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Virus transmission by host-specific strains of *Olpidium bornovanus* and *Olpidium brassicae*

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Abstract

Zoospores of 12 isolates *O. bornovanus* from geographically diverse sites and representing the three host specific cucurbit strains were tested as vectors for seven viruses using watermelon bait plants and the in vitro acquisition method. All isolates of the cucumber, melon, and squash strains transmitted melon necrotic spot carmovirus (MNSV) and cucumber necrosis tombusvirus (CNV) but none transmitted petunia asteroid mosaic tombusvirus (PAMV) or tobacco necrosis necrovirus (TNV). The isolates varied as vectors of three other carmoviruses: cucumber leaf spot virus (CLSV); cucumber soil borne virus (CSBV); and squash necrosis virus (SqNV). All cucumber isolates transmitted CLSV and SqNV but not CSBV. Some of the melon isolates transmitted CLSV and SqNV but none transmitted CSBV. Two squash isolates transmitted CSBV and SqNV but not CLSV. Two isolates of *O. brassicae* transmitted only TNV and a third did not transmit any of the viruses. The species of bait plant sometimes affected transmission. The most efficient vector strains of *O. bornovanus*, as determined by reducing zoospores and virus in the inoculum, were the cucumber strain for CLSV; the cucumber strain for CNV if cucumber was the bait plant or melon strain if watermelon was the bait plant; and the squash strain for SqNV. The plurivorous strain of *O. brassicae* was the most efficient vector of TNV. *Olpidium bornovanus* is the first vector reported for CSBV and is confirmed as a vector of SqNV. It is proposed that all carmoviruses may have fungal vectors. *Ligniera* sp. did not transmit any of the viruses in one attempt.

Abbreviations: CLSV = cucumber leaf spot virus; CNV = cucumber necrosis virus; CSBV = cucumber soil borne virus; MNSV = melon necrotic spot virus; PAMV = petunia asteroid mosaic virus; SqNV = squash necrosis virus; TNV = tobacco necrosis virus; TBSV = tomato bushy stunt virus.

Introduction

The carmo- and tombusviruses share many biophysical characteristics [Martelli *et al.*, 1988; Morris and Carrington, 1988] and may have similar fungal vectors [Campbell *et al.*, 1991]. The carmoviruses found on cucurbit hosts [Morris and Carrington, 1988] include: cucumber soil borne virus (CSBV), cucumber leaf spot virus (CLSV), cucumber fruit streak virus (CLSV-FS) that is serologically related to CLSV [Weber *et al.*, 1986], and melon necrotic spot virus (MNSV). Squash necrosis virus (SqNV) [Lin *et al.*, 1983] is a probable member of this group because it was serologically related to CSBV in double diffusion tests [R. Koenig, pers. comm.] although not in DAS-ELISA [Campbell *et al.*, 1991]. Cucurbit-infecting tombusviruses include cucumber necrosis virus (CNV) that was listed as a carmovirus [Morris and Carrington, 1988] but

has been transferred to the tombusvirus group [Rochon and Tremaine, 1988] and petunia asteroid mosaic virus (PAMV) that was isolated from cucumber [Campbell *et al.*, 1990].

The zoospores of *Olpidium bornovanus* (Sahtiyanci) Karling (syn.: *O. radicale* Schwartz & Cook fide Lange & Insunza, *O. cucurbitacearum* Barr & Dias) transmit CNV [Dias, 1970a], MNSV [Furuki, 1981], SqNV [Lin and Palagi, 1983], CLSV and CLSV-FS [Campbell *et al.*, 1991]. Although Gerhardson suggested to Lange and Insunza [1977] that this fungus may be the vector of red clover necrotic mosaic dianthovirus (RCNMV), his experiments have not been reported. In all cases, the zoospores acquired the viruses in the in vitro manner as described for tobacco necrosis necrovirus (TNV) and *O. brassicae* (Wor). Dang. [Campbell, 1979].

Although *Olpidium* spp. are obligate parasites, techniques for obtaining single sporangial cultures that are the equivalent of single spore cultures of other fungi have been developed [Campbell, 1988; Dias, 1970b; Lin *et al.*, 1970; Sahtiyanci, 1962]. Such cultures are essential for studies to identify fungal vectors and to test their host range or vector specificity. Isolates of *O. brassicae* generally are plurivorous but some have more restricted host ranges [Campbell and Sim, 1994; Temmink *et al.*, 1970]. Two groups differing in host range were postulated in *O. bornovanus* [Lange and Insunza, 1977]. The plurivorous type was based on isolates from red clover [Lange and Insunza, 1977] and from lettuce [Sahtiyanci, 1962] whereas the narrow host range type was based on cucurbit isolates [Barr, 1968]. Host specificity among cucurbit isolates from diverse geographical sites was narrower than described by Barr [1968] and three strains that reproduced readily in cucumber, melon, or squash were recognized [Campbell and Sim, 1994]. Watermelon was an intermediate host in which all strains grew moderately well.

