Effect of Temperature on Transmission, Translocation, and Persistence of the Lettuce Big-Vein Agent and Big-Vein Symptom Expression

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ABSTRACT

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Lettuce big-vein agent (BVA) was transferred to lettuce soon after protoplasts of *Olpidium brassicae* zoospores infected lettuce root cells. The BVA was translocated to the top of plants 1 to 4 days prior to symptom expression; the most rapid translocation occurred at 18 and 22 C, and the slowest at 10 C. Translocation occurred at temperatures unfavorable for symptom expression (24 C), and BVA persisted in some shoot tips at this temperature for about 1 mo. Big-vein symptom expression was affected by the temperature of the tops of plants. It was severe if the tops were at 14 C regardless of whether roots were at 14 C or 24 C; virtually no symptoms developed in infected plants if tops were at 24 C and the roots were at 14 C or 24 C. Further attempts to characterize or mechanically transmit BVA were unsuccessful.

Additional key words: *Lactuca sativa*, soil-borne vectors, soil-borne pathogens.

The big-vein disease of lettuce is caused by an infectious, graft-transmissible agent (BVA). *Olpidium brassicae* (Wor.) Dang., a holocarpic chytrid, is the natural vector of this disease agent (2, 3, 4, 5, 8, 17). Campbell et al. (5) concluded that *O. brassicae* survives adverse conditions in the absence of the host as thick-walled resting sporangia and that BVA is borne internally in resting sporangia. Later Campbell and Grogan (4) showed that acquisition of BVA by BVA-free *O. brassicae* isolates occurred in a single vegetative generation. It was postulated that BVA transmission to lettuce also could occur in a single *O. brassicae* generation (4), but this possibility could not be tested until a means for eradication of *O. brassicae* without damaging host roots was developed.

The usual procedure for inoculation with BVA used in this laboratory is to inoculate 4- to 7-day-old lettuce seedlings in a 100-ml pot with 1×10^6 zoospores of a BVA-transmitting isolate of *O. brassicae* (BVA-*O. brassicae*). After 3-4 wk at 16-18 C, vein-banding symptoms are clearly visible. During this period, BVA evidently is released from the *O. brassicae* thallus, presumably multiplies, and is translocated upwards into the tops. The length of the latent period for big-vein symptom development can be shortened by application of larger numbers of zoospores in the inoculum. An inoculum dilution-endpoint for big-vein symptoms and *O. brassicae* infection was reported to be approximately 140-730 zoospores per plant (4). In those trials a mass culture of BVA-*O. brassicae* was used in which it was later shown that only about 50% of the thalli transmitted BVA (8).

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Lettuce growers have observed that big-vein symptoms are more severe in the Imperial Valley than in the Salinas Valley of California. Variability in symptom expression also is observed commonly in plants from the same field and in plants located not more than 25-30 cm apart in the same row. Variability in severity of symptoms produced by strains of BVA has been suggested to account for this variation. Another possible explanation is the influence of temperature on symptom development. Severe symptoms of big vein have been associated with cool temperatures in greenhouse experiments in which both roots and tops of the plants were kept at the same temperature (7, 10, 16). Constant temperatures greater than 22 C prevented big-vein symptom development. No studies have been done to determine whether either soil or air temperatures or both are critical for development of big-vein symptoms or whether BVA is translocated and survives in lettuce when the temperature is too high for symptom development.

Several workers have attempted to characterize BVA, but its nature has not yet been elucidated (3, 4, 8, 17) despite the report by Ragozzino and Furia (11) that mycoplasma-, rickettsia-, and virus-like particles are associated with lettuce plants with big-vein symptoms.

This paper presents data on the time required for *O. brassicae* to transmit BVA to lettuce, and on the effect of temperature and BVA-zoospore concentrations on the time required for the upward translocation of BVA, and for symptom development. The possibility that different strains of BVA are responsible for differences in symptom severity also was examined. Additional attempts were made to transmit mechanically, to characterize, and to visualize with the electron microscope the BVA in infected lettuce, in BVA-*O. brassicae*, and in preparations from infected lettuce tissues.

