

Vector-Assisted Seed Transmission of Melon Necrotic Spot Virus in Melon

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ABSTRACT

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Seed lots prepared from fruits collected from systemically infected melon plants were tested for seedborne melon necrotic spot carmovirus (MNSV). The hypothesis of "vector-assisted" seed transmission (VAST) was tested in trials with rigorous control of the vector fungus, *Olpidium bornovanus*. The vector also formed the basis for the most sensitive assays of seedborne virus, enzyme-linked immunosorbent assay (ELISA) was intermediate, and infectivity was the least sensitive assay. Although extracts of

seeds not treated with acid reacted in ELISA, the assay did not discriminate between viral antigens and infectious virus. The superficial viral antigens and virions were removed by acid treatment of seeds, but infectious virus remained within the seed coat at levels not usually detectable by ELISA. Seedborne virus rarely infected seedlings unless the vector was present, confirming VAST as a novel means of seed transmission that is possible because of the in vitro method of virus acquisition by *O. bornovanus*. The incidence of internally borne MNSV ranged from 0.1 to 5.3% in the vector-based assays. In one seed lot, MNSV was primarily on the surface of seeds, with an incidence of >50% on nontreated seeds and 0% on acid-treated seeds.

A fungal vector has been demonstrated for at least one of the viruses belonging to each of the genera *Necrovirus*, *Carmovirus*, and *Tombusvirus*, and the possibility that all carmoviruses have a fungal vector should be considered (8). These viruses are acquired in vitro by zoospores of *Olpidium* spp., but overseasoning of the viruses is independent of the vector (5). Seed transmission could provide a mechanism for perennation, as well as for long-distance transport of the virus independent of the vector.

Seed transmission of several carmo- and tombusviruses has been studied, but the current survey covers only the cucurbit-infecting viruses that have a fungal vector. Cucumber leaf spot carmovirus was transmitted through 1% of cucumber seeds (17). Cucumber necrosis tombusvirus was not seed transmitted (16). Reports of seed transmission of melon necrotic spot carmovirus (MNSV) are inconsistent with incidence, ranging from 20 (1,15) to 2% (13) to none (Yoshida et al. [18] cited by Bos et al. [2]). Most of these reports were done without knowledge of the role of fungal vectors. Furuki (11) identified the vector, which is now called *Olpidium bornovanus* (Sahtiyanci) Karling (7), and demonstrated 10 to 40% seed transmission of MNSV when externally contaminated seeds were sown in the presence of the vector fungus but none in its absence. Thus, the earlier reports of variable incidences of seedborne viruses could be attributed to the presence or absence of the unrecognized vector fungus. Furuki (11) introduced the term "vector dependent" seed transmission. We concur that the fungus plays a critical role in acquiring and transmitting virus to roots of germinating seedlings to initiate primary infection and to continue secondary infection cycles. We propose, however, to use the term "vector-assisted" seed transmission (VAST), because the virus oc-

curs in or on the seed independently of the vector, and it also may infect at a low rate in the absence of the vector.

The objectives of this study were to test for seed transmission of MNSV in naturally infested or infected melon seeds and to test the VAST hypothesis, using techniques that ensure strict control of the fungus.

MATERIALS AND METHODS

Fungal vector. Single-sporangial isolate SS205, a melon strain of *O. bornovanus* from Montfavet, France (7), was maintained by standard methods (3). Resting spores of the fungus were stored in air-dried roots harvested more than 3 weeks after inoculation and stored for 1 to 2 years at 4°C. These resting spores were used as inoculum in some of the trials by finely chopping the roots and using a forceps to place about 6 mg of roots in the sand in each pot. An active culture of the fungus was recovered from resting spores and maintained by zoospore transfer on *Cucumis melo* L. 'Vedrantais' or 'PMR45.' Zoospores were prepared in root washings made with tap water, and 5 ml of the zoospore suspension was added to each experimental unit (pot or leachate). The number of zoospores added to each experimental unit varied from 40 to 235×10^4 , except as noted in two trials. Assays of each pot to confirm fungal infection, or the lack of it, were done by phase microscopy to detect zoospores newly released into 5 ml of tap water when the experiments were harvested.

