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Effects of Dimethipin*, a Defoliant and Desiccant, on Stomatal Behavior and Protein Synthesis

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Abstract. The first visible macroscopic effect of a foliar spray of dimethipin (2,3-dihydro-5,6-dimethyl-1,4-dithiin 1,1,4,4-tetraoxide) on kidney beans (*Phaseolus vulgaris* L. cv. Back Valentine) was a severe loss of leaf turgor followed by desiccation and, ultimately, abscission. Dimethipin-treated leaves had higher rates of transpiration than control leaves when the leaves received treatments that cause stomatal closure (e.g., darkness, water stress, or exogenous abscisic acid). The higher rates of water loss from the dimethipin-treated leaves were not due to a massive nonspecific disruption of leaf cells, since dimethipin-treated leaves maintained turgor for 24 h if the plants were placed in a chamber of 100% relative humidity. These results indicate that the dimethipin-induced loss of leaf turgor is due, at least in part, to a loss in stomatal control.

The earliest detectable biochemical effect of dimethipin was an inhibition of the incorporation of ^{14}C-leucine into protein. In both kidney bean leaf discs and oat (*Avena sativa* L. cv. Garry) coleoptiles, greater than 50% inhibition of ^{14}C-leucine incorporation into protein was observed 1 h after the start of incubation in 1 mM dimethipin. Dimethipin had a substantially smaller effect, however, on the incorporation of ^{3}H-uridine into RNA, suggesting that dimethipin acts primarily on the processes associated with translation rather than transcription. Cycloheximide also caused a loss of stomatal control, and both dimethipin and cycloheximide retarded the deg-

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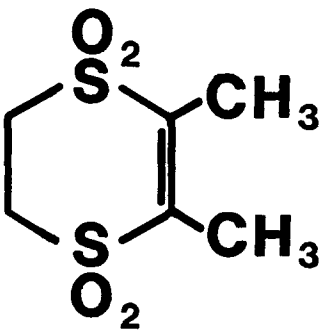


Fig. 1. Structure of dimethipin (2,3-dihydro-5,6 dimethyl-1,4 dithiin 1,1,4,4-tetraoxide).

radation of chlorophyll in senescing oat leaf segments in the dark, indicating similar mechanisms of action for the two compounds. In summary, the evidence suggests that an initial inhibition of protein synthesis is responsible for the loss of stomatal control associated with high rates of transpiration and loss of leaf turgor. The possible role for dimethipin-induced loss of turgor in abscission is discussed.

Dimethipin (2,3-dihydro-5,6 dimethyl-1,4 dithiin 1,1,4,4-tetraoxide) (Fig. 1) is a plant growth regulator that is used as a harvest aid on a variety of crops. It has been used successfully as a defoliant in cotton (Bell et al. 1975) and nursery stock (Knight 1979). Other uses include as a desiccant for potato vines (Murphy 1976) and as a promotor of drying and maturation of both sunflower and rice seed heads (Blem et al. 1983).

The effectiveness of defoliants in agricultural situations is highly dependent on both the prevailing environmental conditions and internal physiological factors (Addicott 1982). Thus, knowledge of the mechanism underlying chemically induced abscission is useful in devising strategies for optimal performance of any given defoliant.

Little is known about the mode of action of dimethipin, although it does appear to be different from other defoliants and desiccants. Paradoxically, dimethipin has been shown to inhibit abscission in petiole explants of *Phaseolus vulgaris* when applied directly to the abscission zone (Reid 1976). Moreover, this inhibition of abscission appeared to be due to an inhibition of cellulase production (Reid 1976). These results suggest that the primary site of action of dimethipin is in the blade. Indeed, other chemical defoliants have a similar requirement that the leaf blade receive the treatment in order to be effective in causing abscission (Addicott 1982).

In preliminary experiments conducted on kidney beans and cotton under controlled environmental conditions, we observed that the first visible macroscopic effect of a foliar spray of dimethipin was wilting of the leaves. Furthermore, we noted that abscission always followed an initial loss of leaf turgor (Metzger and Keng 1983). This suggests that an alteration of leaf water relations is an important link in the chain of events leading to abscission. This paper describes further experiments on the physiological and biochemical basis for the dimethipin-induced loss of leaf turgor.

Materials and Methods

Plant Material

Kidney bean (*Phaseolus vulgaris* L. cv. Black Valentine) seedlings were grown in moist vermiculite at 25°C and under a 16-h photoperiod (420 $\mu E\ m^{-2}sec^{-1}$). The relative humidity was maintained at 50%. After 2 weeks the plants were decapitated, and each of the primary leaves was sprayed with 10 ml of a solution containing the test compound, 1.0% (v/v) acetone, and 0.1% (v/v) Tween 20. Leaves of control plants received a similar treatment, except that no test compound was included. The spray was allowed to dry for 1 h under ambient laboratory conditions, and the plants were returned to the growth chamber.

