Big Vein of Lettuce: Infection and Methods of Control

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ABSTRACT

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Olpidium brassicae, the vector of the lettuce big-vein agent (BVA), is uniformly distributed to a depth of 60–90 cm in big vein-prone soils in the Salinas Valley of California. *Olpidium* infects ≥ 50% of the lettuce seedlings as early as 8 days after summer plantings or 15 days in winter plantings that emerge more slowly. Soil temperatures at the 10-cm depth were not different between big-vein-prone soils and nearby non-infested soils at any season. Fungicides were tested at 100 μg a.i./ml for effects on zoospore motility and infectivity, and on growth and maturation of thalli in vivo. Fenaminosulf generally was ineffective; metalaxyl stopped zoospore motility but not infection or reproduction in vivo; pyroxychlor, captan, or ethazole (5-ethoxy-3-[trichloromethyl]-1, 2, 4-thiadiazole) stopped motility and infection by zoospores, but were ineffective in vivo; triadimefon or benomyl did not stop motility, but did prevent infection or reproduction. Benomyl was systemic in the roots and prevented reproduction in seedlings for >7 days after 1 day of uptake. In four field trials, transplanted lettuce had less big vein than did direct-seeded lettuce and in two of the four trials a benomyl drench prior to transplanting reduced the incidence still further. Annual soil fumigation with methyl bromide (224 kg/ha) was tested in field plots for 3 yr; it controlled *Olpidium*, reduced the incidence of big vein in two succeeding lettuce crops, and increased the rapidity and uniformity of maturity. Chloropicrin (336 kg/ha) or Vorlex (80% chlorinated C_3 hydrocarbons + 20% methylisothiocyanate) (234 or 468 L/ha) did not control *Olpidium* or big vein, but increased plant vigor, size, and the rapidity and uniformity of maturity in some trials.

Olpidium brassicae (Wor.) Dang. is the vector of the graft-transmissible agent that causes big vein of lettuce. This causal agent may be a virus but has not been characterized (12,20); hence it is termed the lettuce big-vein agent (BVA). *Olpidium* has two important functions in the etiology of the disease (5). The zoospores carry the BVA, apparently internally, and inoculate it into the host cells, and the resting spores carry BVA internally and enable its survival in soil from crop to crop.

The best diagnostic criterion for infection by BVA in the greenhouse is the vein banding that is visible on young leaves as they enlarge and mature. This symptom is evident on seedlings of susceptible cultivars (eg, Climax) as early as 18–21 days after inoculation with *Olpidium* zoospores carrying BVA. In the field, a more complex syndrome is ascribed to big vein. The plants are exposed to a more variable environment and the cultivars grown in the Salinas Valley (eg, Calmar and Salinas) have some resistance to big vein. Thus, vein banding is seldom seen before the plants are 35 days old and ready for thinning to the standard 30-cm spacing. In addition, the leaves are thicker with an upright habit of growth and frilly margins. Infected plants are delayed in heading and in severe cases may fail to head.

In the Salinas Valley, crisphead lettuce is sown on about 20,000 ha between December and July. The individual fields are about 8 ha in area and are irrigated. Harvest begins 70–120 days after planting and continues from April through October. Big vein is more severe and incidence is greater in lettuce grown and harvested during the cooler part of the year. If the weather is favorable for symptom expression varying amounts of big vein can be found on a high percentage of the lettuce acreage, but on certain soils it is a consistent problem that affects virtually all the early lettuce. These soils (about 10% of the Salinas Valley lettuce acreage) were termed "big-vein-prone" soils (21). These soils are uniformly infested; big vein can affect virtually every plant in a field. Such fields are often double-cropped; one field studied had had 23 crops of lettuce, two crops of sugar beet, one crop of onion, and three barley cover crops in a 15-yr span.

The possibility of chemical control of big vein was tested before the vector was identified. Treatment of infested soil in closed containers with chloropicrin or with D-D at more than 0.12 gm/L prevented big vein (3). Grogan et al (9) reported similar results in similar trials with these chemicals as well as with CBP-55 and CS_2. In field trials, fumigation with chloropicrin at 655 L/ha under a tarp or incorporation of 78 or 224 kg/ha of PCNB reduced disease incidence (13). We observed an apparent reduction in big vein when lettuce followed strawberry crops planted on soil fumigated with a mixture of methyl bromide and chloropicrin. Methyl bromide (MB) killed *Olpidium* and prevented transmission of tobacco necrosis virus to cucumbers and beans in glasshouses in New Zealand (17). Fumigation with MB is common in European glasshouses for control of soilborne pathogens, but has sometimes resulted in excessive bromide residue in crop tissues (19).

