Soil Factors Affecting the Reproduction and Survival of Olpidium brassicae and its Transmission of Big Vein Agent to Lettuce

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ABSTRACT

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A semi-quantitative method was developed to estimate the relative numbers of resting sporangia of *Olpidium brassicae* in the soil. There were more propagules in a big-vein-prone (BV-P) soil than in a big-vein-intermediate (BV-I) soil and virtually none in a big-vein-suppressive (BV-S) soil. Similar numbers of resting sporangia were produced in roots of inoculated lettuces in BV-P or BV-S soils at matric potentials (ψ_m) fluctuating between 0 and −150 millibars (mb), whether the soils were fumigated with methyl bromide or not. Zoospores of *O. brassicae* remained motile for 12-24 hr in sand-soil mixtures of either BV-P or BV-S soil and they remained infective for 48 hr. Thus, no biological or chemical factors were detected to account for the differences in big-vein occurrence between BV-P and BV-S soils. Resting sporangia in naturally infested, air-dried soils required wetting in nearly saturated soil for about 6 days before they were capable of germination. Once rewetted the resting sporangia germinated whether host roots were present or not; they germinated rapidly at 18 and 22 C and slowly at 10 C. Germination of resting sporangia in BV-P soil only occurred at $\psi_m \geqslant -60$ mb and the number of lettuce plants infected was reduced as ψ_m decreased from 0 to −60 mb. Zoospores were released from vegetative sporangia only when the water content of the sand was equivalent to that at saturation ($\psi_m = 0$). From a point source zoospores moved at least 40 mm in sand at $\psi_m \geqslant -20$ mb and 30-40 mm in BV-P and BV-S soils at $\psi_m = 0$. At lower ψ_m the distance zoospores moved was reduced especially in BV-P soil in which they moved only 10 mm at $\psi_m = -40$. Thus, the most important factor to account for the big-vein proneness of soils seemed to be the soil-water relations and the rapidity with which water drained from the soils.

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

Big vein of lettuce described by Jagger and Chandler (11) is an important problem in many areas of lettuce production. *Olpidium brassicae* (Wor.) Dang., a holocarpic chytrid, is the vector of a graft-transmissible agent that causes big vein (3, 19). Although presumed to be a virus or virus-like, the causal agent has not been characterized and is referred to here as the lettuce big-vein agent (BVA) (12).

Lettuce grown in some fields in the Salinas Valley, California, has been severely affected by big vein, but in other nearby fields the incidence of disease has been consistently lower. In general, the higher incidence of disease has occurred in fields with heavy, poorly drained soils (clay or clay loams) whereas plants growing in sandy or sandy-loam soils with better drainage have had less disease. For convenience, soils associated with a history of big vein will be referred to as big-vein-prone (BV-P) and those nearly free of big vein will be referred to as big-vein-suppressive (BV-S). Low air and soil temperatures and high soil moisture increase both the severity of symptoms and the incidence of big vein (15, 16, 18) presumably because cool, wet soil conditions favor the vector. This study examines the behavior of *O. brassicae* in soils from the Salinas Valley with and without a history of big vein, and the influence of soil moisture on the various stages of the life cycle of *O. brassicae*.

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MATERIALS AND METHODS

Biological materials.—Isolates of *Olpidium brassicae* (Wor.) Dang. carrying the big vein agent (BVA-*O. brassicae*) were trapped from various soils. Approximately 25 g of soil was incorporated into pasteurized quartz sand in 100-ml pots and lettuce (*Lactuca sativa* L. 'Climax') seeds were sown and incubated at 16-18 C for 3 wk. The soil and sand were rinsed from the roots of the plants and the roots were immersed in tap water which was examined after 15 min for zoospores of *O. brassicae*. The water in which roots were immersed (root washings) was transferred to 100-ml pots containing sand and 3- to 7-day-old lettuce seedlings. Single-sporangial isolates were obtained from these bulk cultures of BVA-*O. brassicae* and either were maintained on living lettuce plants or stored as resting sporangia in air-dried roots (4, 12).

Zoospore suspensions usually were prepared in tap water and kept in an ice bath or refrigerated at 4 C prior to estimation of numbers of zoospores or use as inocula. The numbers of zoospores were counted in a hemocytometer using phase-contrast microscopy (1). Quantitative root washings were made to determine the number of zoospores produced in the roots of a pot of seedling plants

inoculated with a zoospore suspension. The inoculated plants were irrigated on the 2nd day after inoculation, the excess water was discarded, and the plants were covered with plastic bags to avoid desiccation and the need for additional watering until the 5th day. Then the number of zoospores released into a known volume of water in a standardized time (usually 15 min) was counted and divided by the number of plants. These zoospores represent nearly the total zoospore production from the first generation of vegetative reproduction (8, 12). The presence of resting sporangia in zoospore preparations was determined by adding samples of the spore suspension to dry sand in pots and allowing them to air dry for 3 days or more before sowing bait plants. Resting sporangia, but not zoospores, can survive air drying and infect the bait plants (4). The detection of infection of lettuce plants by *O. brassicae* and BVA was done by three methods: (i) root washings were examined for the presence of motile zoospores of *O. brassicae* after the roots were immersed in water for 15 min. If a root washing contained too few zoospores for immediate visual detection it was transferred to sand containing lettuce plants and incubated for 3 days or more at 16 ± 2 C before a final determination of infection by *O. brassicae* was made; (ii) epidermal root cells were examined with a microscope for the presence of vegetative sporangia or resting sporangia; or (iii) leaves were examined for symptoms of big vein.

