

perennial shrub, becomes unsuitable<sup>10,11</sup>. Thus, it will be interesting to examine the possibility that the beetle may establish on the more continuously available parthenium. It will also be worthwhile to ascertain the impact *L. vittata* may have on the recently introduced biocontrol agent *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae). As *Z. bicolorata* undergoes diapause in dry months and emerges from diapause with the onset of heavy rains<sup>12</sup>, will *L. vittata* displace *Z. bicolorata* in the event of delayed or inadequate or erratic rainfall for a couple of years? Long-term studies on the biologies and interactions of these two beetles may provide an answer.

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Received 25 September 1990; accepted 8 November 1990

## Effect of polyamine biosynthesis inhibitors on polyamine levels in bean seedlings

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$\alpha$ -Difluoromethylornithine (DFMO) and  $\alpha$ -difluoromethylarginine (DFMA), inhibitors of polyamine (PA)

biosynthesis, are known to control fungal infections of plants. To see if these inhibitors affect plant polyamine metabolism and growth, changes in endogenous polyamine (PA) pools of bean plants (*Phaseolus vulgaris* L. var Pinto) were examined following a single spray of DFMO and DFMA onto unifoliolate leaves. The level of PAs did not decline after inhibitor treatment; rather putrescine and spermidine levels rose in plants sprayed with inhibitor. PA concentrations were greater in unexpanded trifoliolate leaves, compared with unifoliolate leaves, suggesting that PA biosynthesis is greatest in rapidly growing parts of the plant. There was no effect of DFMO or DFMA spray on growth of the plants. These findings further substantiate the idea that DFMO and other PA inhibitors might be used to protect crop plants from pathogenic fungi.

POLYAMINE (PA) research has increased greatly since the specific and enzyme-activated irreversible inhibitors of PA biosynthetic enzymes, such as  $\alpha$ -difluoromethylornithine (DFMO) and  $\alpha$ -difluoromethylarginine (DFMA) were synthesized. The availability of these and other inhibitors has not only enabled researchers to confirm the role of PAs in cell growth and differentiation in many systems<sup>1,2</sup>, but has also had very exciting spin-offs<sup>3-5</sup>. For example, DFMO, an inhibitor of the PA biosynthetic enzyme ornithine decarboxylase (ODC), has proved to be an effective drug against cancer and protozoan infections<sup>3,4</sup>. Furthermore, Rajam *et al.*<sup>5,6</sup> showed that DFMO at 0.5 mM or higher completely controlled rust infection of french bean (*Phaseolus vulgaris* L. var. Pinto) caused by *Uromyces phaseoli* L. with systemic action. This novel approach has attracted the attention of several workers, who have also reported the protective effect of DFMO in the control of several other plant diseases caused by pathogenic fungi. For instance, rust infections of broad bean<sup>7</sup>, wheat leaf and stem<sup>8</sup>, oat and corn<sup>9</sup>; powdery mildews of wheat<sup>8</sup>, barley<sup>10</sup>, bean and apple<sup>9</sup>; leaf blight of corn<sup>9</sup>; and wilt of tomato<sup>11</sup> might be controlled by using DFMO and other inhibitors of PA biosynthesis. Thus the use of DFMO and other PA biosynthesis inhibitors for control of phytopathogenic<sup>3-15</sup> and human-pathogenic fungi<sup>16-18</sup> appears to be a promising area for future research. In the present investigation, we examined the effect of DFMO and DFMA on PA levels in bean seedlings to establish the extent to which DFMO and other PA biosynthesis inhibitors can be used for plant protection.

Unifoliolate leaves of 10-day-old greenhouse-grown bean (*P. vulgaris* L. var Pinto) seedlings (12 seedlings per treatment) were sprayed with three concentrations (0.01, 0.1 and 1.0 mM) of DFMO and DFMA in 0.01% Tween-20 at pH 7.0. Control plants were sprayed similarly with Tween-20 without inhibitor. PAs were determined in unifoliolate bean leaves 1 and 3 days later, while the first trifoliolate leaves, unexpanded at

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the time of spray and themselves unsprayed, were examined 8 days later following a single inhibitor spray onto the unifoliolate leaves (~400 µl per leaf). Samples from three different plants were used for PA determinations in each treatment. Leaf samples were ground in prechilled mortars with 10% (w/v) HClO<sub>4</sub> at a ratio of 200 mg fresh weight leaf per ml HClO<sub>4</sub>, and the homogenates centrifuged at 26,000 *g* for 20 min at 4°C. The clean supernatant fractions were used for dansylation according to a procedure previously described<sup>19</sup>. The dansylated PAs were extracted with 0.25 ml benzene and the clear benzene layer was used for PA determinations by TLC on LK6D high-resolution silica gel plates (Whatman). After development in chloroform: triethylamine (25:2 v/v) for about 1 h, and location by fluorescence under a UV lamp, the dansylpolyamine bands were scraped off, eluted in 4 ml of ethyl acetate, and quantified using an Aminco-Bowman fluorimeter.