The objectives of the research reported in this paper were to study big-vein-prone soils in the Salinas Valley with regard to the depth of infestation by *Olpidium,* the timing of *Olpidium* infection of lettuce seedlings, and the efficacy of two measures for control of big vein: transplanting, with or without fungicidal treatment, rather than direct seeding and fumigation. Results from laboratory tests of fungicides against *Olpidium* are included.

MATERIALS AND METHODS

Field locations. Plant and soil samples were collected from, and plots were located in, fields with big-vein-prone soils near Gonzales and Soledad, California, in the Salinas Valley. These soils are classified as Cropley silty clay and Salinas clay loam types. To determine the depth profile of *Olpidium*, soil samples were collected from depths of 15 cm and 30 cm by digging a hole with a shovel and removing undisturbed soil from the side of the hole with a trowel. Samples from greater depths were removed with a soil sampling tube (2 cm diameter) pushed through the bottom of the shovel hole. Care was taken to prevent surface soil from falling into the holes and to wash the instruments with 1% sodium hypochlorite between each sampling site. Each sample was assayed in duplicate

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for *Olpidium* using the equivalent of 5 gm of air-dried soil in the standard test (21). Noninfested pots of lettuce were interspersed among the assay pots to detect *Olpidium* contamination.

Field infection of lettuce by *Olpidium*. The semiquantitative method for estimating the number of resting spores in the soil (21) was modified to determine when lettuce seedlings were infected by *Olpidium*. A random sample, usually of 10 seedlings, of Calmar or Calmar-related cultivars in commercial fields were dug from each field starting as early as 7 days after the spring and summer plantings or 15 days after the winter plantings. A second collection was made 2–3 wk after the first sampling. Additional samples were unnecessary because results from the first samples were available and plants in the field began to show big-vein symptoms so it was obvious that infection had occurred earlier. Most of the root system of the small seedlings was returned to the laboratory in a ball of soil. The plants were washed free of soil with a water spray and treated as bait plants that were tested for zoospores 2 wk later (21).

Soil temperature in representative fields was measured with a 45-day thermograph (Model H, Ryan Instruments, Kirkland, WA 98033) buried with the sensing element placed horizontally 10 cm below the soil surface and centered between two rows of lettuce on a bed.

Fungicide testing. *Olpidium* was propagated and tested in plants of lettuce cultivar Climax grown in sand culture (5,20). Zoospore suspensions were prepared by washing the roots free from sand and immersing them in tap water, or in 0.05 M glycine-NaOH (pH7.6) for experiments involving zoospore motility. Fungicides were tested for effect on zoospore motility and infectivity by mixing equal volumes of a 2× concentration of fungicide in distilled water and a zoospore suspension. Drops were removed at intervals up to 45 min and examined with a phase-contrast or a dark-field microscope for motile zoospores. Infectivity was tested when the mixture had incubated for 10 min by pipetting 10 ml into each of three replicate pots each of about 100 ml capacity and with about 50 lettuce seedlings sown 4–7 days earlier in pasteurized quartz sand. The in vivo activity of fungicides was tested by pipetting 10 ml of a 1× concentration of fungicide solution into similar pots of seedlings inoculated with *Olpidium* 1 day previously. After the seedlings in either test were incubated 4 days or more without further irrigation, viable thalli had formed sporangia, but zoospores were not discharged until a quantitative root washing was made (21). The number of zoospores released reflected the in vivo activity of the fungicides when compared to the nontreated controls that had been given distilled water.

Transplanting lettuce. Seed were sown in a peat-vermiculite potting mix (Jiffy-Mix, George Ball Pacific, Inc., Sunnyvale, CA 94088) in Todd planter flats (Speedling, Inc., Sun City, FL 33586) which provided a 33-ml cavity for each seedling and were maintained in a glasshouse at 13 C minimum. Approximately 1 mo after planting, each seedling was drenched with 5 ml of a 1 mg/ml solution or 10 ml of a 0.25 mg/ml solution of benomyl. One to 13 days after drenching, the seedlings were transplanted into the plot area in a commercial field direct-seeded with the same seed lot. Each replicate consisted of 25–70 plants in two rows on one bed; there were four replications with a complete randomized block design. Two nontreated controls were included in each trial: nontreated transplants and plants direct-seeded by the grower and thinned to the same spacing at the time of transplanting. Plants were rated positive for big vein if the typical vein-banding symptom was visible on any leaves of the intact plant just before harvest and in some trials about 2 wk prior to harvest.

