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Tests for Transmission of Prunus Necrotic Ringspot and Two Nepoviruses by *Criconemella xenoplax*[1]

W.-Q. YUAN, O. W. BARNETT, S. W. WESTCOTT III, AND S. W. SCOTT[2]

Abstract: In two of three trials, detectable color reactions in ELISA for Prunus necrotic ringspot virus (PNRSV) were observed for *Criconemella xenoplax* handpicked from the root zone of infected peach trees. *Criconemella xenoplax* (500/pot) handpicked from root zones of peach trees infected with PNRSV failed to transmit the virus to cucumber or peach seedlings. The nematode also failed to transmit tomato ringspot (TomRSV) or tobacco ringspot viruses between cucumbers, although *Xiphinema americanum* transmitted TomRSV under the same conditions. Plants of peach, cucumber, *Chenopodium quinoa,* and *Catharanthus roseus* were not infected by PNRSV when grown in soil containing *C. xenoplax* collected from root zones of PNRSV-infected trees. Shirofugen cherry scions budded on Mazzard cherry seedling rootstocks remained symptomless when transplanted into root zones of PNRSV-infected trees. Virus transmission was not detected by ELISA when *C. xenoplax* individuals were observed to feed on cucumber root explants that were infected with PNRSV and subsequently fed on roots of *Prunus besseyi* in agar cultures. Even if virus transmission by *C. xenoplax* occurs via contamination rather than by a specific mechanism, it must be rare.

Key words: Criconemella xenoplax, nematode transmission, Prunus necrotic ringspot virus, *Prunus persica,* ring nematode, tobacco ringspot virus, tomato ringspot virus, *Mesocriconema xenoplax.*

The ability of nematodes to transmit certain plant viruses has been well documented only for some Dorylaimida nematode species of *Trichodorus* Cobb, *Paratrichodorus* Siddiqi, *Xiphinema* Cobb, and *Longidorus* (Micoletzky) Thorne & Swanger, Siddiqi, Hooper & Khan (25). However, two reports (11,14) implicate ring nematodes, *Criconemella* DeGrisse & Loof, as plant-virus vectors. In the first report on nematode transmission of a plant virus (11), *Xiphinema index* Thorne & Allen transmitted grapevine fanleaf virus (GFLV) and grapevine fanleaf symptoms developed on 1 of 13 grapevines (*Vitis vinifera* L.) in soil infested with *Criconemella xenoplax* (Raski) Luc & Raski handpicked from soil from the rhizosphere of grapes infected with GFLV. Klos et al. (14), in Michigan, reported that two of eight peach (*Prunus persica* (L.) Batsch) seedlings exhibited typical peach rosette mosaic symptoms after 1 or 2 years in soil infested with *Criconemella* sp. handpicked from soil from the root zone of peach trees infected with peach rosette mosaic virus (PRMV). In 1987 J. M. McGuire (pers. comm.) suggested that large populations of some Tylenchida, such as ring, stunt, and spiral nematodes, might occasionally transmit viruses by a contamination mechanism but at a low level compared with Dorylaimida nematodes that have specific mechanisms for transmission of plant viruses (10).

Prunus necrotic ringspot virus (PNRSV) reportedly is transmitted by *Longidorus macrosoma* Hooper (6). Both PNRSV and *C. xenoplax* are widespread in peach orchards throughout the southeastern United States and symptoms of PNRSV infection can resemble those of peach tree short life (19). The long stylet of *Criconemella* nematodes allows them to reach cortical cells below the root epidermis (16). PNRSV was detected in the peach cortex parenchyma tissue by immuno-gold-silver staining (9). Therefore, *C. xenoplax* could logically have a role as a vector of PNRSV (19).

Tomato ringspot virus (TomRSV), a nepovirus readily transmitted by nematode vectors, also was transmitted to roots reliably by a "knife-slash" method (1). If *C. xenoplax* can transmit a virus by a contamination mechanism, perhaps it might transmit TomRSV.

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[2] Graduate student, Professor, Visiting Assistant Professor, and Associate Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

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The objectives of this study were to determine if PNRSV could be transmitted through soil and if *C. xenoplax* could vector PNRSV, TomRSV, or tobacco ringspot virus (TRSV).