Zoospores were detected in soil, or in sand, or a 1:1 (w/w) mixture of soil and sand incubated at 16-18 C in 100 ml pots by sampling the water that drained from the bottom of pots after watering. The 3- to 5-mm diameter opening in the bottom of each plastic pot was covered with a cellulose acetate or polypropylene filter that was glued in place. The maximum pore size of these filters was 10 μm which permitted passage of zoospores, but not resting sporangia. At predetermined intervals, enough tap water was added to the top of each pot to completely displace the void volume of the soil and/or sand. Water that drained from the pot was collected and the number of motile zoospores was estimated with a hemocytometer. One-fourth of the drainage water from each pot was added to each of two pots of lettuce seedlings to assay for the presence of *O. brassicae* and the other half was divided and added to two replicate pots of sand and allowed to air dry prior to assay for the presence of resting sporangia.

The standard method used for detection of *O. brassicae* in soil has been to sow lettuce seeds in 5-25 g of air-dry soil mixed with 50 g of sand, incubate for 3 wk at 16 ± 2 C, and make a root washing to detect zoospores of *O. brassicae*. Although this method consistently detects *O. brassicae* in the soil, it is not quantitative. Thus, the following semi-quantitative method was developed. Lettuce seeds that had germinated on filter paper or sand were transferred onto the surface of air-dried soil that was rewetted and maintained at saturation by daily additions of water or that was maintained at a constant matric potential (ψ_m) of 0 millibars (mb) by the methods described below. The bait plants were incubated at 16 ± 2 C for the desired period of time, removed, thoroughly washed to remove adhering soil, and transplanted individually into 100-ml pots of pasteurized sand. After additional growth for 2-3 wk at 16 ± 2 C to permit increase of *O. brassicae*, the plants were assayed for *O. brassicae* infection. The numbers of infected seedlings were used as a relative measure of the numbers of resting sporangia in the initial soil sample.

Soils.—Soil was collected at depths between 1-30 cm from three fields on which at least one crop of lettuce had been grown during each of the past 20-30 yr. The three soils had the following history and physical make-up: (i) big-vein-prone soil (BV-P) (Salinas clay, containing 48% clay, 38% silt, and 14% sand) in which 80-100% of the lettuce plants in spring crops consistently have had severe big vein symptoms; (ii) big-vein-intermediate soil (BV-I) (Mocho silty clay loam, containing 33% clay, 59% silt, and 8% sand) in which about 4% of the lettuce plants in spring crops have had big vein; (iii) big-vein-suppressive (BV-S) (Metz fine sandy loam, containing 18% clay, 24% silt, and 58% sand) in which nearly all lettuce plants in spring crops have been symptomless. The soil was sieved through screens (1.7-mm openings) and used immediately ("fresh" field soil) or air-dried. Samples of each soil were freed of *O. brassicae* by fumigation with methyl bromide (112 g/m^3) or by autoclaving for 1 hr at 121 C on two successive days. After aeration for several days, the samples were stored at room temperature (approximately 20 C). Soil extracts were prepared by mixing soil and distilled water (1:2,w/v), shaking overnight on a rotary shaker, and centrifuging.

Soil moisture.—The effect of moisture on *O. brassicae* was examined by using Büchner funnels with fritted-glass plates to control the matric (ψ_m) component of soil water potential (ψ). Matric potential is equivalent to but opposite in sign from soil moisture suction or tension, and ψ_m values are given in millibars (mb). The ψ values of soil adjusted to known values of ψ_m were measured with thermocouple psychrometers (5) and the solute potentials (ψ_s) of the soil solution was calculated as $\psi_s = \psi - \psi_m$. The mean ψ_s values of BV-P, BV-I, and BV-S in two experiments were −380, −370, and −410 mb, respectively.

Each 90-mm-diameter funnel was filled to a depth of 1.5 to 2.5 cm with air-dried soil or sand. The porous plate that supported the soil was the reference point for adjusting ψ_m values with water columns. Once the soil or sand was in place, it was wetted from the top and maintained at saturation for 24 hr. Soils then were consolidated to bulk densities of 1.2 to 1.3 g cm^{-3} by raising the funnels of saturated soil and allowing the soil water to drain until ψ_m became −150 or −300 mb. Before soil was adjusted to the desired ψ_m values, the funnels were once again lowered so that water would rise through the porous plates to saturate the soil before funnels were set to their desired height. If only $\psi_m = 0$ was desired, 60-70 g of air-dried soil was placed in open petri dishes and kept saturated by addition of tap water daily. The final bulk densities and water contents of soils were determined by removing known volumes of soil from the funnels and drying to a constant dry weight at 105 C. The relationship between ψ_m from 0 to −150 mb and water content of the three soils and sand is shown in Fig. 1.

Zoospore movement.—To determine the extent of active horizontal movement of zoospores, funnels were filled to a depth of 1.5 cm with samples of pasteurized sand or methyl-bromide-fumigated BV-P or BV-S soil. The funnels were adjusted to $\psi_m = 0$ and 2-day-old bait plants were transplanted into the consolidated sand or soil. A drop of water was added to determine the size of the future inoculum site and the roots were placed at

measured distances from the edge of the inoculum site in spiral patterns so that plants near the site of inoculation would not interfere with zoospore movement to plants at greater distances. After 2 days, the funnels with sand were set at $\psi_m = -30$ mb and those with the soils at $\psi_m = -50$ mb. Then the zoospore suspension was added dropwise so that it moved immediately down into the sand or soil before the next drop was added. At least 5×10^5 zoospores were added in 1.5-2.5 ml of suspension at each inoculation site. Soon after inoculation, moisture potentials in the funnel were adjusted to the desired ψ_m values. After 24-48 hr, pairs of bait plants from each distance being tested were removed, washed thoroughly, transplanted together into pots of sand, and incubated for 2 wk before assaying for infection by *O. brassicae*. The 24- to 48-hr period used for zoospore movement was too short for the production of secondary inoculum of *O. brassicae*.