Etiolated oat (*Avena sativa* L. cv. Garry) seedlings were grown between moist paper towels at 27°C. When the seedlings were 4 days old, a 13-mm segment of the coleoptile was excised 2 mm from the tip.

Leaf Water Potential Measurements

Leaf water potential was determined with a dew point hygrometer (HR-33 Dew Point Microvoltmeter with a C-52 Sample Chamber, Wescor, Inc., Logan, Utah, USA). A 9-mm leaf disc was obtained with a sharp cork borer and placed in a 1-cm sample holder. The sample was allowed to equilibrate at 21 ± 2°C for 8 h before the measurement was taken. Each treatment had two replicates and the results were expressed as the mean.

Transpiration Measurements

Instantaneous rates of transpiration from primary leaves on intact bean plants were obtained with a steady state porometer (LI 1600, LI-COR, Inc., Lincoln, Nebraska, USA). In each treatment, transpiration rates were measured on both primary leaves from three plants for a total of six measurements.

Capacity for Protein Synthesis

The ability of a tissue to incorporate ^{14}C-leucine into protein was taken as a measure of its capacity for protein synthesis. In a typical experiment, 10 leaf discs (1 cm diameter) from primary bean leaves or 10 oat coleoptile segments (13 mm in length) were incubated in a 50-ml Erlenmeyer flask with 3 ml of a solution containing 0.5 μCi of ^{14}C-leucine (54 mCi $mmole^{-1}$, Amersham, Arlington Heights, Illinois, USA), 50 mg L^{-1} chloramphenicol, and various concentrations of a test compound. The incubations were made in the dark at 35°C in a reciprocating water bath. After prescribed times, the tissues were removed from the incubation medium, rinsed thoroughly with distilled water, and quickly frozen in liquid N_2. The frozen tissue was then homogenized in 10 ml of ice cold 80% (v/v) aqueous ethanol with a Polytron homogenizer (Brinkman Instruments, Westburg, New York, USA) at ½ maximum speed for 30 sec. The

homogenate was centrifuged at 20,000 × *g* for 20 min at 2°C. The supernatant was decanted and the pellet was extracted twice more as described above. The resulting pellet following the third extraction was resuspended in 5 ml of water and transferred to a scintillation vial. Ten milliliters of Instagel scintillation cocktail (Packard Instrument Co., Inc., Downers Grove, Illinois, USA) was added and the amount of radioactivity insoluble in 80% aqueous ethanol determined by liquid scintillation spectrometry. Treatment of the pellet with pronase converted most of the radioactivity (>75%) to a form soluble in 80% ethanol; this was taken as evidence that the radioactivity insoluble in 80% aqueous ethanol represented the incorporation of ^{14}C-leucine into protein.

An aliquot of the first homogenate was taken to determine the amount of ^{14}C-leucine taken up by the tissue. The data are expressed as the percentage of the amount of the total radioactivity taken up by the tissue that was incorporated in the 80% ethanol insoluble pellet. Each treatment had three replicates.

Capacity for RNA Synthesis

The ability to incorporate ^{3}H-uridine into 80% ethanol insoluble products was taken as a measure of a tissue's capacity for RNA synthesis. Ten oat coleoptile segments (13 mm in length) were incubated with 5 μCi of ^{3}H-uridine (20.7 Ci mmol^{-1}, New England Nuclear) under conditions identical to those for the protein synthesis experiments. The tissues were extracted with 80% aqueous ethanol as described before. Treatment of the 80% ethanol insoluble pellet with RNAase released most (>80%) of the radioactivity to a form soluble in 80% ethanol. Each treatment had three replicates.

Membrane Integrity

The loss of electrolytes was used as a measure of membrane integrity. Fifteen bean leaf discs (8 mm in diameter) were preincubated for 1 h on a solution containing 0.2 M mannitol. The leaf discs were transferred (abaxial side down) to a 5-cm glass Petri dish with 5 ml of a solution containing 0.2 M mannitol and a test compound dissolved in glass distilled water. Periodically, the conductivity of the incubation solution was measured with a Markson Electromark conductivity meter (Markson Science, Phoenix, Arizona, USA). Each treatment had three replicates.

Respiration

Rates of respiration were measured using a Clark type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA). Fifteen oat coleoptile segments (13 mm in length) were incubated for various lengths of time in a test solution at 35°C and in the dark. The coleoptiles were then removed

and placed in a sample chamber with 5 ml of air-saturated distilled water. The consumption of O_2 was measured for 10 min. Each treatment had three replicates.

Measurement of Chlorophyll Loss

Segments of light grown oat leaves, 1 cm in length, were excised 3 cm from the tip of the first leaf of 2-week-old plants. These plants were grown under conditions identical to those described for the kidney beans. Four segments were placed adaxial side down in a scintillation vial containing 2 ml of a test solution. The scintillation vials were capped and placed in the dark for 3 days at 25°C. The chlorophyll was extracted from the leaf segments by placing them into 10 ml of 80% (v/v) aqueous ethanol at 80°C for 20 min. The extract was allowed to cool and was made up to 10 ml with fresh 80% ethanol. Relative chlorophyll content was estimated by measuring the absorbance at 665 nm. Each treatment had three replicates.