The objective of this paper was to study the ability of 15 host-specialized, single-sporangial isolates of *O. bornovanus* and *O. brassicae* to transmit seven viruses of the carmo-, tombus-, or necrovirus groups of which CSBV and PAMV have no known vector.

Materials and methods

Fungal isolates

The origin, single sporangial isolation, and host specificity of 12 isolates of *O. bornovanus* and 3 isolates of *O. brassicae* was described [Campbell and Sim, 1994]. The cucumber (C) strain was maintained on *Cucumis sativus* L. cv. Poinsett 76, the melon (M) strain on *C. melo* L. cv. PMR-45, the squash (S) strain on *Cucurbita pepo* L. cv. Small Sugar pumpkin, and a red clover (R) strain on *Trifolium pratense* L. cv. Violetta. An earlier lettuce isolate of *O. brassicae*, SS58L, was recovered from dry resting spores and maintained on *Lactuca sativa* L. cv. Climax. A unifungal culture of a *Ligniera* sp. isolated from and maintained on pumpkin [Campbell and Sim, 1994] was used for one trial.

All plants were grown in sand culture and incubated in growth chambers operating at 24 °C day/18 °C night with a 14–16 hour photoperiod. Preparation of zoospore suspensions and counting of zoospore numbers were as described [Campbell and Sim, 1994].

Viruses

The seven viruses were described previously [Campbell *et al.*, 1991]. Partially purified preparations of CLSV, CSBV, PAMV, and SqNV were stored at –20 °C in 50% glycerol. A similar preparation of CNV was used for the screening trials but for later trials a 1:10 wt:vol extract of cucumber cotyledons was used. This extract was prepared in 0.05 M phosphate buffer, pH 7.4, and frozen in 1 ml aliquots so that a new tube was thawed and used for each experiment. Similar extracts of infected cucumber leaves or cotyledons were used for MNSV and TNV.

Bioassays were done on the four youngest, fully-expanded leaves of *Chenopodium quinoa* Willd., except that MNSV was bioassayed on the fully expanded cotyledons of two or three cucumber seedlings. If MNSV was used in a trial, the fungus/no virus controls were assayed on both hosts.

Transmission experiments

The techniques were modified slightly from previous experiments [Campbell *et al.*, 1991]. Watermelon (*Citrullus lanatus* (Thunb.) Matsum.

& Nakai cv. Sugar Baby) was the bait host because it was the common host for all fungal isolates [Campbell and Sim, 1994]. Seedlings were germinated on wet paper towels for two days at 31 °C and then for 3 days at room temperature at which time two seedlings were transplanted into sand in 100 ml plastic pots and watered with dilute nutrient solution. The plants were incubated in growth chambers for approximately six days before they were used.

Experiments were done by adding 100 µl of a diluted stock of each virus in 50% glycerol per 10 ml of zoospore suspension (or of water for the virus/no fungus control). The final dilution of virus was approximately one 10 fold dilution step less than its dilution end point. The mixtures were gently swirled and 10 ml was dispensed into each of 2 replicate pots of bait seedlings. Plants were inoculated near the growth chambers with each pot in a separate saucer and labelled externally to avoid damage to, and mechanical inoculation of, the roots. The pots were placed randomly in the growth chambers with one replicate in each of two chambers. The inoculations were completed between 15 and 45 min after preparing the mixtures.

Three control treatments were included in each trial. The virus/no fungus control contained virus in tap water; the fungus/no virus control consisted of zoospores to which no virus was added; and the no virus/no fungus control had neither virus nor zoospores. A 0.8 ml sample of the virus/no fungus control was bioassayed to confirm the presence of virus. The number of local lesions/leaf varied from 2 to 40 for the viruses assayed on *C. quinoa* and 1–2 with MNSV on cucumber. In a given trial the number of local lesions on *C. quinoa* was low or high for all viruses irrespective of the numbers in earlier or later trials due to differences in the sensitivity of assay hosts from one trial to the next.

Three types of experiments were done: screening trials, host range trials, and vector efficiency trials. Screening trials compared transmission of seven viruses by four fungal isolates. Each of the first six screening trials included an isolate of each of the three strains of *O. bornovanus* and one isolate of *O. brassicae*. Based on the results of the first six trials, PAMV, TNV and *O. brassicae* were omitted from the last three trials that each compared four isolates of *O. bornovanus* and five viruses. Thus, each *O. bornovanus* isolate was tested at least two times [SS205M was tested three times, SS179S five times, and SS232S four times] with each of five viruses and once with PAMV and TNV. Each *O. brassicae* isolate was tested two times with all seven viruses. The number of zoospores of *O. bornovanus* was not adjusted among the isolates in each trial and ranged from 8 to 74×10^4 zoospores/ml. Although melon isolates had few zoospores (8 to 14×10^4 zoospores/ml) in four trials, they transmitted CNV, MNSV, and SqNV as well as other isolates in the same trial or as well as they transmitted the same viruses in other trials.

