Phytochemistry, Vol. 24, No. 10, pp. 2185–2190, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00
Pergamon Press Ltd.

EFFECT OF PACLOBUTRAZOL ON WATER STRESS-INDUCED ETHYLENE BIOSYNTHESIS AND POLYAMINE ACCUMULATION IN APPLE SEEDLING LEAVES

SHIOW Y. WANG and GEORGE L. STEFFENS

Fruit Laboratory, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

(*Revised received* 17 *December* 1984)

Key Word Index—*Malus domestica*; Rosaceae; apple; water stress; plant bioregulator; paclobutrazol; ethylene; polyamines.

Abstract—Ethylene biosynthesis and polyamine content were determined in [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol] (paclobutrazol) pre-treated and non-treated water-stressed apple seedling leaves. Paclobutrazol reduced water loss, and decreased endogenous putrescine and spermidine content. Gibberellic acid (GA) counteracted the inhibitory effect of paclobutrazol on polyamine content. Paclobutrazol also prevented accumulation of water stress-induced 1-aminocyclopropane-1-carboxylic acid (ACC), 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC), ethylene production and polyamines in apple leaves. α-Difluoromethylarginine (DFMA), but not α-difluoromethylornithine (DFMO), inhibited the rise of putrescine and spermidine in stressed leaves. *S*-Adenosylmethionine (SAM) was maintained at a steady state level even when ethylene and the polyamines were actively synthesized in stressed apple seedling leaves. The conversion of ACC to ethylene did not appear to be affected by paclobutrazol treatment.

INTRODUCTION

Plant tissue shows a rapid increase in ethylene production under water-stress [1]. Apelbaum and Yang [2] found that the pathway for ethylene biosynthesis in wilted wheat leaves was the same as that in ripening fruit, derived from methionine via *S*-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). They also found that the step enhanced by wilting was the synthesis of ACC, the immediate precursor of ethylene [2, 3].

Most metabolic processes in living organisms are controlled by hormones, which act via their receptors and generation of second messengers. Pretreatment of wheat leaves with benzyladenine or indole-3-acetic acid prior to water stress caused further increase in ethylene production and ACC level, whereas pretreatment with abscisic acid reduced ethylene production and decreased ACC content [4, 5]. This suggests that plant hormones could regulate stress-induced ethylene. Plant bioregulators are known to alter the biosynthesis of plant hormones, and in turn regulate metabolic activities and reaction to stress [6].

Recently, the plant bioregulant [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol] (paclobutrazol; PP333) has been shown to be an inhibitor of gibberellin biosynthesis [7]. Although gibberellic acid (GA) plays no particular role on water stress-induced ethylene production [4], paclobutrazol may affect stress-induced ethylene production through its inhibition of growth [8–12] and its reduction in water use [13, 14]. Paclobutrazol was also shown to regulate various metabolic processes in apple seedlings [15]: shifting assimilate partitioning from leaves to roots, increasing carbohydrate content in all parts of apple seedlings, increasing chlorophyll content on a leaf area basis, increasing soluble protein in leaves, increasing mineral element concentration in leaf tissue, and increasing root respiration. Foliar application of GA counteracted the effects induced by paclobutrazol [15]. Ethylene and polyamine biosyntheses utilized the same substrate, *S*-adenosylmethionine (SAM), and produce the same by-product, methylthioadenosine (MTA) [16, 17]. In higher plants, however, the functions of polyamines and ethylene are different. In general, ethylene is considered to be a plant-senescence hormone, initiating fruit ripening, inducing chlorophyll loss in leaves, promoting senescence and retarding plant growth [18]. On the contrary, polyamines have been shown to be senescence inhibitors, to inhibit the rise in RNase and protease, to decrease the rate of senescence of leaf protoplasts, to induce DNA synthesis and mitotic activity, to promote the synthesis of macromolecules, to stabilize thylakoid membranes, to maintain high protein content and to prevent the loss of chlorophyll in leaf discs [19]. The purpose of this research was to study the effect of paclobutrazol on water-stress and stress-induced ethylene production and polyamine content in apple leaves.

