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EFFECT OF POLYAMINES ON ETHYLENE PRODUCTION

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Key Word Index—*Tradescantia*; Commelinaceae; *Glycine*; Leguminosae; soybean; polyamines; ethylene production; senescence.

Abstract—The polyamines putrescine, cadaverine, spermidine and spermine reduced the amount of ethylene produced by senescing petals of *Tradescantia* but they did not prevent anthocyanin leakage from these same petals. These polyamines also inhibited auxin-mediated ethylene production by etiolated soybean hypocotyls to less than 7% of the control. The basic amino acids lysine and histidine reduced the amount of auxin-induced ethylene produced by soybean hypocotyls by *ca* 50%. In the hypocotyls, methionine was unable to overcome the inhibition caused by the polyamines. The polyamines spermidine and spermine inhibited ethylene production induced by the application of 1-aminocyclopropane-1-carboxylic acid and they also reduced the endogenous content of this amino acid in the treated tissues.

INTRODUCTION

The polyamines putrescine, cadaverine, spermidine and spermine are of widespread occurrence in the plant kingdom [1, 2]. While much is known about the biosynthesis and catabolism of these amines, very little is known about their physiological roles in plants.

Recently, the diamines putrescine and cadaverine were shown to reduce significantly both the rise of nuclease activity and the incidence of spontaneous lysis in isolated oat leaf protoplasts [3]. Further studies have shown that the addition of polyamines to the oat protoplast incubation medium stimulates incorporation of thymidine into DNA and also increases the frequency of mitosis [4]. Polyamines also retarded the loss of chlorophyll in excised oat leaves maintained in the dark [5]. Similarly, chlorophyll loss was also retarded in barley leaf discs maintained in darkness [6]. These last investigations suggested that polyamines may prevent senescence-associated changes in other tissues as well. Since increased ethylene production is a characteristic of senescing tissues [7], polyamines may affect this process as well. This study was undertaken to determine the effects of polyamines on ethylene production and loss of membrane integrity in senescing petals of *Tradescantia*. In addition, the effect of polyamines on auxin-induced ethylene production in soybeans was examined.

RESULTS

During senescence, isolated petals of *Tradescantia* exhibit an increased rate of ethylene production and a loss of vacuolar integrity which leads to a release of anthocyanin pigments [8]. Table 1 shows that at a

Table 1. The effect of various polyamines on ethylene production and anthocyanin leakage from senescing petals of *Tradescantia*

Compound	Concentration	Ethylene Produced (%)	Anthocyanin Efflux (%)
None	—	100 a*	100 a*
Putrescine	1 mM	94 a b	84 a
	10 mM	34 c d	98 a
Cadaverine	1 mM	82 b	82 a
	10 mM	40 c	93 a
Spermidine	1 mM	42 c	85 a
	10 mM	19 d	87 a
Spermine	1 mM	33 c d	91 a
	10 mM	22 d	83 a

*Values given are the means of 7 experiments. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

concentration of 10 mM all the polyamines tested inhibited ethylene production. With the exception of putrescine, this inhibitory activity was also manifested at a concentration of 1 mM. Spermidine and spermine were more effective than the diamines putrescine and cadaverine in inhibiting ethylene production. In spite of their ability to inhibit ethylene production, none of the polyamines at either 1 or 10 mM reduced significantly the amount of anthocyanin lost during petal senescence (Table 1). These data suggested that the polyamines do not stabilize cellular membranes of senescing petals of *Tradescantia*.

In order to investigate more fully the inhibitory activity of polyamines on ethylene production, the effect of polyamines on IAA-induced ethylene production in etiolated soybean hypocotyl segments was examined. This system was chosen because of its reproducibility and ease of manipulation.

All of the polyamines tested inhibited auxin-induced ethylene biosynthesis (Table 2). As in the flower petals, inhibition was most pronounced at concentrations of 10 mM but concentrations of 1 mM were also inhibitory. Of the polyamines tested, spermidine appeared to be the most effective inhibitor. Spermidine inhibition of ethylene production was detected at a concentration of 1×10^{-6} M and was saturated at 1×10^{-2} M (Fig. 1).

The specificity of the polyamine response was examined by comparing the effects of spermidine and spermine with the effects of other cations and the basic amino acids arginine, histidine and lysine. Mg^{2+} was without effect while Ca^{2+} stimulated auxin-induced ethylene production (Table 3). Of the basic amino acids tested, arginine stimulated ethylene production but histidine and lysine were inhibitory.

