Piperonyl butoxide as a Protectant Against Potato Spindle Tuber Viroid Infection

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ABSTRACT

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Piperonyl butoxide (PB), two mineral oils, and two vegetable oils were tested for their effect on local lesions in *Scopolia sinensis* plants inoculated with the potato spindle tuber viroid (PSTV). Local lesions were strongly inhibited by PB; sesame oil inhibited them less, and other oils inhibited them only weakly. In tests with potato, PB at a concentration of 1% (v/v) prevented infection of PSTV in sprayed leaves up to 4 days, whereas sesame oil at a concentration up to 4% (v/v) failed to prevent infection. Inhibition by PB was observed in several potato cultivars, but not in the tomato plants. In one greenhouse trial equal numbers of PSTV-infected and healthy potato plants were intermingled and 'cultivated' regularly. In one group of potato plants sprayed each week with PB, three of the healthy plants became infected with PSTV whereas seven did so in the control group.

Additional key words: insecticide synergist, methylene dioxyphenyl compound.

Piperonyl butoxide (PB), an insecticide synergist containing the methylene dioxyphenyl group (13), is so effective in preventing infection of *Scopolia sinensis* Hemsl. by potato spindle tuber viroid (PSTV) that it may be useful as a protectant (12). I have, therefore, tested whether PB also inhibits PSTV infection in potatoes and tomatoes, two hosts for which a protectant would be of potential value.

Oils have been used to curtail spread of aphid-transmitted viruses in potatoes (1, 2, 7) and can also inhibit mechanical transmission of viruses in other plants (5, 6). Among the various vegetable and essential oils, sesame oil, like PB, contains the methylene dioxyphenyl group (3, 4). Therefore, some vegetable and mineral oils were tested as inhibitors of PSTV, with the aim of finding an oil which might control both aphid-transmitted viruses and PSTV in potatoes.

MATERIALS AND METHODS

Each substance tested was emulsified in water containing 0.1% Triton-X100. They were: PB, technical grade (80%) (S. C. Johnson and Son Limited, Brantford, Ontario); sesame oil (unrefined, Arrowhead Mills, Incorporated, Hereford, Texas); corn oil (Canada Starch Company, Montreal); paraffin oil, viscosity 125-135 (Fisher Scientific Company, Montreal); mineral oil (Eastern Drug Services Limited, Fredericton, New Brunswick). The emulsions were sprayed on the leaves with a hand-held sprayer using Freon as propellant. Only the upper surfaces of the half-leaves were wetted with the liquid (about 0.5-1.0 ml/half-leaf). The control half-leaves were sprayed with water and emulsifier mixture. Spraying was done 24 hr prior to inoculation.

A partially purified ribonucleic acid (11) of a severe strain of PSTV, at a concentration of 10-15 mg/ml, was used to inoculate *S. sinensis* (8), or potato leaves (10). Ten half-leaves of *S. sinensis* (two from each of five different plants) or four leaves of each virus-free potato (10) plant that was used were rubbed in each test. All tests except the last were repeated three times. Environmental conditions and viroid infectivity for *S. sinensis* and potato plants were as described (9, 10).

TABLE 1. Effect of piperonyl butoxide and oils on local lesion development of potato spindle tuber viroid in *Scopolia sinensis*

Treatment[a]	Local lesions (no.)[b]		Inhibition[c] (%)
	Treated	Untreated	
Piperonyl butoxide	0	166	100
Sesame oil	75	202	63
Corn oil	106	207	48
Mineral oil	75	130	45
Paraffin oil	119	169	30

[a]All the oils were applied as 2% emulsion and the piperonyl butoxide was applied as 1% emulsion. Untreated plants were sprayed with 0.1% emulsifier in water. Inoculation was done 24 hr after treatment.

[b]Average number of local lesions from 30 half-leaves. Treated and untreated halves were from the same leaf.

[c]Per cent inhibition was calculated:

$$100 - \left(\frac{\text{local lesions on 30 treated half-leaves}}{\text{local lesions on 30 untreated half-leaves}} \times 100 \right)$$

RESULTS

Effect of piperonyl butoxide and oils on development of local lesions in Scopolia **sinensis.**—As found previously (12) no local lesions developed on PB-sprayed leaves, whereas varying degrees of inhibition were observed with oils (Table 1). Among the oils, sesame oil inhibited lesion development most and was used together with PB in further tests for their effect on potato plants inoculated with PSTV.

Effect of applying piperonyl butoxide and sesame oil on potatoes before inoculation with potato spindle tuber viroid.—A solution of 1% PB or 4% sesame oil was sprayed on two lots of 28 plants of Netted Gem potato. At various intervals four plants sprayed with each solution were inoculated with PSTV. Infection was prevented up to 4 days in PB-sprayed plants or up to 10 days if only sprayed leaves were inoculated. By the sixth day, new unsprayed leaves were present and infection resulted when these leaves were inoculated (Table 2). Thus, the effect of PB was limited to sprayed leaves only and was not systemic. The PSTV infection was not prevented with sesame oil (Table 2).

Piperonyl butoxide and sesame oil applied on potato after inoculation with potato spindle tuber viroid.—A solution of 4% of sesame oil, or 1% of PB was sprayed on 32 potato plants 24, 48, and 96 hr after inoculation with PSTV. All the inoculated plants developed typical PSTV symptoms, irrespective of spraying. Thus, these compounds did not prevent infection when applied sometime after inoculation with the viroid.

