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Potentiation and Inhibition of the Effects of 2-Chloroethylphosphonic Acid by Malformin[1]

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ABSTRACT

Malformin completely inhibited Ethrel-induced swelling and fresh weight increase on the basal stem portion of *Phaseolus vulgaris* L. cuttings, but markedly potentiated Ethrel- or ethylene-induced abscission. With regard to abscission, malformin reacted synergistically with ethylene and dark aging, and in a manner which appeared to differ from that of ethylene and dark aging. The numerous effects of malformin on plant growth and development cannot be explained in simple terms of enhanced ethylene production.

Malformin is a highly active growth regulator, produced by the fungus *Aspergillus niger,* which induces striking malformations in the growth of higher plants (12) and pronounced root curvatures (10). Roots of *Zea mays* have been observed to literally tie themselves into knots in response to malformin. Malformin also stimulates abscission (5) and stimulates production of either ethylene or ethane, depending on method of application (6). Because of these effects, malformin may be a useful tool in studies of normal and abnormal growth, abscission, and ethylene or ethane biosynthesis.

I recently proposed a role of ethylene in the induction of growth disturbances by malformin in stems and petioles of *Phaseolus vulgaris* and stimulation of abscission of *P. aureus* (5). Because stimulation of abscission by Ethrel, a mixture of 2-chloroethylphosphonic acid and its ethyl ester, is mediated by ethylene (11), I compared the biological activity of malformin and Ethrel. In preliminary experiments I treated cuttings of *P. vulgaris* with mixtures of malformin and Ethrel and observed that malformin markedly potentiated and inhibited the biological activity of Ethrel. These observations, reported here, are significant with regard to the mode of action of malformin and may be of value for studies of ethylene activity, abscission, and stem growth.

MATERIALS AND METHODS

Malformin A, isolated from culture filtrates of *Aspergillus niger* van Tiegh. strain 58-883, was used (15). Ethrel, formulation 66-329, was a gift from Amchem Products, Inc., Ambler, Pa.

Seedlings of *Phaseolus vulgaris* L. cv. Black Valentine or *P. vulgaris* cv. Resistant Asgrow Valentine were grown in vermiculite to the primary leaf stage and prior to elongation of the apical bud. Cuttings were obtained by removing the cotyledons and cutting the stems 2 cm below the cotyledonary node. Usually, 8 to 10 cuttings were placed in 50-ml beakers containing 30 to 35 ml of test solution, incubated in the light (800 ft-c) for 24 hr, rinsed, transferred to deionized water, and maintained in the light up to 6 days at 26 to 27 C. Some cuttings were treated with ethylene in 250-mm desiccators containing 10% KOH in a separate beaker. Other variations of method and time of treatment are described later.

Two-centimeter stem sections were removed from the basal end of treated and control cuttings at various intervals. Roots, which usually appeared after 4 days, were not included in the fresh weight of these sections. Petiole sections were 10-mm portions from the blade end of the primary leaf petiole.

Abscission of the primary leaves was determined after applying sufficient pressure to the petioles to move the petioles through an arc of 10 to 15°.

The method for ethylene analysis by gas chromatography has been described (5). Samples of four basal stem sections or eight petiole sections were sealed for 2 to 4 hr in 5-ml glass syringes, analyzed for ethylene liberation, and weighed.

Replicated experiments were performed at least three times. Confidence intervals were calculated at the 5% level.

RESULTS

Ethylene liberation from basal stem and petiole sections, fresh weight of basal stem sections, and primary leaf abscission on cuttings of *P. vulgaris* were increased by treatment with Ethrel (Table I). Although basal stem sections from cuttings treated with 0.69 μM Ethrel liberated more ethylene than controls, the amount liberated was insufficient to increase the fresh weight. At this concentration of Ethrel, ethylene liberation by petiole sections and abscission of primary leaves were not increased. Basal stem sections from cuttings treated with 6.9 μM Ethrel liberated much less ethylene than those from cuttings treated with 69 μM Ethrel, but the amount of ethylene liberated by the lower concentration induced significant increases in the fresh weight.

When cuttings were treated simultaneously with Ethrel and malformin, malformin completely inhibited Ethrel-induced swelling and fresh weight increase of basal stem sections (Table II). Malformin alone slightly inhibited the fresh weight of basal stem sections, compared to water controls, and did not induce abscission. But abscission by cuttings treated with Ethrel *plus* malformin was markedly stimulated 48 hr after treatment was initiated.