The host range trials tested the ability of selected fungal isolates to transmit one virus to four cucurbit hosts (watermelon, cucumber, and melon each with 2 seedlings per pot; and pumpkin with 1 seedling per pot). The melon seedlings were usually 7 days old when transplanted. Watermelon was the only host inoculated with the fungus/no virus controls in these experiments. The zoospore concentrations of the isolates within a trial were adjusted so that they were within a 2-fold range.

Vector efficiency trials compared transmission of one virus, usually to watermelon bait seedlings, by selected fungal isolates when virus and zoospore concentrations were varied. A 1:20 dilution of the initial zoospore suspension was made in tap water. The virus was added to samples of each concentration of zoospores at double the amount used in previous trials or at one-tenth that concentration (H and L, respectively) to give four combinations for each isolate.

Evaluation of transmission

Bait seedlings were harvested six days after inoculation and their roots were washed thoroughly in running tap water and triturated in a mortar with five ml of 0.05 M phosphate buffer, pH 7.4. A 0.8 ml sample of the extract was placed in a tube with a small amount of charcoal for bioassay and the remainder was placed in another tube for serological testing. The extracts were refrigerated for use within a few days or frozen for later use. A small sample of the roots from a fungus/no virus control was mounted in tap water on a slide for microscopic examination. Fungal

infection was confirmed for each isolate in each trial by the presence of either abundant thalli in the root or zoospores released from zoosporangia in the root.

The double antibody sandwich ELISA was done with antiserum to the inoculated virus [Campbell *et al.*, 1991] with two wells per experimental sample. The plates were read with a Ceres 900 autoreader (Bio-Tek Instruments Inc., Winooski, VT 05404) which read absorption linearly to 3.0 and to a maximum of 4.3. The average absorbance at 405 nm (A405) of the fungus/no virus controls for the same fungal isolate (two samples each with two wells) was subtracted from the A405 of two wells for each fungus + virus sample. Similarly the A405 of the no virus/no fungus controls was subtracted from the virus/no fungus controls. In the host range trials the A405 of watermelons inoculated as fungus/no virus controls was subtracted from the A405 of the fungus + virus samples regardless of the host species. For the screening and vector efficiency trials the results are presented as mean A405 with the positive value of the standard deviation. The results from the host range trials usually were analyzed by SAS with 2-way ANOVA with Weller-Duncan minimum significant difference and with 1-way ANOVA when significant interactions were found.

Bioassays confirmed the relative virus titers obtained by ELISA in fungus + virus treatments which gave transmission but the bioassays were not quantitative because the local lesions saturated the assay host when the virus titer was high. The bioassays of the three controls supplemented the serological assays in several ways. Bioassays of the virus/no fungus controls detected low levels of residual viral inoculum and/or mechanical inoculation (non-vector soil transmission). Bioassays of the fungus/no virus controls and of the noninoculated controls detected viral contamination of the fungal cultures or experimental plants.

Results

Six viruses were acquired in vitro and transmitted by zoospores of at least one isolate of *Olpidium* spp. (Fig. 1). These results will be presented individually later. None of the 15 isolates of either fungal species transmitted PAMV with which each was tested at least once.

The A405 of the virus/no fungus controls for the combinations in Fig. 1 (N = 102) ranged from 0.077 to –0.068 with an average of 0.002 and a standard deviation of ± 0.0204. The threshold for virus transmission was set at the average plus 10 times the standard deviation or A405 = 0.206. The bioassays of the virus/no fungus controls were virtually free from virus; a total of 5 local lesions developed from 114 samples. Bioassays of the fungus/no virus and no virus/no fungus controls produced no local lesions in any experiment.

Transmission of CLSV

The five cucumber isolates of *O. bornovanus* transmitted CLSV whereas the squash isolates did not (Fig. 1A). The melon isolates were variable and only SS205M and SS220M which originated at Montfavet, France, and Brawley, CA, respectively, were vectors. The non-transmitting isolates SS201M, SS277M, SS179S and SS232S were each included in two or three additional trials and failed to transmit CLSV.

Isolate SS205M was compared with either SS92C or SS145C in host range and vector efficiency tests. Watermelon, cucumber, and melon were equally good hosts (mean A405 = 0.67 to 0.80) but pumpkin was significantly poorer (mean A405 = 0.12, M.S.D. = 0.32) in two host range trials (Fig. 2A). In these trials isolate SS145C gave significantly higher virus titers (mean A405 = 0.97) than did SS205M (mean A405 = 0.69, M.S.D. = 0.26). Isolate SS92C was a more efficient vector than SS205M as shown by the ability of low concentrations of zoospores to transmit low concentrations of virus in two trials of which one is presented (Fig. 2B).