There was no significant reduction of endogenous PA pools in any of the inhibitor-treated leaves (Table 1); in fact, putrescine and spermidine levels were significantly increased by the highest concentration of DFMO and DFMA. No significant change in spermine levels was observed. Walters<sup>7</sup> observed that DFMO at 0.4 mM had no effect on either growth of or total intracellular PA levels in *Vicia faba* plants. Spermidine and spermine levels were highest in the young unifoliolate and unexpanded trifoliolate leaves, and fell sharply as leaves aged. The mature unifoliolate leaves showed slightly

more putrescine than spermidine and spermine. This suggests that PA biosynthesis is very active in rapidly dividing tissues, and gradually declines with age of the tissue<sup>20</sup>. Furthermore, the increased levels of spermidine and spermine in rapidly growing regions of the plant indicates that these PAs might be involved in cell replication and induction of DNA synthesis<sup>21</sup>.

There was no effect of DFMO or DFMA spray on growth of the plants. Plants sprayed with inhibitors at all concentrations looked green and healthy, exhibiting no malformation or reduction in growth rate compared to unsprayed controls (visual observations). In earlier work, Rajam *et al.*<sup>5,6</sup> and Weinstein *et al.*<sup>9</sup> observed that DFMO and DFMA had no apparent effects on growth of bean and wheat plants respectively. Mussell *et al.*<sup>8</sup> showed that DFMO even at 20 mM had no apparent effects on growth and development of tomato plants for the duration of the 28-day observation period.

The present findings show that DFMO and DFMA did not affect intracellular PA pools; rather putrescine and spermidine levels actually rose in plants sprayed with the highest concentration of inhibitor (1 mM). This may be due to the known but paradoxical stimulation of arginine decarboxylase (ADC) activity by DFMO or ODC activity by DFMA (an inhibitor of ADC) in higher plants. Activation of one pathway for putrescine synthesis may result from inhibition of the other<sup>22</sup>.

Therefore, in the absence of any depression in the PA pools or in growth of the plants, we believe that DFMO and other PA biosynthesis inhibitors, singly or in conjunction, may prove to be useful for the control of many plant diseases caused by pathogenic fungi through selective inhibition of fungal putrescine biosynthesis<sup>3,4,15</sup>. Furthermore, the ODC inhibitors like DFMO are non-toxic to human beings and animals<sup>23</sup>; hence they may be safe fungicides and present an interesting alternative to traditional fungicides.

Table 1. Polyamine levels following DFMO and DFMA application to bean plants.

Day	Treatment	Putrescine	Spermidine	Spermine
(nmol/g fresh wt <sup>a</sup> )				
1 <sup>b</sup>	Control	170 ± 34	413 ± 23	208 ± 29
	DFMO, 0.01 mM	160 ± 18	527 ± 54	224 ± 32
	DFMO, 0.1	181 ± 6	578 ± 53**	228 ± 6
	DFMO, 1.0	212 ± 24	634 ± 18*	227 ± 17
	DFMA, 0.01	291 ± 20**	648 ± 95**	299 ± 52
	DFMA, 0.1	319 ± 34**	536 ± 43**	205 ± 17
	DFMA, 1.0	301 ± 46	703 ± 57**	287 ± 25
3 <sup>b</sup>	Control	163 ± 32	149 ± 2	51 ± 3
	DFMO, 0.01	122 ± 44	159 ± 6	60 ± 18
	DFMO, 0.1	160 ± 22	183 ± 7**	45 ± 5
	DFMO, 1.0	301 ± 1**	248 ± 21**	51 ± 8
	DFMA, 0.01	215 ± 29	301 ± 25*	81 ± 12**
	DFMA, 0.1	160 ± 21	252 ± 3*	53 ± 3
	DFMA, 1.0	198 ± 39	294 ± 33**	68 ± 12
8 <sup>c</sup>	Control	178 ± 20	479 ± 39	219 ± 14
	DFMO, 0.01	231 ± 17	616 ± 72	239 ± 19
	DFMO, 0.1	195 ± 18	552 ± 37	255 ± 34
	DFMO, 1.0	184 ± 21	619 ± 17**	261 ± 14
	DFMA, 0.01	220 ± 15	638 ± 45**	287 ± 65
	DFMA, 0.1	196 ± 20	559 ± 19	246 ± 11
	DFMA, 1.0	214 ± 17	623 ± 28**	218 ± 12

<sup>a</sup>Each value is mean ± SE of three replicates.

<sup>b</sup>Samples for analysis from unifoliolate leaves.

<sup>c</sup>Samples for analysis from trifoliolate leaves.

Significance of difference. \**P* < 0.01, \*\**P* < 0.05

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ACKNOWLEDGEMENTS. M. V. R. is much indebted to the Ministry of Education and Culture, New Delhi, for the award of a National Scholarship for Study Abroad. We are grateful to Dr Peter McCann of Merrell Dow Research Institute, Cincinnati, USA, for a generous gift of DFMO and DFMA.

Received 10 August 1990; accepted 28 August 1990

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