Malformin stimulates or inhibits ethylene production, or stimulates ethane production, depending on the treatment (6). To determine if malformin altered the liberation of ethylene from Ethrel in the tissues, cuttings were treated simultaneously

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Table I. *Ethylene Liberation, Basal Stem Weight, and Abscission of P. vulgaris Cuttings Treated with Ethrel*

Treatment	Days after Treatment			
	1	2	3	6
Ethylene from basal section (nl/hr·g fresh wt)				
H_2O	1.0 ± 0.2	1.6 ± 0.3	1.6 + 0.3	...
Ethrel, 69 μM	78.3 ± 11.9	54.7 ± 7.4	52.1 ± 4.6	...
Ethrel, 6.9 μM	10.3 ± 1.9	8.4 ± 1.3	6.5 ± 0.9	...
Ethrel, 0.69 μM	2.1 ± 0.3	2.0 ± 0.2	2.2 ± 0.4	...
Ethylene from petiole sections (nl/hr·g fresh wt)				
H_2O	5.5 ± 1.5	3.7 ± 0.6	3.6 ± 0.6	2.7 ± 0.9
Ethrel, 69 μM	49.7 ± 8.8	28.8 ± 6.2	19.6 ± 2.7	8.5 ± 3.7
Ethrel, 6.9 μM	10.6 ± 2.6	7.3 ± 0.8	5.8 ± 0.9	5.3 ± 1.4
Fresh wt per 4 basal stem sections (g)				
H_2O	0.49 ± 0.04	0.48 ± 0.06	0.50 ± 0.05	0.55 ± 0.05
Ethrel, 69 μM	0.47 ± 0.03	0.49 ± 0.02	0.57 ± 0.06	0.80 ± 0.07
Ethrel, 6.9 μM	0.48 ± 0.03	0.48 ± 0.06	0.56 ± 0.06	0.67 ± 0.06
Primary leaf abscission (%)				
H_2O	0	0	0	0
Ethrel, 69 μM	1.1	16.5	24.1	35.5
Ethrel, 6.9 μM	0	0	0	7.6

Table II. *Effect of Malformin on Ethrel-induced Stem Swelling and Abscission of P. vulgaris Cuttings*

Treatment	Fresh wt per 4 Basal Stem Sections[1]	Abscission	
		2 days	6 days
	g	%	%
H_2O	0.55	0	0
Ethrel, 69 μM	0.90	22.9	48.0
Ethrel, 6.9 μM	0.79	0	6.5
Malformin, 10 μM	0.51	0	0
Malformin, 10 μM, + Ethrel, 69 μM	0.53	58.3	72.5
Malformin, 10 μM, + Ethrel, 6.9 μM	0.56	28.2	35.7

[1] After 6 days, without roots.

with Ethrel and malformin, and ethylene liberation from basal stem and petiole sections was determined (Table III). Malformin reduced the amount of ethylene liberated from both basal stem and petiole sections. However, reduction of ethylene liberation cannot explain the inhibition by malformin of fresh weight increase induced by Ethrel, because the ethylene released is more than sufficient to induce a substantial increase in fresh weight (Table I). Since Ethrel-induced abscission is mediated by ethylene (11), malformin potentiates the ability of Ethrel to induce abscission *despite* a reduction in ethylene liberation. Malformin alone stimulated ethylene production slightly in basal stem and petiole sections, but had no effect on ethane production.

Swelling of basal stem sections from cuttings treated with Ethrel is presumably mediated by ethylene which is liberated within 4 hr after treatment (Table III). Within 24 hr after treatment, ethylene stimulated RNA, DNA, and protein synthesis in basal hypocotyl tissue of *Glycine max* (8), and within 3 hr it increased the capacity of chromatin for RNA synthesis (9). To determine if malformin inhibited Ethrel-induced fresh weight increases by inhibiting the early action of ethylene, cuttings were treated with malformin after they were pretreated 24 hr with Ethrel. Malformin completely inhibited further increase in fresh weight induced by Ethrel (Fig. 1) but stimulated abscission 48 hr after the initiation of *malformin* treatment (Fig. 2). Whether applied simultaneously, or 24 hr after initiation of Ethrel treatment, malformin requires 48 hr to potentiate Ethrel-induced abscission. In similar experiments, cuttings were pretreated with Ethrel between 0 and 24 hr, and with malformin between 96 and 120 hr. Malformin again required 48 hr to potentiate Ethrel-induced abscission but immediately inhibited further Ethrel-induced fresh weight increase of basal stem sections. When cuttings were pretreated with malformin (0–24 hr) and subsequently treated with Ethrel (48–72 hr), malformin potentiated Ethrel-induced abscission 24 hr after initiation of *Ethrel* treatment (Table IV).