RESULTS

Inoculum levels in natural soils.—The number of infected seedlings in the semi-quantitative test for *O. brassicae* in soils was highest in the BV-P soil, intermediate in the BV-I soil, and lowest in the BV-S soil (Table 1). The test was repeated three more times with similar results. A larger proportion of infected bait plants was interpreted to be evidence for a relatively larger number of resting sporangia in the BV-P soil. The standard method consistently detected *O. brassicae* in BV-P and BV-I soils, but did not indicate the relative numbers of resting sporangia in the soils (Table 1).

Big vein development in natural soils.—Nontreated BV-P soil was mixed with nontreated BV-S soil in the proportions shown in Table 2. Each mixture was placed in two replicate 10 × 30-cm trays, saturated with tap water, and held for 24 hr. Ten healthy plants were transplanted into each tray and the length of time required for big vein symptoms to develop was recorded. A longer time was required for symptom development in soil mixtures with increased proportions of BV-S soil, but the number of plants with big vein symptoms after 50 days was nearly the same in all treatments (Table 2). The delay in symptom development was not longer than that expected from dilution of the inoculum in the BV-P soil by the lightly infested BV-S soil (2).

Behavior of O. brassicae in soil extracts.—Soil extracts were made from fumigated, autoclaved, or nontreated BV-P and BV-S soils. A portion of the extract from the nontreated soil was autoclaved for 15 min at 121 C and allowed to cool prior to use. Extracts were mixed 1:1 v/v with a zoospore suspension and incubated at 8 or 20 C. The duration of zoospore motility was estimated by periodically removing samples of the mixtures and observing motile zoospores with phase microscopy.

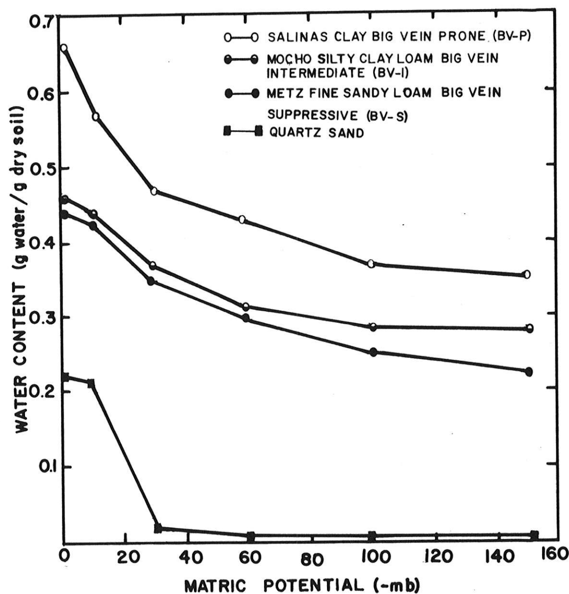

Fig. 1. Relationship between water content and matric potential determined during the release of water from big-vein-prone (BV-P), big-vein-intermediate (BV-I), big-vein-suppressive (BV-S) soils, and quartz sand (QS).

TABLE 1. Presence of *Olpidium brassicae* in big-vein-prone (BV-P), big-vein-intermediate (BV-I), and big-vein-suppressive (BV-S) soils

Soil type	Standard[a] method	Semi-quantitative method[b]			
		Exposure time of bait plants			
		6 days	8 days	12 days	21 days
BV-P	12/12[c]	2/5[d]	5/5	5/5	10/10
BV-I	10/12	1/5	2/5	1/5	8/10
BV-S	0/12	0/5	0/5	0/5	1/10

[a]Lettuce seeds were sown in 5-25 g of air-dry soil mixed with 50 g of sand and incubated at 16 ± 2 C for 3 wk; infection was detected by examination of root washings for zoospores.

[b]Germinated lettuce seeds were transferred onto the surface of soil that was maintained at saturation by daily addition of water or that was maintained at a constant matric potential of 0 mb in Büchner funnels; seedlings were removed, washed free of soil, planted in sand and grown for 2 wk before assay for *O. brassicae* infection.

[c]Results are expressed as the number of pots of plants infected by *O. brassicae*/the total number of pots tested.

[d]Results expressed as the number of plants infected by *O. brassicae*/ total number of plants tested.

TABLE 2. Incidence of lettuce big-vein in plants grown in different proportions of naturally infected big-vein-prone (BV-P) and big-vein-suppressive (BV-S) soil

Proportion of suppressive/ prone soil (wt/wt)	Incubation period (days)		
	30	40	50
	Number of plants infected[a]		
0:4	15	17	19
1:3	11	13	16
2:2	12	13	16
3:1	7	10	16
4:0	0	0	0

[a]Total number of plants with big-vein symptoms in two 10-plant replications.

Zoospores remained motile for 25-26 hr at 8 C and 4-6 hr at 20 C regardless of the soil type, treatment of the soil, or treatment of the extracts. Zoospores in suspensions diluted with pure distilled water remained motile for 19 hr at 8 C and for 4-6 hr at 20 C.