Effect of concentration of piperonyl butoxide and sesame oil on infection with potato spindle tuber viroid.—Several concentrations (0.1, 0.5, 1.0, and 2.0%) of PB and (0.5, 1.0, 2.0, and 4.0%) of sesame oil were sprayed on potato plants. Within 24 hr of spraying, necrotic spots were observed with 2.0% PB. No phytotoxicity was observed with the oils or with lower concentrations of PB. One per cent and 2% PB applied before inoculation prevented PSTV infection, but lower concentrations did not (Table 3). Sesame oil did not prevent PSTV infection at any concentration (Table 3). Various PB concentrations also were applied to tomato (*Lycopersicon esculentum* Mill. 'Sheyenne') plants prior to inoculation. Phytotoxicity was severe at all concentrations and most sprayed leaves became necrotic. Yet all inoculated plants later showed symptoms characteristic of PSTV.

Effect of piperonyl butoxide on different potato cultivars.—In view of the effect of PB in preventing PSTV infections in potato and *Scopolia* but not in the tomato plants, it was of interest to determine how different potato cultivars would react. A 1.0% emulsion of PB was sprayed on eight plants each of cultivars Avon, Sebago, Kennebec, Keswick, and Katahdin. Twenty-four hr after the spraying, each plant was inoculated with PSTV. An

TABLE 2. Effect of applying piperonyl butoxide and sesame oil on potato leaves before inoculation with potato spindle tuber viroid

Parts inoculated	Inoculation[a] after spraying (days)	No. of plants infected/ no. of plants inoculated with	
		Sesame oil	Piperonyl butoxide
Sprayed leaf	1	4/4	0/4
Sprayed leaf	2	4/4	0/4
Sprayed leaf	3	4/4	0/4
Sprayed leaf	4	4/4	0/4
New growth	6	4/4	4/4
New growth	9	4/4	4/4
Sprayed leaf	10	4/4	0/4

[a]The potato plants were sprayed with piperonyl butoxide (1%) emulsion, then the sprayed leaves were inoculated with the viroid. By the sixth day usually new leaves were present and inoculation of such unsprayed leaves with viroid resulted in the infection.

TABLE 3. Effect of various concentrations of piperonyl butoxide and sesame oil on potato spindle tuber viroid infection in potato plants

Treatment[a]	No. of plants infected/ no. of plants inoculated with		
	Test 1	2	3
Water + emulsifier (control)	4/4	8/8	8/8
0.1% piperonyl butoxide	4/4	8/8	8/8
0.5% piperonyl butoxide	4/4	8/8	5/8
1.0% piperonyl butoxide	0/4	0/8	0/8
2.0% piperonyl butoxide[b]	0/4	0/8	0/8
0.5% sesame oil	8/8		
1.0% sesame oil	8/8		
2.0% sesame oil	8/8		
4.0% sesame oil	8/8		

[a]The treatments were applied 24 hr before inoculation with the viroid.
[b]The application of 2% piperonyl butoxide caused phytotoxicity on the potato leaves.

TABLE 4. Attempted control of potato spindle tuber viroid by piperonyl butoxide sprays on potato plants[a]

Row (no.)	Infected source plants (no.)	Sprays (no.)	No. of plants newly infected/ no. of healthy plants
1	9	0	7/9
2	9	15	3/9
3	4	15	2/15
4	2	15	1/17

[a]Selected plants in each row (19 plants) were inoculated with viroid before spraying with piperonyl butoxide. First row was not sprayed while others were sprayed weekly with 1% piperonyl butoxide. Attempts were made to maximize contact between infected source plants and healthy plants throughout the growing period. The new infections at the end of the growing season constituted the "spread" of viroid.

equal number of healthy controls was sprayed with water and emulsifier, and were inoculated with PSTV. As observed with Netted Gem, all PB-sprayed potato cultivars remained free of symptoms, and all controls developed symptoms typical of PSTV.

Attempted control of PSTV spread with piperonyl butoxide.—To simulate field conditions, potato cuttings were grown in rows in a 20 cm depth of soil in greenhouse benches. Different levels of initial infection were created in each row by inoculating selected plants with PSTV. Every alternate plant, every fourth plant, or every sixth plant in each row (19 plants) was rubbed with PSTV-RNA. The first row with nine infected source plants was not sprayed with PB. The second row with nine infected source plants, the third with four infected source plants, and the fourth row with two infected source plants were sprayed weekly with 100 ml of 1% PB emulsion. Plants were not staked and were hilled and cultivated liberally to maximize contact between healthy and infected source plants. In the second row with nine infected source plants and receiving PB sprays, PSTV spread to three new plants, whereas in the first row with nine infected source plants and not receiving PB sprays there were seven new infections (Table 4). In the third and fourth rows with fewer infected source plants, the spread was considerably lower. Usually a plant next to an infected source plant became infected. From the foregoing, it appears that some infection of PSTV occurred in spite of PB sprays.

DISCUSSION

The inhibitory effect of PB appears to be host-specific because it failed to prevent infection in tomatoes but did inhibit it in the potato and *S. sinensis* plants.

The narrow concentration range at which PB can be applied to prevent PSTV infection and the need of frequent applications during the growing season may be limiting factors in its use as a protectant for potato crops, but it may be useful on a limited basis for minimizing spread in the nuclear stocks or parent plants in a breeding unit. Piperonyl butoxide is available commercially and is manufactured in different countries. Its price compares well with those of common pesticides.

Although there was some inhibition with oils, none appeared promising enough for use on potatoes to curtail both aphid-transmitted viruses and spindle tuber viroid.

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