A role of ethylene in accelerating certain aging precesses was discussed (3). Because malformin stimulates ethylene production in petioles, the rapid potentiation of Ethrel-induced abscission by cuttings pretreated with malformin might be the result of aging caused by malformin-induced ethylene production. However, pretreatment of cuttings with low concentrations (6.9 μM) of Ethrel, which liberate even more ethylene in petioles than that resulting from malformin treatment, did

Table III. *Ethylene from P. vulgaris Cuttings Treated with Malformin and Ethrel*

Treatment	Ethylene		
	4 hr	24 hr	48 hr
	nl/hr·g fresh wt		
Basal stem sections			
H_2O	1.3	1.1	1.6
Ethrel 69 μM	18.4	84.5	87.6
Ethrel 6.9 μM	2.4	11.7	10.8
Malformin 10 μM	1.0	2.2	1.9
Malformin 10 μM + Ethrel 69 μM	12.8	59.1	44.2
Malformin 10 μM + Ethrel 6.9 μM	2.2	10.0	7.5
Petiole sections			
H_2O	4.9	4.6	4.7
Ethrel 69 μM	25.1	77.1	44.3
Ethrel 6.9 μM	6.3	11.2	10.4
Malformin 10 μM	4.6	5.3	6.5
Malformin 10 μM + Ethrel 69 μM	19.1	47.4	34.0
Malformin 10 μM + Ethrel 6.9 μM	6.4	11.2	10.1