The inhibitory activity of histidine and lysine could have resulted from their intrinsic inhibitory activities or the inhibition could have resulted from the action of their respective decarboxylation products histamine and cadaverine. To test this latter possibility, comparisons were made between the inhibitory activities of lysine and cadaverine as well as those of histidine and histamine. At equal concentrations the polyamines cadaverine and histamine were more effective in inhibiting ethylene production than were their corresponding amino acids (Table 4). These data suggested that the inhibitory action of lysine and histidine was caused by their respective decarboxylation products [1] rather than by the amino acids themselves.

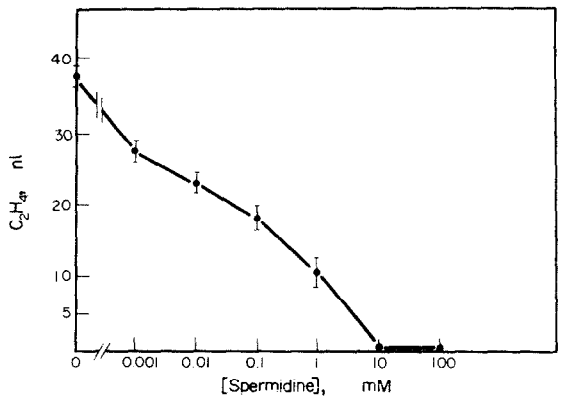

Fig. 1. Effect of spermidine concentration on the inhibition of IAA-dependent ethylene production by etiolated soybean hypocotyls. Bars indicate standard error.

The ability of ethylene precursors to reverse the polyamine inhibition of ethylene biosynthesis was investigated. Methionine (10 mM) was unable to overcome the inhibition of ethylene biosynthesis caused by spermidine (10 mM) (Table 5). Methionine alone stimulated ethylene production when compared to control values.

Spermidine and spermine inhibited the last step in the biosynthetic pathway of ethylene formation, namely the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Table 6).

The effects of spermidine and two other inhibitors of ethylene production, cobalt and aminoethoxyvinylglycine (AVG), on the endogenous level of the ethylene precursor ACC were compared (Table 7). Application of cobalt to soybean hypocotyl tissue increased the level of ACC (Table 7). Treatments with spermidine or AVG substantially reduced the level of ACC in the same tissue.

Table 2. Effects of various polyamines on IAA-induced ethylene production by etiolated soybean hypocotyls

Treatment	Concentration	Ethylene produced (nl)
None	—	55.4 a*
Putrescine	1 mM	29.7 b
	10 mM	4.4 c d
Cadaverine	1 mM	37.4 b
	10 mM	16.5 c
Spermidine	1 mM	17.2 c
	10 mM	3.2 d
Spermine	1 mM	15.8 c d
	10 mM	10.0 c d

*Values presented are the means of 2 separate experiments with 3 replications of each. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 3. The effects of polyamines, basic amino acids and inorganic cations on IAA-induced ethylene production in etiolated soybean hypocotyls

Treatment	Concentration	Ethylene produced (nl)
None	—	34.9 c
Mg	10 mM	32.4 c d
Ca	10 mM	78.0 a
Arginine	10 mM	51.1 b
Histidine	10 mM	25.3 d
Lysine	10 mM	17.2 e
Spermidine	10 mM	0.3 f
Spermine	10 mM	2.3 f

Values presented are the means of 5 replications of one experiment. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 4. Comparison of the inhibitory activities of lysine, histidine, cadaverine and histamine (all at 10 mM) on IAA-induced ethylene production in etiolated soybean hypocotyls

Treatment	Ethylene produced (nl)
None	32.9 a
Lysine	15.6 c
Cadaverine	4.1 d
Histidine	23.8 b
Histamine	15.6 c

Values are the means of 5 replications of one experiment. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Inhibition of 1-aminocyclopropane-1-carboxylic acid-induced ethylene production in etiolated soybean hypocotyls by spermidine and spermine (all at 10 mM)

Treatment	Ethylene produced (nl)
None	565 a*
Spermidine	85 b
Spermine	33 b

*Values are means of 5 replications of one experiment. Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

DISCUSSION

The inability of methionine to reverse the polyamine inhibition (Table 5) suggested that the polyamines were not reducing methionine availability. The fact that spermidine inhibited the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Table 6) and reduced the endogenous level of ACC (Table 7) indicated that this polyamine inhibits at least two steps in the biosynthetic pathway of ethylene formation. A similar response is exerted by *n*-propyl-gallate, which inhibits the conversion of ACC to ethylene [9] and also inhibits the activity of the enzyme responsible for ACC formation [10].

