Effect of Abscisic Acid on Rishitin and Lubimin Accumulation and Resistance to *Phytophthora infestans* and *Cladosporium cucumerinum* in Potato Tuber Tissue Slices

J. W. D. M. Henfling, R. Bostock, and J. Kuć

Department of Plant Pathology, University of Kentucky, Lexington 40546.
Present address of senior author: The International Potato Center, Apartado Postal 5969, Lima, Peru.

ABSTRACT

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A number of terpenes similar to the potato sesquiterpenoid stress metabolites (SSM) were tested for their effect on the accumulation of rishitin and lubimin in potato tuber slices treated with an elicitor preparation from cultures of *Phytophthora infestans*. Abscisic acid (ABA) was found to be the only compound that consistently and strongly inhibited rishitin and lubimin accumulation. ABA also markedly inhibited the accumulation of rishitin and lubimin in slices inoculated with incompatible races of *P. infestans*. Incompatible races grew and sporulated on slices treated with ABA prior to inoculation, and the reaction was indistinguishable from that induced by compatible races. ABA did not affect the accumulation of rishitin and lubimin or fungal growth in slices inoculated with compatible races of the fungus, and it did not stimulate fungal growth in defined media. *Cladosporium cucumerinum*, a pathogen of cucumber but not of potato, grew well and sporulated abundantly on tuber slices treated with ABA prior to inoculation. ABA, however, increased the accumulation of rishitin and lubimin in slices inoculated with *C. cucumerinum*. The reduced accumulation of rishitin and lubimin in potato slices treated with ABA did not appear to be responsible for the conversion of incompatible to compatible interactions with *P. infestans*. The data cast some doubt on the role of the SSM as primary agents responsible for containing the fungus in incompatible interactions of potato and *P. infestans*.

Additional key words: phytoalexins, late blight, *Solanum tuberosum* L., plant hormones.

Accumulation of potato sesquiterpenoid stress metabolites (SSM) results from increased synthesis via the acetate-mevalonate pathway and/or decreased degradation (13,17–19,21–23, 28–30,32). The major fungitoxic SSM which accumulate in potato are rishitin and lubimin (3,6,9). Significant accumulation of the SSM occurs only under rather specific conditions of stress; eg, incompatible fungi and biotic elicitors. Hence, the accumulation of SSM appears to be under tighter metabolic control than is the accumulation of isoflavonoids and furanoterpenes in legumes and sweet potato, respectively (12).

In animals, the transformations leading from squalene to the various steroid hormones are under tight metabolic control. Induction of the synthesis of steroid hormones is under the influence of the pituitary hormones and end-products cause a feedback inhibition of synthesis (4).

Earlier investigations reported that the occurrence of hypersensitive death of potato leaf petiole cells penetrated by an incompatible race of *P. infestans* was delayed and hyphal elongation was increased by post-infectional treatment of the host tissues with either sodium azide or 2,4-dinitrophenol or pre-infectional sublethal heat treatment (24–27). The investigators did not consider the effect of these treatments on the accumulation of SSM or disease reaction of the tissues.

The purpose of this investigation was to determine the effect of exogenously applied ABA on the accumulation of SSM and disease reaction in potato tuber tissues infected with compatible or incompatible races of *P. infestans*, and *C. cucumerinum*, a nonpathogen of potato.

MATERIALS AND METHODS

General. The accumulation of SSM was determined by a semi-micro method (7,9). In all experiments, Kennebec (R1) tuber slices were used. Kennebec potatoes are incompatible with races 0 and 4 and compatible with race 1.2.3.4 of *Phytophthora infestans* (Mont.) de By. In most experiments, 0.1 ml of solution containing the test substance was applied to the upper surface of potato tuber slices (3 cm in diameter and 5 mm thick) within 1 hr after slicing. Slices were then aged for 8–10 hr at room temperature and elicitor or fungal inoculum was applied to the same surface as the test substance. The slices were then incubated at 19 C for 72–96 hr. The upper millimeter of the slices was harvested after the incubation period and the SSM were extracted and quantitated as described previously (7,9). All treatments were done in duplicate and all experiments were performed at least three times unless otherwise indicated.