Soil extracts from nontreated BV-P and BV-S soils were used to examine their effect on the infection and replication of *O. brassicae* in lettuce. Seedlings in small pots of sand were drenched with sufficient BV-P or BV-S extracts to replace twice the void volume of the sand. Equal numbers of zoospores suspended in the same extracts used to flush the pots were added to each of three replicate pots of seedlings that were incubated at 16 ± 2 C. After 2 days the pots were irrigated with the soil extracts and the excess was removed. A quantitative root washing was made on the 5th day. An average of 3.8×10^5 and 3.5×10^5 zoospores were liberated per plant grown in the BV-P and BV-S extracts, respectively. Additionally, five seedlings were transplanted from each tray and incubated for big-vein symptoms. After 5 wk, 12 of 15 plants from the BV-P treatment and 11 of 15 plants from the BV-S treatment had developed big-vein symptoms.

Duration of zoospore motility in soil.—The time that zoospores remained motile in saturated sand, or in mixtures of sand and nontreated BV-P, BV-I, or BV-S soils without bait plants was determined in two experiments. Sufficient zoospore suspension was pipetted into each pot of sand or mixture of sand and soil to replace the void volume. The drainage water that passed through a 10-μm (pore-size) filter in the bottom of each pot was collected from two different replicate pots at each sampling time. Motile zoospores were observed in the water from all three soils for up to 12 hr and from sand for up to 24 hr (Table 3). Nonmotile zoospores with flagella or thicker walled, cyst-like structures were observed at each sampling through 48 hr, and *O. brassicae* was infectious in all samples through 48 hr. It was not possible to determine whether the infectivity in the 48-hr samples with no motile zoospores was due to: (i) nonmotile zoospores; (ii) encysted zoospores; or (iii) a few, nondetected, motile zoospores. Infectivity was not due to resting sporangia because assays to detect resting sporangia were all negative. A second experiment was done to obtain a more quantitative estimate of the number of infections resulting from the original inoculum. The method described above was used, except that after the drainage water was inoculated onto lettuce seedlings, a quantitative root washing of these plants was made 5 days later. The number of zoospores liberated was directly related to the number of motile zoospores contained in the original water drained from the soil (Table 4).

Formation of Olpidium brassicae resting sporangia in various soils.—The ability of *O. brassicae* to become established in BV-P, BV-I, and BV-S soils was tested. One portion of each soil was fumigated with methyl bromide

TABLE 3. Comparison of the time zoospores of *Olpidium brassicae* remained motile and infectious in three nonsterilized field soils or in pasteurized quartz sand

Sampling time (hr)[a]	Zoospores[b] Sand ($\times 10^{-4}$)	BV-S[c] ($\times 10^{-4}$)	BV-I[c] ($\times 10^{-4}$)	BV-P[c] ($\times 10^{-4}$)
0	91.3	59.2	61.2	56.3
4	47.5	39.1	41.3	30
8	33.3	7.2	10.1	6.1
12	21.3	1.1	2.3	1.5
24	4.9	0	0	0
48	0	0	0	0

[a]Time from addition of zoospores to substrate until water was added to flush zoospores into the drainage water.

[b]Number of motile zoospores/ml $\times 10^{-4}$ observed in drainage water. All drainage water samples assayed positive for *O. brassicae* infectivity when transferred directly to lettuce bait seedlings, but were negative for *O. brassicae* resting sporangia.

[c]Abbreviations: Big-vein-prone soil (BV-P); big-vein-intermediate soil (BV-I); and big-vein-suppressive soil (BV-S).

TABLE 4. Retention of motility and relative infectivity (RI) of zoospores in three nonsterilized field soils

	Soils[b]					
	BV-S		BV-I		BV-P	
Sampling time (hr)[a]	Zoospores[c] ($\times 10^{-4}$)	RI[d] ($\times 10^{-4}$)	Zoospores[c] ($\times 10^{-4}$)	RI[d] ($\times 10^{-4}$)	Zoospores[c] ($\times 10^{-4}$)	RI[d] ($\times 10^{-4}$)
0	59.4	37.1	40.6	171.3	44.4	102.5
2	40.6	67.5	20.4	58.8	38.8	140.6
4	28.2	70	17.1	95	13.4	57.5
8	7.9	43.1	8.0	63.1	2.7	33.8
12	1.1	2.5	3.8	7.3	0.88	2.7
24	0	1/2[e]	0.31	0.063	0.06	1/2[e]
48	0	2/2[e]	0	2/2[e]	0	1/2[e]
72	0	1/2[e]	0	2/2[e]	0	2/2[e]

[a]Time from addition of zoospores to substrate until water was added to flush zoospores into the drainage water.

[b]Abbreviations under "soils" mean: big-vein-suppressive soil (BV-S), big-vein-intermediate soil (BV-I), and big-vein-prone (BV-P).

[c]Number of motile zoospores observed per milliliter in drainage water.

[d]Relative infectivity (RI) is expressed as the average number of zoospores $\times 10^{-4}$/ml released from first generation sporangia produced by inoculation of two replicate pots of bait plants with zoospores in drainage water.

