The Role of Sporangia in the Epidemiology of Pearl Millet Downy Mildew

S. D. Singh and R. J. Williams

Plant pathologist and principal pathologist, Millet Improvement Program, International Crops Research Institute for the Semi-Arid Tropics, ICRISAT P.O., Patancheru, 502 324, Andhra Pradesh, India.

The authors are grateful to M. N. Pawar and P. Malla Reddy for technical assistance in this study.
Journal Series Article 132 of the International Crops Research Institute for the Semi-Arid Tropics.
Accepted for publication 3 June 1980.

ABSTRACT

SINGH, S. D., and R. J. WILLIAMS. 1980. The role of sporangia in the epidemiology of pearl millet downy mildew. Phytopathology 70:1187-1190.

Several experiments were conducted at ICRISAT Center on the role of sporangia in the epidemiology of pearl millet downy mildew (DM). Exposure of field-grown plants to sporangial inoculum provided by earlier planted "infector rows" resulted in considerably more DM on all cultivars tested than was obtained by exposure of these cultivars to oospores by several other methods. In large isolated field plots of DM-susceptible hybrids, DM incidence and severity were considerably greater in plots with a central source of sporangia (potted plants with sporulating DM-infected leaves) present from crop emergence than in plots with no such initial sporangial source. Sporangia remained infective for up to 340 m downwind from a sporangial source during the rainy season, but during the dry post-rainy season no infection occurred at distances more than 80 m from a sporangial source. The significance of these results in the epidemiology of pearl millet DM and their application for large scale field screening for DM resistance are discussed.

Additional key words: Sclerospora graminicola, zoospores.

Downy mildew (DM) caused by *Sclerospora graminicola* (Sacc.) Schroet., caused severe and widespread yield losses in the Indian hybrid pearl millet (*Pennisetum americanum* [L.] Leeke) crop during the period 1970 to 1976 (7). Although this disease is now recognized as one of the main factors limiting the achievement of high yield potentials of improved millet hybrids and cultivars in India and Africa, several basic details of its biology and epidemiology remain uncertain (5).

In the laboratory, high levels of systemic downy mildew infection can be obtained by exposure of young pearl millet seedlings to oospores or sporangia (and thus, zoospores) of *S. graminicola* (8,9), but the relative role of these infective propagules under field conditions is a matter of controversy. Bhat and Safeeulla (2) reported that the role of asexual spores in pearl millet DM epidemiology was limited in the region of Mysore and neighboring districts of south India. Studies on the production, liberation, and dispersal of sporangia indicated that secondary spread in this DM is negligible under the ecological conditions prevalent in Mysore (6). Safeeulla (8) comments that while zoospores can cause secondary infection under artificial conditions, their role in the spread of the disease in the field is not clear. Nene and Singh (5), in their review of pearl millet DM, comment that the reasons for the apparent ineffectuality of asexual inoculum under natural conditions were not fully understood. Some workers, however, suggest that sporangia can be important in the epidemiology of pearl millet DM. On the basis of his work with this disease in Senegal, Girard (3) presented several pieces of indirect evidence supporting sporangial involvement in the buildup of pearl millet DM in the field. King (4) stated that in the more humid areas of millet production, sporangia probably do contribute significantly to pearl millet DM increase and spread.

During the past 4 yr we investigated many aspects of this disease, and in this paper we present our findings on the role of *S. graminicola* sporangia in the buildup and spread of pearl millet DM in the field.

MATERIALS AND METHODS

Comparison of inocula and inoculation methods. Two highly susceptible pearl millet hybrids (HB-3, NHB-3), one highly susceptible male-sterile line (5071-A), and three less-susceptible cultivars (PHB-10, A-836, and B-282) were inoculated directly and indirectly with oospores and sporangia of *S. graminicola* by various techniques in field plots at the ICRISAT Center during the rainy season in 1976. Inoculation treatments were: (i) dry oospore powder incorporated in the furrows (0.2 g/m) immediately prior to planting, (ii) dry oospore powder applied to the seed (0.1 g/g) immediately prior to planting; (iii) dry oospore powder applied to furrows and seed (0.2 g/m and 0.1 g/g respectively) immediately prior to planting; (iv) oospores in aqueous suspension (5 g/L) injected into whorls of plants 23 days after planting (DAP); (v) sporangial suspension injected into the whorls of test plants 23 DAP; (vi) sporangia-producing, infected leaf-bits scattered in the rows 18 DAP; (vii) test cultivars exposed to sporangia produced by the highly susceptible hybrid HB-3 planted 5 and 2 wk prior to the test cultivars in a 1-m band at both ends of the block of test cultivar rows (end-infector); (viii) test cultivars exposed to sporangia produced by the highly susceptible hybrid HB-3 planted 5 and 2 wk prior to the test cultivars in alternate rows (alternate-infector); and (ix) no inoculation. Downy mildew was promoted in the infector rows by injecting plants with sporangia at the seedling stage. Seed in treatments iv to ix were treated with Agrosan GN (5 g/kg) immediately prior to planting to minimize oospore infection from naturally occurring soilborne oospores.