Materials and Methods

Culture of plants, viruses, and nematodes: Seed of Nemaguard peach were stratified, germinated, and transplanted into steamed sand (60 C for 30 minutes). Seeds of cucumber (*Cucumis sativus* L. cv. Chicago Pickling) were planted in steamed sand. All plants were grown in a growth chamber at 25 C and 12 hours light per day or in a greenhouse at 20–30 C. The soil was kept moist but not flooded by watering one to several times each day. All plants were fertilized weekly with Hoagland's solution (12).

A peach tree that was positive for PNRSV by enzyme-linked immunosorbent assay (ELISA) was used as the PNRSV source in nematode transmission tests. TomRSV and TRSV were maintained by sap inoculation in *Nicotiana clevelandii* Gray and *N. tabacum* L., respectively. *Criconemella xenoplax* was cultured on Nemaguard peach seedlings in the greenhouse. *Xiphinema americanum* was extracted from naturally infested garden soil; its identification was confirmed by S. A. Lewis, Clemson University. Nematodes were extracted from soil by decanting-sieving (2) or with an elutriator (3) followed by centrifugal flotation (13).

Assays for virus in plants: Plant tissues were ground in 30 mM phosphate buffer (pH 7.0) with 20 mM sodium diethyldithiocarbamate (DIECA) and 0.05% polyoxyethylene sorbitan monolaurate (Tween 20). The extract of the plant tissues was assayed by direct, double antibody sandwich ELISA (4) with rabbit polyclonal antibodies against PNRSV strain G (ATCC PVAS 22), TomRSV type strain (ATCC No. 13, isolate from R. W. Fulton), or TRSV-F (isolate from R. W. Fulton). Each extract was placed in three wells. If 2 hours after adding substrate the absorbance at 410 nm was more than twice that of the mean of negative controls, it was considered positive for viruses (23).

Assay for PNRSV in nematodes: One hundred nematodes of *C. xenoplax* were handpicked from a population extracted from the root zone of a peach tree infected with PNRSV. The nematodes were ground in PBS buffer (0.01 M phosphate, 0.15 M NaCl, and 0.03 M KCl; pH 7.3) with 0.05% Tween 20 and 0.02 M DIECA with 600-mesh corundum (20). The extract was then handled in the same way as plant extracts in an ELISA assay. Absorbance at 410 nm (after 5 hours) for nematodes exposed to virus-infected plants was compared with that for unexposed nematodes by the Student's *t*-test.

Nematode population on cucumber: Chicago Pickling cucumber planted in half-liter pots developed first leaves before the soil was infested with 600 adults and juveniles of *C. xenoplax.* Nematode inoculation and determination of final nematode populations followed the method of Zehr et al. (31) except that the experiment was terminated after 63 days instead of 90 days. Nemaguard peach seedlings and soil with no plants were infested with *C. xenoplax* as controls. The experiment was replicated six times and repeated once.

Transmission of PNRSV through soil: In July, September, and October 1987, soil was collected 10–20 cm deep under three PNRSV-infected peach trees and a healthy peach tree at the Sandhill Research and Education Center (SREC) near Pontiac, South Carolina, and placed in sterilized pots in a greenhouse. Healthy seedlings of Nemaguard peach, *Chenopodium quinoa* Willd., and *Catharanthus roseus* (L.) Don. were transplanted and seeds of cucumber were planted into these field soils. Roots and leaves of the cucumber, *Chenopodium quinoa,* and *Catharanthus roseus* plants were assayed for PNRSV by ELISA 9 weeks after planting. Leaves of the peach plants were removed and some shoots were pruned five times at 3-month intervals. After new leaves developed on stripped trees,

young leaves and roots were assayed for PNRSV.

Six PNRSV-infected and six healthy peach trees were selected in an orchard at SREC. Mazzard cherry (*Prunus avium* L.) rootstocks were budded with Shirofugen cherry (*P. serrulata* Lindl.) scions and transplanted into the soil under the tree canopies, five under each infected tree and four under each healthy tree. The indicator trees were monitored for necrosis at the Shirofugen–scion junction which is a symptom indicative of PNRSV infection. Roots of each Mazzard cherry were sampled and assayed for PNRSV three times over 12 months.