[e]No zoospores were detected from first generation sporangia, but each duplicate set of bait plants were repotted and incubated for 8 days. Figures indicate number of replicates infected (numerator) per the total number tested (denominator).

whereas another portion was not fumigated. After fumigation and aeration, all soils were checked for viable *O. brassicae* by the standard method. *Olpidium brassicae* was not detected in any of the fumigated soils or in the nonfumigated BV-S soil, but it was present in the nonfumigated BV-P and BV-I soils. The treated and nontreated samples of each soil were divided into thirds that were each placed in a 15-cm diameter clay pot. Three 2-wk-old lettuce plants infected with BVA-*O. brassicae* were transplanted into each pot. During incubation for 8 wk at 16-18 C, soil moisture was monitored with tensiometers and maintained at between 0 and −150 mb ψ_m by adding water or, once a week, nutrient solution. After incubation the tops were removed; each soil and root mass was broken up, mixed, and air-dried for 2 wk at 16-18 C. The relative number of *O. brassicae* resting spores in each replicate of each treatment was determined by the semi-quantitative method (five bait plants/replicate/ infection period). Only one bait plant was infected after 6 days in fumigated BV-P soil, but nearly every bait plant in all the soil samples was infected after 8 or 10 days. Thus, there was no evidence for biological differences between the soils, because zoospores remained motile for the same time in each soil or soil extract, and about the same number of resting sporangia were produced in the roots of lettuce plants in all three soils whether fumigated or not.

Influence of soil moisture and temperature on the germination of Olpidium brassicae resting sporangia.—The effect of temperature on the germination of *O. brassicae* resting sporangia and infection of lettuce roots was determined by using the natural inoculum in air-dried BV-P soil. The semiquantitative test was done in controlled-temperature growth chambers at 10, 14, 18, and 22 C. Resting sporangia of *O. brassicae* did not germinate and infect the bait plants until the 6th day, and infection was more rapid at 18 and 22 C than at 14 and 10 C (Table 5).

The effect of ψ_m on the germination of *O. brassicae* resting sporangia in air-dried BV-P soil was studied in several experiments. The resting sporangia of *O. brassicae* germinated and bait plants were infected after incubation at constant ψ_m values from 0 to −60 mb at 18 ± 2 C, but not at greater tensions (Table 6). In another experiment, naturally infested BV-P and BV-I soils were compared. Of the eight bait plants incubated in BV-P soil for 10 days, *O. brassicae* infected eight, three, one, zero, and zero plants at ψ_m = 0, −40, −60, −100, or −150 mb, respectively. In the BV-I soil, only three of eight plants were infected at ψ_m = 0, and none was infected at the greater tensions. To test further the effect of ψ_m on germination of resting sporangia, funnels with BV-P soil and eight bait plants were incubated at ψ_m = 0. At daily intervals up to 14 days, one replicate funnel was adjusted to ψ_m = −150 mb, a tension at which infection had not occurred in the previous experiments. After the 14th day, all the plants were removed from soil, washed thoroughly, and transplanted individually into sand and incubated for 10 days before *O. brassicae* infection was determined. None of the plants kept at ψ_m = 0 mb for 1 to 6 days was infected, but all the plants held at ψ_m = 0 for 8 or more days were infected.

The combined effects of rewetting air-dry soil for various periods of time, and of ψ_m on germination of the resting sporangia and infection were tested. Air-dried BV-P soil was saturated and kept at ψ_m = 0 for either 1 day or 6 days; the soil then was adjusted to ψ_m = 0, −40, −60, −100, or −150 mb, the bait plants were added and grown for 4 or 8 more days. If soil was rewetted for 1 day and bait plants were exposed for 4 days, none was infected at any ψ_m tested. However, six, two, and zero of eight bait plants exposed for 8 days were infected at ψ_m of 0, −40, −60 mb, respectively. If soil was rewetted for 6 days, bait plants exposed for either 4 or 8 days were infected. There were eight, two, and zero infected plants in 4 days at ψ_m of 0, −40, and −60 mb, respectively, whereas eight, four, and one were infected in 8 days. None of the bait plants at −100 or −150 mb was infected. The results confirmed that decreasing the ψ_m value of BV-P soil increased the time required for infection of *O. brassicae* and decreased the probability of infection. Also resting sporangia in air-dried soils required rewetting for several days before they were capable of germination and infection.

In these trials lettuce bait plants were not infected until the 6th day in soil that had been stored air dry and rewetted just prior to testing. To determine if air drying affected germination of resting sporangia, air-dry soil and freshly collected soil from the same field were compared. Bait plants were added to soil kept at ψ_m = 0 mb and removed after 2, 4, 6, or 8 days of incubation at 18 ± 2 C. Ten of 10 bait plants were infected after 2 or more days in freshly collected field soil, but in previously air-dried soil, zero, zero, six, and 10 of 10 bait plants were infected after 2, 4, 6, and 8 days of incubation, respectively.

To determine if host roots were necessary for germination of resting sporangia, air-dried BV-P soil was placed in funnels, wetted and kept at ψ_m = 0. Bait plants were transplanted into half of the funnels on day 0; the

TABLE 5. Effect of temperature on time required for *Olpidium brassicae* resting spores in naturally infested, big-vein prone soil to germinate and infect lettuce plants

	Exposed period						
Temperature (C)	4 (days)	6 (days)	8 (days)	10 (days)	12 (days)	14 (days)	16 (days)
10	0[a]	0	0	0	1	3	7
14	0	0	1	6	7	8	8
18	0	2	5	8	6	8	8
22	0	1	4	7	8	7	8

[a]Number of lettuce plants infected by *O. brassicae* out of eight exposed plants. Soils were maintained saturated during exposure of seedlings by daily addition of water.