The vector ability of SS205M was surprising because it originated from a bulk culture (90–3) that did not transmit in three earlier trials [Campbell *et al.*, 1991]. Three sibling isolates of SS205M were tested using cucumber and watermelon as bait hosts in two trials. None of the three sibling isolates (SS207M, SS208M, or SS210M) transmitted to either host in either experiment except that one replicate of watermelon inoculated with SS207M had A405 = 0.38 and low infectivity

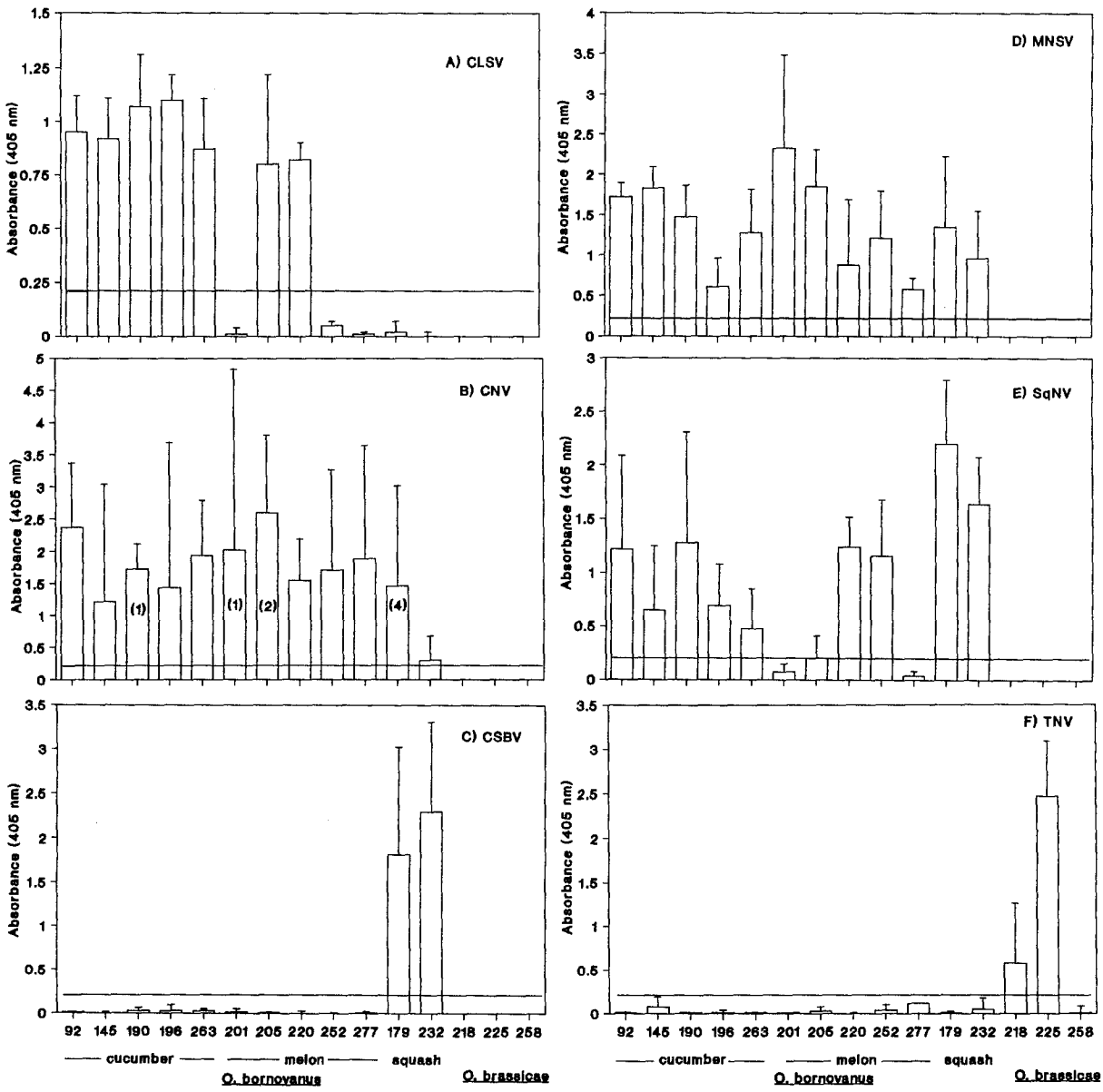

Fig. 1. Absorbance values (A405) in double antibody sandwich ELISA of extracts of watermelon roots inoculated with in vitro mixtures of one of six viruses and zoospores of 15 single-sporangial isolates of *Olpidium* spp. Viruses and corresponding antisera used for ELISA: A) cucumber leaf spot virus (CLSV); B) cucumber necrosis virus (CNV); C) cucumber soil borne virus (CSBV); D) melon necrotic spot virus (MNSV); E) squash necrosis virus (SqNV); F) tobacco necrosis virus (TNV). Horizontal line at A405 = 0.206 represents the threshold value for indicating positive transmission; error bars indicate positive value of standard deviation. Each combination was tested in 2 trials with the following exceptions: all *O. bornovanus* isolates were tested once with tobacco necrosis virus; with the other viruses isolate 205M was tested in 3 trials, 232S was tested in 4 trials, and 179S was tested in 5 trials; four isolates were tested fewer times with cucumber necrosis virus as shown by numbers inserted in the bars in B).

