatments (Table 1). In the second 2-month period, the rate of appearance was significantly greater in those treatments than in the control (Table 1). We attribute the lower leaf appearance rate in the first 2 months to inhibition of leaf elongation rather than to a reduced rate of initiation.

The effect of CPA on plant leaf area and FM and dry mass (DM) was not measured because of the above mentioned injury to some plants. Leaf area and FM and DM of the young leaves, including the M-leaf, were reduced significantly by uniconazole and paclobutrazol (Table 2). Leaf area and FM and DM of leaves older than the M-leaf were not different from the control. Stem FM of the paclobutrazol-treated plants was significantly greater than that of the control and uniconazole-treated plants, but there was no significant treatment effect on stem DM. Thus, the increase in stem DM was less affected by uniconazole and paclobutrazol treatments than was that of the leaf.

Table 3. Effects of 25 mg of paclobutrazol (Pac-25), 2.5 mg of uniconazole (Uni-2.5), or 2.5 mg of CPA (CPA-2.5) per plant on ethylene production, ACC oxidase activity, and ACC and MACC contents of plant tissues.

	Treatment				
	Control	Pac-25	Uni-2.5	CPA-2.5	LSD (0.05)
Nov. 7, 1994 (31 DAT)[a]					
S-Ethylene[b]	1.8	2.5	1.8	7.7	0.9
S-ACC oxidase	24.9	49.9	30.5	62.6	20.0
S-ACC	2.2	3.5	5.1	4.6	N.S.[c]
S-MACC	1.2	1.3	N.D.	53.6	13.9
Dec. 13, 1994 (67 DAT)					
S-Ethylene	3.1	6.4	8.3	—	N.S.
S-ACC oxidase	29.6	86.3	77.0	—	20.8
S-ACC	0.9	1.0	1.0	—	N.S.
S-MACC	3.2	1.9	1.2	—	N.S.
M-L-Ethylene	10.2	3.0	2.9	—	2.5
M-L-ACC oxidase	71.7	12.4	8.7	—	29.4
Jan. 20, 1995 (105 DAT)[d]					
S-Ethylene	3.6	2.2	3.0	—	N.S.
S-ACC oxidase	72.1	42.8	50.3	—	21.5
S-ACC	1.1	0.9	1.6	—	N.S.
S-MACC	2.4	4.6	6.1	—	N.S.
M-L-Ethylene	0.5	0.6	0.5	—	N.S.
M-L-ACC oxidase	1.6	1.7	2.0	—	N.S.
M1-L-ACC	0.4	1.1	1.2	—	N.S.
M1-L-MACC	0.7	1.9	1.9	1.9	N.S.
M6-L-Ethylene	3.1	0.7	0.5	—	N.S.
M6-L-ACC oxidase	38.2	2.27	1.6	—	31.5

[a] Plant sampling dates and days after treatment (DAT), respectively. Tissue incubation times were 2.25 h for the Nov. 7 sampling, 3.75 h on Dec. 13, and 4 h on Jan. 20.
[b] Sample sources were stem apical tissue (S) and leaf basal white tissue of the fourth youngest leaf marked at the time of treatment (M-L), the first leaf younger than M-L (M1-L), and the 6th leaf younger than M-L (M6-L). The units on a fresh mass basis for ethylene and ACC oxidase activity are nL g^{-1} h^{-1} and those for ACC and MACC are nmol g^{-1}.
[c] N.S., not significant at the 0.05 level of probability by F-test; N.D., not detectable; —, not measured.
[d] The control plants had young inflorescences that were estimated to be 1–2 weeks old at the time of sampling; all paclobutrazol and uniconazole-treated plants were vegetative.

Effects of PGRs on Ethylene Metabolism

Ethylene production by and ACC and MACC levels in stem tissue were not significantly affected by paclobutrazol or uniconazole at any sampling date (Table 3). ACC oxidase activity in stem tissue was significantly higher in plants treated with paclobutrazol in the first and second samplings and with uniconazole in the second sampling. By January 20, 1995, all three control plants had floral primordia that, based on a study of inflorescence development (Bartholomew 1977), were estimated to be approximately 2 weeks old. At that sampling, the ACC oxidase activity in the stem tissue of the control was

Table 4. Ethylene production and ACC oxidase activity of plant tissue for vegetative and reproductive pineapple plants. Data are means of either nine reproductive plants or six vegetative plants ± S.D.