RESULTS AND DISCUSSION

Reduction of water loss

The rate of water loss was much slower in the paclobutrazol-treated leaves than in the control leaves (Fig. 1). Control leaves lost 15% of their initial fresh weight within 1 hr while it took more than 2 hr for the paclobutrazol-treated leaves to reach the same weight loss. This suggests that paclobutrazol may modify the leaves of apple seedling in such a way that they can better withstand water stress.

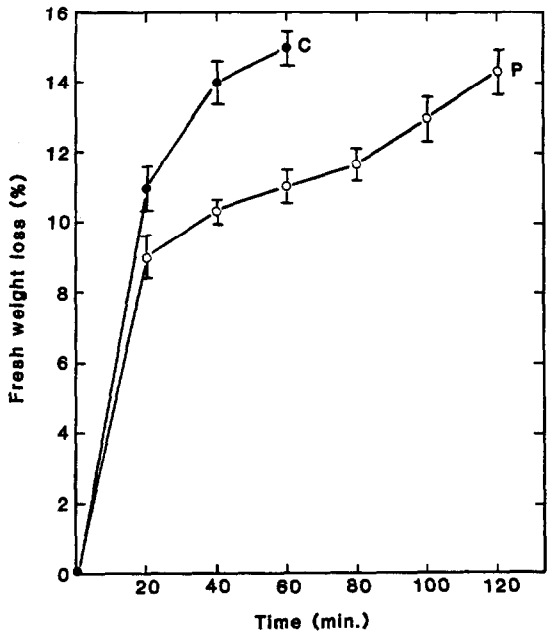

Fig. 1. Comparison of water loss between control (C) and paclobutrazol (P) treated leaves of apple seedlings. Apple leaves were treated with paclobutrazol 7 days before subjected to water stress. Bars represent ± s.e.m.

Ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) content

In apple leaves, water-stress also induced substantial increases in ethylene production. Ethylene production increased progressively with an increasing degree of the water stress and reached a maximum rate (0.24 nmol/g/hr) when water loss reached 15% of the initial fresh weight and then declined (Fig. 2A). This elevated stress-induced ethylene production was suppressed by paclobutrazol. In stressed leaves, the rate of ethylene production was 22 times higher than that produced by non-stressed leaves whereas in the paclobutrazol-treated stressed leaves ethylene production was only 10 times higher than the non-stressed ones (Fig. 2A). The longer the duration of paclobutrazol treatment was before the wilting, the greater the reduction of stress-induced ethylene production became (Fig. 3). Increased ethylene production was also observed in wheat leaves when they were subjected to water-stress [2, 4, 5].

ACC content followed a pattern similar to ethylene production during the process of water stress (Fig. 2B). The ACC content of non-stressed apple leaves was very low (0.05 nmol/g/hr). It started to rise when the leaves were subjected to water-stress and reached a peak value (0.5 nmol/g) when weight loss was 15% of the initial fresh weight. Further weight loss due to water deficit caused a decline in both ethylene production and ACC content. This was also observed in wheat leaves [2]. Paclobutrazol substantially suppressed the increase of ACC in the wilted leaves (Fig. 2B).

Along with ethylene production and ACC content, MACC, the major ACC conjugate [20, 21] also increased as water was lost up to 15% of the initial fresh weight, and then remained at this high level as the water loss