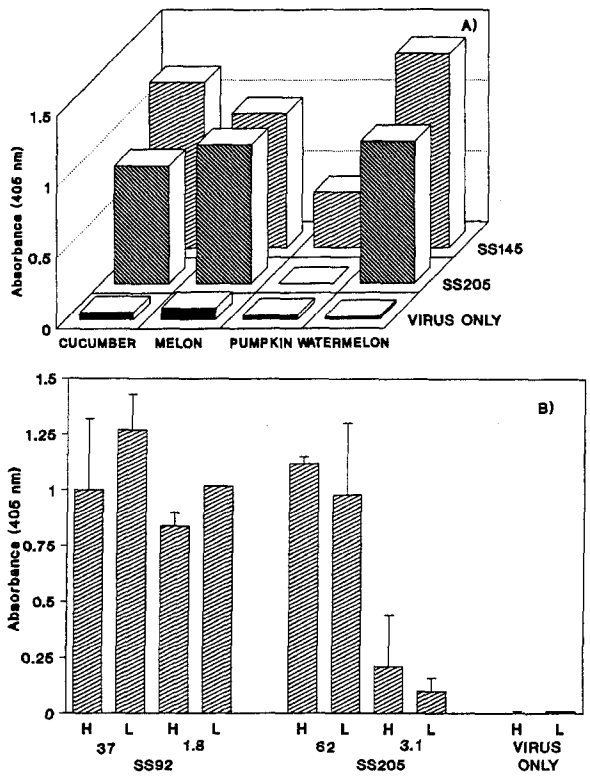

Fig. 2. Absorbance values (A405) with cucumber leaf spot virus (CLSV) antiserum in double antibody sandwich ELISA of extracts of roots inoculated with in vitro mixtures of CLSV and zoospores of *O. bornovanus*. Virus only refers to virus/no fungus control. A) Transmission to four hosts, average A405 values of two replicates in two trials. B) Transmission efficiency experiment with virus and zoospores at two concentrations; virus concentrations were 2X (H) or 0.1X (L) those used in Fig. 1A and the zoospore concentrations were as indicated (no. $\times 10^4$/ml). Error bars indicate positive value of standard deviation.

(34 local lesions/leaf). By contrast SS205M transmitted to both hosts in both trials (A405 = 0.77 ± 0.37) and SS145C transmitted to both hosts in one trial (A405 = 0.95 ± 0.12).

Transmission of CNV

All isolates of *O. bornovanus* transmitted CNV but the variability was high as shown by the standard deviations (Fig. 1B). In the last trial CNV was not transmitted by any of four *O. bornovanus* isolates, each of which had transmitted it in previous tests. The results with CNV in this trial were omitted from Fig. 1B and a new source of viral inoculum was used for later trials. Isolate SS232S was the poorest vector; it failed to transmit in three trials, in which other isolates transmitted well, but transmitted well in a fourth trial.

Isolate SS196C, that originated from the site where CNV was found, isolate SS205M, and either isolate SS179S or SS232S were used for host range and efficiency tests. The combinations of SS196C + CNV inoculated to cucumber or SS205M + CNV inoculated to watermelon transmitted CNV significantly better than other host-fungus combinations (Fig. 3A). There was no transmission to pumpkin by any isolate or to melon by SS179S. All other host-fungus combinations gave intermediate to low transmission levels.

Because of the host effect on transmission of CNV, the vector efficiency trials were done either with watermelon or with cucumber plants. With watermelon as the host, isolate SS205M was the most efficient vector as shown by transmission with 1.8×10^4 zoospores/ml (Fig. 3B). Similar results were obtained when this trial was repeated (data not presented). With cucumber as the host, isolate SS196C was the most efficient vector in two trials of which one is presented (Fig. 3C).

Transmission of CSBV

Two isolates of the squash strain of *O. bornovanus* were the only vectors of CSBV (Fig. 1C). Both isolates transmitted CSBV equally well to each of the four cucurbit hosts in two experiments (data not shown).

Transmission of MNSV

All strains and isolates of *O. bornovanus* transmitted MNSV (Fig. 1D). No additional tests of host range or of vector efficiency were done.

Transmission of MNSV by the melon isolates, except SS252M, or isolate SS179S provided the only instances in which bait seedlings had symptoms of virus infection. The cotyledons were wilted, plants were smaller than in other treatments because the primary leaf had not expanded, and necrosis of the vascular tissue extended into the lower hypocotyl.