Chemicals

Abscisic acid (ABA), chloramphenicol, cycloheximide, and cordycepin were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Dimethipin (analytical grade) was a gift from Uniroyal Chemical Co., Bethany, Connecticut, USA.

Results

Effect of Dimethipin on Leaf-Water Relations

In preliminary experiments, we always noted that the first visible effect of a foliar spray of dimethipin was wilting of the leaves, indicating that an alteration of leaf-water relations had occurred. Leaf water potential (Ψ) was used as a quantitative measure of dimethipin-induced changes in leaf-water relations. In the first experiment, primary bean leaves received a 1-mM spray of dimethipin and were placed in a darkened growth chamber at 25°C and 50% relative humidity (Rh). After 24 h, the leaf was measured. At this point, the dimethipin-treated leaves were visibly wilted, and this was reflected in a substantial reduction in leaf Ψ (Table 1).

The dimethipin-induced loss of turgor could have been due to a generalized disruption of cellular integrity similar to that caused by bipyridyl desiccants such as paraquat (Fedtke 1982). In an experiment to test this possibility, primary bean leaves were sprayed with 1 mM dimethipin as before and then placed in a dark growth chamber at 25°C and 100% Rh. After 24 h, the dimethipin-treated leaves appeared turgid, and as shown in Table 1, had a substantially smaller reduction in leaf Ψ than when plants were maintained at 50%

Table 1. The effect of dimethipin on leaf water potential at two different relative humidities.

	Leaf Ψ (bars)	
	Relative humidity (%)	
Treatment	30	100
Control	−5.6	−5.2
Dimethipin	−14.4	−6.5

Primary bean leaves were sprayed with 10 ml of a solution with or without 1 mM dimethipin and 1% (v/v) acetone and 0.1% (v/v) Tween 20. After 24 h, leaf Ψ was measured on a 1-cm disc with a dew point hygrometer. Leaf Ψ values are the mean of two measurements.

Rh. However, even in 100% Rh, the dimethipin-treated leaves consistently had a lower leaf Ψ than control leaves.

These results suggest that the loss of turgor is not caused by a massive disruption of cellular integrity. Alternatively, it is possible that dimethipin causes a loss of stomatal control. This in turn would be expected to be associated with higher rates of transpiration from plants exposed to environmental conditions that induce stomatal closure.

Effect of Dimethipin on Stomatal Control

Normally, darkness causes a decrease in transpiration rates because the stomates close (Raschke 1975). Therefore, it was of interest to examine the effects of dimethipin on transpiration from plants maintained in the dark. Primary bean leaves received a spray containing various concentrations of dimethipin and were then placed in a dark growth chamber at 25°C and 50% Rh. After 24 h, the transpiration rates were estimated with a steady state porometer. The results are shown in Fig. 2. As expected, transpiration from control plants in the dark was low. In contrast, transpiration from leaves receiving treatments of 0.1 and 1.0 mM dimethipin was significantly higher than controls.

Water stress is another factor that has an impact on transpiration rates. Usually as the leaf Ψ becomes more negative, a point is reached at which the stomates close and transpiration is greatly diminished (Raschke 1975). The rate of water loss from excised primary bean leaves was used to examine the effects of dimethipin on water stress-induced stomatal closure. Leaves received a spray with or without 1 mM dimethipin, and the plants were then placed in a dark growth chamber at 25°C and 100% Rh. After 24 h, the leaves were excised, weighed, and placed on a laboratory bench with the abaxial side facing up. The leaves were weighed every 15 min thereafter. Fig. 3 shows the effect of dimethipin on water loss from excised bean leaves. During the first 30 min, the rate of water loss from control and dimethipin-treated leaves was about the same. After 30 min, however, the rate of water loss from the control leaves was sharply curtailed, suggesting that the stomates had closed. Water loss from the dimethipin-treated leaves continued essentially unabated. Apparently, dimethipin prevented the normal water stress-induced closure of the stomates.

It is well known that application of ABA causes rapid closure of the stomates

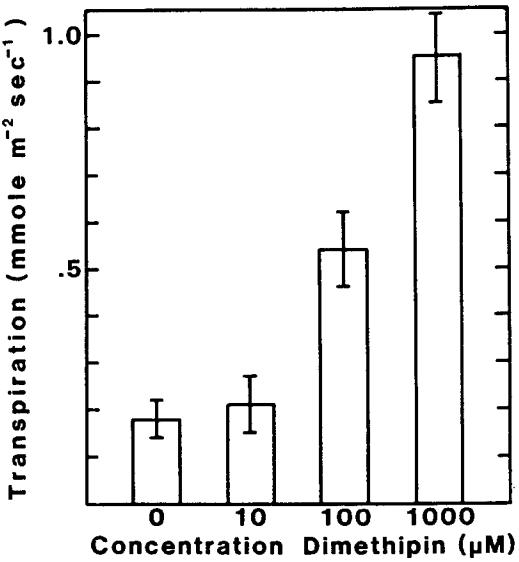


Fig. 2. The effect of various concentrations of dimethipin on transpiration from leaves maintained in the dark. Each value represents the mean of six measurements on three plants. Vertical bars refer to the standard deviations.