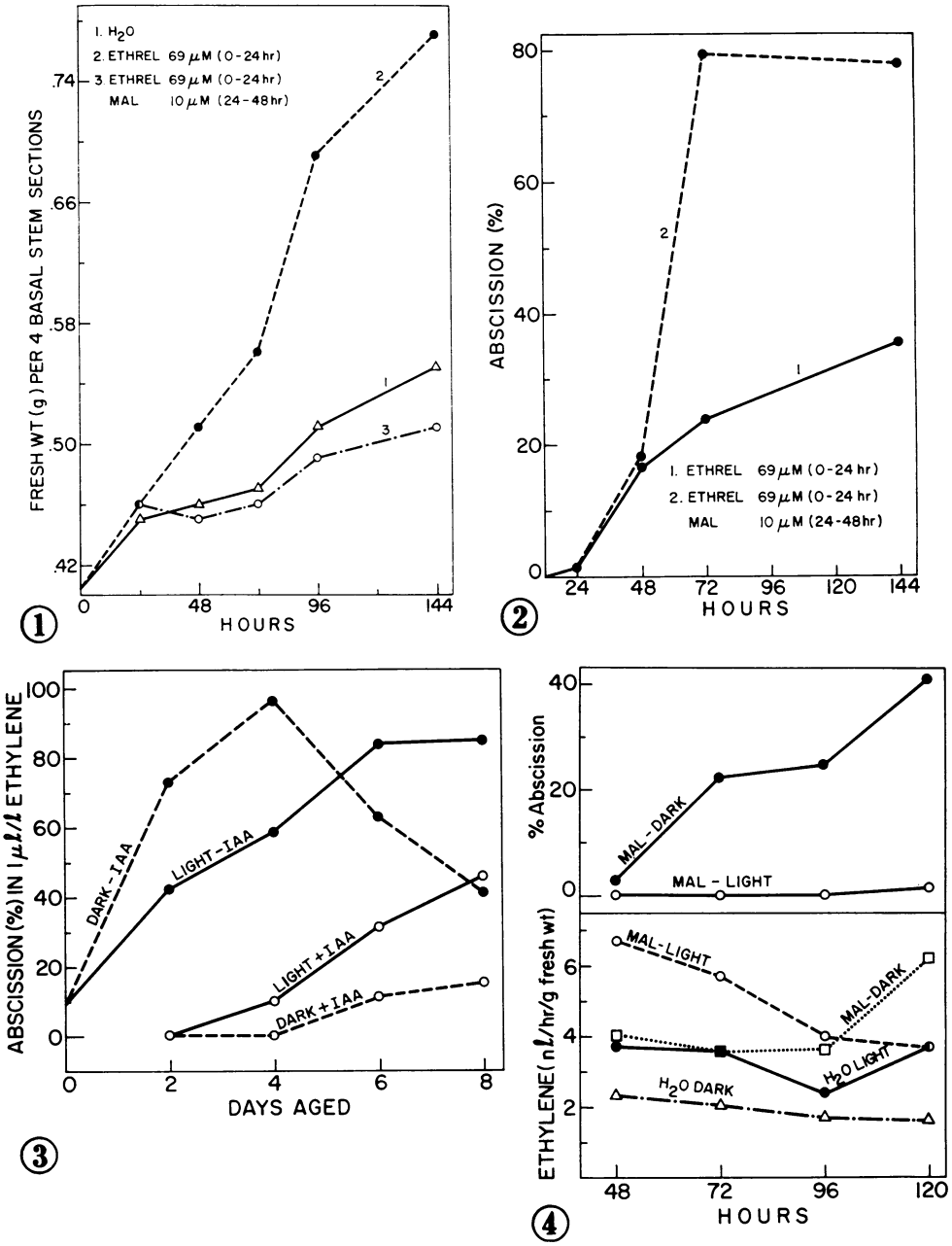

FIG. 1. Malformin (MAL) inhibition of fresh weight increase of basal stem sections from *P. vulgaris* cuttings pretreated with Ethrel.

FIG. 2. Malformin potentiation of abscission by cuttings of *P. vulgaris* pretreated with Ethrel.

FIG. 3. Effect of IAA on ethylene-induced abscission by cuttings of *P. vulgaris* aged in light and dark. IAA (60 μM) was sprayed on leaves and petioles to run-off prior to ethylene treatment.

FIG. 4. Effect of malformin on abscission and ethylene production by cuttings of *P. vulgaris* in light and dark. Cuttings treated with water and maintained in light or dark did not abscise. Ethylene determinations employed petiole sections from the primary leaves.

not enhance abscission induced by subsequently applied and higher concentrations (69 μM) of Ethrel (Table V).

Rubinstein and Leopold (14) found that as petiole explants age they pass through two abscission stages, an induction period (stage I) which is prolonged by auxin, and a later stage (stage II), which is stimulated by auxin. Abeles has also shown that ethylene induced more abscission in aged than unaged explants (1). If potentiation of Ethrel-induced abscission by malformin is the result of malformin-induced aging, similar to the stage I-stage II transition of explants, then IAA should inhibit malformin action when applied simultaneously with malformin, but not when applied 48 hr after malformin treatment. But IAA blocked potentiation of Ethrel-induced abscission by malformin whether applied with or 48 hr after malformin (Table VI). To determine if cuttings of *P. vulgaris* pass through two abscission stages similar to that of explants, the effect of IAA on abscission of light- and dark-aged cuttings treated with 1 μl/liter ethylene was investigated (Fig. 3). The sensitivity of dark-aged cuttings to ethylene increased more rapidly than that of light-aged cuttings up to 4 days, but the sensitivity of dark-aged cuttings to ethylene decreased after 4 days. The ability of IAA to inhibit ethylene-induced abscission decreased as the cuttings aged, but IAA always inhibited abscission substantially. Cuttings of *P. vulgaris* behave dif-

Table IV. *Effect of Premalformin Treatment on Ethrel-induced Abscission*

Treatment		Abscission (72 hr)
0–24 hr	48–72 hr	
		%
H_2O	H_2O	0
Malformin, 10 μM	H_2O	1.4
Malformin, 10 μM	Ethrel, 69 μM	52.4
Malformin, 10 μM	Ethrel, 6.9 μM	16.6
H_2O	Ethrel, 69 μM	5.6
H_2O	Ethrel, 6.9 μM	0

Table V. *Effect of Pretreatment with Ethrel on Subsequent Ethrel-induced Abscission of P. vulgaris Cuttings*

Treatment		Abscission	
0–24 hr	48–72 hr	72 hr	96 hr
		%	%
H_2O	H_2O	0	0
Ethrel, 6.9 μM	H_2O	0	0
Ethrel, 6.9 μM	Ethrel, 69 μM	0.4	14.0
H_2O	Ethrel, 69 μM	0.6	17.6

Table VI. *Effect o, Indoleacetic Acid on Malformin-Ethrel-induced Abscission by P. vulgaris*