Test cultivars were planted in single 5-m rows. To reduce interplot movement of sporangia, each plot of six cultivars was surrounded by a 0.5-m belt of maize, planted 5 wk prior to the test cultivars. All the test cultivars within one plot were subjected to the same inoculation treatment. Each inoculation treatment-cultivar combination was replicated four times in randomized complete blocks.

The total number of plants was recorded 15 DAP and the number of systemically infected plants was recorded 15, 24, and 36 DAP.

Isolation plot study. During the monsoon season 1977 we compared DM buildup in isolated plots of susceptible hybrids with or without a source of sporangia placed in the centers of the plots at emergence. Two susceptible hybrids, NHB-3 and HB-3, were sown in 30 × 30 m plots with each plot at least 500 m from any other pearl millet planting. Rows were 0.75 m apart and the spacing within the row was approximately 5 cm. Two plots were sown with NHB-3 and two were sown with HB-3. A single pot of DM-infected pearl millet plants producing abundant sporangia was placed in the center of one plot of each hybrid when the hybrid seedlings were

0031-949X/80/12118704/$03.00/0

emerging. The second plot of each hybrid was not provided with a sporangial source. Three times during the crop growth (Figs. 1 and 2) the numbers of infected plants and total plants were counted in each 1-m segment of each row. At the final scoring, the severity of infection was assessed by counting numbers of plants with less than one-half of the tillers infected (light infection) and those with more than one-half of the tillers infected (severe infection).

Sporangial dispersal study. During the post-rainy season 1978 (PRS-78) and the rainy season 1979 (RS-79) experiments were conducted to determine the distance over which sporangia can travel and remain infective. Surface sterilized seed (treated with 0.1% $HgCl_2$ for 10 min) of pearl millet cultivar 7042 (very highly DM-susceptible) were planted in pots, in a mixture of red soil and farmyard manure (3:1, v/v) that had been sterilized at 1.1 kg/cm^2 (15 psi) for 2 hr per day for 3 days. The pots were transferred to the field immediately after planting and were placed at various distances downwind from a field of pearl millet severely infected with DM (the ICRISAT DM resistance screening nursery). During PRS-78 five pots were maintained at each of five positions at 20-m intervals up to 100 m from the field. During RS-79 five pots were maintained at each of 11 positions up to 340 m from the field. DM incidence was recorded 11 and 20 DAP during PRS-78, and 13, 17, and 27 DAP during RS-79. At each scoring time, infected seedlings were removed to minimize the opportunity for secondary inoculum spread from pot to pot. During both experiments, five pots of seedlings were maintained as checks in a screenhouse about 1 km from the field.

RESULTS

Comparison of inocula and inoculation methods. The data on percent DM incidence (Tables 1 and 2) indicate significant effects of both inoculation treatments and cultivars. At each scoring date, plots with the alternate infector treatment had greater DM incidence than did plots with end infector treatment, and these were the only treatments to develop DM incidence significantly greater than the untreated check ($P = 0.05$).

In Table 2, percent incidence values are shown for the individual cultivar treatment combinations at 36 DAP. For each cultivar the alternate infector inoculation system gave the greatest DM incidence. However, the differences between the infector-plant treatments and other treatments were not statistically significant for the two most resistant cultivars, PHB-10 and B-282.

Isolation plot study. For each plot at each scoring date there were 1,200 incidence records. In order to more easily represent DM incidence in the plots, we subdivided each isolation plot into 16 equal-sized subplots, and calculated a mean DM incidence value for each subplot.

At the first observation, all 16 subplots of the two main plots with a central sporangial source had DM-infected plants. Incidence ranged from 0.1 to 18.0% for HB-3 and from 4.0 to 39.4% for NHB-3. The greatest incidence occurred in the subplots located on the northeast sections of the plots (Figs. 1A and 2A). The two mainplots without a central sporangial source had only slight DM incidence— 0.2–0.9% of the plants in three subplots of HB-3 and 0.1–1.7% of those in four subplots of NHB-3 were infected (Figs. 1D and 2D).