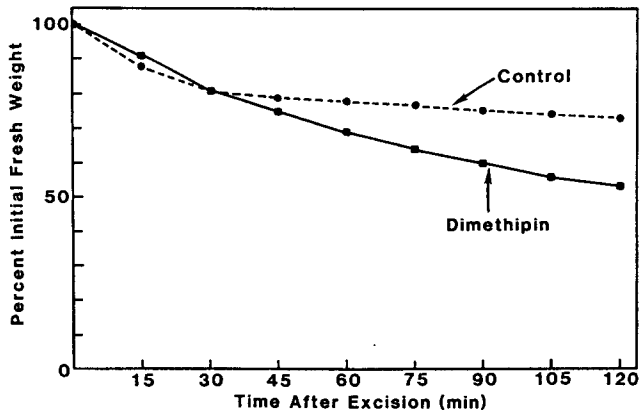


Fig. 3. The effect of dimethipin on water loss from excised bean leaves. Six primary leaves from three plants were used. The values represent the percentage of initial fresh weight of six leaves.

(Raschke 1975). Therefore, the effects of dimethipin on the reduction of transpiration resulting from exogenous ABA were investigated. In this experiment, primary bean leaves were treated as in the previous experiment. After 24 h at 25°C and 100% Rh, half of the control plants and half of the dimethipin-treated plants were sprayed with 10 ml of a solution containing 100 μM ABA. The other plants were similarly sprayed except that the solution contained no ABA. The leaves were excised 1 h later and the rate of water loss from the excised leaves was determined as in the previous experiment. The effect of the various treatments on the rate of water loss is shown in Fig. 4. As expected, ABA

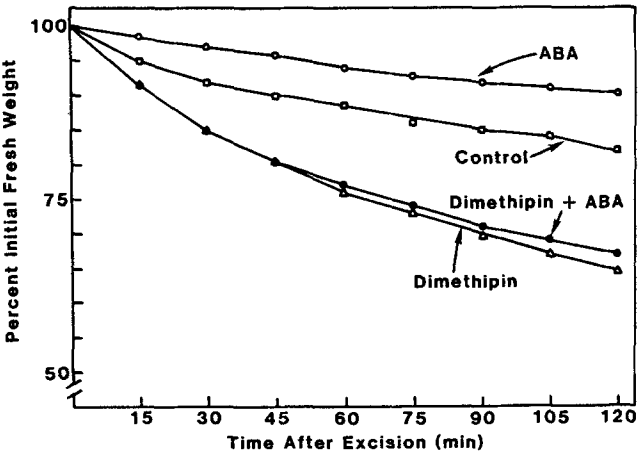


Fig. 4. The effect of dimethipin pretreatment on ABA-induced reduction of transpiration. The values represent the percent of initial fresh weight of six leaves.

significantly slowed the loss of water from control plants. However, ABA had little effect on water loss from the dimethipin-treated leaves. This indicates that dimethipin caused a loss in the guard cells' ability to respond to ABA.

Biochemical Effects of Dimethipin

Reid and Marsh (1976) reported that dimethipin inhibited protein synthesis in petiole explants of kidney beans. Furthermore, Thimann and Satler (1979) have shown that the protein synthesis inhibitor cycloheximide will cause the opening of oat leaf stomates in the dark. Therefore, it is possible that the biochemical basis for dimethipin-induced wilting and desiccation lies in an inhibition of protein synthesis.

The effect of dimethipin on a tissue's ability to incorporate ^{14}C-leucine into protein was characterized. A time course study of the effect of 1 mM dimethipin on the ability of bean leaf discs and oat coleoptile segments to incorporate ^{14}C-leucine into protein is shown in Fig. 5. "Percent incorporation" refers to the percentage of total radioactivity taken up by the tissue that was incorporated into the 80% ethanol insoluble pellet. Dimethipin inhibited protein synthesis in both tissues; this inhibition could be detected within 1 h. The inhibition was not due to an effect on the uptake of the ^{14}C-leucine, since the total radioactivity taken up by the tissue was not affected. Only the amount incorporated into the 80% ethanol insoluble pellet was diminished, indicating that only processes associated with protein synthesis were affected.

Next, it was important to establish whether the inhibition of protein synthesis is the primary biochemical event or whether it is secondary, resulting from a deleterious effect on some other basic process. We compared the kinetics of inhibition of protein synthesis of dimethipin and its effects on other basic biochemical processes.