	Reproductive plants (nL g^{-1} h^{-1})	Vegetative plants (nL g^{-1} h^{-1})
Leaf ethylene	21.3 ± 8.6	5.1 ± 0.5
Stem ethylene	3.0 ± 0.6	2.9 ± 0.1
Leaf ACC oxidase	131.2 ± 27.7	52.9 ± 7.4*[a]
Stem ACC oxidase	68.5 ± 7.1	43.6 ± 2.1**

[a] * and ** denote significance at the 0.05 and 0.01 levels of probability, respectively, by the *t*-test.

Table 5. Effect of AOA, daminozide, paclobutrazol, STS, or uniconazole on mean days ± S.D. to inflorescence appearance and fruitlets per fruit for pineapple that flowered naturally.

Treatment	Days	Fruitlets
Experiment 2[a]		
Control	141 ± 34	128 ± 18
AOA-1.0	128 ± 18	142 ± 8
Uniconazole-1.0	219 ± 26	133 ± 15
STS-19	170 ± 61	135 ± 17
LSD (0.05)	48	N.S.[b]
Experiment 3[c]		
Control	181 ± 11	128 ± 4
AOA-1.0 + 1.0	139 ± 22	125 ± 7
Daminozide-20	192 ± 17	129 ± 7
Paclobutrazol-10	368 ± 44	116 ± 3
Uniconazole-1.0	387 ± 40	116 ± 7
STS 9.5 + 9.5	228 ± 12	131 ± 8
LSD (0.05)	167	N.S.

[a] Plants in Experiment 2 were treated 266 days after planting. In both experiments, there were six plants each in the control and uniconazole treatment and five plants each in AOA and STS treatments. The numbers adjacent to the treatment indicate the amount of active ingredient in mg applied per plant.

[b] N.S., not significant at 0.05 level of probability by *F*-test.

[c] In Experiment 3, plants were treated 168 days after planting (n = 4). Second applications of AOA and STS were made 53 days after the first one.

significantly higher than that in stems of the paclobutrazol- and uniconazole-treated plants (Table 3).

Because a number of plants treated with 2.5 mg of CPA were injured, the effect of this PGR on ethylene metabolism was measured only once (Table 3). Plants treated with 2.5 mg of CPA had significantly higher stem tissue ethylene production, there was no effect on ACC level, ACC oxidase activity was significantly higher, and there was a 44-fold increase in the MACC content (Table 3). The large amount of MACC in these plants indicates that ACC production greatly exceeded its oxidation to ethylene. Therefore, it seems likely that the stimulation of ethylene production by CPA resulted mainly from an increase in ACC synthase activity.

Ethylene production and ACC oxidase activity of the M-leaf basal tissue were reduced significantly in the paclobutrazol and uniconazole treatments in the second sampling (Table 3); ACC and MACC contents were not determined. There were no significant treatment effects on any parameter measured on the M-leaf basal tissue on January 20, 1995. The ACC oxidase activity of the sixth leaf younger than the M-leaf (M6-L) in the control was significantly higher than those of the paclobutrazol and uniconazole treatments (Table 3). ACC and MACC contents measured in basal tissue of the leaf just younger than the M-leaf (M1-L) and stem apical tissues were not significantly affected by the treatments (Table 3).

In Experiment 5, some plants in all treatments had initiated inflorescences at the time of sampling. There were no significant treatment effects on ethylene production or ACC oxidase activity of the M-leaf basal-white or stem apical tissues of vegetative plants (data not shown). Ethylene production by leaf tissue of reproductive plants averaged four times higher than that of vegetative plants, but because of the large variation the difference was not significant (Table 4). Ethylene production by stem tissues of vegetative and reproductive plants did not differ. However, reproductive plants had significantly higher leaf and stem ACC oxidase activities than did vegetative plants (Table 4). The higher ACC oxidase activity of the reproductive plants may indicate that increased ethylene production is one of the factors involved in EI of pineapple.

