J Plant Growth Regul (1987) 6:33–40

Journal of
Plant
Growth
Regulation

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Cellular Basis for Dimethipin-Induced Loss of Leaf Turgor and Desiccation

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Received September 5, 1986; accepted January 9, 1987

Abstract. Dimethipin-induced increase in transpiration from kidney bean leaves (*Phaseolus vulgaris* L. cv. Black Valentine) was not correlated with stomatal aperture. From analysis of the kinetics of water loss from excised kidney bean leaves, it was concluded that the increase in transpiration was due almost entirely to an increase in the cuticular component. Both light and scanning electron microscopic analysis of dimethipin-treated leaves indicated that the first cells to be affected by dimethipin were the epidermal cells. These results suggest that dimethipin causes a loss of leaf turgor and desiccation by disrupting epidermal cells, thereby removing a major barrier for water loss from the leaf.

Dimethipin (2,3-dihydro-5,6 dimethyl-1,4-dithiin 1,1,4,4-tetraoxide) is a plant growth regulator that is finding increasing use as a harvest aid in a number of crops (Bell et al. 1975, Murphy 1976, Blem et al. 1983). Previous work by us showed that the first visible macroscopic effect of a foliar spray of dimethipin was a loss of leaf turgor followed by desiccation and abscission (Metzger and Keng 1984). Furthermore, the loss in leaf turgor was due to increased transpiration, and we attributed this to a loss in stomatal control (Metzger and Keng 1984). However, since stomatal apertures were not directly measured in our earlier work, the possibility that the dimethipin-induced increase in transpiration was due to an increase in cuticular transpiration by disrupting the cuticle and/or epidermal cells could not be excluded. In this communication, we ex-

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amined the cellular basis for the dimethipin-induced increase in water loss. The results indicate that a general disruption of the epidermal layer is responsible for water loss rather than a specific effect on guard cell activities.

Materials and Methods

Plant Material

Kidney beans (*Phaseolus vulgaris* L. cv. Black Valentine) were grown in the greenhouse at ~25°C. After 2 weeks the trifoliate leaves were removed, and both sides of each of the primary leaves were sprayed with a solution (10 ml total per leaf) containing various concentrations of dimethipin, 1.0% (v/v) acetone, and 0.01% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20, Sigma Chemical Co., St. Louis, MO). Leaves of control plants received a similar treatment except that no test compound was included. Once the leaves dried, the plants were then placed in a controlled environmental chamber at 25°C and ambient relative humidity with a 16-h photoperiod (150 μE m^{-2} s^{-1}, PAR) unless otherwise stated.

Measurement of Stomatal Apertures

Stomatal apertures were determined by the method described by Waisel et al. (1969). Basically, a thin coat of a commercially available butyl acetate cement (Duco; DuPont Chemical Co., Wilmington, DE) was applied to the abaxial side of primary bean leaves. After drying (about 30 s), the butyl acetate layer was carefully peeled away from the epidermis and mounted on a glass slide, and the widths of the stomatal openings were determined with the aid of a microscope equipped with an eyepiece micrometer. The widths of 25 randomly selected stomata imprints were measured from each leaf.

Light Microscopy

Leaf disks (1 cm diameter) were excised and fixed in formalin:acetic acid:ethanol (3:1:1, v:v:v), dehydrated with a graded series of aqueous *tert*-butanol solutions, and embedded in paraffin (Tissue-Prep, Fisher Scientific Co., Pittsburgh, PA). Cross sections (10 μm thick) were mounted on a microscope slide and stained with safranin and fast green (Johansen 1940). The sections were viewed and photographed at × 138. In each treatment, at least 3 sections from 3 separate leaves (9 sections total) were examined and gave qualitatively similar results.

Scanning Electron Microscopy

Leaf squares (3 × 3 mm) were fixed in 2.5% (v/v) glutaraldehyde in 50 mM

Table 1. Comparison of stomatal apertures and rates of transpiration in dimethipin-treated kidney bean leaves.

Treatment	Stomatal aperture[a]	Transpiration[b]
Dark control	0.22 ± 0.08	0.09 ± 0.03
Dark + 0.1 mM dimethipin	0.19 ± 0.05	0.62 ± 0.09
Dark + 1.0 mM dimethipin	0.29 ± 0.10	1.46 ± 0.16
Light control	7.21 ± 1.4	3.74 ± 0.24

Primary leaves were sprayed with 10 ml of a solution containing 1% (v/v) acetone, 0.1% (v/v) Tween 20, and various concentrations of dimethipin. Measurements were taken after 24 h in a darkened or lighted growth chamber.
[a] μm ± SD.
[b] mmol m^{-2} s^{-1} ± SD.