Seed lots. Experimental melon seed lots produced in France or Spain were provided by cooperators and given code numbers G?. These lots were stored in the laboratory for 1 to 2 years prior to the trials. Each seed lot was prepared from one to many fruits produced on plants systemically infected by MNSV. Commercial seed lots of cv. Vedrantais or PMR45 were used as virus-free controls. Superficial viral antigens and virions were removed from seeds by acid treatment. Seeds were immersed in 0.1 N HCl for 30 min

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with continuous stirring, followed by washing in running water for 30 min and drying on a laboratory bench.

Melon plants. In one type of trial, 2 or 10 seeds were sown per pot to test for virus transmission to seedlings of experimental seed lots. In other trials, one to four seeds of cv. Vedrantais or PMR45 were sown in pots to produce healthy bait seedlings that were inoculated about 7 days later. The pots were 100-ml disposable plastic pots half full of sterilized sand that had been saturated with nutrient solution. After sowing, the seeds were covered with additional sand. In a few trials, two healthy bait seedlings, pregerminated on paper towels for 8 to 10 days, were transplanted into each pot and inoculated 3 to 7 days later.

Pots were kept in individual saucers on a transite bench pre-washed with hypochlorite solution. The pots were arranged with 10 pots of a treatment in each row, with the treatments randomly interspersed by rows. Pot labels were placed under each pot to avoid wounding roots. The plants were irrigated as needed with tap water or weak nutrient solution applied through a low pressure, gravity flow system (3). When the plants were harvested, the tops were removed, and the roots were thoroughly washed in running tap water. All the roots from a pot, and in some cases the tops, were kept as an individual sample.

Assays for MNSV. Virions or viral antigen in and on dry seeds were assayed by crushing 10 seeds in a ball shaker (broyeur Danguomau, Prolabo, Paris) in 4 ml of 0.05 M phosphate buffer, pH 7.1, for a total of two replicates per seed lot. Two 200- μ l aliquots were tested in double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA), and the remainder was used for infectivity assays. ELISA was done as previously described, with a

polyclonal antiserum to MNSV (6). An ELISA A_{405} value from 0.00 to 0.09 was negative; an A_{405} value from 0.1 to 1.0 was weakly positive; and an A_{405} value >1.0 was positive. Virtually all the bait seedling roots to which the vector transmitted MNSV gave A_{405} values >2.0 . Infectivity assays were done by mechanical inoculation of the expanded cotyledons of cv. Vedrantais melons, which produced necrotic local lesions in about 6 days.

Vector assays of virus in experimental seed lots used one of the protocols described below. At the end of each trial, the roots of each sample unit were checked to confirm fungal infection, and sap was extracted with a roller press and 3 ml of 0.05 M phosphate buffer, pH 7.1. A 0.8-ml sample was placed in a tube with charcoal and Carborundum and used for the infectivity assay, and the remainder was used for ELISA or frozen. Each of the 483 root extracts was assayed for infectivity in the first trial; only two samples that were scored as positive in ELISA produced local lesions. Thereafter, only extracts that were positive in ELISA were assayed for infectivity. These extracts produced numerous local lesions and will not be reported further.

Four vector-based protocols were developed to assay for seedborne MNSV (Fig. 1). The usual number of replicates shown in Figure 1 was reduced for some seed lots because neither of two seeds in a pot germinated or because there were not enough seeds in the lot to provide a full compliment. Protocol 1 was considered a direct test of transmission, although two seeds were sown in each pot (Table 1, footnote e). The other protocols were indirect tests that increased the sampling intensity. When leachates were prepared, the seeds imbibed water, but germination at 10°C was delayed to the extent that emerging root tips were visible only on a few seeds.

The percent incidence of seedborne MNSV was calculated from the number of positive seeds per total number of seeds in the direct test. With indirect tests, the incidence (I) was calculated from the number of seeds in the sample unit (N) and the percent contaminated units (P_C) by the formula $P_C = 1 - e^{-NI}$ (12).