MATERIALS AND METHODS

Lettuce (*Lactuca sativa* 'Climax') was used throughout these studies because *O. brassicae* reproduces well and big-vein symptoms are readily detected on this cultivar. The plants were grown in pasteurized quartz sand with nutrient solution, or in a pasteurized greenhouse-potting soil. The methods used for maintaining and inoculating with BVA-*O. brassicae*, for making root washings to detect *O. brassicae* infection, for counting zoospores, and for detecting resting sporangia in inocula have been described (3, 6). Graft transmission of BVA to healthy lettuce was done as previously described (5) except that the tobacco necrosis virus assays were omitted.

Elimination of Olpidium brassicae from infected roots. Benomyl (obtained from E. I. duPont de Nemours and Company, Wilmington, DE 19898) at 500 mg/liter was applied to lettuce seedlings previously inoculated with at least 1×10^6 zoospores per seedling. The times chosen for application of benomyl spanned the stages in a vegetative generation of *O. brassicae* (14). Sufficient benomyl solution was applied as a drench from the top of each pot to replace twice the void volume. After 24 hr, nutrient solution was added from the top to flush out the benomyl and individual plants were transplanted into pots of pasteurized sand. In some tests benomyl was applied as a soak by removing the inoculated seedlings from the sand, placing their roots in 10 ml of benomyl solution for 24 hr, rinsing in cool tap water, and planting in pasteurized sand. Root washings were made to check plants for *O. brassicae* survival 6 days and 30 days after the benomyl treatment.

The time required for BVA to move from the roots into the shoots was determined by an excision-rooting method (19) in which the tops of plants were excised by cutting through the hypocotyl, and the top portion was washed free of sand and placed in moist pasteurized sand to allow formation of adventitious roots. The excised tops were covered to reduce transpiration and incubated at 16 ± 2 C. If BVA had been translocated into the excised parts, symptoms of big vein developed in the young leaves that grew from the excised top. Symptoms of big vein were recorded after 10 wk and symptomless plants were incubated for an additional 3 wk before final results were recorded.

RESULTS

Influence of BVA isolates on symptom severity.— Ten lettuce plants with big-vein symptoms ranging from very severe vein clearing and stunting to very mild symptoms were collected from the Imperial Valley, California. The BVA in each plant was graft-transmitted to a healthy lettuce plant. These plants developed symptoms of big vein after 3-4 wk in growth chambers maintained at 16-18 C. A second and third consecutive series of graft transmissions were made in the same manner. All graft-inoculated plants developed symptoms of big vein, but symptom severity was similar in all plants; differences observed in the original field samples were not evident in the controlled environment.

Time required for transmission of BVA to lettuce by Olpidium brassicae.— In eight trials, assays for *O. brassicae* survival were negative at 6 and 30 days after benomyl treatment of infected roots indicating that the fungus had been eradicated. Assuming that the fungus was killed soon after application of benomyl, transmission of BVA occurred in some plants very soon after the fungus had penetrated the host cell; i.e., within about 4 hr after inoculation (Table 1). There was some transmission at all treatment times up to 72 hr at which time the sporangia had matured and formed zoospores (14). Symptom expression in benomyl-treated plants was always less frequent and often less severe (Fig. 1) and the time required for symptom expression was longer (6 to 8 wk as compared with 3 to 4 wk for nontreated controls).

In five other trials in which benomyl was applied as a drench, *O. brassicae* was not detected after 6 days but was detected in assays made after 30 days. Although there were too few zoospores for detection in 6 days, *O. brassicae* had multiplied and was readily detectable after 30 days; apparently a few sporangia of *O. brassicae* had survived the treatment. These results are not included in Table 1.

Influence of temperature and numbers of Olpidium brassicae zoospores on time required for root-to-shoot movement of BVA.— Big-vein symptoms develop most rapidly at 14 or 18 C, more slowly at 10 C, and not at all at 22 C (R. N. Campbell, *unpublished*). Experiments were done to determine the effect of these temperatures on the rate of BVA translocation from roots to shoots. Small pots of healthy seedlings were inoculated with approximately 1×10^6 zoospores per plant and placed in growth chambers operating at constant temperatures. At 2-day intervals from 14 to 30 days after inoculation, the tops of eight replicate plants were tested for the presence of BVA by the excision-rooting technique. The BVA moved into the shoots at all the temperatures tested (Table 2), including 22 C, a temperature at which symptoms were not expressed in the intact, inoculated

