

Effect of Benomyl on *Xiphinema americanum* and Tobacco Ringspot Virus Infection

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ABSTRACT

Infection of cucumber and blackeye cowpea with tobacco ringspot virus (TRSV) was not affected by benomyl seed treatment. Feeding access to cucumber grown from benomyl treated seed killed or inactivated *Xiphinema americanum* and its ability to transmit TRSV was almost eliminated. Nematode transmission of TRSV to treated bait plants was also greatly reduced. Powdery mildew was controlled. *Phytopathology* 60:1150-1151.

Cucumber (*Cucumis sativus* L.) is used as a virus acquisition host, bait plant, and indicator plant in experiments on transmission of tobacco ringspot virus (TRSV) by *Xiphinema americanum* Cobb and blackeye cowpea (*Vigna sinensis* [L.] Endl.) is used as an indicator plant. When these hosts are grown in the greenhouse, powdery mildew caused by *Erysiphe cichoracearum* DC. on cucumber and *E. polygoni* DC. on cowpea is often severe if not controlled.

Since the systemic chemical benomyl controls powdery mildews of cucurbits (1, 3, 6, 7, 8) and cowpea (2), tests were made to determine whether its use for mildew control would affect TRSV infection or *X. americanum* used in transmission experiments.

Untreated seeds of Model cucumber and Monarch blackeye cowpea and seeds treated with 0.5% by wt of active benomyl applied as Benlate 50% WP were planted in soil in 75-mm clay pots and grown in the greenhouse. When cotyledons of cucumber and primary leaves of cowpea were fully expanded they were mechanically inoculated with sap from TRSV-infected cucumber leaves ground in PO₄ buffer, pH 7.2. At the time of virus inoculation, half the plants from untreated seed were sprayed with a solution containing 350 ppm active benomyl (2.3 g 50% Benlate/3.75 liters of water). There was no apparent effect of benomyl on infection of either cucumber or cowpea by TRSV. Systemic chlorotic mottling in cucumber and localized necrotic lesions in cowpea were produced at the same time and were equally severe in benomyl-treated and untreated plants.

Techniques for testing acquisition and transmission of TRSV by *X. americanum* were those previously reported (4), except that some of the acquisition plants and bait plants were grown from seed treated with 0.5% active benomyl by wt. Cucumber acquisition plants were grown from treated or untreated seed in fine river sand in 100-ml beakers, and mechanically

inoculated with TRSV. Handpicked nematodes were added to the root zones of these plants and given a 10-day virus acquisition access period at 28 C. Single live nematodes from treated and untreated acquisition plants were then transferred to root zones of treated and untreated cucumber bait plants grown in fine sand in 250-ml plastic cups. The cups were put in a heated sandbed in the greenhouse to maintain a root zone temp of 28-30 C. They were watered 2-3 times weekly with a soluble fertilizer solution. After 30 days, roots of all bait plants were ground separately in PO₄ buffer, pH 7.2, and assayed for presence of TRSV by mechanical inoculation of cowpea and cucumber. The test was conducted twice.

Approximately 50% of the nematodes added to untreated acquisition plants were recovered alive in each test. These nematodes were vigorous and appeared healthy, and their intestines were filled (Fig. 1-A). On the other hand, less than 30% of the nematodes added to acquisition plants grown from benomyl-treated seed were recovered alive, and those which were alive were inactive and lying in a "C" shape (Fig. 1-B) characteristic of approaching mortality in *X. americanum*. A striking contrast was evident in the internal structures of the esophageal regions. In nematodes from untreated plants the stylet, stylet extension, esophagus, and esophageal lumen were distinct (Fig. 2-A). In nematodes from treated plants the esophagus was granular and the structure indistinct (Fig. 2-B). The stylet and stylet extension were usually displaced to the side, and the stylet sometimes protruded slightly. Similar results were obtained when nematodes had feeding access to cucumber plants grown from benomyl-treated seed but not infected with TRSV.

The effect of benomyl on *X. americanum* was also reflected in the ability of the nematodes to transmit TRSV (Table 1). A much larger number of single nematodes from untreated acquisition plants transmitted TRSV to untreated than to benomyl-treated bait plants. In only one case in the two tests did a nematode from benomyl-treated acquisition plants transmit TRSV, and that was to an untreated bait plant.

Powdery mildew developed early on untreated cucumber bait plants in both nematode transmission tests, and was a serious problem by the end of the test period. Only isolated spots of powdery mildew developed on older leaves of bait plants from treated seed in the last week of each test.