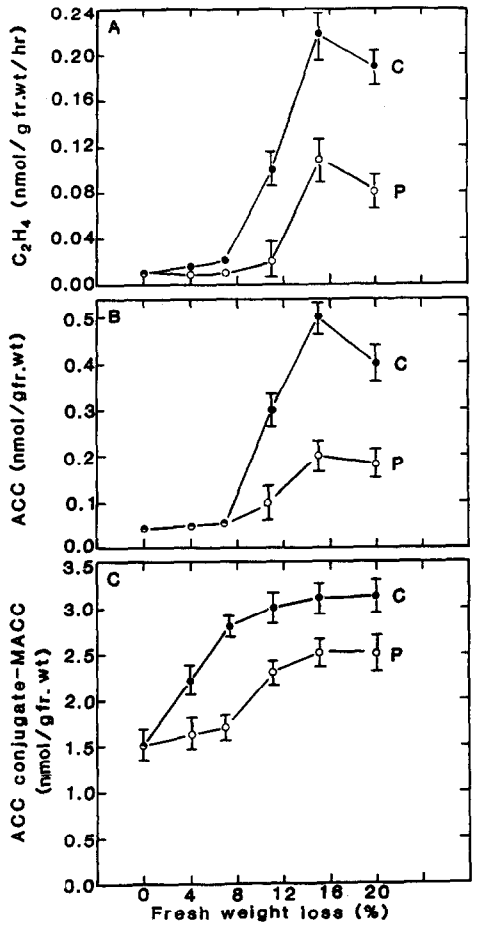

Fig. 2. Ethylene production (A), ACC (B) and MACC (C) content in apple seedling leaves in response to paclobutrazol pretreatment following different degrees of water stress. Apple leaves were treated with 0.1 mM paclobutrazol 6 days before subjected to water stress. Bars represent ± s.e.m. Control (C); paclobutrazol (P).

continued, even though ethylene production and ACC levels declined (Fig. 2C). Paclobutrazol also resulted in reduced levels of the stress induced MACC. However, the reduced level of MACC in the paclobutrazol-treated tissue resulted from the inhibited synthesis of ACC, rather than from the inhibited synthesis of MACC. The magnitude of the increase in ethylene production, ACC and MACC levels under water deficit stress was much smaller for paclobutrazol-treated leaves than for non-treated leaves.

Similar amounts of ethylene were produced from the control and from the paclobutrazol treated leaves when exogenous ACC was added to both stressed and non-stressed leaves (Table 1). This indicates that paclobutrazol has no significant effect on the conversion of ACC to ethylene in either stressed or non-stressed leaves. Thus, the synthesis of ACC appears to be the key step where paclobutrazol exerts its effect resulting in inhibition of ethylene production and ACC content.

Polyamine content

Free polyamine concentrations in leaves were reduced by paclobutrazol treatments and these changes were

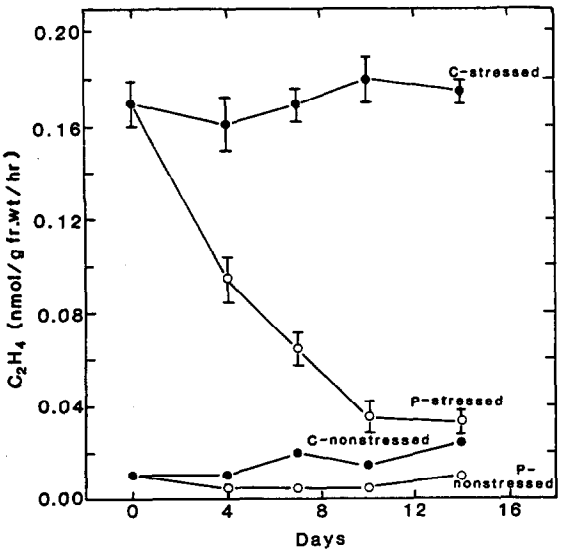

Fig. 3. Effect of the duration of 0.1 mM paclobutrazol pretreatment on ethylene production of water stressed (15% of weight loss) and non-stressed apple seedling leaves. Bars represent ± s.e.m. Control (C); paclobutrazol (P).