Treatment[1]		Abscission
		%
Simultaneous treatment (0–24 hr)		*48 hr*
Ethrel		12.0
Ethrel + Malformin		83.8
Ethrel + Malformin + IAA		14.1
Premalformin treatment		
(0–24 hr)	(48–72 hr)	*72 hr*
Malformin	Ethrel	28.3
Malformin	Ethrel + IAA	5.7
H_2O	Ethrel	0.0

[1] Concentrations of Ethrel, Malformin, and IAA were 69, 10, and 60 μM, respectively. IAA was sprayed on leaves and petioles to run-off at 0 and 24 hr for the simultaneous treatment, and at 48 and 56 hr for the premalformin treatment.

ferently than explants. Cuttings treated with water and aged in the dark up to 8 days, but not treated with ethylene, did not abscise.

Malformin stimulated abscission of the primary leaves of *P. aureus* in the dark but not in the light (5). Since malformin both stimulates ethylene production in petioles and increases the sensitivity of *P. vulgaris* cuttings to ethylene-induced abscission in the light, why is abscission stimulated by malformin only in the dark? To determine if malformin stimulates ethylene production and abscission in cuttings to a greater extent in the dark than in the light, cuttings were treated with malformin for 24 hr in the light, transferred to water, and incubated in light or dark. Abscission and ethylene production were determined at regular intervals (Fig. 4). Malformin stimulated abscission by cuttings in the dark after 72 hr, but not in the light. Differences in the magnitude of malformin-induced ethylene production in light and dark cannot explain these results, because malformin stimulated ethylene production to a greater extent in light up to 96 hr. The pronounced rise in stimulation of ethylene production by malformin in the dark after 120 hr occurs after abscission began.

IAA completely inhibited ethylene-induced abscission by cuttings aged up to 4 days in the dark (Fig. 3) and, when applied simultaneously with malformin, inhibited over 90% malformin-induced abscission of *P. aureus* seedlings in the dark (5). The ability of IAA to inhibit ethylene-induced abscission after cuttings of *P. vulgaris* had been treated with malformin and subsequently aged in the dark was examined. Cuttings were treated with malformin in light (0–24 hr), transferred to water and aged in light or dark (24–72 hr), sprayed with water or IAA (60 μM), dried, and treated with 1 μl/liter ethylene (72–96 hr). Abscission was determined at 96 hr (Fig. 5). Although IAA completely inhibited ethylene-induced abscission by cuttings treated with water and aged in light or dark and markedly reduced ethylene-induced abscission by cuttings treated with malformin and aged in light, the ability of IAA to inhibit ethylene-induced abscission by cuttings treated with malformin and aged in the dark was considerably diminished. In this respect, malformin and dark aging are synergistic in reducing the ability of IAA to inhibit ethylene-induced abscission.

If malformin and dark aging are synergistic with regard to their ability to increase the sensitivity of cuttings to ethylene-induced abscission, then the increase in sensitivity induced by treatment of cuttings with malformin *plus* dark aging should be greater than the increase in sensitivity induced by malformin

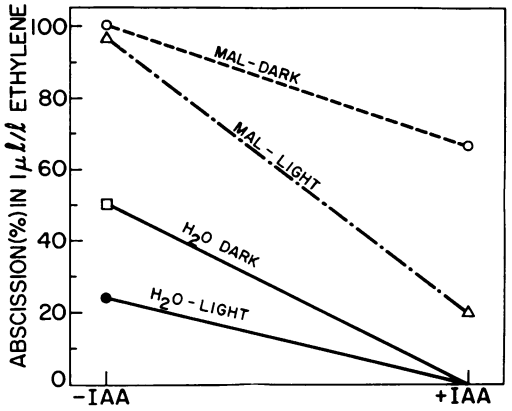

FIG. 5. Effect of IAA on ethylene-induced abscission by cuttings of *P. vulgaris* previously treated with malformin (MAL) and maintained in light and dark. IAA (60 μM) was sprayed on leaves and petioles to run-off prior to ethylene treatment.