Soil fumigation. Fumigants applied by a commercial operator were injected 20 cm deep in field soils in October or November when the soil temperature was above 13 C and before beds were formed. The treatments were covered with 0.0254-mm (1-mil) polyethylene film for 3 days, thereafter the plots received the same management as the rest of the field. The following fumigants and dosage per hectare were used: chloropicrin (336 kg) + DD® (224 kg) = Pic-DD, (DD® = 1,3 dichloropropene and related C_3 hydrocarbons); chloropicrin (168 kg) + DD (224 kg); chloropicrin (112 kg) + MB (224 kg) = Pic-MB; MB (224 kg). The incidence of big vein at harvest, and in some plots at 2–3 wk before harvest, was determined by visual inspection of 25 or 100 plants in each of four samplings randomly located in each replicate. The yield is the number of cartons, each with 24 heads of lettuce, per hectare.

Vorlex® (80% 1,3-dichloropropene and related C_3 hydrocarbons; 20% methylisothiocyanate), tested at broadcast rates of 234 and 468 L/ha in two plots, was injected 20 cm deep into preformed beds using two shanks 30 cm apart or three shanks 20 cm apart in each bed. The soil surface was sealed by compaction with a roller and planted about 2 mo later. The plots were a randomized block design with four replications that were two beds wide × 30 m long in 1976 and four beds wide × 172 m long in 1977. Vorlex equivalent to 327 L/ha (30 cm deep) was injected into a sealed 15.5-L container and incubated 4 days at 25 C before aeration and testing in the laboratory.

Bromide content of soil or lettuce tissues was determined by the bromide-specific ion electrode method (1). Each sample of lettuce tissue was collected as a composite from four plants at one site, weighed, oven-dried at 105 C, and pulverized in a Wiley mill prior to being subsampled for analysis. The bromide content is expressed as micrograms per gram of fresh weight.

RESULTS

Infection of lettuce in big-vein-prone soils. To determine the spatial and depth distribution of *Olpidium*, a big-vein-prone field was sampled at 10 sites evenly spaced around the field. At each site, soil samples were taken at depths of 15, 30, 60, and 75–90 cm where a hard pan was encountered. Duplicate assays for *Olpidium* were done for a total of 20 assays at each depth. *Olpidium* was found in 20 assays at 15 and 30 cm, in 19 assays at 60 cm, in 14 assays at 75–90 cm, but in none of the 20 noninfested controls.

Lettuce seedlings were assayed for *Olpidium* infection in 26 fields planted between December 1975 and January 1977. The spring crop was represented by fields planted from December through early May. In three fields planted in December 1975, there was no *Olpidium* infection in 70 plants collected 20–45 days after planting but seven of 10 were infected at 54 days in one field. Because of this slow rate of infection, two of these fields were sampled again after they were planted in December 1976 and eight or 10 of 10 plants were infected in 25 or 27 days. In 15 other plantings made in January through early May 1976 or in January 1977 more than 50% of the seedlings usually were infected at the first sampling date (15–32 days after planting). The exceptions were in two fields where none of the seedlings were infected in 13 or 17 days, but all were infected at 33 or 37 days.

Summer crops were represented by eight fields planted from mid-May to early July 1976. Most seedlings in five fields were infected at the first sampling (8–20 days after planting). In three fields only one or two seedlings were infected even at the second sampling (24–29 days after planting).

Two to four thermographs were kept in the fields being sampled to relate seasonal temperature changes to the infectivity data. The weekly mean temperature increased from 7–10 C in January–February, to 18 C in early May, and to 21 C in July (1976). The minimum temperature ranged from 4 to 13 C whereas the maximum ranged from 19 to 27 C. Temperatures from one or two thermographs kept in nearby fields with big-vein-intermediate or big-vein-suppressive soils (21) were within 1 C of those in big-vein-prone soils.

Laboratory evaluation of fungicides. Three criteria used to evaluate the effect of five fungicides on *Olpidium* were the effect on zoospore motility and infection, and on maturation of thalli in vivo (Table 1). Although fenaminosulf reduced zoospore infection and thallus maturation in three of five tests, fungicidal activity was not adequate for effective control. Pyroxychlor stopped zoospore motility and infection, but not maturation of thalli. Captan was fungicidal in all tests, but it caused a faint yellow discoloration of the roots. Although triadimefon and benomyl did not stop zoospore motility, they prevented infection and/or maturation of thalli. Triadimefon caused the lettuce to grow more slowly and to develop a darker green color than the controls. Data for copper sulfate and arasan are omitted from Table 1. Although both

stopped zoospore movement, they caused necrosis of lettuce roots.

Later, two other fungicides were used in similar tests. An emulsifiable concentrate of ethazole® was phytotoxic, presumably due to xylene in the formulation. In a wettable powder formulation, it stopped motility and infection of zoospores within 5 min at 100 μg/ml. At 50 μg/ml it did not stop all zoospores within 10 min, but it prevented infection. It had no effect on zoospore motility or infection at 25 μg/ml and no effect on maturation of thalli at 25 to 100 μg/ml. Metalaxyl, which was tested once, stopped zoospore movement within 10 min at 50 or 100 μg/ml, but not at 10 μg/ml. Zoospore infection was reduced only at 100 μg/ml. There was no effect on maturation of thalli at 10–100 μg/ml.

