The Effect of Tentoxin on Stomatal Aperture and Potassium Content of Guard Cells

R. D. Durbin, T. F. Uchytil, and L. Sparapano

Research Leader and Professor, Research Assistant, and C.N.R. Fellow (Italy), respectively, U.S. Department of Agriculture and Department of Plant Pathology, University of Wisconsin, Madison 53706.

Research supported in part by N.I.H. Grant No. GM-19311-01, the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and the Agricultural Research Service, U.S. Department of Agriculture.

Mention of a trademark does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture.

ABSTRACT

Tentoxin induced closure of stomata on broad bean leaflets and epidermal strips kept in light. The extent of closure in leaflets was directly correlated with tentoxin concentrations between 100-10 μM. The toxin also retarded stomatal opening in the dark when CO_2 concentration was reduced. The uptake of potassium by guard cells was inhibited in the presence of tentoxin.

Phytopathology 63:1077-1078.

Additional key words: Alternaria tenuis, Vicia faba.

Tentoxin, a cyclic tetrapeptide produced by the fungus *Alternaria tenuis* Nees, induces chlorosis in the cotyledons of germinating seedlings of many dicotylendonous species (5, 7). Ultrastructural studies have shown that it affects development of the prolammellar bodies and lamellae in the chloroplast, but that it has no observable effect on mitochondrial or nuclear integrity, or the conversion of protochlorophyll to chlorophyll. From these and other data, it was concluded that tentoxin acts by disrupting plastid development (4).

Recently, we found that tentoxin induced a reduction in the transpiration rate of bean, mung bean, and cucumber seedlings within 1 hr after the stems were immersed in an aqueous solution of 200 μM tentoxin (1). This, and the further finding that tentoxin treatment suppressed the opening of stomata in CO_2-free air in the dark, suggested that at least part of its mode of action in plants involves a relatively immediate effect related to guard cell turgor. Accordingly, we investigated in greater detail the relationship between tentoxin and stomatal aperture.

Tentoxin was prepared separately from 8-liter lots of culture filtrate and mycelium using the extraction method of Saad et al. (6) except that before extraction the pH was adjusted to 3.0 with 1.0 N HCl and chloroform was substituted for diethyl ether. After partitioning, the organic phase was evaporated to dryness under reduced pressure in the presence of aluminum oxide, neutral (activity 1). The residue was placed on the top of a column (2.5 X 12 cm) of the same absorbant, equilibrated with ethyl acetate:benzene (9:1), and the column developed using this solvent system. Fractions which eluted between 75-975 ml were pooled and evaporated to dryness under reduced pressure. The residue was dissolved in chloroform, applied to TLC plates (Merck Silplate 60 F-254, 0.25 mm), and developed with ethyl acetate:benzene (99:1, v/v). Spots containing 1 μg or more tentoxin (R_F 0.20) were detected under UV light. The tentoxin-containing regions were removed, chloroform added, and the silica gel removed by filtration. Tentoxin concentration was determined spectrophotometrically in chloroform using a Cary 15 (ϵ=12,050 at 284 nm).

A 3-hr treatment time and 100 μM tentoxin were used in all experiments unless otherwise stated. Before treatment, leaflets or epidermal strips from broad bean, *Vicia faba* L., were kept in light 8,060 lux (750 ft-c) for 3 hr if the experiments were to be done in the light. All plant material to be used for experiments in the dark was kept in the dark for 12 hr before treatment.

Stomatal aperture was determined by the silicone-rubber impression technique (8). The impressions were made on the abaxial side of both leaflets, whose petiole was immersed in the test solution. Fifty randomly chosen stomata from four leaflets were counted in each treatment. Light 8,060 lux (750 ft-c) was provided by an incandescent lamp; the air temperature was 25 C and 15% relative humidity.

The potassium content of the guard cells was determined by use of Macallum's cobaltinitrite staining test (3). Epidermal strips from the abaxial side of broad bean leaflets were floated on 10 mM KCl with or without tentoxin. Staining was done for 30 min.

As shown in Table 1, tentoxin induced stomatal closure on broad bean leaflets in the light, the extent of which was directly related to concentration. A solvent-processed blank medium had no effect on stomatal aperture. The significant effect of low tentoxin concentrations is in keeping with the previous data in which 4 μM tentoxin was found to reduce significantly the rate of transpirational water loss (1). Using a 4-day treatment time, Saad et al. (6)

TABLE 1. Effect of different tentoxin concentrations on stomatal aperture of broad bean leaflets

Tentoxin concentration (μM)	Stomatal aperture (μ)[a]
300	1.2 ± 1.8[b]
100	1.8 ± 2.9
50	3.0 ± 3.0
10	5.4 ± 4.4
1	11.2 ± 2.6
0	11.5 ± 2.3

[a]The data are the means of 50 stomatal measurements/treatment.

[b]± Standard deviation.