RESULTS

When dry seeds were assayed by ELISA, seed lots G3, G6, G7, and G9 had high titers of viral antigen ($A_{405} > 2.0$); G10 had an intermediate titer ($A_{405} = 1.4$); G4, G5, and G11 had low titers ($A_{405} = 0.2$ to 0.7); and the controls had no titer ($A_{405} = 0.0$). None of the samples were positive in infectivity assays. To localize the site of the antigen, 10 individual seeds of lot G6 were dissected into three parts (seed coat, papery layer, and embryo) that were

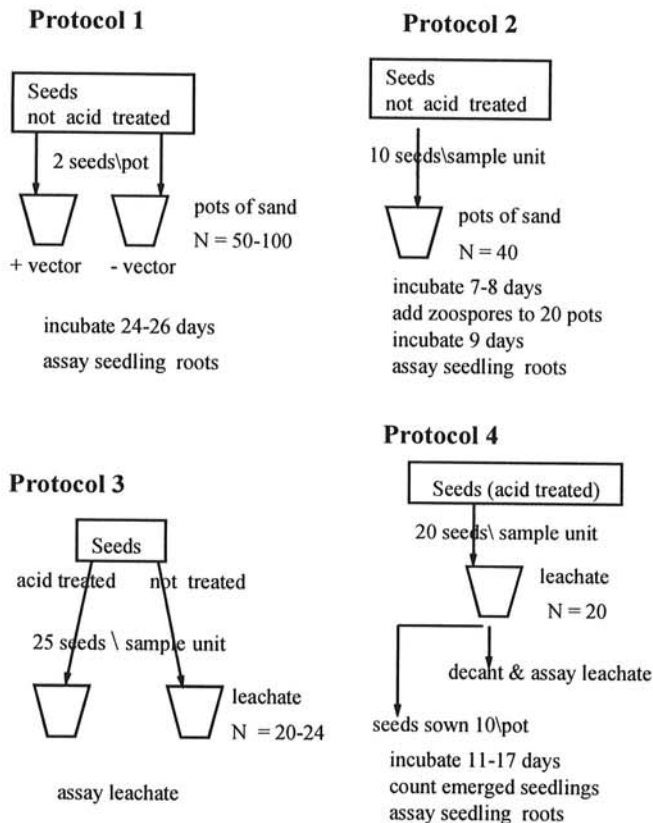


Fig. 1. Protocols developed to detect seedborne melon necrotic spot virus (MNSV). In protocol 1, + vector indicates fungal resting spores in dry roots were added to sand before sowing seeds. Seedling roots were assayed for infection by the vector and MNSV as described in text. Leachates were prepared by incubating seeds in 5 ml of tap water for 2 days at 10°C. Leachates were assayed for MNSV by infectivity, enzyme-linked immunosorbent assay, and vector zoospores. Plants inoculated in the vector assays were incubated 13 to 15 days (protocol 3) or 7 to 10 days (protocol 4) and tested for MNSV and vector infection.

TABLE 1. Direct seedling test for melon necrotic spot virus (MNSV) in four melon seed lots in the presence or absence of the vector *Olpidium bornovanus*^a

| Seed lot | Trial no. | Infested pots ^b | | | Noninfested pots | |
|----------------------|-----------|----------------------------|-------------------|----------------------|------------------|-------|
| | | Fungus ^c | MNSV ^d | I (%) ^e | Fungus | MNSV |
| G3 | 2 | 47/47 | 0/47 | 0.0 | 0/46 | 0/46 |
| G5 | 1 | 99/100 | 1/100 | 0.50 | 0/100 | 0/100 |
| G6 | 1 | 88/88 | 1/88 | 0.76 | 0/97 | 0/97 |
| G6 | 2 | 44/44 | 1/44 | | 0/46 | 0/46 |
| G7 | 2 | 91/91 | 1/91 | 0.55 | 0/89 | 0/89 |
| Control ^f | 1 & 2 | 96/98 | 0/98 | 0.0 | 0/98 | 0/98 |

^a Protocol 1: two seeds (nontreated) were sown in each of 50 or 100 pots.

^b Resting spores of *O. bornovanus* were placed in sand before seeds were sown.

^c Number of replicates in which *O. bornovanus* was detected at harvest/total number of replicates.

^d Number of replicates positive for MNSV in enzyme-linked immunosorbent assay/total number of replicates.

^e Percent incidence (I) of MNSV, assuming that either seed in a pot could have introduced the virus irrespective of its viability and that only one of the two seeds carried the virus at the observed level of transmission.