Benomyl was selected for further testing because it prevented *Olpidium* development in lettuce roots and had a low phytotoxicity. The first tests were to determine whether benomyl prevented infection by motile zoospores or affected later stages in thallus maturation. Zoospores were mixed with 100 μg/ml benomyl for 10 min and inoculated to lettuce seedlings that were removed from sand after 8 hr and examined microscopically for stages in the normal infection process; ie, encysted zoospores, papillae (2), and zoospore protoplasts in the host cells. Treated zoospores infected normally in two trials, but quantitative comparisons of infection frequency were not made.

Other tests determined the length of time that benomyl remained active in lettuce roots. About 25 lettuce seeds were germinated on 2.5 cm^2 pieces of plastic screen placed on vials with weak nutrient solution. Five days later the treated seedlings were given a 24-hr pulse of benomyl by immersing the roots in 100 μg/ml benomyl in aqueous solution while the nontreated controls were immersed in distilled water. After 24 hr, the screens and seedlings were washed with running water and transplanted as a unit into 100-ml pots of sand. At intervals from 1 to 14 days after the removal from the fungicide, seedlings in replicate pots (usually two) were inoculated with a zoospore suspension and maintained while *Olpidium* sporangia matured. Quantitative root washings were made, as in previous trials, to assess systemic fungicidal action. Nontreated controls always released zoospores normally. Benomyl prevented growth of *Olpidium* for about 1 wk after the uptake pulse. In the first three tests, the longest times tested were 5, 7, and 13 days and benomyl was effective in all cases. In the fourth test benomyl was effective for 7 days, but not for 14 days. In this experiment benomyl was also tested at 25 and 50 μg/ml. At 50 μg/ml it prevented growth for 1 and 3 days, but not longer. At 25 μg/ml it reduced multiplication for only 1 day. In the last two trials, 10 seedlings from each of two replicates treated with 100 μg/ml benomyl were transplanted to individual pots and incubated for 5 wk to observe the effect of the treatment on big vein symptom development. From 0 to 20% of the benomyl-treated plants developed big-vein symptoms, whereas from 55 to 100% of the controls had symptoms. Pyroxychlor was included in two of these trials and slightly reduced zoospore release for 1–3 days but not longer.

Transplanting and benomyl drenches for control of big vein. Lettuce transplanted into fields 2–5 wk after the fields had been sown had less incidence of big vein than did the direct-seeded plants in four trials done in 1975 and 1976 (Table 2). Transplants also were drenched with benomyl to determine if the incidence of big vein could be reduced further. In trials 1 and 2 benomyl added at 5 mg per plant 1 day before transplanting was not phytotoxic but only reduced the incidence of big vein slightly in trial 2. Pyroxychlor at 100 μg/ml also was tested as a drench (5 ml per plant) or as a foliar spray applied to run-off in trial 1, but the incidence of big vein was not significantly reduced. In another trial, benomyl drenched at 5 mg per plant 3 or 6 days before transplanting was phytotoxic. The plants were yellow and stunted and the data are omitted here. Thus, benomyl was used at 2.5 mg per plant in trials 3 and 4 in 1976 without phytotoxicity. In trial 3, benomyl significantly reduced big vein when applied 9 or 13 days before transplanting. In trial 4, the incidence of big vein in transplants 2 wk before harvest was not reduced by benomyl treatment. At harvest time, plants treated with benomyl had more big vein than did nontreated controls, but this result is suspect because the big vein incidence in nontreated transplants did not increase during the last 2 wk as ordinarily occurs.

Soil fumigation for control of big vein. In the 1975 tests all fumigants were applied to two replicate plots. The plot size for the high rate of Pic-DD was 34 × 190 m and all other plots were

TABLE 1. Toxicity to *Olpidium brassicae* of fungicides at 100 μg/ml

	Fungicide tested against					
	Zoospores					
		Infectivity[b] (×10^{-4})		Thalli in vivo[b]		
Fungicide	Motility[a]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 3
Fenaminosulf	+	22.9 z	326.1 y	13.3 z	128.4 y	45.3 y
Pyroxychlor	−	0 z	0 z	110.9 y	165.3 y	36.7 y
Captan	−	0 z	0 z	3.7 z	2.7 z	4.2 z
Triadimefon	+	0 z	0 z	0 z	0 z	0 z
Benomyl	+	0 z	0 z	0 z	0 z	0 z
Nontreated control	+	84.8 y	285.0 y	168.0 y	213.0 y	67.5 x

[a] Results from two trials were combined: + = zoospores remained motile; − = zoospores became nonmotile in <10 min.