KH_2PO_4 (pH 7.0) for 2 h at room temperature followed by three 20-min washes with 200 mM KH_2PO_4 (pH 7.0). The tissues were then dehydrated in a graded series of aqueous ethanol solutions and critical-point-dried with CO_2 (Hayat 1981). The tissue specimens were then sputter-coated with Au-Pd for 21.5 min using a Hummer V instrument (Anatech LTD, Alexandria, VA) set at 10 mA and 9.5 V. This resulted in a 125-Å thick coating of Au-Pd. Observations of the abaxial leaf surfaces were made with an Amray AMR 1000 scanning electron microscope at 20 kV. Reproducibility within treatments was assessed by examining 3 squares from 3 separate leaves.

Transpiration Measurements

Instantaneous rates of transpiration were determined with a steady-state porometer as described before (Metzger and Keng 1984).

Results and Discussion

Relationship between Stomatal Aperture and Transpiration

The efffects of dimethipin on stomatal aperture and transpiration rates were compared. Primary bean leaves were sprayed with 0, 0.1, or 1.0 mM dimethipin and placed in the dark. After 24 h, transpiration rates were measured, and a leaf impression of the abaxial surface was taken from each leaf. As expected, plants maintained in the light had higher rates of transpiration that were correlated with increased stomatal apertures than the dark controls (Table 1). In contrast, there was no significant difference in stomatal aperture in dimethipin-treated and control plants maintained in the dark despite a dimethipin-induced increase in transpiration rates (Table 1).

The lack of correlation between the dimethipin-induced increase in transpiration and size of stomatal aperture can be rationalized if it is assumed that dimethipin treatment results in damage to the cuticle and/or epidermis, thereby leading to an increase in cuticular transpiration. Support for this possibility

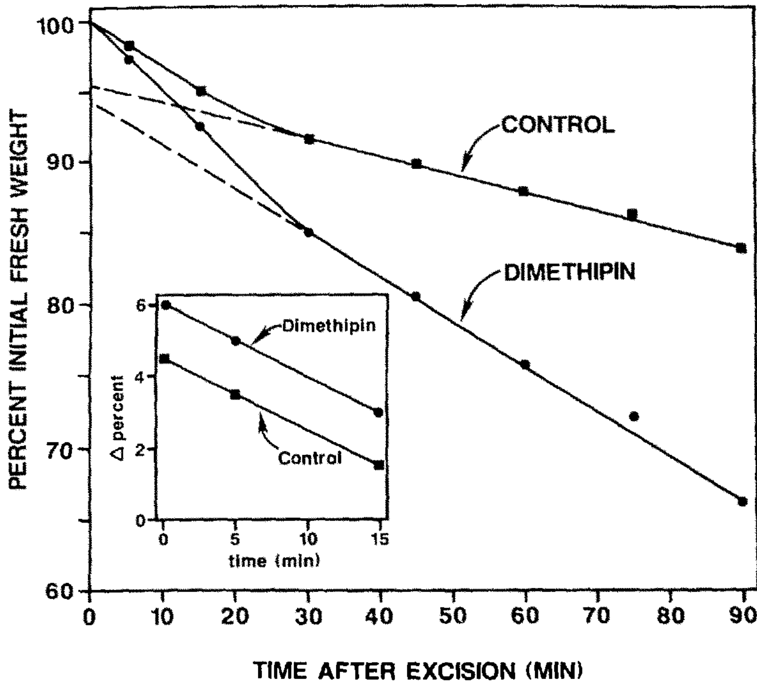

Fig. 1. Kinetics of water loss from excised kidney bean leaves treated with 1 mM dimethipin. Six primary leaves from three plants were used. The values represent the percent of initial fresh weight of six leaves. Inset shows the lines derived by subtracting the extrapolated line of the second linear phase (dashed line) from the line of the first linear phase. More details described in the text.

was obtained by examining the effect of dimethipin on the rate of water loss from excised leaves. Primary bean leaves were sprayed with a solution containing 0 or 1.0 mM dimethipin and then placed in a dark growth chamber at 25°C and 100% relative humidity (Metzger and Keng 1984). The leaves were excised 24 h later, weighed, and placed on a laboratory bench at 21°C and normal laboratory lighting with the abaxial side facing up. The amount of water loss was determined every 15 min. In agreement with our previous results (Metzger and Keng 1984), dimethipin greatly accelerated water loss from excised leaves (Fig. 1). From this kind of data, it is possible to determine the approximate relative contributions of cuticular and stomatal transpiration to overall water loss from the leaves (Waisel et al. 1969). The two curves in Fig. 1 have in common three characteristic phases: an initial linear phase representing the sum of water loss from both the stomatal and cuticular components; a nonlinear phase (15–30 min) in which the rate of water loss has decreased probably because of stomatal closure; and, finally, a second linear phase most likely due to cuticular transpiration (Waisel et al. 1969). Therefore, the slope of the second linear phase is an estimate of the rate of water loss due to cuticular transpiration. As can be seen from Fig. 1, the rate of water loss from the dimethipin-treated leaves during the second linear phase (−0.32%