Virus transmission by nematodes: The procedures described by Teliz et al. (27) were used to test for virus transmission by nematodes. Each treatment had 3–5 replications. In tests for transmission of TomRSV and TRSV by *C. xenoplax*, ca. 1,000 nematodes extracted from a culture on a healthy peach seedling were added to pots near the roots of each healthy cucumber seedling as a control and near roots of each seedling recently infected by sap inoculation with either TomRSV or TRSV. All the seedlings were potted in 80-ml plastic pots sealed at the bottom with plaster of Paris (25). After a 2-week acquisition access period, the soil was carefully separated from the roots and the *C. xenoplax* were extracted and handpicked. About 500 nematodes per pot were added near roots of healthy cucumber seedlings in a sealed 80-ml pot. Likewise, 50 *X. americanum* were transferred from each cucumber seedling infected with TomRSV to healthy cucumber seedlings. Distilled water was added to the control plants. After 2–3 weeks for transmission, roots and leaves of the bait plants were assayed for virus. This experiment was repeated four times.

Procedures were similar in tests for PNRSV transmission, but a 5-month acquisition access time on infected peach was allowed. About 500 *C. xenoplax* were handpicked and added to soil around the root system of a healthy cucumber seedling in an 80-ml plastic pot and to the root system of a healthy peach seedling in a 500-ml plastic pot. Roots and leaves of the cucumber plants were assayed for PNRSV after 2 weeks. Peach leaves were removed three times at 4-month intervals. After new leaves developed on the stripped seedlings, young leaves and roots were assayed for PNRSV.

Transmission tests with PNRSV were conducted in vitro from National Pickling cucumber to *Prunus besseyi* L. H. Bailey. Cucumber seeds were disinfested in aqueous sodium hypochlorite (5.2 mg/ml) for 15 minutes and then rinsed in sterile distilled water. Seeds were germinated on water agar (15 g/liter) and sterile seedlings were grown under lights (40 watt cool white fluorescent) in culture dishes (8 cm deep × 10 cm d) in a tissue-culture medium containing Gamborg's B-5 salts plus vitamins (8), 20 g/liter sucrose, and 10 g/liter agar. Temperatures ranged between 24 and 30 C. After cotyledons were fully expanded, they were dusted with sterile 600-mesh corundum and inoculated aseptically by gentle rubbing with glass spatulas. Virus inoculum was obtained by grinding PNRSV-infected cucumber tissue in sodium phosphate buffer (0.03 M; pH 8) filtered (0.45-μm pores) to remove microorganisms. After 2 weeks, shoots and roots were collected from cucumbers and assayed for PNRSV by ELISA. A single cucumber plant was selected on the basis of a positive test for PNRSV, and root-explant cultures were prepared by transfer of root segments from this plant to the tissue-culture medium in petri dishes.

Sterile *C. xenoplax* from in vitro cultures were added to the cucumber root cultures and allowed to feed at 26 C in the dark. During periodic observations, nematodes that were actively feeding, as evidenced by insertion of stylets into roots, were transferred aseptically in groups of 5–7 to each of 10 axenic rooted shoot tips of *P. besseyi* grown under lights on the tissue-culture medium in petri dishes (24–30 C). Daily observations of these nematodes were continued to verify that at least one nematode

TABLE 1. Population densities of *Criconemella xenoplax* from soil around roots of peach and Chicago Pickling cucumber seedlings and from fallow soil 9 weeks after adding 600 adults and juveniles.

Host	Nematodes/pot	
	Total	Gravid females
	Repetition 1	
Peach	7,617 ± 565	120 ± 29
Cucumber	850 ± 76	13 ± 2
Fallow	471 ± 42	0
	Repetition 2	
Peach	8,275 ± 779	346 ± 34
Cucumber	519 ± 87	7 ± 3
Fallow	293 ± 43	0

Values are the mean populations of *C. xenoplax* from five or six replications ± standard errors.

had fed upon roots of each *P. besseyi* plant. Shoots from the *P. besseyi* plants were collected after 2–5 weeks and assayed by ELISA for presence of PNRSV.