[b] Results given as average number of zoospores released in standard root washings from three replicates of each treatment. Numbers followed by the same letter in each column are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Effect of transplanting and benomyl drenches on the incidence of big vein in field-grown lettuce

		Percentage of plants with big vein[a]						
		Trial 1	Trial 2		Trial 3		Trial 4	
Benomyl (mg/plant)	Days from treatment to transplant	At harvest	2 wk before harvest	At harvest	2 wk before harvest	At harvest	2 wk before harvest	At harvest
2.5	3						8.3 y	37.4 y
2.5	7						9.7 y	38.2 y
2.5	3 & 7						5.4 y	43.6 y
2.5	9				2.5 x	8.2 x		
2.5	13				0.0 x	0.7 x		
5	1	27.6 y	40.5 x	51.0 x				
Transplanted, not treated		31.2 y	49.7 y	67.0 y	17.6 y	31.2 y	16.2 y	20.1 x
Direct-seeded control		48.4 z	91.2 z	96.8 z	51.5 z	65.6 z	58.8 z	65.0 z

[a] In trials 1–4 the total number of transplants in four replicates of each treatment was 100, 200, 280, and 280 and the interval from planting date to transplanting date was 20 days, 15 days, 37 days, and 15 days, respectively. In each column, numbers followed by the same letter do not differ significantly by Duncan's multiple range test ($P = 0.05$).

7 × 190 m. Results from the low rate of Pic-DD were similar to those from the high rate and are omitted here.

Composite soil samples were collected at the 2–8 cm depth from sites spaced across each replicate at planting time in February 1975 and four subsamples of 25 gm each were assayed for *Olpidium*. *Olpidium* was not found in any assays from the MB plots or in five of eight assays from the Pic-MB plots, but a few zoospores were found in the other three assays of this treatment. *Olpidium* was recovered readily in all assays from Pic-DD and nontreated plots.

Big-vein incidence in the lettuce crop was closely correlated with the assays for *Olpidium* (Table 3). The plants were more vigorous in all the fumigated plots than in the nontreated area and the difference in vigor, (measured as plant weight at 6 wk) could be seen throughout the growth of this crop. All fumigants produced earlier and more uniform maturity of plants with the consequent high yield from the first cutting regardless of their effect on *Olpidium* or big vein. Furthermore, *Olpidium* had not been eradicated from the soil because all soil samples from the MB-treated plots assayed positive for *Olpidium* at harvest.

Within 2 wk after harvest the field was disked, the beds were reshaped, and a second crop of lettuce was sown. When this crop matured, there was less big vein and more rapid and uniform maturation on the plots treated with MB or Pic-MB (Table 3). There was no difference in growth or big-vein incidence in the third lettuce crop planted the following year.

Methyl bromide was selected for further testing in 1976, but the fumigation was unsatisfactory because of drought and the yield data are incomplete because either poor market prices or rain interfered with the harvest of the first two crops. The poor fumigation was shown by the recovery of *Olpidium* in nearly every sample collected either from the surface, 30 cm deep, or 60 cm deep at each of four sites. Nevertheless, the *Olpidium* population apparently was reduced enough to reduce big vein from 76% in the nontreated plot to 2% in the treated plot (based on 12 or 18 samplings each of 100 plants, respectively). Lettuce on the fumigated area grew more uniformly and matured earlier so that harvest began 5 days earlier on the treated plot.

A third trial done in 1977 had two replicates of 0.6 and 0.9 ha fumigated with MB and two nontreated replicates each of about 0.5 ha. Assays for *Olpidium* were done from each of four depths at three sites in each replicate. *Olpidium* was not recovered in any of 12 assays of soil from depths of 0–5, 15, or 30 cm in the fumigated plots and was recovered in only three of 12 assays from the 60 cm depth. It was present in all corresponding assays from the nontreated plot except two from 0–5 cm and three from the 15-cm depth. In the first lettuce crop, less than 1% of the plants in the treated plots had big vein at harvest compared to 82% in the nontreated plots. Although there was no obvious difference in plant size, the first crop of lettuce in fumigated soil matured earlier, and more uniformly, and yielded 1,621 cartons per hectare in the first cutting compared to only 188 cartons per hectare in nontreated plots. There was a second cutting for which yield data were not obtained. Observations a few days later showed that yield from the nontreated plots had improved considerably. The second crop, planted 6 wk later, had a significantly lower incidence of big vein (25%) in the treated plots than in the nontreated plots (40%) at harvest. Likewise, the yield from the first cutting was 1,223 cartons per hectare from the treated plots, significantly better than the 568 cartons per hectare from nontreated plots. Although the nontreated plots yielded more than the treated plots (608 vs 420 cartons per hectare) in the second cutting, the second cutting and total yields did not differ significantly. After two crops of lettuce, however, *Olpidium* was detected in 12 of 12 assays of soil from the 0–5 cm depth at six sites in the treated plots.