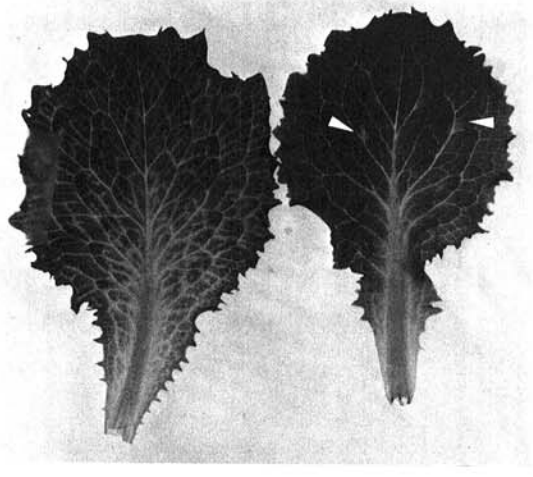

Fig. 1. Comparison of big-vein symptoms on leaves from lettuce plants inoculated with *Olpidium brassicae* zoospores and either drenched with benomyl (500 μg/ml) 24 hr after inoculation (right), or not treated with benomyl (left). Note that there are only a few isolated, chlorotic, vein-banded areas (arrows) on the leaf from the benomyl-treated plant.

TABLE 1. Big-vein infection of lettuce plants after inoculation with *Olpidium brassicae* zoospores and the big-vein agent, followed by treatment with 500 mg/liter of benomyl at times indicated

Treatment time (hours)[a]	Trial number[b]								
	1	2	3	4	5	6	7	8	Total
4	...[b]	...	...	0/4	1/4	0/4	0/8	0/4	1/24
8	...	1/4	...	...	1/4	1/4	1/8	...	4/20
12	...	1/4	...	...	0/4	1/4	0/8	0/4	1/24
16	...	1/4	...	1/4	...	0/4	2/8	...	4/20
20	3/20	1/4	...	0/4	...	1/4	1/8	1/4	7/44
24	...	0/4	0/4	1/4	1/4	0/4	1/8	1/4	4/32
36	...	...	0/4	0/4	1/4	1/4	...	0/4	2/20
48	...	...	1/4	0/4	1/4	1/4	2/8	0/4	5/28
60	...	1/4	1/4	1/4	...	0/4	0/8	...	3/24
72	2/20	1/4	2/4	...	1/4	1/4	2/8	1/4	10/48
Nontreated[c]	18/20	4/4	4/4	3/4	7/8	4/4	8/8	4/4	52/56
Noninoculated[d]	0/20	0/4	0/4	0/4	0/8	0/4	0/8	0/4	0/56

[a]Plants were inoculated with at least 2.5×10^5 zoospores of BVA-*O. brassicae*; 4-72 hr later benomyl was applied as a drench in trials 1-5 or as a 24-hr soak in trials 6-8, and seedlings were transplanted individually into pots. In all trials assays for *O. brassicae* infection at 6 and 30 days after benomyl treatment were negative, which indicated that the fungus had been eradicated.

[b]Results expressed as number of plants with big-vein symptoms during incubation at 16 ± 2 C for 6-8 wk/number tested. Blank = not tested.

[c]Inoculated with BVA-*O. brassicae*, but not treated with benomyl.

[d]All noninoculated plants were free of *O. brassicae* infection and big-vein symptoms at the end of the experiments.

TABLE 2. Effect of constant soil and air temperature on the time required for the big-vein agent to move from *Olpidium brassicae*-inoculated roots to the tops of plants

Sample time (days after inoculation)[a]	Number of plants infected at temperatures of:			
	10 C	14 C	18 C	22 C
14	0	0	0	0
16	0	0	4	2
18	0	1	6	2
20	0	2	6	4
22	1	8	6	6
24	4	8	8	4
28	6	8	8	6
30	8	8	8	7

[a]Test plants were inoculated with $\sim 1 \times 10^6$ zoospores/plant and placed in growth chambers at the indicated temperature. On the indicated sample day, tops of eight plants were excised and transferred to moist pasteurized sand and allowed to root and develop symptoms at 16 ± 2 C for 6-8 wk. Results are expressed as number of plants that developed big-vein symptoms.