Transmission of SqNV

All isolates of the cucumber and squash strains of *O. bornovanus* transmitted SqNV but only melon isolates SS220M from Brawley, CA, and SS252M

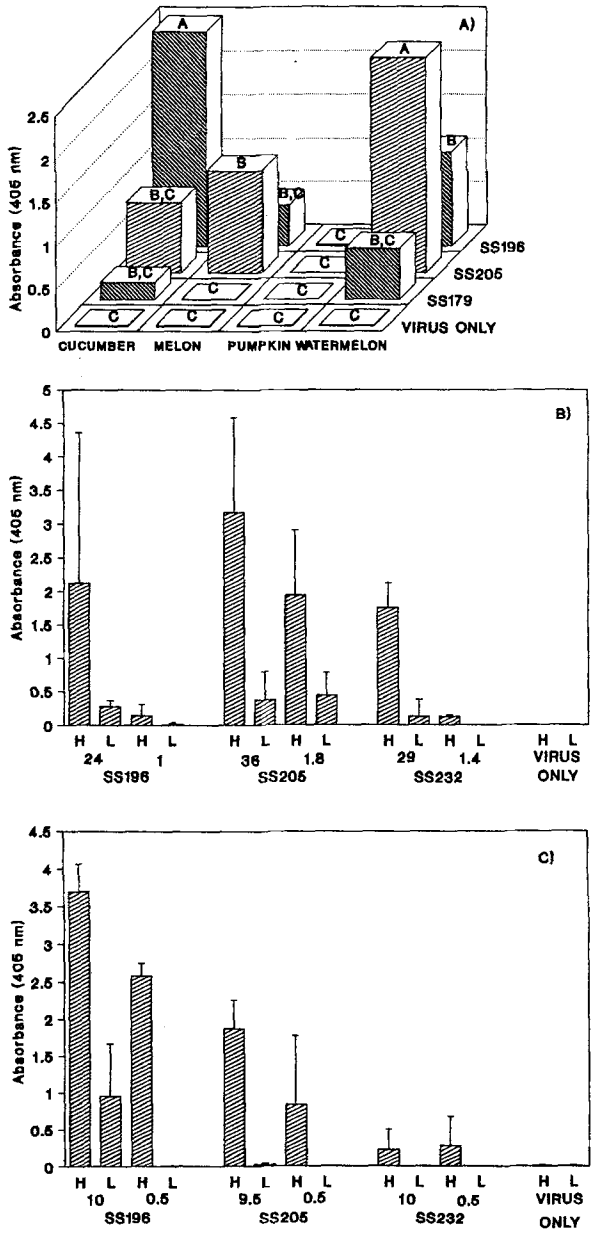

Fig. 3. Absorbance values (A405) with cucumber necrosis virus (CNV) antiserum in double antibody sandwich ELISA of extracts of roots inoculated with in vitro mixtures of CNV and zoospores of *O. bornovanus*. Virus only refers to virus/no fungus control. A) Transmission to four hosts, average A405 values of two replicates in two trials. Bars labelled with the same letter did not differ by ANOVA (LSD = 1.02) B) Transmission efficiency experiment with virus and zoospores at two concentrations using watermelon as the host; virus concentrations were 2X (H) or 0.1X (L) those used in Fig. 1B and the zoospore concentrations were as indicated (no. × 10^4/ml). Error bars indicate positive value of standard deviation. C) As in B, except host was cucumber.

from Montfavet, France, were vectors (Fig. 1E). Isolate SS205M from Montfavet, France, was a poor vector; it produced A405 values that ranged from near zero to low positive levels.

Three isolates were selected for further testing: SS145C had previously failed to transmit SqNV to cucumber seedlings [Campbell *et al.*, 1991], SS205M was a poor vector, and SS179S was associated with SqNV [Lin and Palagi, 1983]. In three host range experiments isolate SS179S was a significantly better vector than the other two isolates and transmitted well to all four hosts (Fig. 4A) whereas SS145C and SS205M transmitted only to watermelon. Isolates SS92C,