Whenever investigating the action of inhibitory compounds, the question of general toxicity of these compounds arises. For example, it can be argued that the inhibition of ethylene production by polyamines is merely a reflection of the fact that these compounds inhibit cellular metabolism in general. It has been shown [9, 11] that treatment of *Tradescantia* petals with either cycloheximide or cordycepin completely blocks the increase in anthocyanin efflux normally observed during petal senescence. The fact that none of the polyamines tested reduced the amount of anthocyanin leakage from senescing petals of *Tradescantia* (Table 1) indicates that the polyamines are not inhibiting RNA or protein synthesis. Also, it has been shown that treatment of excised oat leaves with 10 mM cadaverine or putrescine actually enhances the incorporation of thymidine into TCA-insoluble material [3]. Still other work has shown that treatment of isolated oat leaf protoplasts with polyamines increased the frequency of mitoses of these protoplasts [4]. Thus, it is unlikely that the polyamines tested in this study are inhibitory to cellular metabolism in general.

It can also be argued that the inhibition of IAA-induced and ACC-induced ethylene production (Tables 2 and 6) occurs as a result of the ability of the polyamines to inhibit IAA or ACC uptake. Ethylene production by senescing petals of *Tradescantia* is not dependent upon exogenous IAA or ACC. The ability of the polyamines to inhibit ethylene production in this tissue (Table 1) clearly indicates that the polyamines inhibit ethylene production in general, and not just IAA- or ACC-dependent ethylene production. Therefore, while inhibition of IAA or ACC uptake could account for a portion of the inhibitory action of the polyamines in tissues dependent upon an exogenous supply of these compounds, it cannot account for the observed inhibition of ethylene production in tissues such as senescing *Tradescantia* petals.

The inhibition of ethylene production in senescing flower tissue was not accompanied by a reduction in the rate of membrane deterioration (Table 1). This indicated that the polyamines exert a selective influence on the senescence process, inhibiting one aspect of senescence while not affecting another. A similar situation was found in senescing barley leaf tissue [6]. In that tissue, the polyamines inhibited the loss of chlorophyll that normally accompanies senescence but they did not reduce the loss of photosynthetic ability that is also characteristic of senescing leaf tissue.

Lastly, ethylene has been shown to be involved in the regulation of leaf senescence in tobacco [12]. If ethylene is

Table 5. Effect of L-methionine on the inhibition of IAA-induced ethylene production in etiolated soybean hypocotyls by spermidine (all concentrations 10 mM)

Treatment	Ethylene produced (nl)
None	19.7 b*
Methionine	29.9 a
Spermidine	0.6 c
Spermidine + Methionine	0.4 c

*Values are the means of 5 replications of one experiment. Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. Effect of spermidine, cobalt and aminoethoxyvinylglycine (AVG) on the amount of ethylene produced and on the endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in IAA-treated etiolated soybean hypocotyls

Treatment	Ethylene produced (pmol)	ACC content (pmol)*
None	326.0	415
Cobalt (5×10^{-4} M)	0.0	587
AVG (1×10^{-4} M)	0.0	73
Spermidine (1×10^{-2} M)	11.8	68

*Average of 2 independent determinations.

also involved in the regulation of leaf senescence in monocots, it is conceivable that the reduction in the rate of chlorophyll disappearance in monocot leaves by the various polyamines [5, 6] could be attributed to their ability to reduce endogenous ethylene production. Such a hypothesis should be easy to test.

EXPERIMENTAL

Plant material and ethylene analysis. Clone 02 of *Tradescantia* was grown as previously described [9]. Soybean seeds (*Glycine max* cv Wilkin) were sown in flats containing vermiculite and were germinated in the dark for 5 days at 26°. Hypocotyl segments (1 cm) were excised 0.5 cm below the hypocotyl hook and were floated on H_2O. C_2H_4 was analysed in 1 ml gas samples by GC as previously described [13].