^f Two seeds of cv. Vedrantais were sown per pot as a control for splash contamination.

submerged in 10% Na₃PO₄ for 30 min, rinsed in tap water, triturated, and assayed. Viral antigens were detected only in the seed coats ($A_{405} = 1.41 \pm 0.85$). Absorbance values for the embryo and papery layer were 0.04 ± 0.02 and 0.01 ± 0.006 , respectively. None of 30 extracts were infective.

The VAST hypothesis was supported when MNSV infections occurred only in the presence of the vector (Table 1), but the distribution of infested seeds also could be attributed to chance at this low incidence. Indirect tests were used to increase sample sizes, and the VAST mechanism was demonstrated by protocol 2 (Table 2). The incidence of MNSV ranged from 0.5 to >50% when *O. bornovanus* was present. Confirmation of vector infection was problematic in some cases (Table 2, e.g., seed lot G9) for reasons discussed later. Also, a low level of seed transmission was detected in 2 of 180 sample units in the absence of *O. bornovanus*. The antigen titers of these samples were $A_{405} = 0.11$ and 0.12 , and the extracts were not infective. The presence of virions was confirmed by immunosorbent electron microscopy (ISEM) and "decoration" of the trapped particles (data not shown).

Two trials were done with protocol 3 to compare acid- and nontreated seeds of lot G5 to determine whether the virus was protected within seeds. The results of both trials were similar and have been combined. Infection by the fungus was confirmed for each pot of seedlings. The 44 leachates of nontreated seeds had ELISA A_{405} values of 0.09 to 1.9, but no local lesions formed in the infectivity tests. Virus was detected by zoospore assay in 11 leachates, including 2 for which the bait plants had weakly positive ELISA readings ($A_{405} = 0.1$ and 0.4). When seeds were acid treated, the A_{405} values of leachates were reduced to 0 in 34 leachates and to between 0.01 and 0.15 in 10 leachates, of which 2 were regarded as weakly positive ($A_{405} = 0.1$ and 0.15). None of the leachates, however, produced local lesions in infectivity assays. Virus was detected by zoospore assay in 15 leachates, including 2 for which the bait plants had weakly positive ELISA readings ($A_{405} = 0.5$ and 0.6). Controls comprised 12 pots of seedlings inoculated with zoospores and were interspersed among the experimental pots. These controls assayed positive for fungus infection and negative for MNSV. Combining the results from acid- and nontreated seeds, the incidence of MNSV in seed lot G5 was 1.4%.

Four trials were done (protocol 4) to determine the incidence of MNSV in seeds of five lots and to test the VAST hypothesis by a

TABLE 2. Vector-assisted seed transmission of melon necrotic spot virus (MNSV) in melon seedlings in the presence or absence of the vector *Olpidium bornovanus*

| Seed lot | Inoculated sample units ^a | | | Noninoculated sample units | |
|------------|--------------------------------------|-------------------|--------------------|----------------------------|-------------------|
| | Fungus ^b | MNSV ^c | I (%) ^d | Fungus | MNSV |
| G4 | 39/40 | 2/40 | 0.5 | 0/40 | 0/40 |
| G5 | 39/40 | 2/40 | 0.5 | 0/40 | 0/40 |
| G6 | 10/10 | 1/10 | 1.05 | 0/10 | 1/10 ^e |
| G9 | 4/40 | 40/40 | >50 | 0/40 | 0/40 |
| G10 | 30/40 | 19/40 | 6.4 | 0/40 | 1/40 ^e |
| G11 | 10/10 | 2/10 | 2.2 | 0/10 | 0/10 |
| Vedrantais | 38/40 | 0/40 | 0 | 0/40 | 0/40 |
| PMR45 | 40/40 | 0/40 | 0 | 0/40 | 0/40 |

^a Combined results of two trials (one trial only in the case of G6 and G11), using protocol 2: 10 nontreated seeds sown per sample unit, and half the sample units were inoculated 7 to 8 days later with zoospores of *O. bornovanus* SS205.

^b Number of sample units positive for *O. bornovanus* at harvest/total number of sample units.

^c Number of sample units positive for MNSV/total number of sample units.

^d Percent incidence (I) of MNSV in seeds, calculated based on the number of sample units inoculated with *O. bornovanus*.