TABLE 3. Effect of zoospore concentration in the inoculum on the time required for the big-vein agent to move from BVA-*Olpidium brassicae*-inoculated roots to the tops of lettuce plants

Incubation period prior to excision	Infected plants							Not inoculated
	Approximate number of zoospores per plant							
	7×10^6	7×10^5	7×10^4	7×10^3	700	70	7	
(days)								
18	0[a]	0	0	0	0	0	0	0
22	2	1	3	0	1	1	0	0
26	5	3	3	1	1	1	0	0
30	8	8	6	2	1	1	1	0

[a]Ten test plants in a pot were inoculated with the indicated zoospore concentrations and placed in a growth chamber at 16 ± 2 C for the indicated days at which time the tops were excised, transferred to moist pasteurized sand and allowed to root and develop symptoms at 16 ± 2 C for 4-6 wk. The results are expressed as the number of plants that developed big-vein symptoms/10 replicates.

control plants. Movement of BVA into the upper parts of the plants was most rapid at 18-22 C and slowest at 10 C. At 10, 14, and 18 C, BVA had moved into the shoots of excised plants only 1 to 4 days before symptoms were evident in intact inoculated control plants.

The relationship between the concentration of zoospores used as inoculum and the movement of BVA to the tops of plants was examined with a single-sporangium isolate of BVA-*O. brassicae*. Four 100-ml pots, each with 10 lettuce seedlings, were inoculated with 10 ml of a series of 10-fold dilutions of a zoospore suspension and incubated at 16-18 C. The tops of plants in one pot from each dilution were excised and rooted 18 to 30 days after inoculation. Upward movement of BVA in plants was slower and the number of plants that subsequently developed big-vein symptoms was less when the numbers of zoospores in the inoculum were decreased, but it occurred in one plant inoculated with the highest dilution (seven zoospores/plant, Table 3).

The effect of temperature on symptom development and persistence of BVA.— The effect of soil vs. air temperatures on the development of big-vein symptoms was determined by transplanting infected lettuce plants into containers placed in controlled temperature baths in controlled-environment chambers with light intensity of approximately 10,000 lux for 12 hr daily. Thus, the tops and roots of the plants could be exposed to the same or different temperatures. A temperature of 14 C that permits big-vein symptom development was compared with 24 C that does not. Thermocouples were placed in soil or in leaves and the temperatures were recorded with a recording potentiometer. The actual combinations of air/soil temperatures that were obtained during the light periods were: 24 ± 2 C/24 ± 2 C; 24 ± 2 C/14 ± 1 C; 14 ± 1 C/24 ± 1 C; and 14 ± 1 C/14 ± 1 C. The same temperatures and variations were maintained during the dark periods except that leaves near the soil surface of the 24/14 C treatment were at 20 ± 2 C. Five trials were done; in four trials the lettuce seedlings were transplanted 14 to 21 days after inoculation, and in the fifth trial they were transplanted 5 days after inoculation. The results from all trials were similar and have been combined. Big-vein symptoms did not develop on any of 33 plants when both the air and soil temperatures were 24 C. Mild big-vein symptoms developed on 29 of 33 plants in the 24 C air/14 C soil treatment, but the symptoms were evident only on the leaves near the soil surface. During the dark period the temperature of these leaves dropped to near 20 C. Later, symptoms were milder and even more difficult to detect on leaves borne further above the surface of the 14 C soil; in these leaves the surface temperatures were 22 ± 2 C during the dark period and 24 ± 2 C during the light. Severe big-vein symptoms developed on 27 of 33 plants and 30 of 33 plants in the 14 C/24 C and 14 C/14 C treatments, respectively. Although no symptoms were expressed by intact plants during 6 wk in the 24/24 C treatment, BVA was detected in the tops of three of nine plants by the excision-rooting method. This confirmed that BVA was translocated upwards at 24 C and showed that BVA can persist at this temperature even though symptoms did not develop.