Table VII. *Effect of Dark Aging and Malformin on Ethylene-induced Abscission of P. vulgaris Cuttings*

Treatment[1]	Abscission	Increase in Abscission over Control
	%	%
H_2O-light (control)	18.0	...
H_2O-dark	65.8	47.8
Malformin-light	38.5	20.5
Malformin-dark	97.6	79.6

[1] Cuttings were treated from 0–24 hr in the light, transferred to water, maintained in the light or dark from 24–72 hr, and treated with 1 μl/liter ethylene from 72 to 96 hr. Abscission was evaluated after 96 hr. Malformin was 1.0 μM.

in light plus the increase in sensitivity induced by dark aging. Because cuttings treated with 10 μM malformin abscised almost 100%, whether subsequently aged in light or dark, in response to ethylene, it was necessary to decrease the concentration of malformin. Cuttings were treated in light (0–24 hr) with water or malformin (1.0 μM), transferred to water, maintained in light or dark (24–72 hr), treated with 1 μl/liter ethylene (72–96 hr), and evaluated for abscission (96 hr). In each of three experiments, the increase in ethylene-induced abscission by cuttings treated with malformin *plus* dark aging (79.6%) was greater than the increase induced by dark aging (47.8%) *plus* the increase induced by malformin in light (20.5%, Table VII).

DISCUSSION

Malformin and ethylene induced epinasty, reduced growth rate and swelling in shoots, reduced growth rate and curvatures in roots, and leaf abscission (2, 5). Malformin-induced root curvatures, abscission, and epinastic curvatures are inhibited by CO_2 (5), a competitive inhibitor of ethylene action (4). Malformin-induced changes in the biochemical composition of *P. vulgaris* seedlings are also similar to those induced by ethylene (7). In addition to inducing curvatures on corn roots, malformin stimulates root hair and lateral root formation, promotes radial expansion, and inhibits elongation and fresh weight increase (10). These effects also follow treatment of roots with ethylene (2). Malformin stimulates ethylene production of both seedlings and explants of *P. vulgaris* (6) but does not stimulate ethylene production by roots (5, 10).

When the apical bud of *P. vulgaris* seedlings is treated with malformin, subsequent growth is disturbed (12). Stem and petioles exhibit severe curvatures, increased diameter, reduced length, and irregular protrusions. It was proposed that these disturbances were mediated, in part, by ethylene (5). But in the present experiments malformin completely inhibited Ethrel-induced swelling, presumably mediated by ethylene, on the basal stem portion of *P. vulgaris* cuttings. Unless the response of the upper portion of seedlings to ethylene and malformin differs from that of the basal portion of cuttings, malformin-induced stem swellings and protrusions may not be mediated by ethylene.

Several factors, including ethylene, appear to contribute toward malformin-induced abscission. These factors include (*a*) stimulation of ethylene production by malformin in light or dark, (*b*) a synergistic response by cuttings to malformin and ethylene in light or dark, and (*c*) a synergistic response by cuttings to malformin and dark aging. Although malformin stimulates ethylene production more in light than in dark and increases the sensitivity of the cuttings to ethylene-induced abscission, the amount of ethylene produced is apparently insufficient to induce abscission in light. Only when an additional factor, dark aging, is added do the leaves abscise.

With regard to abscission, the response of cuttings to malformin and dark aging suggests that the mode of action of malformin and dark aging differ. When cuttings were aged in the dark to such an extent (4 days) that almost all leaves abscised in response to ethylene (Fig. 3), IAA completely inhibited abscission. On similar cuttings treated with malformin *plus* dark aging, which abscised 100% in response to ethylene, IAA reduced abscission only 34% (Fig. 5). Furthermore, since malformin markedly potentiated Ethrel-induced abscission despite a reduction in ethylene liberation, the mode of action of malformin and ethylene also appears to differ. Whether applied with or after Ethrel, malformin required 48 hr to potentiate Ethrel-induced abscission. But when cuttings were pretreated with malformin, Ethrel-induced abscission was potentiated in 24 hr. Although these results may reflect differences in translocation rates for malformin and Ethrel, they also suggest that malformin induces changes in petioles which precede those induced by ethylene.

Evidence for and against a single primary event catalyzed by ethylene was discussed (3, 13). It is unlikely that malformin could both potentiate and inhibit a single chemical reaction. Clearly, malformin does not interfere with early events catalyzed by ethylene (Ethrel) which lead to stem swelling, because malformin inhibits ethylene-induced swelling when applied as much as 4 days after treatment with Ethrel. But with regard to malformin potentiation of ethylene-induced abscission, malformin appears to act prior to ethylene action. A bimodal action of malformin is also suggested by the cubic growth curve of *Zea mays* roots treated with malformin (10), and by the ability of malformin to stimulate either ethane or ethylene production (6). Although the mode of action of malformin appears to be related to that of ethylene, the numerous effects of malformin on plant growth and development cannot be explained in simple terms of enhanced ethylene production (5).

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