At the second observation (41 and 46 DAP for HB-3 and NHB-3, respectively), substantial DM increase was recorded in all subplots of mainplots with a central sporangial source (Figs. 1B and 2B). Incidence for HB-3 ranged from 10.8 to 55%, and for NHB-3 from 19.0 to 54.6%. In the check plots, all HB-3 subplots had DM ranging from 2 to 29.9% and 14 NHB-3 subplots had DM ranging from 0.3 to 11.1% (Figs. 1E and 2E).

The final observations were made from 82 to 87 DAP. The DM

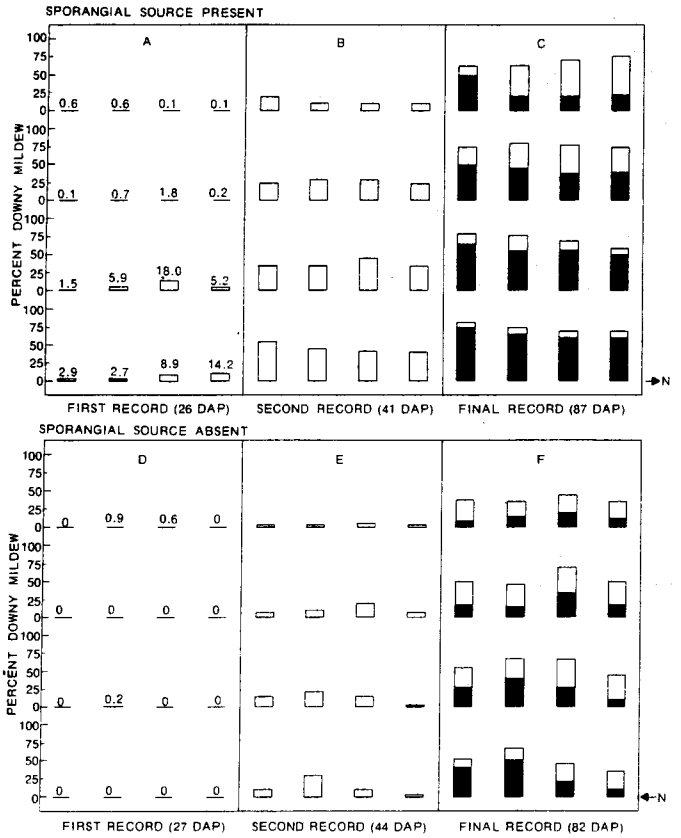

Fig. 1. Downy mildew incidence in 16 equal-sized subplots of two 30 × 30-m field plots of pearl millet hybrid HB-3 which had (A, B, and C) or did not have (D, E, and F) a source of sporangia placed in the center of the plot at crop emergence. For the final record, incidence is given separately for "severely infected plants" (incidence of plants with more than 50% of the tillers infected [indicated by the shaded area]) and for lightly infected plants (incidence of plants with less than 50% of the tillers infected [indicated by the unshaded area]). N indicates north.

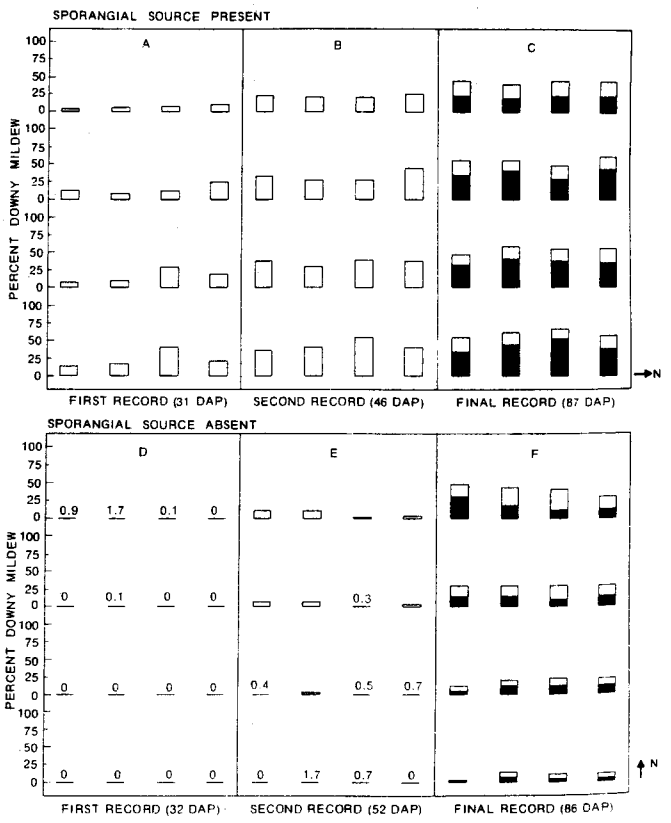

Fig. 2. Downy mildew incidence in 16 equal-sized subplots of two 30 × 30-m field plots of pearl millet hybrid NHB-3 which had (A, B, and C) or did not have (D, E, and F) a source of sporangia placed in the center of the plot at crop emergence. For the final record, incidence is given separately for "severely infected plants" (incidence of plants with more than 50% of the tillers infected [indicated by the shaded area]) and for lightly infected plants (incidence of plants with less than 50% of the tillers infected [indicated by the unshaded area]). N indicates north.