^e One sample in each lot had a weakly positive reaction for MNSV: $A_{405} = 0.11$ and 0.12 , respectively. The presence of MNSV in these samples was confirmed by immunoelectron microscopy.

different method. Each trial consisted of 20 sample units of two or three experimental seed lots plus healthy seed lots in various combinations; each experimental lot was tested twice, and the results were combined. All seeds were acid treated, and the leachates had A_{405} values rated as negative in most cases. Vector assays gave incidences of MNSV comparable to those obtained in Table 2, except no virus was detected in lot G9 (Table 3). Vector assays detected virus in 17 samples that had leachate A_{405} values <0.03. Lot G10 had seven leachates with A_{405} values from 0.04 to 0.09, a range normally regarded as negative, and seven weakly positive leachates (A_{405} values from 0.10 to 0.79). MNSV was detected by infectivity assay in 3 of the latter leachates and by vector assay in all 14 leachates. On the other hand, two samples of lot G4 produced leachates with weakly positive A_{405} values of 0.14 and 0.63, but virus was not recovered by vector assay.

The seeds from which the leachates had been decanted were germinated in vector-free conditions to provide an additional test of the VAST hypothesis. A total of 3,427 seedlings were produced, and their roots were assayed as free from *O. bornovanus* and MNSV. Furthermore, the tops of 537 plants corresponding to the sample units known to contain virus based on the vector assays completed earlier were tested by ELISA and assayed as negative.

The reduction in the incidence of MNSV in lot G9 from the earlier assays with nontreated seed was striking. Apparently the virus contaminating this seed lot, in contrast to that of the other lots, was primarily external on the seed and was removed by acid treatment. Two tests were done using protocol 4 to confirm the external location of the virus by comparing treated and nontreated seeds in the same trial and by testing for virus in the leachate after short periods of soaking. The soaking periods in each trial were adjusted so they ended at the same time and were assayed with one zoospore preparation. Within 2 h, the leachates of nontreated seeds of G9 were antigenically positive in ELISA, and infectious virus was demonstrated in vector assay (Table 4). As expected, the leachates from acid-treated seeds were free from viral antigens detectable by ELISA, except for one 48-h leachate of G10. Vector assay, however, detected MNSV in six of the leachates that were soaked 5 to 48 h, including one of G9 that had a weakly positive ELISA titer.

TABLE 3. Enzyme-linked immunosorbent assay (ELISA) and vector-based assays for seedborne melon necrotic spot virus (MNSV) in leachates of acid-treated melon seeds^a

| Seed lot | Leachate A_{405} ^b | | | I (%) ^c | Fungus ^d | Seedlings ^e |
|---------------|---------------------------------|-------|------|--------------------|---------------------|------------------------|
| | Nil | Trace | Weak | | | |
| G4 | 3/37 | 0/0 | 0/2 | 0.4 | 39/39* | 743 |
| G5 | 1/40 | 0/0 | 0/0 | 0.1 | 40/40 | 746 |
| G6 | 1/32 | 0/0 | 0/0 | 0.2 | 32/32* | 421 |
| G9 | 0/39 | 0/0 | 0/0 | 0 | 39/39 | 783 |
| G10 | 12/26 | 7/7 | 7/7 | 5.3 | 32/40* | 734 |
| Vedrantais | 0/40 | 0/0 | 0/0 | 0 | 40/40 | 701 |
| PMR45 | 0/79 | 0/0 | 0/0 | 0 | 79/79* | 954 |
| Noninoculated | nt | | | 0 | 0/77 | nt |

^a Protocol 4: 20 seeds per sample unit; results combined from four trials.

^b Number of sample units positive in vector assay/number of sample units inoculated. Sample units are grouped according to ELISA values (A_{405}) of the leachates used for inoculations: nil = 0.00 to 0.03; trace = 0.04 to 0.09; and weak = 0.1 to 0.8. nt = not tested.

^c Calculated percent incidence (I) of MNSV from vector-based assays of seed lots.

^d Number of sample units positive for *O. bornovanus*/total number of sample units harvested in two trials, except for PMR-45 and noninoculated controls, which were harvested in four trials. In one trial, the vector inoculum was 15×10^4 zoospores per sample unit; the four lots in this trial are marked with an asterisk.