Table 1. Effect of exogenous ACC (0.5 mM) on ethylene production in non-stressed and water-stressed apple leaves in response to paclobutrazol pretreatment*

Treatment	Ethylene production (nl/g fr. wt/hr)	
	no ACC	ACC
Control		
non-stressed	0.0 a	21.9 a
water-stressed	5.3 c	22.8 a
Paclobutrazol		
non-stressed	0.0 a	22.6 a
water-stressed	2.1 b	23.0 a

*Apple leaves were treated with 0.1 mM paclobutrazol for 6 days prior to water stress. Control or paclobutrazol-treated leaves were pre-incubated for 3 hr in 10 mM 2-[*N*-Morpholino]ethanesulphonic acid (MES) buffer (pH 6) or in buffer containing 0.5 mM ACC before subjected to water stress. Ethylene was determined when 15% fresh weight loss was reached. Mean separation in columns by Duncan's multiple range test, 0.05 level.

correlated with duration of paclobutrazol treatment (Fig. 4). Paclobutrazol-treated apple leaves were lower in endogenous putrescine and spermidine (Fig. 4A–D). Putrescine decreased by 44% and spermidine declined by 38%, 14 days after paclobutrazol treatment (Fig. 4A and C). When expressed on per leaf basis, putrescine and spermidine content were also decreased (data not shown). Application of GA to paclobutrazol-treated leaves counteracted the inhibitory effect of paclobutrazol on polyamine content (Table 2). Paclobutrazol was reported to reduce leaf expansion, but treatment with GA spray could overcome this growth inhibition [8–12]. Thus, there appears to be a correlation between leaf growth and

Table 2. Reversal of the effect of paclobutrazol on polyamine content and leaf growth by GA treatment*

Treatment	Polyamine content (nmol/g fr. wt)		Leaf growth area/leaf (cm^2)
	Putrescine	Spermidine	
Control	236 a	703 a	15.9 a
Paclobutrazol	109 b	276 b	8.7 b
Paclobutrazol + GA	217 a	680 a	14.3 a

*Paclobutrazol at 0.68 μM was continually supplied via the nutrient solution to apple seedlings. GA_3 at 71.4 μM plus 0.01% of the surfactant Regulaid was applied to the foliage 2 weeks later. Polyamines and leaf area were determined 4 weeks after initiation of experiment. Mean separation in columns by Duncan's multiple range test, 0.05 level.

polyamine levels. In higher plants, polyamine metabolism has been associated with the action of plant growth substances and the regulation of plant growth and development [19, 22]. GA treatment of pea seedlings increased activity of arginine decarboxylase (ADC), a key rate-limiting enzyme in the conversion of arginine to putrescine [23], and induced higher levels of putrescine and spermidine which paralleled the increased internode elongation. On the other hand AMO 1618, a growth retardant, caused changes in the opposite direction for ADC activity, polyamine content and growth. The ornithine decarboxylase (ODC), an enzyme which decarboxylates ornithine to produce putrescine [23], was not affected by GA or by AMO-1618. Paclobutrazol is one of several triazol derivatives that have shown considerable plant growth retardant activity by inhibiting GA biosynthesis [7, 24, 25]. Although ADC and ODC were not determined in our experiment, decrease in putrescine and spermidine content in treated apple seedling leaves might also be due to inhibition of the activity of ADC. Paclobutrazol treatment did not change the spermine content; and spermine did not seem to be correlated to leaf growth (Fig. 4E, Table 2).

The effect of paclobutrazol on polyamine content under water stress is also shown in Fig. 4. Water stress caused a 34% and 85% rise in putrescine and spermidine, respectively, in the leaves. Pre-treating seedlings with paclobutrazol prevented the stress-induced rise in putrescine and spermidine (Fig. 4A–D). The polyamine ratio of stressed/non-stressed was higher in the control and lower in the paclobutrazol-treated leaves. This indicates that paclobutrazol suppressed the increase in polyamines induced by the stress. The suppression was particularly significant for spermidine.

α-Difluoromethylarginine (DFMA) and α-difluoromethylornithine (DFMO) are highly specific irreversible inhibitors of ADC and ODC, respectively. Both polyamine inhibitors have been used for investigating the cellular role of polyamines [26, 27]. Experiments with DFMA and DFMO demonstrated that elevated putrescine and spermidine levels in water-stressed apple leaves could be substantially reduced in the presence of only DFMA, but not DFMO (Table 3). This indicates that only ADC mediates the water-stress induced rise in polyamines as shown in oat leaves [28].