RESULTS

Assay for PNRSV in nematodes: The absorbance readings from the nematodes extracted from the root zone of the infected peach (0.15, 0.23**, and 0.22**) were higher (** = $P \leq 0.01$) than those from healthy nematodes (0.11, 0.10, and 0.12) in two of three experiments.

Nematode population on cucumber: After 9 weeks, *C. xenoplax* population densities in root zones of Chicago Pickling cucumber were slightly higher than in control pots not containing plants (Table 1), but were 10-fold higher on peach seedlings. Gravid females were observed in the nematode populations from cucumber, but not from soil without plants. Thus, limited reproduction of *C. xenoplax* occurred on cucumber during its period of vigorous growth. Necrotic lesions were observed on the roots of cucumber infested with *C. xenoplax*, but not on the roots without nematodes. Necrotic areas were few and small on cucumber roots initially infested with 100 nematodes, but extensive on cucumber roots infested with 500 *C. xenoplax*.

Transmission of PNRSV through soil: For tests conducted in the greenhouse or field, population densities of *C. xenoplax* ranged from 8 to 80 nematodes per 100 cm^3 soil, with an average of 20–30. No PNRSV was detected in roots or leaves of cucumber, *Chenopodium quinoa*, or *Catharanthus roseus* bait plants after 9 weeks, or in the peach seedlings tested over 16 months while growing in soil collected from the root zone of either PNRSV-infected or healthy peach trees. In the field test, no PNRSV was detected in Mazzard cherry rootstocks during the 12-month period after transplanting bait plants into the root zone of either healthy or PNRSV-infected peach trees, and necrosis was not observed at the rootstock–Shirofugen scion junctions during this period. Thus, PNRSV apparently was not transmitted into the roots of bait plants in nematode-infested soil either in the field or in greenhouse tests.

Virus transmission by nematodes: TomRSV was detected in cucumber plants after infestation with viruliferous *X. americanum*, but not with virus-free *X. americanum* (Table 2). In comparable tests, however, TomRSV and TRSV were not detected in roots or leaves of cucumber seedlings 3 weeks after infestation with *C. xenoplax* (500/80-ml pot) handpicked from the root zone of cucumber plants infected with TomRSV or TRSV (Table 2). PNRSV was not detected in roots or leaves of cucumber seedlings 2 weeks after infestation or in peach seedlings 10 months after infestation with ca. 500 handpicked nematodes extracted from the rhizosphere of a peach tree infected with PNRSV (Table 3). The other controls with the nematodes but no virus, viruses without nematodes, and no virus or nematodes were all negative for virus when the roots of the bait plants were assayed.

PNRSV was detected in root explants from agar cultures of the National Pickling cucumber by ELISA after all *C. xenoplax* had fed and had been transferred to *P. besseyi* plants. No necrosis was detected at the feeding sites on cucumber roots. At least two and often more nematodes from each group placed in culture with each *P. besseyi* plant fed on roots within 5 days of transfer. Again, no necrosis was detected

TABLE 2. Detection of tomato ringspot virus (TomRSV) and tobacco ringspot virus (TRSV) in cucumber bait plants after infestation with ca. 500 *Criconemella xenoplax* handpicked from a population extracted from the root zone of infected cucumbers.

Treatment	Bait plant assay
TomRSV + *X. americanum*	+
TomRSV + *C. xenoplax*	−
TomRSV + no nematode	−
TRSV + *C. xenoplax*	−
TRSV + no nematode	−
No virus + *X. americanum*	−
No virus + *C. xenoplax*	−
No virus + no nematode	−
Infected cucumber control	+
Healthy cucumber control	−

+ Indicates virus was detected by ELISA; − indicates virus was not detected. The same results were obtained in four experiments.

TABLE 3. Detection of Prunus necrotic ringspot virus (PNRSV) in bait plants after infestation with ca. 500 *Criconemella xenoplax* handpicked from a population extracted from the root zone of infected peach trees.