The effect of high temperatures on the persistence of BVA in the aboveground portions of lettuce plants was determined in three experiments. In the first experiment, 6- to 8-wk-old plants with severe symptoms of big vein were used. Three plants were placed at 27 ± 2 C with continuous light and three other plants were placed at 16 ± 2 C with a 12-hr photoperiod. No symptoms developed on the new growth that developed on plants at 27 C; the symptoms in the older leaves gradually decreased in severity and were not evident after approximately 21 days. After 30 days at 27 C, a 10-cm portion of the newly formed, elongated stem was excised and rooted at 16 ± 2 C. Distinct symptoms of big vein developed in each of the three rooted tops within 21-30 days. After excision of the tops, the bottom portion of plants that had been at 27 C were transferred to 16 ± 2 C. New growth from adventitious buds on these plants also developed symptoms of big vein after 21-30 days of incubation. Control plants maintained throughout at 16 ± 2 C had symptoms of big vein when excised and rooted; symptoms continued to develop on the excised tops and on new growth from adventitious buds on the original plants.

In a second experiment the methods were the same except that plants were incubated at 28 ± 1 C with a 12-hr photoperiod and after 25 days a 2-mm portion of the stem apex was excised. New leaves on four of the six stem apices developed symptoms of big vein within 18-21 days when rooted and incubated at 16 C. The six control plants maintained at 16 C throughout the experiment had symptoms of big vein when excised, and symptoms continued to develop on newly formed leaves on excised stem apices.

In a third experiment the roots of five 7-day-old lettuce plants were inoculated with 6×10^6 zoospores per plant and incubated for 5 days at 16 ± 2 C. The plants then were maintained at 24 ± 2 C for 3 wk after which time 2 to 5 mm of the stem apex was excised and rooted. Two of the five stem apices developed symptoms of big vein within 30 days after excision. The plants from which the apices had been cut were held an additional 3 wk at 22 ± 1 C during which time new elongated stems had formed. These new stems were excised, rooted and incubated at 16-18 C for symptom development. After 4 wk, symptoms of big vein had not developed on the leaves of any of the excised apices, but symptoms had developed on the new growth from the roots after transfer to 16-18 C and incubation for 4 wk.

Attempts to characterize BVA.— Attempts to mechanically transmit or to visualize the BVA with the electron microscope were unsuccessful in this study. Details of methods used for each separate attempt are omitted from the following summary. In most attempts, leaves with symptoms of big vein were homogenized in a Waring Blendor at 4 C. The following buffers, solvents, or methods of preparations were tested: tissue was comminuted in 0.5, 0.05, or 0.01 M phosphate buffer (pH 7.2 or 7.6) or in 0.5, 0.05, or 0.01 M borate buffer (pH 9.0) alone or with addition of 0.001 M 1-phenylthiosemicarbazide, 0.01 M Na_2SO_3, 0.1% or 0.2% mercaptoacetic acid, 0.01 M cysteine-HCl, and/or 10-20% sucrose. Extracts from comminuted tissue, after preliminary clarification in a Sorvall GSA rotor at 5,000 rpm, were centrifuged in a Spinco 30 rotor at 27,500 or a 50 rotor at 49,000 rpm, or were precipitated by addition of two volumes of ethanol. Such preparations were injected into approximately 500 lettuce plants with a Hypospray^R

(R. P. Scherer Corp., Detroit, MI 48213) pressure injector (9). No symptoms of big vein developed on any inoculated plants after 4-6 wk of incubation at 16 ± 2 C. Also, no symptoms developed when extracts made by homogenizing tissue with big-vein symptoms in 0.5, 0.05, or 0.01 M phosphate buffer + 0.1% mercaptoacetic acid or 0.01 M cysteine-HCl were not treated with solvents or sedimented, but were directly injected into an additional 150 lettuce plants.

A pathogenic RNA (viroid) has been demonstrated in spindle tuber of potato and citrus exocortis-diseased tissue (13). In a test utilizing similar methods but with BVA-infected tissue, there were no apparent differences in the number of nucleic-acid bands detected in polyacrylamide gels of BVA-infected and healthy preparations. Thus, there was no evidence for the presence of a viroidlike agent.

Ultrathin sections of big-vein tissue and BVA-*O. brassicae* were processed for the electron microscopic examination as described previously (8, 14, 15). The sections were examined in the electron microscope for viruslike particles, rickettsialike organisms, or mycoplasmalike bodies. None was found in the vascular tissues of roots or parenchyma tissues of leaves of diseased plants or in *O. brassicae* sporangia in epidermal cells of roots.