Bound polyamines consisted of about 20% of the total

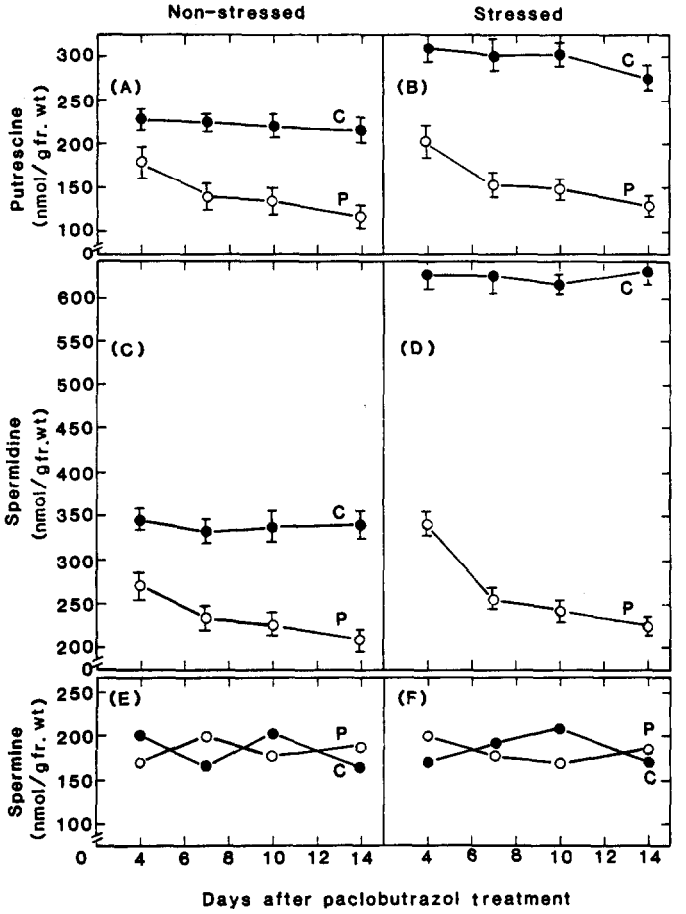

Fig. 4. Effect of paclobutrazol pretreatment on free polyamine titre in stressed and non-stressed apple seedling leaves. Apple seedlings were treated with 0.1 mM paclobutrazol and followed by water stress. When the weight loss in leaves reached 15%, the leaves were homogenized for assay of polyamines as described in the Experimental. Bars represent ± s.e.m. Control (C); paclobutrazol (P).

Table 3. Effect of DFMA (0.5 mM) or DFMO (0.5 mM) on polyamine levels in control or paclobutrazol-treated leaves of water-stressed apple seedling*

	Putrescine		Spermidine	
Treatment	control	paclobutrazol	control	paclobutrazol
	(nmol/g fr. wt)		(nmol/g fr. wt)	
Control	306±18	189±10	698±31	298±21
DFMO	318±21	180±8	712±27	307±19
DFMA	97±10	45±4	143±9	47±6

*Apple seedlings were treated with 0.1 mM paclobutrazol 7 days prior to water stress. Control or paclobutrazol-treated leaves were pre-incubated for 3 hr in 10 mM MES buffer (pH 6) or in buffer containing 0.5 mM DFMO or 0.5 mM DFMA before subjected to water stress. When the weight loss of leaves reached 15%, the leaves were homogenized for assay of polyamines as described in the Experimental. Data represent mean of three analyses ± s.e.m.

polyamines. There was only a trace of bound putrescine, but bound spermidine and spermine levels on a unit fresh weight basis were fairly constant for all treatments (data not shown). Therefore, increases or decreases in free polyamines among the various treatments were not due to degradation or synthesis of bound polyamines.