^e The seeds used to prepare each leachate were sown in 2 sample units, each with 10 seeds. All sample units assayed as negative for MNSV and *O. bornovanus*. The number of seedlings tested is shown.

DISCUSSION

In describing vector-dependent seed transmission, Furuki (11) proposed that the vector acquired and transmitted MNSV that contaminated the surface of melon seeds at high incidence but that would not otherwise infect the seedling. Although we concur that the vector plays an important role in seed transmission, we found that some transmission to seedlings occurred in the absence of the vector, that seedborne virus is not always externally borne, and that the incidence of MNSV is often <2%. Hence, we prefer the term "vector-assisted" seed transmission (VAST). Perhaps Furuki's (11) use in some trials of seeds artificially contaminated with virus contributed to his interpretation. This procedure would be the equivalent of mixing virus and fungus in an *in vitro* transmission experiment if the tests were done soon after contamination. We used seed lots produced on plants naturally and systemically infected with MNSV. The virus was internal in the seeds of four lots, as judged by its recovery from acid-treated seeds at incidences of 0.1 to 5.3%. In a fifth lot (G9), the virus was external and inactivated by acid treatment (Table 3), but in a later trial (Table 4), one acid-treated sample was positive, indicating an extremely low incidence of internally borne virus in this lot as well. MNSV was not detected in one lot (G3) by direct seedling assay. This failure may be attributed to the small probability of having one virus-bearing seed in approximately 100 seeds exposed to the vector.

There was limited evidence of infection in the absence of the vector. We observed 2 sample units among 180, each with 10 seeds, that had weakly positive ELISA readings and in which virions were detected by ISEM. If it is assumed that only one seedling had a local infection in each sample unit and if data from all the lots are combined, the virus incidence in the absence of the vector was 2/1,800 or 0.1%. If one includes the germinated seedlings that were grown free from the vector in Table 3, the incidence of infection might be less than 2/5,227 or 0.04%. Much larger sample sizes from single seed lots are needed to confirm this point. We have not determined whether the non-VAST represents embryos infected as they formed or mechanically inoculated as they germinated in the presence of nonembryonic virus, but we favor the latter possibility. We cannot rule out the possibility that nonembryonic, seedborne virus could be mechanically inoculated to a greater percentage of seedlings raised in a different system or kept for longer incubation times. We doubt, however, that mechanical transmission is as important as VAST in view of the ubiquity of the vector in soils, the efficiency of virus acquisition by zoospores, and the high virus titers in vector-inoculated roots.

VAST is a novel means of seed transmission of viruses and was not included in a recent review on seed transmission (14). There are no other examples in which seedborne, nonembryonic virus is acquired and transmitted by a vector. VAST reflects another unique aspect of the *in vitro* mechanism of fungal transmission in which viruses survive season to season independently of the vector (4). Seed transmission also facilitates long-distance transport of virus independent of the vector. It is likely that the outbreak of MNSV in the melon breeding program in California (13) was due to VAST, because the vector is wide spread in California (7).

The method by which MNSV reaches the interior of seeds is unknown. It might occur during seed development or when seeds are extracted. Virions could be in the papery layer or simply enclosed in the seed coat, but they probably are not in the embryo. We cannot eliminate the possibility that individual seeds from which virus was released into the leachate failed to germinate, for example, because of the necrotic reaction of a virus-infected embryo. The survival of virions on the surface of seeds of some lots probably depends on the seed extraction method. Survival of virus on dry seeds or in the seed coat is not expected, because drying usually inactivates viruses in these locations (14). Inactivation of

contaminating MNSV was demonstrated by acid treatment and by drying for 4 days or longer (9).

Three methods were tested to detect seedborne virus. Their reliability was related both to their sensitivity and to the virus-seed relationship. Infectivity assays of dry seed extracts or seed leachates routinely gave negative results. ELISA was much more sensitive, but produced both false positives and false negatives compared to the vector-based assays. ELISA did not discriminate between viral antigen and infectious virions that occurred on the seed coat and within the seeds. Thus, there was no correlation between ELISA values for dry seeds and the incidence of seedborne MNSV detected by vector assay. Acid treatment of seeds removed both infectious virus and MNSV antigens from the seed coat. Leachates prepared from acid-treated seeds contained infectious virus that frequently was not detected by ELISA. In the same trials, there were also two leachates of lot G4 (Table 3) that were positive in ELISA but negative in vector assay. Although positive ELISA values were obtained for dry seeds and for leachates from nontreated seeds, the assay indicates that MNSV-infected fruits were included in the seed harvest but does not predict the incidence of MNSV in vector-based assays.