plants in one funnel were removed and assayed for *O. brassicae* daily from days 1 to 8. On these same days 3-day-old bait plants were transplanted into another funnel that contained only wet soil. These plants were exposed for 24 hr, removed, washed, and assayed for *O. brassicae* infection. A separate funnel was used for each sample and treatment. None of the plants in any of the treatments became infected until day 6. On day 7, four of six plants and five of six plants were infected from soils incubated the previous 6 days with water only or with plants and water, respectively. Six of six plants from both treatments were infected on day 8. In three other experiments, air-dried soil was mixed with sand and placed in 100-ml pots with a filter covering the drainage hole. The soil-sand mixture was saturated with water for 24 hr before 1-day-old plants were transplanted into half of the pots. At intervals from the 4th to the 14th day after planting, drainage water was collected and examined microscopically for zoospores. It then was added to bait plants to check for infectivity or to sand and allowed to dry to check for resting sporangia. Zoospores were not observed by microscopic examination in any of the collections of drainage water from pots without host plants but were observed in the drainage water from a few replicates with host plants after 10 days and in all replicates with host plants after 14 days. These zoospores evidently had been released from vegetative sporangia in the plant roots. Infectivity was detected in some samples of drainage water from all sampling dates from 4 through 14 days indicating that *O. brassicae* resting sporangia had germinated whether host roots were present or not (Table 7). The infective propagules in the drainage water apparently were zoospores rather than resting sporangia because all assays for resting sporangia were negative.

If resting sporangia germinate in wet soil in the absence of the host roots, it might be possible to induce germination by saturating the soil prior to planting and thereby reduce the incidence of big vein in subsequent crops. This possibility was tested by placing air-dried soil in duplicate funnels that were incubated in a flooded condition ($\psi_m = 0$) or at ψ_m values of -50, and -300 mb or in air dry condition for 3 wk at 20 ± 2 C. Soil was removed from each replicate of each treatment after 1, 2, and 3 wk, air dried for 1 or more wk at 18 ± 2 C and tested for *O. brassicae* by the standard method. *Olpidium brassicae* was detected in all samples regardless of the ψ_m value. Thus, all resting sporangia were not induced to germinate within the short time of 3 wk by wetting the soil.

The effect of matric potential on the movement and infection of lettuce by Olpidium brassicae zoospores in soil and sand.—In two experiments, zoospores added to sand at 18 ± 2 C swam through sand at $\psi_m = 0, -5, -10$, or -20 mb and infected all seedlings located at 2, 5, 7, 10, and 40 mm from the inoculation site. Longer distances were not tested. In BV-S soil, zoospores swam 30-40 mm at $\psi_m = 0$ mb, but their movement was reduced at $\psi_m = -20$ or -40 mb (Table 8). In contrast, zoospores were even more restricted at each ψ_m value in BV-P soil (Table 8).

Effect of matric potential on formation of Olpidium brassicae sporangia.—Funnels containing sand and 25 seedlings were set at $\psi = 0$ mb and 2×10^6 zoospores were added. After 4 hr to allow the zoospores to infect, the funnels were adjusted to ψ_m values between 0 and -40 mb and incubated for 5 days at 16 ± 2 C. The relative numbers of vegetative sporangia produced per root system were estimated by making a quantitative root washing. In one experiment the plants and sand were not initially covered to prevent evaporation and the plants in the sand at $\psi_m \leq -30$ mb wilted within 24 hr after inoculation. Petri dish covers were placed over the funnels and plants regained turgor within 12 hr. The number of zoospores ($\times 10^{-4}$) produced per root system were 4.5, 6.1, 4.3, 4.7, 0.4, and zero at $\psi_m = 0, -10, -15, -20, -30$, and -40 mb, respectively. In a second experiment the same ψ_m values were tested, but all funnels were kept covered to prevent water loss and wilting of the plants; 4.5, 3.6, 4.6, 3.2, 5.1, and 4.3×10^4 zoospores were produced per root system at the respective matric potentials.

The effect of matric potential on the release of zoospores from sporangia.—The effect of ψ_m on zoospore release was tested in three experiments. In the first experiment plants in sand were inoculated with zoospores, incubated for 4-8 hr at $\psi_m = 0$ to allow infection, and then adjusted to $\psi_m = -40$ mb. After 5 days, funnels were set to give soil ψ_m values from 0 to -15 mb.

TABLE 6. The effect of matric potential (ψ_m) on time required for germination and infection of lettuce seedlings by *Olpidium brassicae* resting spores in naturally infested big-vein-prone soil at 18 ± 2 C

Matric potential (millibars)	Exposure period of bait plants 4 (days)	6 (days)	8 (days)	10 (days)	12 (days)
0	0[a]	4	6	8	8
−40	0	2	2	3	3
−60	0	0	0	1	1
−100	0	0	0	0	0
−150	0	0	0	0	0

[a]Number of bait seedlings infected by *O. brassicae* from total of eight that were exposed.

TABLE 7. Effect of lettuce roots and time of incubation at 16-18 C on the germination of *Olpidium brassicae* resting sporangia in naturally infested big-vein-prone soil

Incubation period (days)	Lettuce plants in soil during incubation: Present	Absent
4	7[a]	7
6	13	10
8	12	11
10	18	6
14	18	4

[a]Water was added to three replicate pots of soil infested with *O. brassicae* resting sporangia after different periods of incubation at 16-18 C and the drainage water was collected. The presence of zoospores of *O. brassicae* was detected by inoculating one-fourth of the drainage water from each pot to each of two pots of lettuce seedlings. Results are expressed as number of pots with infected lettuce seedlings from a total of 18 that were inoculated in three trials. The other half was divided and added to two pots of sand and allowed to air dry for 3 days to test for *O. brassicae* resting sporangia; all of these assays were negative.