The effect of 1 mM dimethipin on protein synthesis and respiration on oat

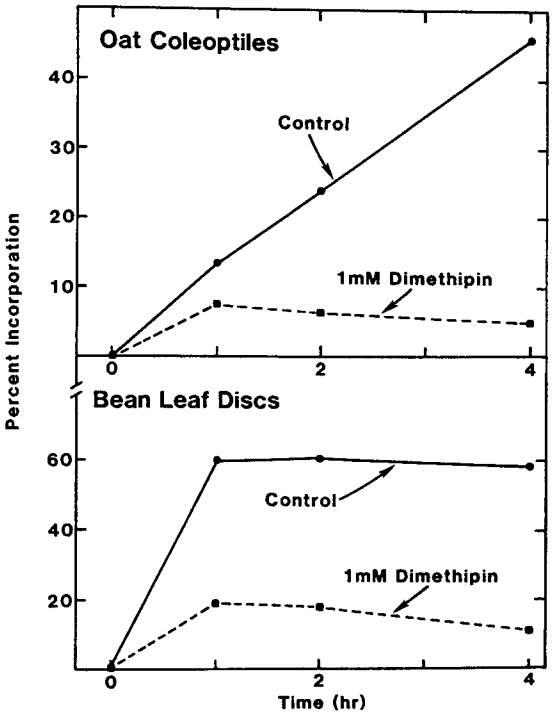
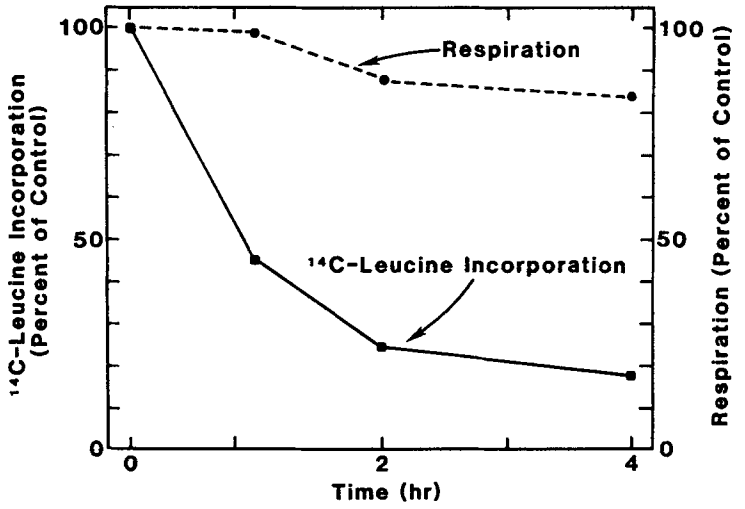


Fig. 5. Time course of the effect of 1 mM dimethipin on the ability of oat coleoptiles and bean leaf discs to incorporate ^{14}C-leucine into protein. Each value represents the mean of three replicates. Percent incorporation is the percent of the total radioactivity taken up by the tissue that was incorporated into the 80% ethanol insoluble pellet.

coleoptile segments was compared. The capacity for protein synthesis was assessed as in the previous experiment, and respiration rates were determined using a Clark-type oxygen electrode. Fig. 6 shows that, as before, dimethipin inhibited protein synthesis within 1 h after the start of incubation. Respiration, on the other hand, was not significantly affected by dimethipin at this time and was only slightly inhibited (15%) after 4 h. In other experiments (data not shown), respiration steadily declined with longer incubations. Thus it does not appear that dimethipin inhibits protein synthesis through an initial effect on respiration.

The effect of dimethipin on membrane integrity as measured by the leakage of electrolytes from bean leaf discs into the incubation medium was examined. The results in Fig. 7 show that dimethipin had little or no effect on the leakage of electrolytes after 1 or 2 h of incubation. A slight increase (35%) in the loss of electrolytes was observed after 4 h. Since dimethipin inhibits protein synthesis in bean leaf disks after 1 h (Fig. 5), the inhibition of protein synthesis cannot be due to a deleterious effect on membranes. On the other hand, the increased leakage of electrolytes could plausibly be due to an initial effect on protein synthesis.