Preparation of test substances. Unless stated otherwise, the test substances were dissolved in methanol and diluted with distilled water to a final concentration of 1 mg/ml. Control solutions included an appropriate concentration of methanol. All solutions were sterilized by passage through a 0.22 μm membrane filter (Millipore Corporation, Bedford, MA 01730). (±) *Cis-trans* abscisic acid was purchased from Sigma Chemical Co., St. Louis, MO 63178. Geranyl acetate, β-ionone, and 3,5,5,8,8-pentamethyl-2-decalone were purchased from Aldrich Chemical Co., Milwaukee, WI 53201. Farnesol was purchased from ICN Nutritional Biochemicals, Cleveland, OH 44128. Phytuberol was isolated from infected potato tuber slices in our laboratory (3,7).

Culture of organisms. *P. infestans* races 0, 4, and 1.2.3.4 were maintained on lima bean agar at 19 C, and *Cladosporium cucumerinum* Ell. & Arth. was maintained on green bean agar at 19 C. Spores were obtained from 10- to 14-day-old cultures of *P. infestans* and 7- to 10-day-old cultures of *C. cucumerinum*. Spore suspensions were prepared by adding sterile distilled water to the cultures and scraping the surface with a bent glass rod. The suspensions were filtered through a double layer of cheesecloth. Suspensions of sporangia from *P. infestans* were placed at 12 C to induce zoospore formation. Approximately 5×10^4 zoospores of *P. infestans* or 2×10^6 conidia of *C. cucumerinum* were applied per tuber slice.

Preparation of elicitor. *P. infestans* race 4 was grown in lima bean broth for 10–15 days. The mycelial mats were collected on a double layer of cheesecloth, thoroughly washed with several liters of tap water and then deionized water, and stored at −20 C. For the preparation of elicitor, the frozen material was homogenized in five volumes (w/v) of distilled water in a Waring blender. The homogenate was sonicated three times in a Model W185 Sonifier Cell Disruptor (Heat Systems-Ultrasonics, Inc., Plainview, NY 11803). The sonicate was autoclaved at 121 C for 15 min and cooled to room temperature. The resulting elicitor preparation either was used immediately or stored at −20 C.

Effect of ABA on growth of *P. infestans*. To test the effect of ABA on the growth of *P. infestans*, separate groups of flasks containing 25 ml of a chemically defined medium (11) in 0.2% methanol or a medium containing various concentrations of ABA were seeded with 2.5×10^5 zoospores per flask of race 4. The concentration of methanol did not exceed 0.2% in any of the flasks treated with ABA. These flasks were then incubated for 14 days at 19 C. The mycelial mats from two flasks were collected on a filter paper using suction, placed in a drying oven at 80 C for 3 hr and then in a dessicator containing P_2O_5 under vacuum overnight. The weight of the dried mycelial mats was determined by using an analytical balance (Mettler Instrument Co., Hightstown, NJ 08502).

RESULTS

Effect of terpenes on the elicitor-induced accumulation of rishitin and lubimin. Both phytuberol and 3,5,5,8,8-pentamethyl-2-decalone were relatively weak inhibitors of elicitor-induced terpene

TABLE 1. The effect of exogenously applied terpenes on the accumulation of rishitin and lubimin in Kennebec tuber slices subsequently treated with an elicitor preparation from *Phytophthora infestans* cultures

Treatment[a]	Terpenes (μg/g fr wt)[b]		
	Rishitin	Lubimin	Total
10% Methanol (control)	292 ± 18	1 ± 3	293 ± 20
Geranyl acetate	264 ± 38	7 ± 5	271 ± 37
β-Ionone	222 ± 22	3 ± 2	225 ± 23
Farnesol	266 ± 84	27 ± 8	293 ± 91
3,5,5,8,8-Pentamethyl-2-decalone	160 ± 41	2 ± 3	162 ± 40
Phytuberol	110 ± 8	1 ± 1	111 ± 8
Abscisic acid	5 ± 6	23 ± 5	28 ± 6

[a] Immediately after being cut, the upper surface of each slice was treated with 0.1 ml of 10% methanol containing 100 μg terpene or with 0.1 ml of 10% methanol. Eight to 10 hr later the elicitor was applied to the same surface.

[b] Values are the mean and standard error determined 72 hr after treatment with elicitor.

accumulation and the latter caused browning and water-soaking of the slice surface (Table 1). Slices treated with β-ionone or farnesol appeared to be slightly water soaked for 3–6 hr after application of the terpene. ABA was consistently the strongest inhibitor of rishitin and lubimin accumulation. The appearance of slices treated with ABA but not treated with elicitor was the same as that of slices treated with 0.1 ml of 10% methanol or water.