At intervals samples of sand were removed to determine water content and samples of water were removed from the sand in each funnel with a Pasteur pipette to detect motile zoospores. Zoospores were released at ψ_m values of 0 and −5 mb (Fig. 2). These ψ_m values resulted in water content of about 0.19 g/g dry sand which is nearly that of sand equilibrated at $\psi_m = 0$ (Fig. 1). Zoospores were not released at the drier values of $\psi_m = -10$ or −15 mb where the water content after 120 min was 0.072 and 0.018 g H_2O/g dry sand, respectively.

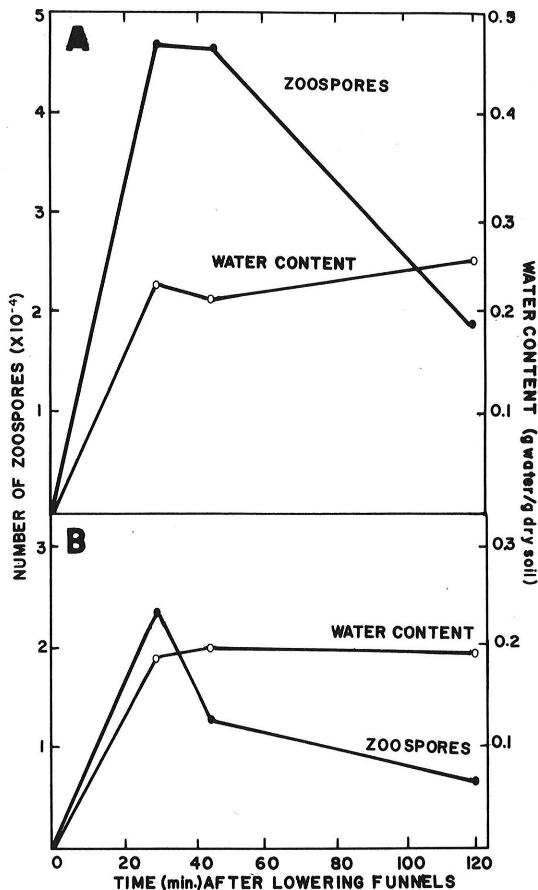

Fig. 2-(A, B). The effect of matric potential on the release of zoospores from sporangia of *Olpidium brassicae*. Infected plants were incubated at $\psi_m = -40$ mb for 5 days then the funnels were lowered to give final ψ_m values of **A**) 0 and **B**) −5 mb. At intervals samples of sand were removed to determine water content and samples of water were removed from the sand in each funnel with a Pasteur pipette to detect motile zoospores.

In a second experiment, the methods were similar except that on the 5th day after inoculation the funnels were set to ψ_m values of 0, −5, −10, or −15 mb and maintained at these ψ_m values for 10 days. The plants then were removed, washed and placed in 0.5 M glycine buffer to prevent further zoospore release (12) and their roots were examined microscopically. If zoospore release and movement occurred at any of the ψ_m values during the 10-day period, there should have been at least two cycles of vegetative reproduction, and *O. brassicae* should have infected the young, distal portions of the roots as well as the older portions which were infected by the initial inoculum. Many *O. brassicae* sporangia were present in the distal portions of roots held at $\psi_m = 0$ or −5 mb; however, there were no sporangia in the distal portions of the roots from treatments at $\psi_m = -10$ or −15 mb.

Because the water content of sand during dewatering was nearly the same at $\psi_m = 0$, −5, and −10 mb (Fig. 1), it seemed likely that zoospores should have been released in sand at $\psi_m = -10$ mb, but were not because the hysteresis effect during water regain prevented a sufficient increase in water content. To test this hypothesis, funnels with plants in sand were inoculated with zoospores and maintained at $\psi_m = 0$, −5, −10, −15, or −20 mb. After 10 days at these ψ_m values the newly developed roots were examined for *O. brassicae*. Sporangia were present in the young distal portions of the roots held at $\psi_m = 0$, −5, or −10, but not in roots held at $\psi_m = -15$, or −20 mb. Thus, *O. brassicae* zoospores were released at $\psi_m \geqslant -10$ mb as long as the water content of sand was about 0.20 g/g or dry sand.

DISCUSSION

Olpidium brassicae survives adverse conditions in the absence of the host as thick-walled, single-celled resting sporangia and BVA is borne internally in the resting

TABLE 8. Effect of matric potential and soil type on the distance zoospores of *Olpidium brassicae* moved from a point source to infect seedlings

Soil	Matric potential (millibars)	Distance from inoculum site to bait plants (mm)											
		Experiment 1[a]						Experiment2					
		10	20	30	60	70	80	10	20	30	40	50	60
BV-S	0	+	+	+	−	−	−	+	+	+	±	−	−
	−20	±	±	±	−	−	−	+	±	−	±	−	−
	−40	+	±	+	−	−	−	±	±	−	−	−	−
BV-P	0	+	+	±	−	−	−	+	+	±	−	−	−
	−20	+	±	−	−	−	−	+	±	−	−	−	−
	−40	+	±	−	−	−	−	+	−	−	−	−	−

[a]Zoospores were added to a small area of BV-prone or BV-suppressive soil and 24 or 48 hr, in Experiment 1 and 2, respectively, were allowed for zoospores to move and infect two replicate pairs of bait plants. Results are given as +, ±, or − to indicate both, one, or none of the replicate pairs of bait plants was infected.

sporangia (1, 4). The method previously used to assay soil for resting sporangia was adequate for detecting the fungus in the soil, but is not quantitative. The improved method described herein allows detection of *O. brassicae* and also provides an estimate of the relative numbers and rate of germination of resting sporangia in the soil. We have been unable to develop a more direct and reliable method for extraction and counting resting sporangia, despite numerous attempts utilizing wet- or dry-sieving, flotation, and density-gradient centifugation.