Effects of PGRs on Inflorescence Induction

In Experiment 1, inflorescence development was forced by ethephon in all control and GA_3-treated plants, whereas only three of six plants treated with 10 mg of uniconazole were forced. A second application of ethephon to the 3 remaining vegetative plants 73 days later also failed to force flowering, but each plant produced 22 shoots at the plant apex. No such shoots were produced on plants treated only with uniconazole. Paclobutrazol promoted shoot production (Suwunnamek 1993), but no reports about uniconazole were found.

For plants of Experiment 1 that were not forced, inflorescences of all control and GA_3-treated plants were visible an average of 150 days after applying the PGRs. In other experiments, none of the compounds that inhibit ethylene production (AOA, AVG, daminozide) or action (STS) delayed inflorescence appearance significantly (Tables 5 and 6). However, both paclobutrazol and uniconazole delayed inflorescence appearance significantly at all concentrations tested, and the delay was greater as the concentration of PGR increased. In Experiment 1, inflorescences of plants treated with 10 mg of unicona-

Table 6. Effects of AVG, CPA, paclobutrazol, STS, and uniconazole on the number of pineapple plants that flowered naturally and means days ± 1. S.D. to appearance of the inflorescence

Treatment	Reproductive plants[a]	Days
Experiment 4[b]		
Control	8	137 ± 2
CPA-0.5	8	175 ± 5
CPA-2.5	8	282 ± 6
Paclobutrazol-5	8	196 ± 22
Paclobutrazol-25	0	
Uniconazole-0.5	8	191 ± 20
Uniconazole-2.5	2	301 ± 6
LSD (0.05)		39
Experiment 5[b]		
Control	10	113 ± 13
AVG-1.0	9	124 ± 19
AVG-1.0 + 1.0[c]	10	118 ± 5
STS[d]	10	127 ± 11
Uniconazole-1.0	7	170 ± 27
LSD (0.05)		35

[a] Plants with a visible inflorescence on August 30, 1995.
[b] The eight plants of Experiment 4 were treated on October 7, 1994 (273 days after planting), and the ten plants of Experiment 5 were treated on December 2, 1994 (238 days after planting). The numbers adjacent to the treatment indicate the amount of active ingredient in mg applied per plant.
[c] A second 1.0 mg of AVG per plant was applied 28 days after the first one.
[d] Three applications of 10 mg of STS per plant were applied at 3-week intervals.

zole appeared 19 (three plants) to about 30 months (two plants) after treatment or did not appear (one plant) at the time the study was terminated on April 15, 1995. When paclobutrazol and uniconazole were compared in the same experiments (Experiment 3, Table 5; and Experiment 4, Table 6), the delay in inflorescence appearance due to treatment with 0.5, 1.0, or 2.5 mg of uniconazole was similar to that associated with ten times as much paclobutrazol. The inflorescences of a number of plants treated with paclobutrazol or uniconazole in the two experiments had not appeared by August 1995, when the study was terminated (Table 6). Treatment of plants with 0.5 mg of CPA delayed inflorescence appearance an average of 38 days, but the difference was not significant; however, treatment with 2.5 mg of CPA delayed inflorescence appearance significantly (Table 6).

Effects of PGRs on Fruitlet Number

Where plants in Experiment 1 were forced with ethephon, mean fruitlets per inflorescence for the control (112) and GA_3 (119) treatments were not different, but uniconazole-treated plants had significantly fewer fruitlets (69) per inflorescence. In studies where the effects of PGRs on EI were evaluated, there were no significant effects of PGRs on fruitlets per fruit (Table 5). Inhibition of EI by a PGR would prolong the vegetative growth period resulting in larger plants when EI did occur. Since fruit size and fruitlet number are highly correlated with plant mass at the time of induction (Bartholomew and Paull 1986), the lack of a significant effect of PGR treatments on fruitlet number causes us to assume that paclobutrazol and uniconazole would reduce fruit size and fruitlet number. If control plants had grown for the same duration as the treated plants, they would have been much larger and would have produced significantly larger fruit than did plants treated with paclobutrazol or uniconazole.