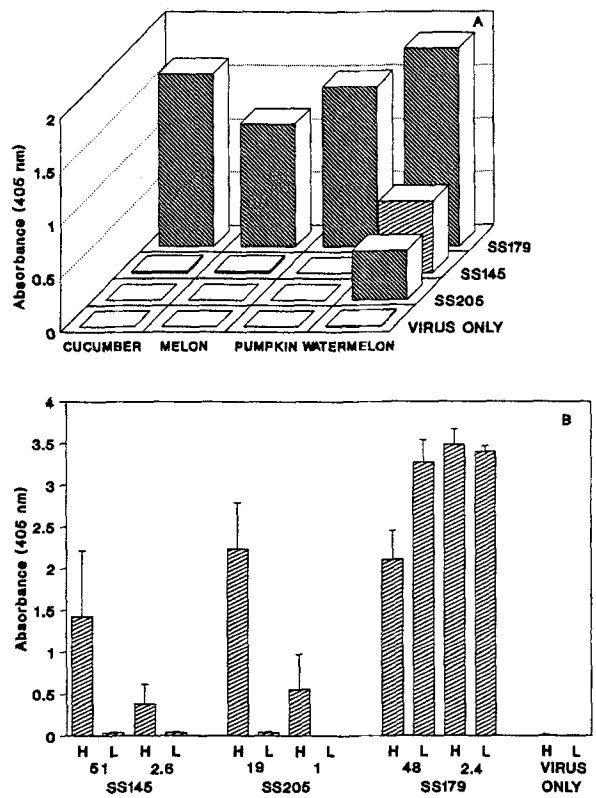

Fig. 4. Absorbance values (A405) with squash necrosis virus (SqNV) antiserum in double antibody sandwich ELISA of extracts of roots inoculated with in vitro mixtures of SqNV and zoospores of *O. bornovanus*. Virus only refers to virus/no fungus control. A) Transmission to four hosts, average A405 values of two replicates in three trials. B) Transmission efficiency experiment with virus and zoospores at two concentrations; virus concentrations were 2X (H) or 0.1X (L) those used in Fig. 1E and the zoospore concentrations were as indicated (no. × 10^4/ml). Error bars indicate positive value of standard deviation.

SS145C, and SS205M transmitted to watermelon but not to cucumber in another experiment (not shown) in which SS179S and SS232S transmitted well to both hosts and SS201M did not transmit to either host.

Vector efficiency was similar in two trials of which one is presented (Fig. 4B). Isolate SS179S efficiently acquired and transmitted SqNV at all combinations of zoospore and virus concentrations while SS145C and SS205M gave similar transmission only at high zoospore and virus concentrations.

Transmission of TNV

Three cucurbit isolates of *O. brassicae* were each tested in two trials and transmitted only TNV with variable efficiency (Fig. 1F). In these trials, an efficient vector, SS225R, transmitted TNV well with 0.8×10^4 zoospores/ml or with 37×10^4 zoospores/ml. An inefficient vector, SS218M, did not transmit in one trial with 7×10^4 zoospores/ml but transmitted well in the next experiment with 34×10^4 zoospores/ml. A non-vector, SS258M, did not transmit with 45 or 60×10^4 zoospores/ml.

The vector ability of three isolates of *O. brassicae* was compared to SS58L in an additional experiment. Isolates SS58L, SS225R, and SS218M transmitted well with 58, 58, and 160×10^4 zoospores/ml, respectively; whereas SS258M with 119×10^4 zoospores/ml did not transmit.

Isolates SS218M and SS225R were compared in two vector efficiency trials as described above except that the high and low virus concentrations were 2 × and 0.5 × or 2 × and 0.2 × those used in Fig. 1F. In both trials, only one of which is presented, isolate SS225R was a more efficient vector than SS218M at all virus and zoospore concentrations (Fig. 5).

Twelve isolates of *O. bornovanus* did not transmit TNV (Fig. 1F). Likewise, three isolates of *O. brassicae* were tested twice with each of the other six carmo- or tombusviruses but did not transmit them.

Transmission attempt with Ligniera sp.

The isolate of *Ligniera* sp. failed to transmit the seven viruses to pumpkin plants in one trial.

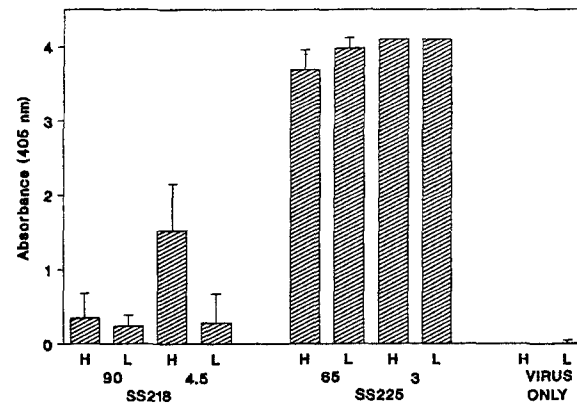

Fig. 5. Absorbance values (A405) with tobacco necrosis virus (TNV) antiserum in double antibody sandwich ELISA of extracts of roots inoculated with in vitro mixtures of TNV and zoospores of *O. brassicae*. Virus only refers to virus/no fungus control. Transmission efficiency experiment with virus and zoospores at two concentrations; virus concentrations were 2X (H) or 0.5X (L) those used in Fig. 1F and the zoospore concentrations were as indicated (no. × 10^4/ml). Error bars indicate positive value of standard deviation.