The length of time zoospores and sporangia survive in the field is not known, but zoospores remained motile in soil extracts for 4-6 hr at 20 C and up to 25 hr at 8 C, and for 12-24 hr in soil-sand mixtures. Although they were no longer motile after 24 hr in soil, a small portion of them apparently remained infective for at least 48 hr. Possibly the zoospores can persist in the soil as encysted zoospores as has been reported for *Phytophthora* spp. (13, 14). Contamination by resting sporangia was not involved because all of the controls specifically designed to detect resting sporangia were negative. It seems unlikely that the infectious propagules were zoospores released from resting sporangia already present in the soil because the soil had been air-dried previously and air-dry resting sporangia require more than 48 hr at saturation before they germinate. Similar results also were obtained when methyl bromide-fumigated soil was used instead of nonfumigated soil (Westerlund, *unpublished*).

Germination of resting sporangia and infection of lettuce occurs most rapidly at 18 and 22 C and only slowly at 10 C. A similar relationship between temperature and the rate of vegetative reproduction of *O. brassicae* was observed in this study (Westerlund, et al., *unpublished*) and earlier by Fry and Campbell (8). Once the induced dormancy due to air drying was overcome, a portion of the resting sporangia germinated in the absence of host roots. Not all of the resting sporangia germinated in soil that was rewetted and maintained at saturation for 3 wk. Thus, the possibility of germinating resting sporangia in the absence of the host and reducing the incidence of big vein in subsequent crops seems remote.

Zoospores of *O. brassicae* swim in a random, jerky manner and apparently are not attracted to host roots in the same directed manner as are zoospores of *Phytophthora* spp. (20). The method used to determine the distance *O. brassicae* moves through soil may have underestimated the distance because the method involves infection and reproduction as well as movement. The distance that the zoospores moved horizontally in the present study was due to their motility and not to water flow in the soil because water equilibration was rapid (6) and the inoculum was added in a manner that reduced the possibility of lateral spread due to flow of water. Zoospores swam up to 40 mm in sand at $\psi_m \geq -20$ mb and 30-40 mm in BV-S and BV-P soils at $\psi_m = 0$, but movement in the soils was restricted at $\psi_m \leq -20$ mb. The lateral distance that zoospores of *O. brassicae* can move is greater than that reported for the zoospores of *Phytophthora cryptogea* (7) presumably because the smaller zoospores of *O. brassicae* are able to utilize smaller, water-filled pores.

Germination of resting sporangia and subsequent infection of lettuce root occurred in BV-P soil wetted to at least −60 mb ψ_m but only occurred in saturated BV-I soil ($\psi_m = 0$). This difference between the two soils may be due to a greater number of resting sporangia in the BV-P soil (Table 1) and/or to the higher water content of the BV-P soil at lower ψ_m values (Fig. 1), thus allowing more activity of *O. brassicae*. Development of sporangia in the epidermal cells of lettuce roots is not affected by soil ψ_m unless plant ψ is depressed to values that result in wilting. Zoospores of *O. brassicae* were released into osmotica of glycine, sucrose, and sodium chloride at ψ_s values as low as −5.5 bars (Westerlund, *unpublished*), but were not released into sand unless it was essentially saturated; i.e., $\psi_m \geq -10$ mb. This indicates that ψ_m and associated changes in water content have a much greater influence on the release of zoospores by *O. brassicae* than does depression of ψ by solutes. Furthermore, release of zoospores from sporangia evidently requires more water than do the other stages in the life cycle of *O. brassicae*. Although there are not enough data to warrant many general statements about the water relation of fungi, it is noteworthy that the water requirements for zoosporic reproduction by *O. brassicae*, *Aphanomyces euteiches* (10), and *Phytophthora* spp. (5, 6, 7, 17) are somewhat similar and are very much greater than the water requirements of most nonzoosporic fungi that have been examined (9).

We detected no chemical or biological differences that affected *O. brassicae* zoospore motility, zoospore infectivity, vegetative reproduction, or resting sporangium formation in BV-P, BV-I, BV-S soils or soil extracts. After artificial infestations of fumigated and nonfumigated soils, *O. brassicae* produced as large a population of resting sporangia in BV-S as in BV-P soil maintained at $\psi_m \geq -150$ mb. Zoospores moved at least as far in BV-S as in BV-P soil, and the percentage of bait plants infected by *O. brassicae* in BV-I soil was nearly the same as in BV-P soil when bait plants in the BV-I soil were incubated for 21 days at $\psi_m = 0$. All of these results indicate that the major differences between BV-P and BV-S soils is attributable to the better water penetration and rapid drainage of water from the BV-I and BV-S soil which reduces the time during which the high soil moisture is maintained and that is necessary for resting spore germination, zoospore release, and zoospore movement. This hypothesis is supported by our repeated observations that lettuce plants in low, flooded placed in a field may be 100% infected by BVA whereas in nearby drier areas plants are nearly free of big vein.

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