The bromide content of lettuce heads at harvest time was determined for each plot. It was significantly higher in heads of the first and second crops from each plot (Table 4), but not in heads of the third crop from the 1976 plot. The maximum bromide content in any sample was 54 μg/g, the tolerance is 60 μg/g. The 1976 and 1977 plots each included a small plot treated with methyl bromide at 448 kg/ha. The first crop of lettuce on those plots had bromide contents that were not significantly different from those of the standard fumigation treatment.

Vorlex in laboratory tests eliminated *Olpidium* in four replicate samples of 25-gm of treated soil, but was ineffective in field trials in 1976 and 1977. There was as much big vein in the treated plots as in the nontreated controls (75% in 1976, 15% in 1977). Assays of soil samples for *Olpidium* in the 1977 plot were all positive, even from the portion of the bed where the fumigant had been injected. The major effect of the treatment was an increase in the size of the plants and uniformity of maturity in the 1976 plot in which big vein was severe. The yield from the treated plots at first cutting was increased but the total yield was not.

DISCUSSION

The first step in the big-vein disease cycle is infection of lettuce roots after germination of soilborne resting spores of *Olpidium*. These spores are distributed throughout the upper 60–90 cm of the soil profile in big-vein-prone soils. Thus, it is not feasible to consider deep plowing or other techniques to bury inoculum and escape infection. Infection from resting spores occurred more rapidly at 18–22 C than at 10 C in laboratory studies (21). The present study shows a similar pattern in response to the seasonal changes in temperature in the field. In winter plantings, the average soil temperature ranged from 7–10 C, the seedlings did not emerge rapidly, and were not sampled until 15 or more days after planting. However, over half of them were infected at this sampling. These plantings matured during the late winter and early summer when air temperatures were cool and 80% or more of the plants had big vein symptoms. By contrast, the summer plantings in soils averaging 18–21 C emerged and were sampled earlier, but had a similar infection pattern. These plantings, however, matured when the air temperatures were warmer and had a low percentage (generally < 25%) with symptoms at maturity. Thus, *Olpidium* infection occurs soon after germination of lettuce seed in big-vein-

TABLE 3. Effect of soil fumigation on the incidence of big vein and yield of lettuce in the first and second crops after treatment[a]

	First crop						Second crop			
				Yield[d]				Yield[f]		
Treatment	Plant weight[b] (g)	Big vein (%)[c] 10 wk	14 wk	First cut	Second cut	Total	Big vein[e] (%)	First cut	Second cut	Total
Nontreated	1.2 w	47.9 y	75.8 y	390 x	618 y	1,008 x	26.7 y	996 xy	269	1,265 y
Chloropicrin-DD	4.3 z	54.5 y	78.9 y	1,542 yz	193 z	1,735 yz	20.7 yz	756 x	311	1,067 y
Chloropicrin-methyl bromide	2.8 y	4.6 z	10.8 z	1,604 yz	198 z	1,802 yz	11.2 z	1,263 yz	334	1,597 z
Methyl bromide	2.0 x	0.8 z	2.2 z	1,937 z	232 z	2,169 z	6.8 z	1,628 z	161	1,789 z

[a] Results are averages from two replicates. Within a column, numbers followed by the same letter do not differ significantly by Duncan's multiple range test ($P = 0.05$).

[b] Mean fresh weight of tops of 25 seedlings per replicate at 6 wk after planting.

[c] Average percentage of plants with symptoms of big vein at 10 and 14 wk after planting, N = 400 plants per replicate.

[d] Average yield (cartons per hectare). The first and second cuttings were made 14 and 15 wk after planting.

[e] Average percentage of plants with symptoms of big vein at 10 wk after planting, N = 400 plants per replication.

[f] Average yield (cartons per hectare). The first and second cuttings were made 10 and 11 wk after planting.

prone soils in all seasons, but early *Olpidium* infection does not correlate with symptom expression that requires cool air temperature regardless of the soil temperature (20). These results have been duplicated in the field by using clear plastic mulch on lettuce beds to raise the daytime soil temperatures by 5–10 C without affecting the incidence or severity of big vein (authors' *unpublished*).

Big-vein-prone soils are regarded by growers as being both heavier (clay loam or silty clay) and colder than BV-suppressive soils. An important attribute of big-vein-prone soils is their slow water drainage which prolongs periods of low moisture tension required for resting spore germination, zoospore release from sporangia, and zoospore movement in soil (21). Although they are not extensive, the present field observations of soil temperature (seasonal changes as well as diurnal patterns) did not show significant differences between big-vein-prone and suppressive soils. Thus, soil texture and physical impediments (eg, hard pans that slow water drainage) are the most important attributes of big-vein-prone soils through their effect on soil moisture tension.