In an effort to distinguish the aspect of protein synthesis, i.e., transcription or translation, that is preferentially affected by dimethipin, the effects of dimethipin on the incorporation of ^{14}C-leucine into protein and ^{3}H-uridine into



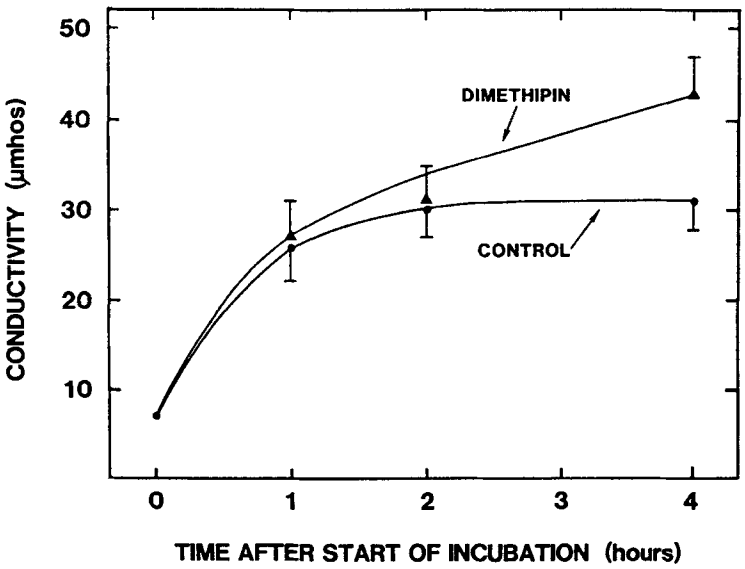


Fig. 6. (top) Time course of the effect of 1 mM dimethipin on protein synthesis and respiration (O_2 consumption) in oat coleoptile segments. Values are expressed as percent of the control value. The values are the mean of three replicates.

Fig. 7. (bottom) Time course of the effect of 1 mM dimethipin on the leakage of electrolytes from ten 1-cm bean leaf discs. Each value represents the mean of three replicates. Vertical bars represent standard deviations.

RNA were compared with the effects of known inhibitors of these processes. Fig. 8 shows the results of a double-label experiment in which 10 oat coleoptile segments were incubated with ^{14}C-leucine, ^{3}H-uridine, and either 1 mM dimethipin, 1 mM cycloheximide (an inhibitor of translation), or 2.5 mM cordycepin (an inhibitor of transcription) (Spiegel and Marcus 1980). Both dimethipin and cycloheximide drastically inhibited the incorporation of ^{14}C-leu-

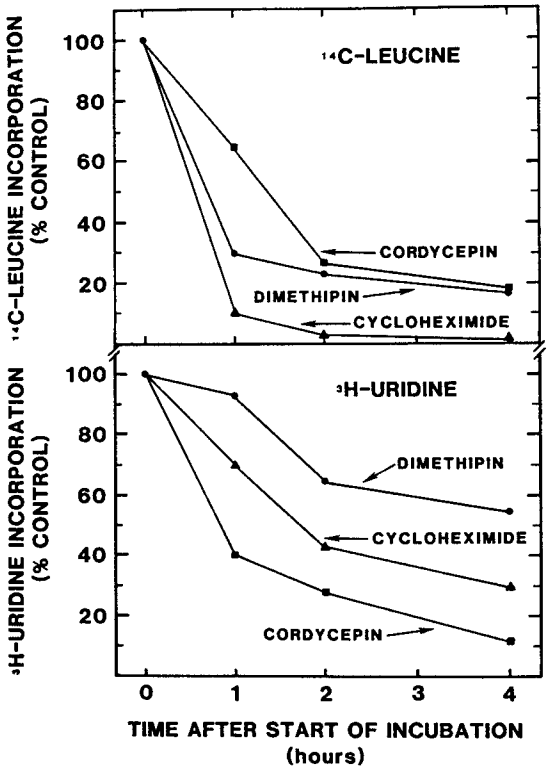


Fig. 8. Time course of the effects of dimethipin (1 mM), cycloheximide (1 mM), and cordycepin (2.5 mM) on the ability of 10 oat coleoptile segments to incorporate ^{14}C-leucine into protein (top) and ^{3}H-uridine into RNA (bottom). Values represent the percent of the control value that was incorporated. Each value represents the mean of three replicates.

cine into protein. Dose-response experiments showed that cycloheximide was about 10 times more effective on a molar basis than dimethipin (data not shown). Cordycepin, on the other hand, initially inhibited protein synthesis less, but its efficacy after 2 h increased almost to a level found with the other two compounds. In contrast, both dimethipin and cycloheximide had less inhibitory effect on the incorporation of ^{3}H-uridine into RNA than their corresponding effects on protein synthesis. Cordycepin had a greater inhibitory effect on RNA synthesis than either dimethipin or cycloheximide. The similarities between the effects of cycloheximide and dimethipin indicate that the primary site of action of dimethipin is at the level of translation.

Comparison of the Biological Effects of Dimethipin and Cycloheximide

The previous results suggest that a possible mechanism underlying the physiological effects of dimethipin is via an initial effect on some aspect of protein synthesis, most likely at the level of translation. If so, it would be logical to expect that dimethipin and cycloheximide would have similar biological properties. This possibility was first tested by comparing the effects of cycloheximide and dimethipin on the rate of transpiration from bean leaves in the dark. Primary bean leaves were sprayed with a solution containing various concentrations of either dimethipin or cycloheximide. The plants were placed in the

Table 2. A comparison of the effects of dimethipin and cycloheximide on transpiration from leaves maintained in darkness.