S-*Adenosylmethionine* (*SAM*) *content*

Water stress caused accumulation of ACC and stimulated ethylene production and also induced polyamine accumulation in apple leaves (Figs 2–4). Pretreatment with paclobutrazol inhibited the stress-induced rise in

Table 4. Effect of water deficit on SAM content in control and paclobutrazol (0.1 mM)-treated apple seedling leaves*

Days after paclobutrazol treatment	Treatment	SAM (nmol/g fr. wt)	
		Non-stressed	Stressed
4	control	46.1	47.5
	paclobutrazol	44.0	48.2
7	control	41.8	39.3
	paclobutrazol	43.8	42.6
10	control	39.8	43.0
	paclobutrazol	43.7	42.8
14	control	42.6	49.4
	paclobutrazol	43.0	41.5

*Batches of excised leaves were allowed to wilt. When the weight loss of leaves reached 15%, the leaves were homogenized for assay of SAM. SAM was determined by isotope dilution as described in the Experimental. Mean separation among treatments by Duncan's multiple range test, 0.05 level showed no significant difference.

these constituents. However, water stress and paclobutrazol treatment caused no change in the level of SAM (Table 4). Similar results on SAM concentration were obtained by spectrophotometric methods or by isotope dilution techniques so only data from isotope dilution data are presented. These data indicate that the conversion step between methionine and SAM is not rate-limiting. It was reported that when the conversion of SAM to ACC was inhibited by aminoethoxyvinylglycine [29] and 2,4-dinitrophenol [30] in auxin-treated mungbean hypocotyl tissue or stimulated in tobacco by tobacco mosaic virus infection [31] or wheat leaves by water deficit stress [2], no change in level of SAM was observed although ACC content and ethylene production were markedly affected. SAM is the substrate for both ACC and polyamine syntheses, but its consumption apparently did not alter the SAM steady state level.

Collectively, the data presented here suggest that paclobutrazol reduced water loss, and decreased endogenous putrescine and spermidine content. GA counteracted the inhibitory effect of paclobutrazol on polyamine content. Paclobutrazol also prevented the accumulation of water stress-induced ACC, MACC, ethylene production and polyamine content in apple seedling leaves possibly by regulating ACC synthase and arginine decarboxylase activity. Both are the rate-controlling enzymes involved in the pathway of ethylene and polyamine biosynthesis in water-deficit stressed apple leaves. SAM is maintained at a steady state level even when the biosynthesis of ethylene and polyamines is active in stressed apple seedling leaves. The conversion of ACC to ethylene does not appear to be affected by paclobutrazol treatment.

EXPERIMENTAL

Plant materials and treatments. The growing conditions and treatments of apple seedlings ('York Imperial' *Malus domestica* Borkh) used in this study have been described previously [11]. Seedlings (except for roots) were immersed for 1 min in soln containing 0.1 mM paclobutrazol and 0.01% surfactant Regulaid at 2 pm and next day at 8 am. In some experiments, paclobutrazol at 0.68 μM was supplied via the nutrient soln to seedlings. GA_3 was supplied as a foliage spray at 71.4 μM [11]. The technique used for H_2O stress was described in refs [2] and [4]. Batches of excised apple seedling leaves (3rd expanded leaf from the tip) were spread out on sheets of filter paper and allowed to wilt at room temp (22°) and 60% RH. When the fr. wt loss in leaves reached 4, 7, 11, 15 and 20%, respectively, of their original wt, they were immediately inserted into a 15 ml glass test tube chamber and were then sealed with serum caps for 3 hr. Non-stressed leaves were inserted in test tubes containing 200 μl H_2O to maintain high RH. The sealed tubes were placed in the room at 22° under light (320 μmol/s/m^2).

Ethylene determination. Gas samples from each tube were taken with a gas-tight hypodermic syringe and analysed for C_2H_4 by GC equipped with an alumina column (FID).

Extraction of polyamines, SAM, ACC and MACC. The tissue was extracted with 5% ice-cold $HClO_4$ for 1 hr, the extracts centrifuged for 20 min at 5000 *g* and the supernatant which contained ACC, MACC, SAM and free polyamines as subjected to further purification. The pellet (perchlorate insoluble material) contained the bound polyamines which were released by a 16 hr hydrolysis in 6 M HCl at 110° and further analysis was done as for free polyamines [32].