Olpidium, a chytrid fungus, has distinctly different response to fungicides than do pathogens in the Oomycetes. *Olpidium* is slightly affected by fungicides (fenaminosulf, pyroxychlor, ethazole, or metalaxyl) with specificity for pathogens in the Peronosporales (10,14,15,22). *Olpidium*, like *Plasmodiophora brassicae* (11), is sensitive to benomyl which is active against the higher fungi but not the Oomycetes (8,16). Motility and infectivity of *Olpidium* zoospores, however, are not affected by benomyl at 100 μg/ml whereas the *Olpidium* thallus is killed after infection of the root. These observations are consistent with the report that the active ingredient, methyl-2-benzimidazole carbamate, is absorbed by the plant and is effective in vivo (16) by interfering with fungal mitosis (7). Our results confirm the systemic movement of the active ingredient in the root system because new roots formed 1 wk after a 24-hr pulse of benomyl did not support growth of *Olpidium*. The sensitivity of *Olpidium* to benomyl in vivo was reported previously (20). A related benzimidazole fungicide, carbendazim, recently has been reported to be nontoxic to zoospores, whereas it was the surfactants in the formulation that killed them (18). This report is probably incorrect for two reasons: the tests evaluated only zoospore motility and neglected zoospore viability or infectivity; and tests with zoospores neglect the in vivo activity of systemic fungicides. Nonmotile zoospores were infective in tests with heat-shocked zoospores (6) and in tests with zoospores incubated in soil without host plants (21) as well as in the present test with metalaxyl at 50 μg/ml. Although captan, copper sulfate, and arasan stopped zoospore movement, their ability to kill either zoospores or thalli in vivo is unknown because they caused moderate to severe damage to the lettuce roots and healthy roots are required for multiplication of the obligately parasitic *Olpidium*. Captan was less phytotoxic, but even so the roots were discolored and only a few zoospores were recovered from in vivo tests.

When *Olpidium* was killed by benomyl treatment during its first vegetative generation in lettuce seedlings, the frequency of infection by the BVA was reduced (20). Similar results were observed in two of the present trials. Some explanations already have been discussed (20).

We have evaluated two measures for control of big vein in the field. Transplantation of lettuce avoids very early infection by *Olpidium* and reduces the amount of big vein at harvest, probably because there is insufficient time for systemic infection by the big-vein agent and for symptom expression. We have observed a similar delay in symptom expression and reduction in the percentage of plants with symptoms when older plants were inoculated in greenhouse experiments. A benomyl drench of transplants might reduce big vein even more, but our results were erratic. Drenches applied 9–13 days before transplanting may have allowed better uptake and systemic distribution in the roots which resulted in better control than that obtained in some of the present trials. Fumigation reduced infection by reduction of the resting spore population. Methyl bromide reduced the *Olpidium* population in the soil and big vein incidence for at least one season. *Olpidium* is deeply distributed in big-vein-prone soils and reinfestation from below or from nearby fields occurs during the first two crops after fumigation. Although technologically feasible, it is doubtful that soil fumigation will be done solely for control of big vein. The economic impact of big vein, and therefore the return from fumigation, are highly dependent on market prices (24) that cannot be predicted at the time of fumigation.

The fumigation plots provided evidence that the symptom complex and yield loss attributed to big vein in the field may not be correct. Plants on plots treated with MB, Pic-MB, or Pic-DD in 1975 or Vorlex in 1976 were large and vigorous, matured uniformly, and were mostly harvested at the first cutting. The plants from the Pic-DD and Vorlex treatments, however, had a high percentage of big vein as judged by the vein banding and erect wrapper leaves with frilly margins. These plants had neither the small heads nor the delayed maturity that reduce yield and that have been ascribed to the BV syndrome. These latter symptoms may have been caused by other factors favored by a cool environment, as big vein is, or they may have been caused by BVA but were masked by the vigorous growth in response to fumigation. A beneficial response to fumigation has been observed for many crops and in many instances responses have occurred when no obvious disease was being controlled (4,23).

TABLE 4. Bromide content of lettuce grown in soil fumigated with methyl bromide at 224 kg/ha

Plot, date, and lettuce crop	Methyl bromide treatment		Nonfumigated control	
	Samples (no.)	Bromide (μg/g)	Samples (no.)	Bromide (μg/g)
1975 crop				
First crop	2	17.5	2	3.5
1976 crop				
First crop	12	33.9 ± 9.8[a]	6	7.9 ± 2.0[a]
Second crop	6	5.6 ± 1.8[a]	3	2.8 ± 0.4[a]
Third crop	3	3.9 ± 0.3	3	4.1 ± 0.4
1977 plot				
First crop	6	25.7 ± 14.2[a]	6	5.6 ± 1.6[a]
Second crop	6	17.3 ± 4.2[a]	6	8.0 ± 1.6[a]

[a]Treatment means differed significantly by *t*-test ($P = 0.05$).