Effect of ABA on the accumulation of rishitin and lubimin and on disease resistance of Kennebec tuber tissue. ABA markedly reduced the accumulation of rishitin and lubimin in potato slices
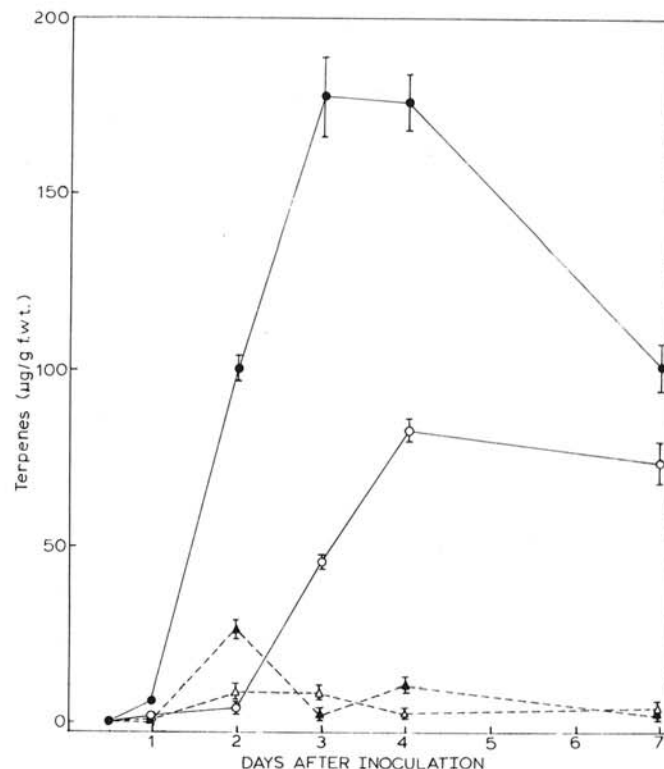

Fig. 1. The effects of abscisic acid on the accumulation of terpenes (rishitin plus lubimin) in Kennebec potato tuber slices as a function of time after inoculation with zoospores of *Phytophthora infestans*. Slices were treated with 0.1 ml of 5% methanol or 0.1 ml of 5% methanol containing 100 μg of abscisic acid and inoculated 10 hr later with 5×10^4 zoospores of race 0 (incompatible) or race 1.2.3.4 (compatible) per slice. Five percent (5%) methanol followed by race 0, ●——●; abscisic acid followed by race 0, ○——○; 5% methanol followed by race 1.2.3.4, ▲------▲; abscisic acid followed by race 1.2.3.4, △------△.

TABLE 2. The effect of abscisic acid (ABA) on rishitin and lubimin accumulation in Kennebec tuber slices subsequently inoculated with zoospores of *Phytophthora infestans*, with conidia of *Cladosporium cucumerinum*, or treated with an elicitor preparation from *P. infestans* cultures

Treatment[a]		Reaction[b]	Terpenes (μg/g fr wt)[c]	
			Rishitin	Lubimin
5% Methanol——————H₂O		0	1 ± 1	0 ± 0
5% Methanol——————Elicitor		HR	283 ± 14	23 ± 1
ABA——————————Elicitor		0	3 ± 0	8 ± 1
5% Methanol——————Race 4, *P. infestans*		HR	350 ± 23	108 ± 4
ABA——————————Race 4		C	96 ± 9	14 ± 1
5% Methanol——————Race 1.2.3.4, *P. infestans*		C	3 ± 2	4 ± 0
ABA——————————Race 1.2.3.4		C	9 ± 6	0 ± 0
5% Methanol——————Race 0, *P. infestans*		HR	278 ± 14	210 ± 5
ABA——————————Race 0		C	92 ± 6	16 ± 2
5% Methanol——————*C. cucumerinum*		0	0 ± 0	21 ± 1
ABA——————————*C. cucumerinum*		C	39 ± 2	34 ± 2

[a] Within 1 hr of being cut, the upper surface of each slice was treated with 0.1 ml 5% methanol containing 100 μg abscisic acid or with 0.1 ml 5% methanol. Eight hours later, elicitor, zoospores of *P. infestans* (5×10^4 spores per slice) or conidia of *C. cucumerinum* (2×10^6 spores per slice) were applied to the same surface.