Treatment	Transpiration (mmole $m^{-2}sec^{-1}$ ± SD)
Control	.14 ± .04
0.01 mM dimethipin	.12 ± .05
0.10 mM dimethipin	.54 ± .11
1.00 mM dimethipin	.91 ± .15
0.01 mM cycloheximide	.59 ± .21
0.10 mM cycloheximide	.87 ± .09
1.00 mM cycloheximide	.64 ± .02

Primary leaves sprayed with 10 ml of a solution containing 1% (v/v) acetone, 0.1% (v/v) Tween 20, and various concentrations of dimethipin or cycloheximide. Transpiration was measured after 24 h in the dark at 25°C and 50% Rh. SD = standard deviation.

dark at 25°C and 50% Rh. After 24 h, the rate of transpiration was measured with a steady state porometer. Both compounds increased transpiration in the dark (Table 2). The rate of transpiration increased with higher concentrations of both compounds except that it was less in leaves treated with 1 mM cycloheximide than those treated with 0.1 mM cycloheximide or 1 mM dimethipin. The leaves treated with 1 mM cycloheximide, however, appeared visually to have suffered a substantial loss in cellular integrity. In general, though, cycloheximide was about 10 times more effective on a molar basis than dimethipin.

Cycloheximide has also been shown to inhibit the loss of chlorophyll in senescing oat leaf segments in the dark (Martin and Thimann 1972). Therefore, if dimethipin has a similar biochemical site of action as cycloheximide, then it is reasonable to expect that dimethipin also should prevent the loss of chlorophyll. Fig. 9 shows that both compounds inhibited the loss of chlorophyll from oat leaf segments in the dark, although, as observed in the previous experiment, cycloheximide was about 10 times more effective on a molar basis. In total, the results presented in Table 2 and Fig. 9 are consistent with the idea that cycloheximide and dimethipin have similar mechanisms of action.

Discussion

The first detectable macroscopic effect of a foliar spray of dimethipin was a loss of turgor, usually observed within 24 h after the treatment (Table 1). The loss of turgor was not due to nonspecific destruction of cellular components, since leaves sprayed with dimethipin had only a small reduction of leaf Ψ if maintained in 100% Rh (Table 1). The evidence that we obtained indicated that the loss of turgor was due to a loss of stomatal control, as the guard cells apparently became insensitive to dark (Fig. 2), water stress (Fig. 3), and exogenous ABA (Fig. 4). Similarly, the desiccation caused by certain herbicides has been attributed to a loss in stomatal control (Rao et al. 1977).

The earliest detectable biochemical effect of dimethipin was an inhibition of the capacity of bean leaf discs and oat coleoptile segments to incorporate ^{14}C-

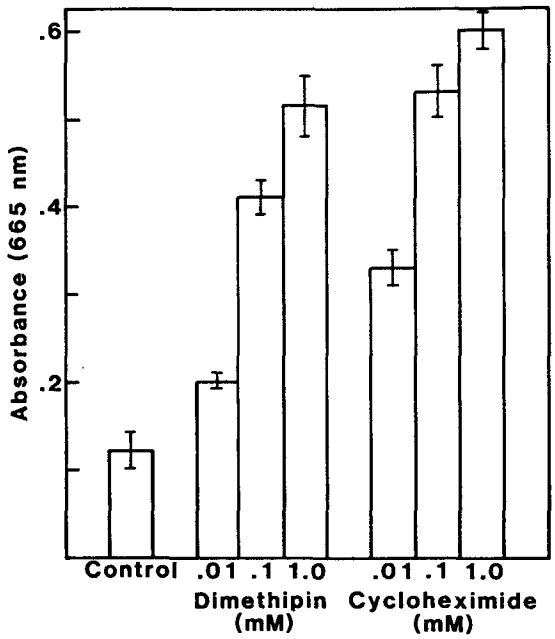


Fig. 9. A comparison of the effects of various concentrations of dimethipin and cycloheximide on chlorophyll degradation in oat leaf segments in the dark. Chlorophyll levels estimated by determining the absorbance at 665 nm of an 80% ethanol extract from 10 oat leaf segments. Each value represents the mean of three replicates. Vertical bars refer to standard deviations.

leucine into protein (Fig. 5). Dimethipin also appeared to act on the processes associated with translation rather than transcription (Fig. 8). The use of cell-free translation systems would help pinpoint the biochemical site of action. The inhibition of protein synthesis was observed 1 h after the start of the incubation with dimethipin (Fig. 5) and before any measurable effect on respiration (Fig. 6) or membrane integrity (Fig. 7). These results suggest that the inhibition of protein synthesis is a very early or perhaps even the primary biochemical event following treatment with dimethipin.