DISCUSSION

Drenching or soaking *O. brassicae*-inoculated plants in benomyl (500 μg/ml) effectively eradicated *O. brassicae*. With this treatment it was shown that BVA is transferred from the *O. brassicae* thallus between zoospore infection of root epidermal cells and maturation of thalli. This is the same time required for acquisition of BVA from infected plants by BVA-free *O. brassicae* isolates (4). Benomyl apparently has other effects than just killing *O. brassicae*. It decreased the number of plants that developed symptoms. In fact, results from some other trials have been omitted here because there was no symptom expression by benomyl-treated plants although nontreated, inoculated controls developed normal symptoms. Benomyl also reduced the severity of symptom expression and the reduction was more pronounced when benomyl was applied as a 24-hr soak than as a drench. The reason for the suppression of symptoms in benomyl-treated plants is not known. It may be due to the effect of benomyl on BVA directly, on multiplication of BVA, on the release of BVA from the *O. brassicae* thallus, on movement of BVA across or through host or vector membranes, on upward translocation, or on the host-BVA interaction which results in symptom production. In this study, as in an earlier one (8), there were fewer chloroplasts with fewer cristae in tissue with big-vein symptoms than in the healthy tissue. Chloroplasts apparently are not destroyed by BVA, but their rate of development is delayed. Thus, benomyl may reduce symptom severity by stimulating chloroplast and/or chlorophyll development or by delaying its destruction (12, 18). The secondary effects of benomyl on frequency and severity of symptom expression should be investigated further.

We have investigated the interrelationship between the effects of temperature on *O. brassicae*, BVA, and lettuce from the time *O. brassicae* infects roots until symptoms are expressed. After introduction, the BVA may multiply in roots and be translocated to tops or perhaps it is translocated to tops where it multiplies. Inability to develop a quantitative assay for BVA or to characterize it in this and earlier studies (3, 4, 8, 17) precludes the determination of sites of multiplication. Nevertheless, it was possible by the excision-rooting method to determine the time when BVA was translocated from roots into tops. The observation that some excised shoots develop symptoms of big vein even before they develop adventitious roots (F. V. Westerlund, *unpublished*) suggests that BVA multiplies in tops once it is translocated there and continual synthesis of BVA in roots and its transport into the top is not essential for symptom expression. The time required for translocation of BVA from roots to tops was affected by temperature and by the concentration of zoospores used for inoculation; the most rapid translocation occurred at 18-22 C and was slowest at 10 C. Translocation occurred even at temperatures at which symptoms were not expressed (22 C), and translocation was most rapid after inoculation with larger numbers of zoospores. This may be similar to the phenomenon reported for curly top virus transmission by leafhoppers in which the percentage of infection was increased by longer acquisition and transmission feeding times (1).

The BVA arrives in the top of the plant only a short time before symptoms are expressed at suitable temperatures. This suggests that its behavior is similar to viruses that cause symptoms following translocation to the growing tip. Also like viruses, BVA can persist for a time at temperatures unfavorable for symptom expression, but excised tops previously held in unfavorable temperatures did not develop symptoms until after incubation at 16-18 C for 21-30 days. This is about the same time required for symptoms to develop on plants inoculated with BVA-*O. brassicae* zoospores. This and the apparent loss of BVA from the tops of some plants kept at high temperatures suggests that the titer of BVA may have decreased in plants kept at 24-28 C. Slower synthesis of BVA may occur at these temperatures than at the 16-18 C optimum for symptom expression, but the explanation for reduction of big-vein symptom severity at the higher temperatures must await development of a method for determination of titer of BVA in plants.

The severity of big-vein symptoms observed in fields is variable. We found no evidence of symptomatological differences among BVA strains. Instead, our results from temperature studies showed that temperatures of shoots of plants was critical for symptom expression. Even if roots were at a warm, suppressive temperature (24 C), severe big vein developed in tops growing at 14 C. This may explain why symptoms on lettuce in Imperial Valley are more severe than in Salinas Valley. During the winter-lettuce season in the Imperial Valley, the ambient temperature, particularly at night, is generally cooler than the ambient temperature in Salinas during the summer-lettuce season.