[b] Slices were evaluated visually 72 hr after inoculation: 0 = no visible change in the appearance of tissue, appears as control; HR = hypersensitive reaction, browned tissue without apparent fungal growth; C = compatible reaction, abundant fungal growth and sporulation.

[c] Values are the mean and standard error determined 72 hr after inoculation or treatment with elicitor.

inoculated with incompatible race 0 or 4 of *P. infestans* (Table 2, Fig. 1). The reduction was evident throughout the period of a time study, 24–168 hr after inoculation with the fungus (Fig. 1). The appearance of tuber slices treated with ABA and subsequently inoculated with an incompatible race of *P. infestans* was indistinguishable from those inoculated with a compatible race
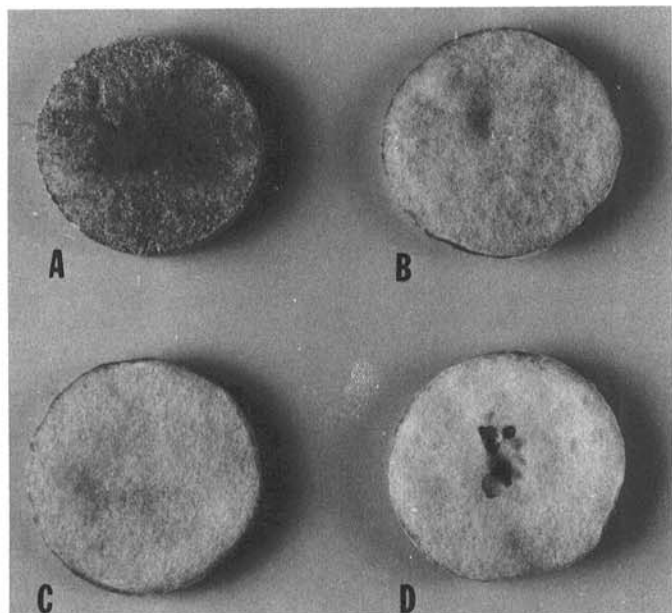

Fig. 2. The effect of abscisic acid on the resistance of Kennebec potato tuber slices to two races of *Phytophthora infestans*. **A,** One-tenth milliliter of 5% methanol per slice followed by inoculation with race 0 (incompatible). **B,** One-tenth milliliter of 5% methanol per slice followed by inoculation with race 1.2.3.4 (compatible). **C,** One-tenth milliliter of 5% methanol containing 100 μg abscisic acid per slice followed by inoculation with race 0. **D,** One-tenth milliliter of 5% methanol containing 100 μg abscisic acid per slice followed by inoculation with race 1.2.3.4.
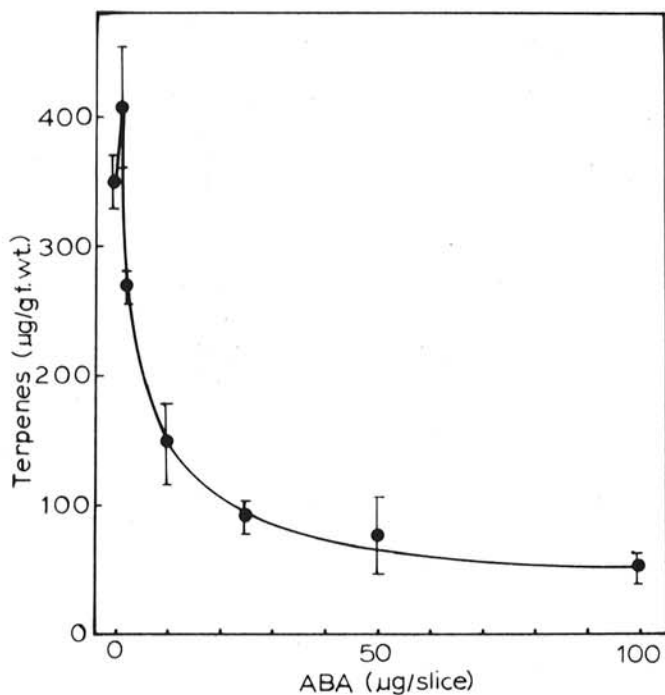

Fig. 3. Dosage-response curve of terpene (rishitin plus lubimin) accumulation vs the concentration of abscisic acid. One-tenth milliliter of 5% methanol or 0.1 ml of 5% methanol containing the appropriate concentration of abscisic acid was applied to the upper surface of Kennebec tuber slices, and 8 hr later slices were treated with elicitor extracted from cultures of *Phytophthora infestans*.