Although we used resting spores as inoculum in the first tests to simulate sowing seeds in fungus-infested soil, the use of zoospores simplified and shortened the duration of the assays. For routine use, either protocol 2 or 4 is suitable, but protocol 4 is preferred because the assay can be completed rapidly and the number of seeds or sample units can be increased easily to detect virus at incidences <0.1% with confidence.

Fungal infection of test plants was confirmed by observing zoospores released from sporangia that developed in roots inoculated with the vector. Thus, a negative assay for virus in the sample was not due to lack of the vector. Although MNSV infected some vector-inoculated samples in Tables 2 and 3, infection by the assay fungus was not confirmed in some cases. The vector doubtless was responsible for virus transmission, because zoospores were uniformly distributed in the inoculum and our techniques avoided mechanical inoculation of the roots. The failure to detect the fungus in certain experiments is attributed to a combination of factors: (i) fungal inoculum was low, in the range of 15 to 40 × 10⁴ zoospores per sample unit; (ii) incubation time was 7 to 9 days, which is after the maturity of many first-generation sporangia and even the roots rated as positive for the vector produced few zoospores; and (iii) MNSV titer in the inoculum and in the inoculated roots was high. The suppression of development of the vector by coinfection with the virus has not been tested experimentally, but it was noted with *O. brassicae* and tobacco necrosis virus (10). The absence of vector in all noninoculated controls demonstrated

TABLE 4. Effect of brief soaking periods on release of melon necrotic spot virus (MNSV) from melon seeds of lot G9 (20 seeds per sample unit) in two experiments^a

| Seed lot | Seed treatment ^b | Leachate soaking period | | | | | |
|------------------|-----------------------------|-------------------------------|-------------------|------------------|------------------|-------------------|------|
| | | 2 h | | 5 h | | 48 h | |
| | | A ₄₀₅ ^c | MNSV ^d | A ₄₀₅ | MNSV | A ₄₀₅ | MNSV |
| G9 | NT | 0.99 | 8/8 | 1.21 | 8/8 | 1.78 | 8/8 |
| G9 | AT | 0.00 | 0/8 | 0.00 | 1/8 ^e | 0.00 | 0/8 |
| G10 ^f | AT | 0.00 | 0/4 | 0.00 | 3/4 | 0.02 ^g | 2/4 |
| PMR45 | NT | 0.00 | 0/14 | 0.00 | 0/14 | 0.00 | 0/14 |

^a The vector assay in the first trial involving melon G9 and PMR45 used 17 × 10⁴ zoospores per sample unit.

^b NT = nontreated; AT = acid treated.

^c Mean enzyme-linked immunosorbent assay A₄₀₅ values of leachates for sample units in 1 or 2 trials.

^d Number of sample units positive for MNSV in vector assay/total number of sample units.

^e One sample unit weakly positive, A₄₀₅ = 0.54.

^f Tested in one trial.

^g One leachate was weakly positive, A₄₀₅ = 0.10.

effective control of the vector. An incubation period of about 2 weeks after zoospore inoculation obviates problems in confirming vector infection, and it may permit detection of very small amounts of virus by permitting two to three infection cycles of the vector. This probably was the case in trials involving lot G5 and protocol 3, in which 4 sample units had weakly positive A_{405} values of 0.1 to 0.6. Irrigation of experimental seedlings kept in small pots for more than 2 weeks is difficult because of the amount of transpiration.

Detection of virus infection in these trials did not rely on systemic symptoms, but occasionally, seedlings were noted with water soaked necrotic lesions on the lower hypocotyls, as reported previously (8). These symptomatic seedlings occurred after a long incubation period (25 days) or with combinations of high virus titer and abundant vector inoculum. ELISA showed higher virus titers in the roots and necrotic hypocotyl than in the upper hypocotyl and cotyledons, indicating root infection and acropetal movement of the virus.

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