TABLE 1. Transmission of tobacco ringspot virus to cucumber by *Xiphinema americanum* with acquisition access on untreated or benomyl-treated cucumber plants

Acquisition plant	Bait plant	Transmission by single nematodes	
		Test 1	Test 2
Untreated	Untreated	6/30 ^a	18/30
Untreated	Treated	0/30	5/30
Treated	Untreated	1/30	0/30
Treated	Treated	0/30	0/30

^a Numerator is number of single nematodes that transmitted; denominator is total number of single nematodes tested.

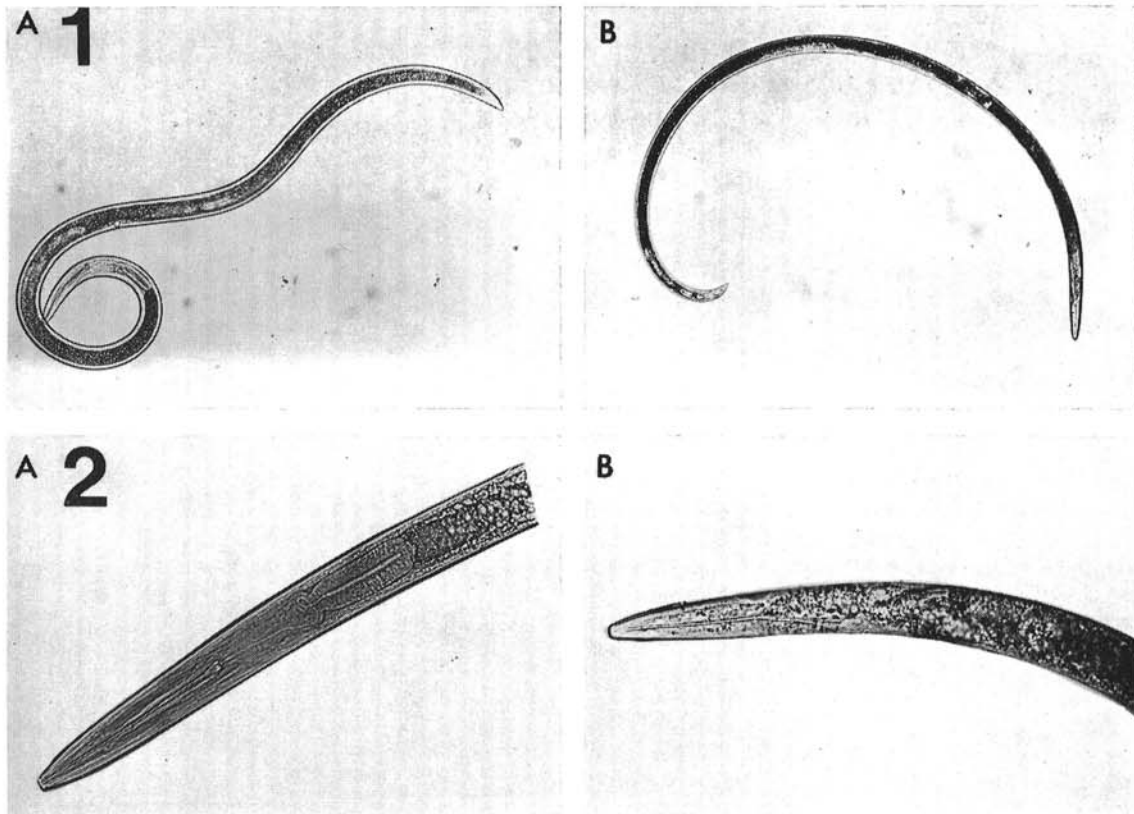


Fig. 1-2. 1) *Xiphinema americanum* with (A) 10-day feeding access to untreated cucumber plants and (B) 10-day feeding access to benomyl-treated cucumber plants. 2) Head and esophagus region of *X. americanum* with (A) 10-day feeding access to untreated cucumber plants and (B) 10-day feeding access to benomyl-treated cucumber plants.

The results of these tests indicate that benomyl used to control powdery mildew, or an active derivative of benomyl in the plant (9), does not affect TRSV directly but is lethal to the vector, *X. americanum*. Therefore, benomyl cannot be used to control powdery mildew in tests for nematode transmission. Perhaps it could be used to decrease nematode transmission of TRSV in the field. Miller (5) recently reported that soil treatment with benomyl inhibits penetration of roots by *Heterodera tabacum* larvae, but he did not find a nematicidal effect. In preliminary tests, we have found no apparent effect of seed treatment with benomyl on production of root knot on cucumber by *Meloidogyne incognita* (Kofoid & White) Chitwood. Further study is required to determine the degree of specificity of benomyl as a nematicide.

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