(Table 2, Fig. 2). Within 72–96 hr, abundant mycelial growth and sporulation were apparent on slices treated with ABA followed by inoculation with an incompatible race. ABA had little or no effect on the interaction between tuber slices and the compatible race 1.2.3.4. If anything, ABA may have enhanced mycelial growth.

The fungus growing on tuber slices treated with ABA and subsequently inoculated with race 4 was reisolated and used to infect Russet Burbank and Kennebec tubers. The fungus caused the typical symptoms of a compatible reaction in Russet Burbank, but was incompatible with Kennebec tubers.

The concentration of ABA at which 50% reduction of rishitin and lubimin accumulation occurred was generally about 15 μg of ABA per slice (Fig. 3). Maximum inhibition of rishitin and lubimin accumulation occurred at approximately 25–50 μg of ABA per slice. The change in reaction type from incompatible to compatible was evident at 25 μg ABA per slice. At the lowest concentration of ABA tested (1 μg ABA per slice), there often appeared to be a small increase in rishitin and lubimin accumulation. The dose-response pattern for elicitor-induced terpene accumulation was similar to that following inoculation with race 0 or race 4 of the fungus.

Since ABA changed the reaction of potato tissue from incompatible to compatible after inoculation with an incompatible race, experiments were conducted to determine how deeply in the slices this change occurred. Tissue cylinders (3 cm × 6 cm) were washed in sterile water, placed upright in a 25-ml beaker and the beaker was placed in a covered 600-ml beaker that was used as a moist chamber. The upper surfaces of the cylinders were treated with 0.1 ml of 5% methanol or 0.1 ml of 5% methanol containing 100 μg of ABA and 8 hr later were inoculated with race 4 of *P. infestans* (5 × 10⁴ zoospores per cylinder). After 96 hr, successive upper 1-mm sections were removed until two consecutive sections were free of visible browning. The sections were then individually extracted.

Mycelial growth was abundant on cylinders treated with ABA, whereas those treated with 5% methanol showed the typical hypersensitive reaction. Browning extended much deeper in infected cylinders treated with ABA than in infected cylinders treated with 5% methanol, and the transition from browned tissue to healthy-appearing tissue was more gradual in the former. Cylinders treated with ABA and not inoculated appeared the same as cylinders treated with water or 5% methanol.

The concentration of rishitin and lubimin declined rapidly as a function of tissue depth in cylinders treated with 5% methanol (Fig. 4). The concentration of rishitin and lubimin was much reduced in
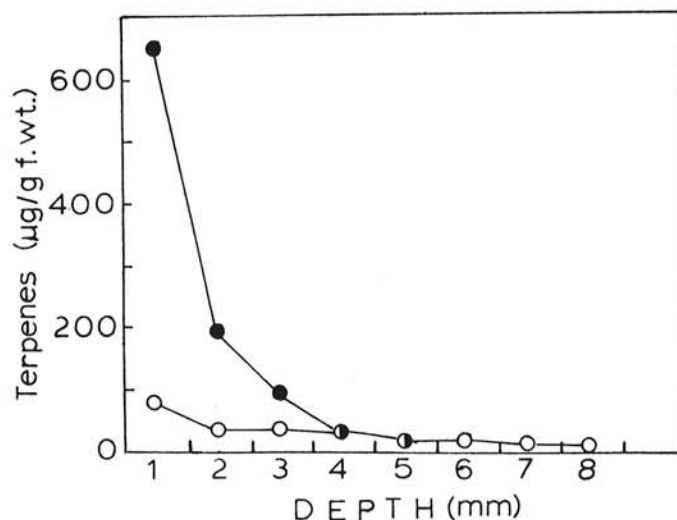

Fig. 4. The effect of abscisic acid on the accumulation of terpenes (rishitin plus lubimin) in Kennebec tuber cylinders inoculated with *Phytophthora infestans*. The upper surface of each cylinder was treated with 0.1 ml of 5% methanol (●——●) or 0.1 ml of 5% methanol containing 100 μg abscisic acid (o——o) within 1 hr after cutting. Eight hr later the upper surfaces were inoculated with zoospores of *P. infestans* race 4 (5 × 10⁴ zoospores per surface) and 96 hr after inoculation terpenes were determined in consecutive 1-mm layers of tissue.

cylinders treated with ABA.