All attempts to characterize BVA have been unsuccessful. A viroid etiology seems unlikely because BVA differs from known viroids in three characteristics: (i) BVA is not sap transmissible, (ii) BVA-diseased plants

have no viroidlike nucleic acids, and (iii) BVA seems to decrease in hosts grown at temperatures too warm for symptom expression. The BVA may be a virus present in small amounts in specific tissues or in specialized cells within tissues such as companion cells in the phloem, differentiating vascular tissues or chloroplasts. Another equally plausable possibility is that BVA is an entirely unique infectious agent. Unfortunately, the nature of BVA is likely to remain a mystery until some method of transmission other than by the natural vector, *O. brassicae*, or by grafting, is developed that permits quantitative assay.

LITERATURE CITES

1. BENNETT, C. W. 1962. Curly top virus content of beet leafhopper influenced by virus concentration in diseased plants. Phytopathology 52:538-541.
2. CAMPBELL, R. N. 1962. Relationship between the lettuce big vein and its vector Olpidium brassicae. Nature (Lond.) 195:675-677.
3. CAMPBELL, R. N., and R. G. GROGAN. 1963. Big-vein virus of lettuce and its transmission by Olpidium brassicae. Phytopathology 53:252-259.
4. CAMPBELL, R. N., and R. G. GROGAN. 1964. Acquisition and transmission of lettuce big-vein by Olpidium brassicae. Phytopathology 54:681-690.
5. CAMPBELL, R. N., R. G. GROGAN, and D. E. PURCIFULL. 1961. Graft transmission of big vein of lettuce. Virology 15:82-85.
6. CAMPBELL, R. N., and M. T. LIN. 1976. Morphology and thermal death point of Olpidium brassicae. Am. J. Bot. 63:826-832.
7. JAGGER, I. C., and N. CHANDLER. 1934. Big vein, a disease of lettuce. Phytopathology 24:1253-1256.
8. LIN, M. T., R. N. CAMPBELL, P. R. SMITH, and J. H. M. TEMMINK. 1970. Lettuce big vein virus transmission by single-sporangium isolates of Olpidium brassicae. Phytopathology 60:1630-1634.
9. MUMFORD, D. L. 1972. A new method of mechanically transmitting curly top virus. Phytopathology 62:1217-1218.
10. PRYOR, D. E. 1946. Exploratory experiments with the big-vein disease of lettuce. Phytopathology 36:264-272.
11. RAGOZZINO, A., and A. FURIA. 1972. Osservazioni preliminari al microscopio electronico di tessuti di lattuga affetta da ingrossamento nervale (Big Vein). Rev. Pathol. Vegetale. Serie IV, 8:321-322.
12. RUFNER, R., F. H. WITHAM, and H. COLE, JR. 1975. Ultrastructure of chloroplasts of Phaseolus vulgaris leaves treated with benomyl and ozone. Phytopathology 65:345-349.
13. SEMANCIK, J. S., and L. G. WEATHERS. 1972. Exocortis disease: evidence for a new species of "infectious" low molecular weight RNA in plants. Nat. New Biol. 237:242-244.
14. TEMMINK, J. H. M., and R. N. CAMPBELL. 1968. The ultrastructure of Olpidium brassicae. I. Formation of sporangia. Can. J. Bot. 46:951-956.
15. TEMMINK, J. H. M., and R. N. CAMPBELL. 1969. The ultrastructure of Olpidium brassicae. II. Zoospores. Can. J. Bot. 47:227-231.
16. THOMPSON, R. C., and S. P. DOOLITTLE. 1942. Influence of temperature on the expression of big vein symptoms in lettuce. Phytopathology 32:542-544.
17. TOMLINSON, J. A., and R. G. GARRETT. 1964. Studies on the lettuce big-vein virus and its vector Olpidium brassicae (Wor.) Dang. Ann. Appl. Biol. 54:45-61.
18. WANG, D., and E. R. WAYGOOD. 1959. Effect of benzimidazole and nickle on the chlorophyll metabolism of detached leaves of Khapli wheat. Can. J. Bot. 37:743-749.
19. WESTERLUND, F. V., and R. N. CAMPBELL. 1975. Germination and infection of Olpidium brassicae and movement of the lettuce big vein agent. Proc. Am. Phytopathol. Soc. 2:119 (Abstr.).