Discussion

We have demonstrated for the first time that the squash strain of *O. bornovanus* is a specific vector of CSBV and strengthened the evidence that *O. bornovanus* is a vector of SqNV [Lin and Palagi, 1983] by using single sporangial isolates. Although SqNV and CSBV are serologically related [R. Koenig, pers. comm.], their vector specificities differ. Transmission of CSBV and SqNV was not detected in earlier trials [Campbell *et al.*, 1991] because of the fungal strains and bait plants that were used. Likewise, when CSBV was discovered in cucumber roots in Lebanon [Koenig *et al.*, 1983], a fungal vector could not have been identified because the squash strain of *O. bornovanus* infects but does not grow in cucumber roots [Campbell and Sim, 1994].

A fungal vector has been demonstrated for each of the cucurbit-infecting carmoviruses listed by Morris and Carrington [1988] plus SqNV [Lin *et al.*, 1983]. It is proposed that all carmoviruses probably have fungal vectors. The plurivorous strains of *O. bornovanus*, frequently found in red clover [Lange and Insunza, 1977], are the most likely vectors of the legume group of carmoviruses and of RCNMV [Lange and Insunza, 1977], but

the cucurbit strains might also be vectors. Other modes of transmission reported for carmo-, tombus-, and dianthoviruses [Bowen and Plumb, 1979; Gerhardson and Insunza, 1979; Martelli *et al.*, 1988; Morris and Carrington, 1988] should be re-evaluated relative to the involvement of fungal vectors.

Vectors, especially fungal vectors, of tombusviruses have only been proven for CNV [Dias, 1970b] which was transmitted herein by all three cucurbit strains of *O. bornovanus*. On one hand, the list of species or isolates that have not transmitted PAMV or tomato bushy stunt virus (TBSV) [Campbell, 1968; Campbell *et al.*, 1975; Teakle and Gold, 1963] has been increased by 12 isolates of *O. bornovanus* and three of *O. brassicae*. On the other hand, it is not possible to prove that TBSV has no vector. Furthermore, the interactions of virus, host, and fungal vectors demonstrated herein provide reason to search for a vector in sites where the tombusviruses are soil borne.

The present results expand the combinations of viruses, isolates, and vector relationships of *O. bornovanus* available for study of the in vitro mechanism of transmission. Three categories of vector efficiency (efficient or good vectors, inefficient or poor vectors, and non vectors) were described previously in isolates of *O. brassicae* and were correlated with the amount of TNV adsorbed to the zoospores [Temmink *et al.*, 1970]. These categories were found in 12 isolates of *O. bornovanus* and in three isolates of *O. brassicae* from cucurbit hosts in the present study. These categories may represent artificial divisions of a continuum, but they are useful with the present status of knowledge about virus-vector relationships. The isolates of *O. bornovanus* within a given strain generally were similar in vector ability (efficiency) for a given virus, especially when the strain was an efficient vector of the virus. Thus, the designation of the efficient vector for a virus can be made at the strain level in *O. bornovanus*: CLSV-cucumber strain, CNV-cucumber or melon strain depending on the host plant, CSBV-squash strain, and SqNV-squash strain. No testing for vector efficiency was done with MNSV but the development of symptoms when several melon or one squash isolate were the vectors is indirect evidence that these isolates were more efficient vectors than cucumber isolates. Efficient isolates of *O. bornovanus* acquired and transmitted virus to cucurbit hosts whether the plants were compatible or incompatible hosts of the isolate [Campbell and Sim, 1994] similar to the situation with *O. brassicae* and TNV [Kassanis and MacFarlane, 1965]. The only virus-host combination which an efficient vector failed to infect the host with the virus was CNV in pumpkin. The roots of this host might be immune to CNV but the virus can be sap inoculated to cotyledons [McKeen, 1959; unpubl. data]. Inefficient vector isolates required more virus or more zoospores or specific hosts or combinations of all items in order to transmit. The most extreme example was with isolates of the melon and cucumber strains which transmitted SqNV only to watermelon and not to their compatible hosts.

Although several isolates of *O. bornovanus* were rated as non-vectors of some viruses by ELISA, bioassays often yielded a few local lesions (avg. 2–5 local lesions per leaf of *C. quinoa*). This low level of infectivity was noted with CLSV and five melon or squash isolates; with CSBV and five cucumber or melon isolates; and with SqNV and two melon isolates. When compared to the virtual absence of local lesions in the virus/no fungus controls, these low levels of infectivity were interpreted as evidence of a possible weak biological interaction that permitted an extremely low level of transmission either of a few virions or by a few zoospores.

The use of single sporangial isolates has shown that inefficient vector isolates, such as SS205M, may occur within a bulk culture (90–3) in which non-vectors predominated. These results emphasize the importance of using single sporangial cultures for critical work [Campbell, 1988; Lin *et al.*, 1970]. Inefficient vectors of CLSV or SqNV may exist in the fungal population at those sites from which single-sporangial, non-transmitting isolates of the melon strain were obtained.

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