An inhibition of protein synthesis by dimethipin could explain why the direct application of dimethipin to the abscission zone of kidney bean petiole explants actually inhibited abscission (Reid 1976). Such an inhibition of protein synthesis would also be expected to inhibit the *de novo* synthesis of enzymes, such as cellulase, that are required for abscission (Addicott 1982).

Consistent with the hypothesis that an inhibition of protein synthesis is an essential feature in the mechanism of action of dimethipin are the observations that dimethipin and cycloheximide evoked similar biological and physiological responses. Both compounds were able to increase transpiration from leaves in the dark (Table 2) and both inhibited the loss of chlorophyll from oat leaves in the dark (Fig. 9). We have also found that cycloheximide, like dimethipin, caused an apparent loss in guard cell sensitivity to both ABA and water stress (data not shown). In dose-response experiments, cycloheximide was, on a molar basis, about 10 times more effective than dimethipin in inhibiting the incorporation of ^{14}C-leucine into protein (data not shown). Cycloheximide was also about 10 times more effective than dimethipin in eliciting several biological responses (Fig. 9 and Table 2). The similarities between the relative effectiveness of both compounds in inhibiting protein synthesis on the one hand and

affecting biological processes on the other are indicative of similar mechanisms of action. Thus, although these results are not unequivocal, they do point strongly to the notion that the dimethipin-induced loss of turgor is due to an initial inhibition of protein synthesis.

There are several possible mechanisms by which an inhibition of protein synthesis could lead to a loss in stomatal control and leaf turgor. First of all, dimethipin could simply prevent stomatal closure and, in effect, arrest the stomates in the open state. Hypoxia and several metabolic inhibitors have been shown to inhibit closing of the stomates, suggesting that stomatal closure, like opening, requires metabolically active guard cells that are able to regulate internal turgor pressure (Weyers et al. 1982, Raschke 1975, and references cited therein). Therefore, it is reasonable to suggest that an inhibition of protein synthesis would also lead to a disruption of the turgor-regulating processes in the guard cells. Since dimethipin was applied to leaves under conditions conducive to stomatal opening, it is possible that dimethipin simply prevented closure when the leaves were subsequently subjected to conditions that normally cause stomatal closure.

On the other hand, cycloheximide has been observed to cause oat leaf stomates to open in the dark (Thimann and Satler 1979). An inhibition of protein synthesis caused by dimethipin could conceivably force the opening of the stomates. However, the promotion of stomatal opening following a foliar chemical treatment need not be explained totally by an effect on the guard cells themselves. It is known that the death of epidermal and/or subsidiary cells can indirectly cause partial stomatal opening by releasing the pressure that is exerted on the guard cells (Glinka 1971, Squire and Mansfield 1972). Since the dew point hygrometer measures the tissue-averaged Ψ, the small reduction of leaf Ψ in dimethipin-treated leaves maintained in 100% Rh (Table 1) could be a reflection of the disruption of mainly the epidermal cells, while the majority of the other leaf cells remained intact. This might be expected if the movement of the dimethipin from the epidermis to the mesophyll cells was restricted and/or if it was rapidly metabolized to inert products before accumulating to concentrations near the mesophyll cells that would be toxic. Thus, it is possible that both dimethipin and cycloheximide cause stomatal opening by the disruption of the surrounding epidermal cells with the attendant release of pressure on the guard cells.

A final possibility is that dimethipin increases cuticular transpiration by disrupting the cutical. This possibility would have been overlooked in the present work, as stomatal apertures were inferred from porometer measurements and were not measured directly. An increase in cuticular transpiration could result directly from the destruction of the epidermal cells without a noticeable effect on the mesophyll cells as suggested before. At present we are actively engaged in determining which of these possibilities is correct.

An important question that remains to be answered is whether or not the dimethipin-induced loss of turgor is an important link in the chain of events leading to abscission. This appears likely since abscission in dimethipin-treated bean plants could be prevented if the plants were maintained in an atmosphere of 100% Rh, although the dimethipin-treated leaves showed signs of senescence, i.e., yellowing (J. D. Metzger, unpublished results). Furthermore, it is

well known that water stress leads to leaf abscission in several species (Addicott 1982).

Currently, it is not well understood how water stress leads to abscission, but stress-induced increases in the endogenous levels of either ethylene, ABA, or both are thought to be contributory factors (Addicott 1982). Preliminary work in this laboratory showed, however, that dimethipin inhibits the normal water-stress-induced increases in the endogenous levels of ABA in kidney beans, cotton, and sunflowers (J. D. Metzger and E. R. Mansager, unpublished results).

A role for ethylene in dimethipin-induced abscission is more plausible. Water stress causes a surge in ethylene biosynthesis in a number of plant tissues (Apelbaum and Yang 1981, and references cited therein). Elevated rates of ethylene production are also often associated with application of many herbicides and abscission-inducing agents (Morgan 1976). Thus, it would be reasonable to expect that ethylene serves as a link between dimethipin-induced loss of turgor and abscission; this possibility remains to be tested.

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