Growth of *C. cucumerinum* and browning were not apparent on the surface of slices treated with 5% methanol and then inoculated with the fungus (Table 2). The fungus grew well and sporulated abundantly on slices treated with 100 µg of ABA prior to inoculation. Treatment with ABA, however, increased the accumulation of rishitin and lubimin in tissues inoculated with the fungus.

Effect of ABA on the growth of *P. infestans*. ABA at all concentrations tested was slightly inhibitory to the growth of *P. infestans* in a chemically defined medium (Table 3).

DISCUSSION

Of the substances tested, ABA was the only one consistently and strongly inhibitory to elicitor-induced accumulation of rishitin and lubimin in potato slices (Tables 1, 2, and Fig. 3). ABA also inhibited the accumulation of rishitin and lubimin in slices inoculated with incompatible races of *P. infestans* (Table 2 and Fig. 1). Slices treated with ABA became compatible to incompatible races of *P. infestans* and supported good growth and abundant sporulation of *C. cucumerinum* (Table 2 and Fig. 2). The effect of ABA was not due to any observable phytotoxicity. When slices were treated with ABA and inoculated with a compatible race of *P. infestans*, terpene accumulation remained low and the appearance of the interaction did not change (Table 2 and Figs. 1 and 2).

ABA induces ethylene production in some plant tissues (1,14) and ethylene enhances the accumulation of phytuberin and phytuberol in potato slices treated with an elictor preparation from *P. infestans* or inoculated with an incompatible race of the fungus (2,10). Indeed, the accumulation of phytuberin and phytuberol often was increased in slices treated with ABA and inoculated with incompatible races of *P. infestans* (7). Ethylene does not, however, reduce rishitin and lubimin accumulation. It is doubtful, therefore, that the ABA effect is mediated by ethylene alone.

Although it is tempting to speculate that the reduced accumulation of rishitin and lubimin in slices treated with ABA is responsible for the growth and sporulation of incompatible races of *P. infestans*, the data with tissue inoculated with *C. cucumerinum* make this unlikely. The enhanced growth and sporulation of *C. cucumerinum* on ABA-treated slices may result in an increased stress on the potato tissues resulting in the accumulation of rishitin and lubimin. This would imply that a mechanism, other than rishitin and lubimin accumulation, is responsible for restricting growth of *C. cucumerinum* on potato slices not treated with ABA. Some doubt also must be cast on the primary role of SSM as agents responsible for restricting the development of *P. infestans* in incompatible reactions. This doubt is supported by recent observations by Henfling (7) and Bostock (*unpublished*) that tubers growing in the field and freshly harvested tubers accummulated little or no SSM when inoculated with incompatible races of *P. infestans*, and at times accumulation was greater in tissues inoculated with compatible races. Though the tubers accumulated low levels of SSM, the fungus did not grow or sporulate on slices inoculated with incompatible races but it did on slices inoculated with compatible races.

TABLE 3. The effect of abscisic acid (ABA) on the growth of *Phytophthora infestans* race 4

Treatment[a]	Weight(mg)[b]
0.2% methanol (control)	155 ± 3 A
10 µg ABA/ml	136 ± 4 B
50 µg ABA/ml	141 ± 3 B
200 µg ABA/ml	122 ± 1 C

[a] ABA was added to the flasks to the concentration listed. The control flasks did not contain ABA. All test solutions contained 0.2% methanol.

[b] Value listed is the weight of the dried mycelial mat from 50 ml of media. The values are the means ± the standard error of five determinations. Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

The role of ABA in diseased plants seems to be indirectly associated with symptoms caused by certain pathogens, but its role as a determinant of resistance or susceptiblity is unclear (15). It has been implicated in wilt diseases (16,20) in which levels increase as in plants under water stress, and in cotton plants infected with a defoliating strain of *Verticillium albo-atrum* (31). Often it is difficult to distinguish the direct effects of ABA from those caused by increased levels of ethylene released under the influence of ABA (1,5).

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