Determination of polyamines. The supernatants from the $HClO_4$ extracts were neutralized to pH 4.5 at 0° with $KHCO_3$, and then centrifuged at 4°, 6000 *g* for 20 min. The neutralized supernatant (pH 4.5) was passed through a Bio-Rex 70 column (H^+ form) which retained SAM and the polyamines. SAM and the polyamines were eluted from the Bio-Rex 70 column with 0.1 M HCl and after neutralization, lyophilized and used for free polyamine determination. Levels of the polyamines were determined after dansylation [22]. The dansylated products were then extracted with 0.5 ml benzene and separated by 2D-TLC on silica gel plates (silica gel 60, without fluorescent indicator, EM reagents) at 5°. The dansyl amines were separated [33] in cyclohexane–EtOAc (5:4) (solvent 1); then the plate was run in the 2nd dimension with $CHCl_3$–Et_3N (5:1) (solvent 2). The fluorescence spots were compared with dansylated standards. The spots were eluted with EtOAc and were quantified in an HTV Fluoroflow Detector V spectrophotofluorimeter, with excitation at 350 nm and emission at 495 nm [22].

Determination of total SAM. An aliquot of the Bio-Rex 70 fraction was chromatographed on prewashed Whatman 3 MM paper in *n*-BuOH–HOAc–H_2O (4:1:5) and the SAM spot eluted with H_2O. The concn of SAM was determined spectrophotometrically, assuming a molar adsorptivity of 15400 at 256 nm [30, 34]. The concn of SAM was also determined by isotope dilution. The sp. act. of the added [3,4-^{14}C]SAM decreases as a result of dilution by endogenous SAM in the tissue extracts. By using ACC synthase (which catalyses the conversion of SAM to ACC), the amount of nonradioactive SAM in the tissue could be calculated from the specific radioactivity of ACC produced and the total radioactivity of SAM added. The ACC synthase was isolated from tomato [35] and ACC was assayed according to the method of ref. [36], which is based on the chemical conversion of ACC to C_2H_4 with NaOCl. The C_2H_4 produced then was transferred to an evacuated 25 ml scintillation vial. A 2-ml gas sample was withdrawn from the vial for C_2H_4 determination by GC. The remainder of the C_2H_4 was absorbed in 0.5 ml of cold 0.25 M $Hg(ClO_4)_2$ for 3 hr. Then, 10 ml of Aquasol scintillation fluid was added and radioactivity was determined via a liquid scintillation counter [17, 30]. The specific radioactivity of ACC was assumed to be identical to the specific radioactivity of the C_2H_4.

Determination of ACC and MACC. The material that was not retained by the Bio-Rex 70 column was passed through a Dowex-50 column (H^+ form). Amino acids, including ACC, were eluted

with 3 M NH_4OH and the eluate was taken to dryness; the residues were dissolved in H_2O, and an aliquot was used for the ACC assay [36]. The effluent from the Dowex-50 (H^+ form) contained MACC. Quantification of the MACC was carried out by hydrolysis in 6 M HCl at 100° for 1 hr [21], and the resulting ACC was assayed as described above.

Chemicals. ACC was purchased from Calbiochem. *S*-Adenosyl-L-[3,4-^{14}C]methionine was obtained from Research Products International Corp. DFMA and DFMO were kindly supplied by Dr. Peter P. McCann (Merrell-Dow Research Center). Putrescine, spermidine and spermine were obtained from Sigma. Technical grade paclobutrazol was generously provided by ICI Americas, Inc.

Acknowledgements—The authors wish to thank Mr. Scott Glassburn for technical assistance, ICI Americas Inc. for the gift of paclobutrazol and Dr. Peter P. McCann of the Merrell-Dow Research Center for the gift of DFMA and DFMO.