Experiments with isolated petals. Tradescantia petals were excised from fully open flowers and immediately after excision were floated on H_2O. Groups of petals were then transferred to glass Petri dishes containing either 5 mM KCl, or 1 or 10 mM of the polyamine soln (pH 7.0). After 2 hr groups of 10 petals were transferred to 25 ml flasks which contained 2 ml of fresh soln. The flasks were then sealed and incubated in the dark for 8 hr at 26°. At the end of this incubation period, the ethylene content of the flasks was determined and an aliquot of the bathing medium was withdrawn for the determination of anthocyanin efflux. Anthocyanin efflux was determined by measuring the *A* of the bathing medium at 575 nm.

Effect of polyamines on IAA-induced ethylene production. 4 hr after excision 5 soybean hypocotyls were transferred to 25 ml flasks which contained 3 ml (pH 7.0) of 5×10^{-6} M IAA and the polyamines at 1 or 10 mM. The flasks were sealed and incubated in the dark at 26°. After 24 hr the C_2H_4 content of the flasks was determined. For this and all the other expts that employed etiolated hypocotyl segments, all manipulations were performed under a dim green safelight and all incubation media contained 1% EtOH to increase IAA solubility.

Dose response to spermidine. Groups of excised soybean hypocotyl segments were transferred to solns (pH 7.0) that contained spermidine at 0.001–100 mM. After 4 hr, groups of 5 hypocotyl segments were transferred to 25 ml flasks which contained 3 ml of the same soln as used in the pre-treatment plus 5×10^{-6} M IAA. The flasks were sealed and incubated in the dark at 26°. After 24 hr, the C_2H_4 content of the flasks was determined.

Effect of basic amino acids, inorganic cations and polyamines. Groups of excised soybean hypocotyl segments were transferred to 10 mM solns (pH 7.0) of the polyamines, inorganic cations or basic amino acids. After 4 hr, groups of 5 segments were transferred to 25 ml flasks which contained 3 ml of the same soln as used in the pre-treatment plus 5×10^{-6} M IAA. The flasks were sealed and incubated at 26°. After 24 hr, the C_2H_4 content of the flasks was determined.

Comparison of basic amino acids and polyamines. Groups of excised soybean hypocotyl segments were transferred to either H_2O or to 10 mM solns (pH 7.0) of the basic amino acids or the indicated polyamines. After 4 hr, groups of 5 segments were transferred to 25 ml flasks which contained 3 ml of the pre-treatment solns plus 5×10^{-6} M IAA. The flasks were sealed and incubated at 26°. After 24 hr, the ethylene content of the flasks was determined.

Methionine reversal of polyamine inhibition. Groups of excised soybean hypocotyl segments were transferred to either H_2O or to 10 mM solns (pH 7.0) of methionine, spermidine or spermidine plus methionine. After 4 hr, groups of 5 segments were transferred to 25 ml flasks which contained 3 ml of the pretreatment soln plus 5×10^{-6} M IAA. The flasks were sealed and incubated at 26°. After 24 hr, the C_2H_4 content of the flasks was determined.

Inhibition of 1-aminocyclopropane-1-carboxylic acid-induced ethylene. Groups of excised soybean hypocotyl segments were transferred to solns (pH 7.0) of either H_2O or to 10 mM solns of spermidine or spermine. After 4 hr, groups of 5 segments were transferred to 25 ml flasks that contained 3 ml of the pre-treatment solns plus 1×10^{-3} M 1-aminocyclopropane-1-carboxylic acid. The flasks were sealed and incubated at 26°. After 24 hr, the C_2H_4 content of the flasks was determined.

Endogenous levels of 1-aminocyclopropane-1-carboxylic acid. Groups of excised soybean hypocotyl segments were transferred to solns (pH 7.0) of the inhibitors at the following concns: spermidine, 1×10^{-2} M; aminoethoxyvinylglycine, 1×10^{-4} M; and cobalt, 5×10^{-4} M. After 4 hr, groups of 10 segments were transferred to 25 ml flasks that contained 3 ml of the pre-treatment soln plus 5×10^{-6} M IAA. The flasks were sealed and incubated in the dark at 26°. After 6 hr, the ethylene content of the flasks was determined and the hypocotyl segments were extracted with 80% EtOH. 1-Aminocyclopropane-1-carboxylic acid was isolated as described in [9] and was quantitated by the method of [10].

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