Discussion

Although AOA inhibited ethylene production of excised plant tissue, Min and Bartholomew (1993) and Mekers et al. (1983) reported that AVG inhibited EI of an ornamental bromeliad, neither chemical delayed or inhibited EI in this study. The failure of AOA and AVG, which block ethylene production, and STS, which inhibits ethylene action, to delay or inhibit EI in this study could be due to several factors. Timing of application could be a problem because there is no way to know whether or when EI will occur. Pineapple is a quantitative but not an obligate short day plant (Gowing 1961), and so EI of pineapple is only weakly coupled to environmental stimuli. It is possible that EI of pineapple treated with these compounds occurred after ethylene production, or action was no longer inhibited. Further, no information is available on the absorption, translocation, dilution, or persistence of AOA, AVG, or STS in pineapple; one or more of these factors could have contributed to the failure of the treatments to inhibit EI. Greater amounts of AVG were not used because it is phytotoxic to bromeliads (Mekers et al. 1983), and concentrations of STS higher than those used were also phytotoxic when poured into the leaf whorl (Min and Bartholomew, unpublished data).

The inhibition of vegetative growth and the duration of inhibition of EI by paclobutrazol and uniconazole were both concentration dependent, which suggests that EI is inhibited because vegetative growth is inhibited. However, the failure of ethephon to force some plants treated with 10 mg of uniconazole suggests that these PGRs directly inhibited the flower initiation process rather than suppressing it by inhibiting vegetative growth. Uniconazole and paclobutrazol inhibited extension growth in a wide range of plant species (Davis and Curry 1991, Davis et al. 1988), and their known mechanism of action is inhibition of GA biosynthesis (Yamaji et al. 1991). Paclobutrazol also inhibited water stress-induced ethylene and polyamine biosynthesis (Wang and Steffens 1985). Uniconazole-P, an active optical isomer

of uniconazole, did not change indoleacetic acid or abscisic acid levels but promoted the biosynthesis of ethylene (1.8 times the control) and cytokinin (approximately 3 times for two zeatin compounds) in rice shoots (Izumi et al. 1988). These triazol growth regulators interfered with sterol metabolism (Grossman 1990, Lurssen 1987) and also inhibited ethylene production by barley and oilseed rape (Grossman et al. 1989), wheat and soybean (Krause et al. 1991), and mung bean (Hofstra et al. 1989). The delay or inhibition of EI of pineapple in the uniconazole and paclobutrazol treatments could be due to decreased ethylene production or to other unknown factors, including altered plant susceptibility to ethylene.

We confirmed the finding of Scott (1993) that CPA inhibited EI of pineapple. Scott (1993) attributed that effect to the inhibition of vegetative growth by CPA, and we found that 2.5 mg of CPA inhibited leaf growth significantly. Pineapple responds to CPA much as it does to NAA in that both PGRs stimulate ethylene production (Table 3; Burg and Burg 1966) and induce reproductive development at low concentrations (Bartholomew and Criley 1983, Gowing and Leeper 1960) but inhibit it at higher concentrations (Table 6; Gowing 1956, Leeper 1965, Millar-Watt 1981, Scott 1993). Higher concentrations of auxins may inhibit EI of pineapple by inhibiting growth, but other factors may also be involved. Induction of flowering of pineapple by geotropic stimulation (Van Overbeek and Cruzado 1948) suggested the involvement of auxin(s) in the control of this process, but the molecular basis for interactions between auxin and ethylene has not been determined (Abeles et al. 1992). Further studies of this interesting effect seem warranted.

The finding that EI plants had significantly greater ACC oxidase activity in leaf and stem tissues and higher ethylene production by leaf tissue no more than 2 weeks after the event (Table 5) could indicate a correlative relationship between ethylene production and EI. However, no consistent variation in ethylene production by pineapple leaf or stem tissue was found in Hawaii or Queensland, Australia (Liu, Sinclair, and Bartholomew, unpublished data). Demonstrating such a relationship could make it possible to predict when EI is likely to occur and increase the chances of suppressing it. Uniconazole, paclobutrazol, and CPA clearly will inhibit pineapple flowering. It would seem worthwhile to conduct field trials with these PGRs under conditions where EI is promoted to assess their efficacy to inhibit EI and to determine their impact, if any, on fruit yield. Prior to the use of PGRs to force induction in Hawaii, EI was promoted by ensuring that plants were at least 1 year old and moderately stressed for nitrogen by December when the incidence of EI is highest. If yield and ratoon sucker development are relatively unaffected, some reduction in leaf length would be beneficial because it would make harvesting easier.

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