REFERENCES

1. McMichael, B. L., Jordan, W. R. and Powell, R. D. (1972) *Plant Physiol.* **49**, 658.
2. Apelbaum, A. and Yang, S. F. (1981) *Plant Physiol.* **68**, 594.
3. Adams, D. O. and Yang, S. F. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 170.
4. Wright, S. T. C. (1980) *Planta* **148**, 381.
5. McKeon, T. A., Hoffman, N. E. and Yang, S. F. (1982) *Planta* **155**, 437.
6. Bruinsma, J. (1982) in *Chemical Manipulation of Crop Growth and Development* (McLaren, J. S., ed.) p. 3. Butterworths, London.
7. Couture, R. M. (1982) *Proc. Plant Growth Reg. Soc. Am.* **9**, 59.
8. Curry, E. D. and Williams, M. W. (1983) *Hort. Sci.* **18**, 214.
9. Greene, D. W. and Murray, J. (1983) *Proc. Plant Growth Reg. Soc. Am.* **10**, 207.
10. Quinlan, J. D. and Richardson, P. J. (1983) *Acta Hort.* **146**, 96.
11. Steffens, G. L., Byun, J. K. and Wang, S. Y. (1985) *Physiol. Plant.* **63**, 163.
12. Williams, M. W. (1984) *Am. Chem. Soc. Sym. Series* **257**, 93.
13. Steffens, G. L., Wang, S. Y., Steffens, C. L. and Brennan, T. (1983) *Proc. Plant Growth Reg. Soc. Am.* **10**, 195.
14. Wample, R. L. and Culver, E. B. (1983) *J. Am. Soc. Hort. Sci.* **108**, 122.
15. Wang, S. Y., Byun, J. K. and Steffens, G. L. (1985) *Physiol. Plant.* **63**, 169.
16. Adams, D. O. and Yang, S. F. (1977) *Plant Physiol.* **60**, 892.
17. Wang, S. Y., Adams, D. O. and Lieberman, M. (1982) *Plant Physiol.* **70**, 117.
18. Yang, S. F. and Hoffman, N. E. (1984) *Ann. Rev. Plant Physiol.* **35**, 155.
19. Galston, A. W. (1983) *Bioscience* **33**, 382.
20. Amrhein, N., Schneebeck, D., Skorupka, H., Tophof, S. and Stockigt, J. (1981) *Naturwissenschaften* **68**, 619.
21. Hoffman, N. E., Yang, S. F. and McKeon, T. (1982) *Biochem. Biophys. Res. Commun.* **104**, 765.
22. Dai, Y., Kaur-Sawhney, R. R. and Galston, A. W. (1982) *Plant Physiol.* **69**, 103.
23. Smith, T. A. (1977) in *Progress in Phytochemistry* (Reinhold, L., Harborne, J. B. and Swain, T., eds) Vol. 4, p. 27. Pergamon Press, Oxford.
24. Graebe, J. E. (1982) in *Plant Growth Substances* (Wareing, P. F., ed.) p. 71. Academic Press, London.
25. Rademacher, W. and Jung, J. (1981) *J. Agron. Crop Sci.* **150**, 363.
26. Kallio, A., McCann, P. P. and Bey, P. (1981) *Biochemistry* **20**, 3163.
27. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. L. and Ververt, J. P. (1978) *J. Am. Chem. Soc.* **100**, 2551.
28. Flores, H. E. and Galston, A. W. (1984) *Plant Physiol.* **75**, 102.
29. Yu, Y. B. and Yang, S. F. (1979) *Plant Physiol.* **64**, 1074.
30. Yu, Y. B., Adams, D. O. and Yang, S. F. (1980) *Plant Physiol.* **66**, 286.
31. Delaat, A. M. M. and Van Loon, L. C. (1982) *Plant Physiol.* **69**, 240.
32. Dumortier, F. M., Flores, H. E., Shekhawat, N. S. and Galston, A. W. (1983) *Plant Physiol.* **72**, 915.
33. Smith, T. A. and Best, G. R. (1977) *Phytochemistry* **16**, 841.
34. Schlenk, F., Zydek, C. R., Ehninger, D. J. and Dainko, J. L. (1965) *Enzymologia* **29**, 283.
35. Yu, Y. B., Adams, D. O. and Yang, S. F. (1979) *Arch. Biochem. Biophys.* **198**, 280.
36. Lizada, M. C. C. and Yang, S. F. (1979) *Analyt. Biochem.* **100**, 140.