TABLE 2. Effect of 100 μM tentoxin (+T) on stomatal aperture of broad bean leaflets

Treatment	Stomatal aperture (μ)[a]
Light[b]	10.4 ± 2.9[c]
Light +T	2.5 ± 3.2
Dark	0.2 ± 0.7
Dark $-CO_2$[d]	16.1 ± 2.8
Dark $-CO_2$ +T	4.9 ± 2.7

[a]The data are the means of 50 stomatal measurements/treatment.
[b]Irradiance = 8,060 lux (750 ft-c).
[c]± Standard deviation.
[d]CO_2-free air ($-CO_2$) was passed over the leaflets in the dark at 30 liters/hr.

had found that chlorosis could be induced in germinating seedlings at a concentration as low as 0.5 μM. Besides inducing closure of stomata kept in the light, tentoxin also inhibited the opening of those kept in the dark when CO_2-free air was passed over the leaflets (Table 2). When epidermal strips were used to obviate the possibility that tentoxin caused stomata to close in the light because of a generalized water deficit, the results were similar to those previously obtained (control, 11.5 ± 2.0 μ; tentoxin-treated, 2.8 ± 3.3 μ). The larger variation in stomatal aperture noted with the tentoxin treatments was not randomly distributed; rather we found scattered clusters of 5-10 stomata were opened wider than normal. This, perhaps, reflected a nonuniformity in tentoxin distribution within the leaflet.

Recently, attention has been directed towards the possibility that active transport of potassium into the guard cells against a concentration gradient may be responsible for subsequent stomatal opening (2). We found by using epidermal strips of broad bean, that potassium accumulated in the guard cells when the stomata were open; i.e., in light and also in darkness when CO_2 concentration was reduced (Fig. 1-A,D). However, tentoxin treatment either abolished potassium accumulation (Fig. 1-B) or severely inhibited it (Fig. 1-E).

These data indicate that tentoxin's effect on transpiration is mediated by means of a reduction in stomatal aperture, and that correlated with closure is an abolishment of potassium accumulation by the guard cells. This supports the idea that in vivo tentoxin may act by inhibiting, in some manner, potassium accumulation. Hence, it would be expected that tentoxin might affect energy-generating reactions.

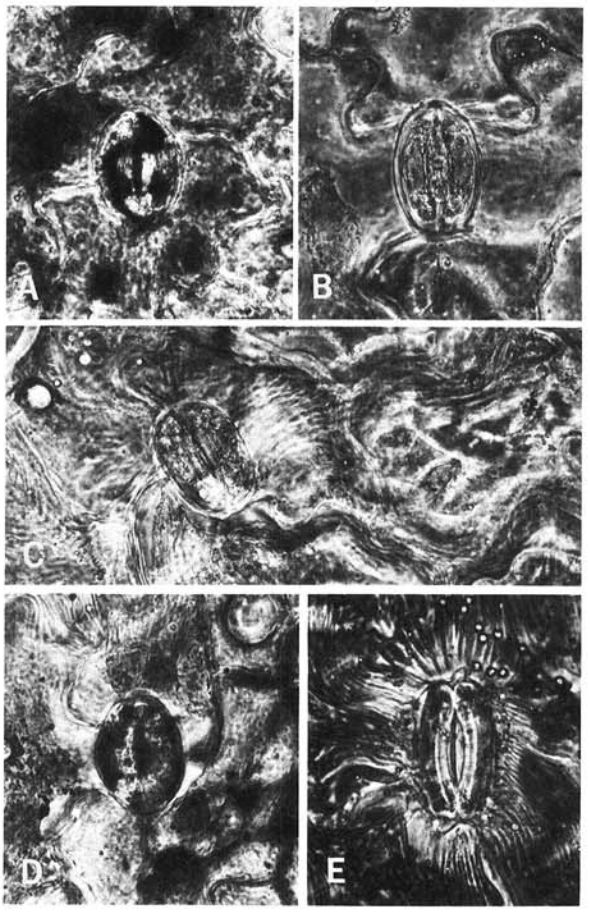

Fig. 1. Effect of 100 μM tentoxin on the potassium content of broad bean guard cells under different environmental conditions. Epidermal strips were floated on 10 mM KCl. After treatment they were stained by use of Macallum's method to visualize potassium. Stomata are closed due to the staining technique. **A)** light 8,060 lux (750 ft-c); **B)** light + tentoxin; **C)** dark; **D)** dark $-CO_2$; and **E)** dark $-CO_2$ + tentoxin.

LITERATURE CITED

1. DURBIN, R. D., & T. F. UCHYTIL. 1972. The effect of tentoxin on transpiration. Phytopathology 62:755 (Abstr.).
2. FISCHER, R. A. 1971. Role of potassium in stomatal opening in the leaf of Vicia faba. Plant Physiol. 47:555-558.
3. GOMORI, G. 1952. Microscopic histochemistry, principles and practice. University of Chicago Press, p. 30.
4. HALLOIN, J. M., G. A. DE ZOETEN, G. GAARD, & J. C. WALKER. 1970. The effects of tentoxin on chlorophyll synthesis and plastid structure in cucumber and cabbage. Plant Physiol. 45:310-314.
5. MEYER, W. L., G. E. TEMPLETON, C. I. GRABLE, C. W. SIGEL, R. JONES, S. H. WOODHEAD, & C. SAUER. 1971. The structure of tentoxin. Tet. Letters 25:2357-2360.
6. SAAD, S. M., J. M. HALLOIN, & D. J. HAGEDORN. 1970. Production, purification and bioassay of tentoxin. Phytopathology 60:415-418.
7. TEMPLETON, G. E., C. I. GRABLE, N. D. FULTON, & K. BOLLENBACHER. 1967. Factors affecting the amount and pattern of chlorosis caused by a metabolite of Alternaria tenuis. Phytopathology 57:516-518.
8. ZELITCH, I. 1961. Biochemical control of stomatal opening in leaves. Proc. Natl. Acad. Sci. (U.S.) 47:1423-1433.