LITERATURE CITED

1. ABDALLA, N. A., and B. LEAR. 1975. Determination of inorganic bromide in soils and plant tissues with a bromide selective-ion electrode. Commun. Soil Sci. Plant Anal. 6:489-494.
2. AIST, J. R., and H. W. ISRAEL. 1977. Timing and significance of papilla formation during host penetration by *Olpidium brassicae*. Phytopathology 67:187-194.
3. ALLEN, M. W. 1948. Relation of soil fumigation, nematodes, and inoculation technique to big vein disease of lettuce. Phytopathology 38:612-627.
4. ALTMAN, J. 1970. Increased and decreased plant growth responses resulting from soil fumigation. Pages 216-221 in: T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. Root diseases and soil-borne pathogens. Univ. Calif. Press, Berkeley. 252 pp.
5. CAMPBELL, R. N., and P. R. FRY. 1966. The nature of the associations between *Olpidium brassicae* and lettuce big-vein and tobacco necrosis viruses. Virology 29:222-233.
6. CAMPBELL, R. N., and M. T. LIN. 1976. Morphology and thermal death point of *Olpidium brassicae*. Am. J. Bot. 63:826-832.
7. DAVIDSE, L. C. 1977. Mode of action, selectivity and mutagenicity of benzimidazole compounds. Neth. J. Plant Pathol. 83 (Suppl. 1):135-144.
8. EDGINGTON, L. V., K. L. KHEW, and G. L. BARRON. 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology 61:42-44.
9. GROGAN, R. G., F. W. ZINK, W. B. HEWITT, and K. A. KIMBLE. 1958. The association of *Olpidium* with the big-vein disease of lettuce. Phytopathology 48:292-297.
10. HILLS, F. J., and L. D. LEACH. 1962. Photochemical decomposition and biological activity of *p*-dimethylaminobenzenediazo sodium sulfonate (Dexon). Phytopathology 52:51-56.

11. JACOBSEN, B. J., and P. H. WILLIAMS. 1970. Control of cabbage clubroot using benomyl fungicide. Plant Dis. Rep. 54:456-460.
12. 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.
13. MARLATT, R. B., and R. T. McKITTRICK. 1963. Fungicidal control of big vein in the irrigated lettuce crop. Phytopathology 53:597-599.
14. NOVEROSKE, R. L. 1975. Dowco 269: a new systemic fungicide for control of *Phytophthora parasitica* of tobacco. Phytopathology 65:22-27.
15. PAULUS, A. O., J. NELSON, M. SNYDER, and J. GAFNEY. 1977. Downy mildew of lettuce controlled by systemic fungicide. Calif. Agric. 31(12):9.
16. SIMS, J. J., H. MEE, and D. C. ERWIN. 1969. Methyl 2-benzimidazolecarbamate, a fungitoxic compound isolated from cotton plants treated with methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl). Phytopathology 59:1775-1776.
17. THOMAS, W. 1973. Control of *Olpidium brassicae*, the vector of cucumber systemic necrosis and bean stipple streak virus diseases. N. Z. J. Exp. Agric. 1:92-96.
18. TOMLINSON, J. A., and E. M. FAITHFULL. 1979. Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. Ann. Appl. Biol. 93:13-19.
19. VANACHTER, A., E. VAN WAMBEKE, and C. VAN ASSCHE. 1975. Investigations on the bromide concentration in Belgian greenhouse lettuce. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 40:1085-1093.
20. WESTERLUND, F. V., R. N. CAMPBELL, and R. G. GROGAN. 1978. Effect of temperature on transmission, translocation, and persistence of the lettuce big vein agent and big vein symptom expression. Phytopathology 68:921-926.
21. WESTERLUND, F. V., R. N. CAMPBELL, R. G. GROGAN, and J. M. DUNIWAY. 1978. Soil factors affecting the reproduction and survival of *Olpidium brassicae* and its transmission of big vein agent to lettuce. Phytopathology 68:927-935.
22. WHEELER, J. E., R. B. HINE, and A. M. BOYLE. 1970. Comparative activity of Dexon and Terrazole against *Phytophthora* and *Pythium*. Phytopathology 60:561-562.
23. WILHELM, S. 1965. *Pythium ultimum* and the soil fumigation growth response. Phytopathology 55:1016-1020.
24. ZINK, F. W., and R. G. GROGAN. 1954. The interrelated effects of big vein and market price on the yield of head lettuce. Plant Dis. Rep. 38:844-846.