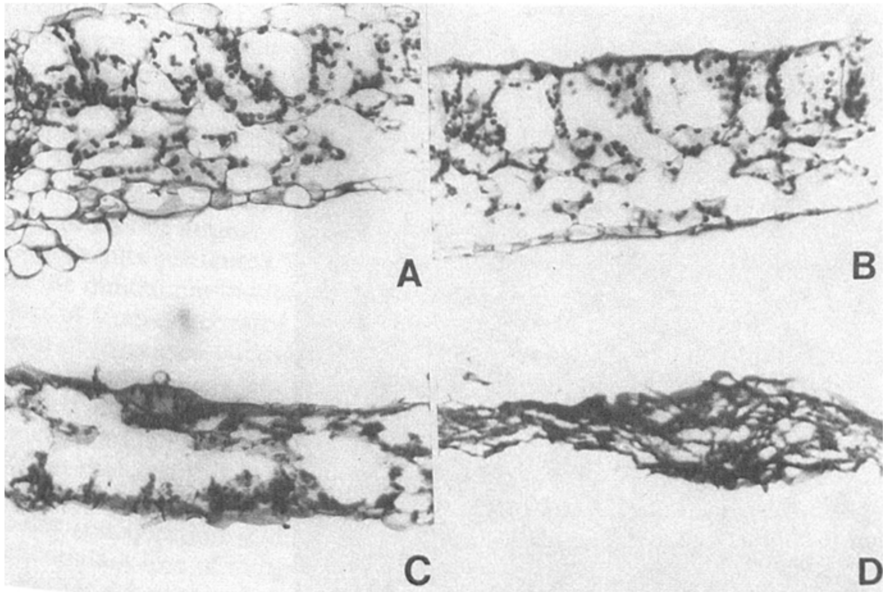

Fig. 2. Light micrographs of cross sections of primary kidney bean leaves at various times after spraying with 1.0 mM dimethipin. (A) control; (B) 24 h; (C) 48 h; (D) 72 h. Original magnification ×138.

min^{-1}) was nearly 2.5 times greater than the rate of water loss from control leaves (−0.13% min^{-1}) during the comparable period.

It is also possible to determine graphically from these data the relative contribution of stomatal transpiration to total water loss from the leaves. Such calculations involve first extrapolating the line representing the second linear phase back to the ordinate (Fig. 1, dashed lines) and then subtracting that line from the line representing the first linear phase (0–15 min). The resulting values form a straight line, the slope of which is an estimate of stomatal transpiration. When this was done for the data obtained in Fig. 1, two parallel lines were obtained with a slope of −0.2% min^{-1} (Fig. 1, inset). Thus, the relative contribution of stomatal transpiration to total leaf water loss was the same in both the dimethipin-treated and the control leaves.

Anatomical Effects of Dimethipin

The effects of dimethipin on leaf anatomy were examined to gain some insights on the mechanism(s) by which dimethipin induces changes in cuticular transpiration. Primary bean leaves were sprayed with 1.0 mM dimethipin and placed in a growth chamber. After various periods of time, leaf sections were excised, and cross sections were examined by light microscopy (Fig. 2). Cross sections of control tissue showed typical anatomical features of healthy leaves:

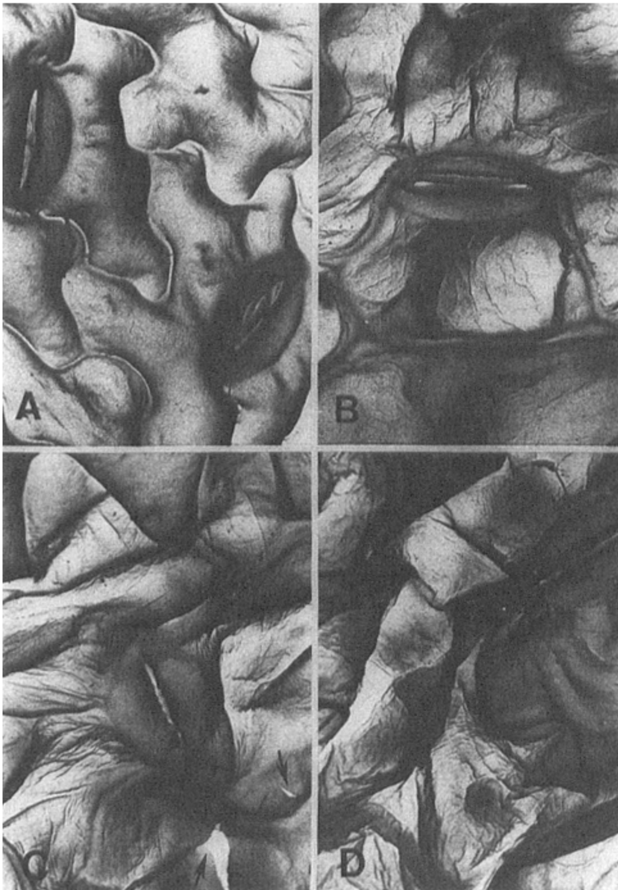

Fig. 3. Scanning electron micrographs of the abaxial surface of primary bean leaves at various times following treatment with 1.0 mM dimethipin. (A) control; (B) 24 h; (C) 48 h; (D) 72 h. Arrows in C point to fissures in epidermis. Original magnification × 1000.

turgid epidermal cells and distinct palisade and spongy mesophyll tissues containing many chloroplasts (Fig. 2A). Subsequent examination of the treated tissue showed that visible cellular damage appeared to progress from the epidermis to the mesophyll cells. After 24 h, the epidermal cells were either collapsed or shrunken, whereas the mesophyll cells, for the most part, appeared healthy (Fig. 2B). However, damage to even these cells was apparent 48 and 72 h following dimethipin treatment (Fig. 2C and D, respectively). Also obvious was the progressive shrinkage of the tissue due to loss of water.

These observations indicate that the initial site of dimethipin-induced cellular damage was the epidermal layer and suggest that dimethipin causes an increase in cuticular transpiration by disrupting the epidermal layer. Further

evidence for this was obtained by examining the effects of dimethipin on the leaf surface by scanning electron microscopy. Figure 3 shows the effects of dimethipin on the leaf surface. Epidermal cells and stomata were normal in appearance in control tissue (Fig. 3A). However, on dimethipin-treated leaves, the epidermal cells became progressively more wrinkled with increasing time (Fig. 3B, C, and D). In some instances, fissures in the epidermal layer were observed (e.g., Fig. 3C). This cellular wrinkling was probably due to dehydration and loss of turgor.

The results presented in this paper do not support our original hypothesis that the dimethipin-induced reduction in leaf turgor and desiccation are due to a loss of stomatal control (Metzger and Keng 1984) but rather that they are the result of increased cuticular transpiration. First, dimethipin-induced increases in transpiration were not correlated with stomatal aperture (Table 1). Second, detailed analysis of the kinetics of water loss from excised leaves showed that dimethipin increased the cuticular component of transpiration but had no effect on the stomatal component (Fig. 1). Microscopic examination of dimethipin-treated leaf tissue suggested that the cellular basis for the increase in cuticular transpiration is due to the disruption of the epidermal cells with the concomitant loss of integrity of the barrier to water loss from the leaf (Fig. 3).

Earlier we reported that the effects of dimethipin on leaf turgor and water potential could be substantially reduced if the treated plants were maintained at 100% relative humidity, indicating that the phytotoxic effects of dimethipin were not simply due to a generalized disruption of cellular integrity (Metzger and Keng 1984). However, a small but consistent reduction in leaf water potential was observed in dimethipin-treated leaves, indicating that at least a small subpopulation of cells was experiencing deleterious conditions (Metzger and Keng 1984). This could be interpreted as an indication that the direct cellular effects of dimethipin are manifested only in the cells that are in direct contact with the chemical, viz. the epidermal cells; the gross macroscopic effects—i.e., loss of turgor and desiccation—are the indirect result of disruption of the epidermis. Consistent with this interpretation is the fact that the epidermal cells are the first to show significant toxic effects of dimethipin (Figs. 2 and 3) and that dimethipin has limited mobility when applied to leaves (A. R. Blem, Uniroyal Chemical Co., Bethany, CT; personal communication).

In a previous report, we noted that the earliest biochemical effect of dimethipin that we could observe was an inhibition of protein synthesis (Metzger and Keng 1984). Thus, our present working hypothesis on the mechanism of action of dimethipin is that it blocks protein synthesis in the epidermal cells, leading to their death and consequently disruption of the epidermal layer. This in turn results in the loss of a major barrier for the evaporation of water from the leaf, resulting in increased cuticular transpiration. The leaf, unable to control water loss, loses turgor, desiccates, and ultimately dies.

Acknowledgments. We are deeply indebted to Uniroyal Chemical Company, Bethany, CT, for the generous financial support of this project. We also thank Dr. Sandy Newman for his help with the scanning electron microscopy.

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