Treatment	Bait plants	
	Peach	Cucumber
PNRSV + *C. xenoplax*	−	−
PNRSV + no nematode	−	−
No virus + *C. xenoplax*	−	−
No virus + no nematode	−	−
Infected plant control	+	+
Healthy plant control	−	−

+ Indicates virus was detected by ELISA; − indicates virus was not detected.

at the feeding sites on *P. besseyi* plants. PNRSV was not detected in any shoots or roots of the *P. besseyi* plants by ELISA after at least 2 weeks from the first nematode-feeding event recorded.

DISCUSSION

For a valid test of a nematode's ability to transmit a virus, source and test plants should be hosts for both the virus and nematode. Cucumber is a good systemic host for PNRSV, TomRSV, and TRSV (7,21,22). Lownsbery (15) reported cucumber as a nonhost for *C. xenoplax* because on National Pickling cucumber the nematode population was similar to that in soil with no plants after 6 months in the greenhouse. In this experiment, however, *C. xenoplax* showed limited reproduction on vigorously growing Chicago Pickling cucumber after 9 weeks and nematodes were observed to feed on National Pickling cucumber root explants in agar cultures. Nematode-related necrosis on roots of cucumber plants also suggests feeding by the nematode.

Acquisition and transmission of a virus by its nematode vector requires from several minutes to a few days (17). In nematode transmission of a plant virus, a vector must feed on both virus-source plants and test plants in order to acquire and transfer the virus. Although a poor host for *X. americanum* (15), cucumber has been a popular test plant for nematode transmission of nepoviruses because the nematodes feed on its roots (5,18). Therefore, cucumber should be an appropriate test plant for the transmission of PNRSV, TomRSV, and TRSV by *C. xenoplax*.

The soil-borne nature of a pathogen is usually confirmed before studies of nematode transmission are done for verification because it is much easier to test soil transmission than to test a putative vector. Transmission of PNRSV through soil was not detected in this experiment. However, the population densities of *C. xenoplax* in the soil (< 80/100 cm^3) could have been insufficient to accomplish transmission of PNRSV.

Transmission of TomRSV by *X. americanum* in this experiment indicates that test conditions were suitable for nematode transmission. The lack of symptoms on, and detectable virus in, bait plants indicates that *C. xenoplax* does not transmit PNRSV from peach, either to cucumber or peach, or from cucumber to *P. besseyi*, nor does it transmit TomRSV or TRSV between cucumber plants.

Root cultures were established from the root tips of the infected cucumber seedling. PNRSV could not be detected by ELISA or bioassay in these subsequent root cultures. Therefore virus may not occur in the tips of cultured cucumber roots, and it is upon these root tips that *C. xenoplax* most often feeds in culture.

The more intense color reaction observed for extracts of nematodes from

PNRSV-infected peach trees than for nematodes from healthy trees may indicate the presence of low levels of PNRSV in the nematodes that had fed on virus-infected trees. This conclusion is tentative because only a few nematode samples have been examined and corroborative tests, such as bioassays, were not done. If present, however, PNRSV concentration must be low, and this could account for the failure of *C. xenoplax* to transmit the virus. It appears likely that a virus must be retained in the esophagus for transmission to occur (25); therefore, virus in the nematode's intestine that could be detected by ELISA would not be available for transmission (26). Other factors, such as failure of the nematode to release virus particles from specific sites into bait plants (24,29), limitation of virus replication and movement due to necrosis caused by nematode feeding on roots of bait plants (18), and the finding that graft inoculations of peach roots with PNRSV failed to establish infections (30), could also explain the failure of *C. xenoplax* to transmit PNRSV.

Criconemella xenoplax is reported to be a vector of GFLV (11) and peach rosette mosaic virus (14), but authors of both reports suggested that confirmation of these reports was desirable. In both cases transmissions by *C. xenoplax* could have been due to a nonspecific mode of transmission involving large numbers of nematodes. Indeed, a second experiment with GFLV and only 10 *C. xenoplax* per plant gave no virus transmission (11). When 3,000 *C. xenoplax* from root zones of PNRSV-infected plants were added to Nemaguard peach seedlings, all six seedlings from one of six sites died (19). It is possible that PNRSV could have caused the death of these six seedlings, but in our trials no transmission of PNRSV occurred even with 500 nematodes in an 80-ml volume of soil. If transmission of PNRSV by *C. xenoplax* does occur it must be a rare event.

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