TABLE 1. Effect of inoculation method on downy mildew incidence 15, 24, and 36 days after planting (DAP) averaged for six pearl millet cultivars

Inoculation treatment	Downy mildew incidence (%) 15 DAP	24 DAP	36 DAP
Alternate infector[a]	21.7	39.5	49.3
End infector[b]	11.1	32.2	44.7
Sporangia injected[c]	3.2	11.9	25.2
Leaf bits applied[d]	8.4	11.7	23.0
Oospores to seed and soil[e]	5.3	13.1	22.5
Oospores to soil[e]	3.7	15.4	20.4
Oospores to seed[e]	5.6	13.2	18.7
Oospores injected[f]	3.8	11.7	19.1
Non-treated check	5.2	14.8	19.3
LSD ($P = 0.05$)	4.4	6.3	7.4

[a] Susceptible hybrid HB-3 planted 5 and 2 wk before test cultivars in alternate rows.
[b] Susceptible hybrid HB-3 planted 5 and 2 wk before test cultivars at the end of the test rows.
[c] Sporangial suspension injected into the whorls of test plants 23 DAP.
[d] Sporangia-producing leaf-bits scattered in the test rows 18 DAP.
[e] Oospores applied to soil and/or seed immediately prior to planting.
[f] Oospore aqueous suspension injected into the whorls of the test plants 23 DAP.

TABLE 2. Effect of inoculation method on downy mildew incidence 36 days after planting (DAP) in six pearl millet cultivars

Inoculation treatment	Downy mildew incidence (%) in cultivar[a] 1	2	3	4	5	6
Alternate infector[b]	82.6	83.4	68.3	29.1	23.4	9.2
End infector[c]	81.8	69.2	77.0	17.4	17.5	5.2
Sporangia injected[d]	51.0	47.4	29.1	16.5	4.9	2.2
Leaf bits applied[e]	47.3	36.1	35.2	8.2	8.3	3.1
Oospores to seed and soil[f]	38.3	33.7	39.2	14.0	3.8	6.2
Oospores to soil[f]	40.1	37.0	24.2	10.7	7.3	3.4
Oospores to seed[f]	32.7	29.4	32.7	11.9	3.5	2.1
Oospores injected[g]	49.0	22.8	25.7	10.3	4.3	2.6
Nontreated check	39.0	31.7	28.5	6.8	7.2	2.4
LSD ($P = 0.05$)	18.0					

[a] Cultivars: 1. NHB-3; 2. HB-3; 3. 5071-A; 4. A-836; 5. PHB-10; 6. B-282.
[b] Susceptible hybrid HB-3 planted 5 and 2 wk before test cultivars in alternate rows.
[c] Susceptible hybrid HB-3 planted 5 and 2 wk before test cultivars at the end of the test rows.
[d] Sporangial suspension injected into whorls of test plants 23 DAP.
[e] Sporangia-producing leaf bits scattered in the test rows 18 DAP.
[f] Oospores applied to soil and/or seed immediately prior to planting.
[g] Oospores suspension injected into the whorls of the test plants 23 DAP.

incidence range was 63.3 to 83.8% in the HB-3 plot with a central sporangial source (Fig. 1C) and 33.9 to 67.8% in the HB-3 check plot (Fig. 1F). For NHB-3, the DM incidence in the plot with a central sporangial source ranged from 40.9 to 68.3% (Fig. 2C) while in the check plot of this hybrid DM incidence ranged from 2.7 to 46.4% (Fig. 2F). The differences between sporangial source plots and check plots appear greater when the severity of infection is examined (Figs. 1C and 2C). In both plots with a central sporangial source, the overall ratio of severe to light infection at final scoring was 2:1 whereas the ratio in the check plots was approximately 1:1.

Sporangial dispersal studies. In the PRS-78 study there was little DM infection 20–80 m downwind from the sporangial source, and no infection occurred 100 m away or in the check pots. In contrast, during RS-79, high levels of infection occurred downwind for up to 340 m with the highest levels of infection within the first 120 m (Table 3).

TABLE 3. Downy mildew (DM) incidence in potted plants of pearl millet cultivar 7042 exposed at various distances downwind from a field of highly DM-infected pearl millet during the post-rainy season 1978 and the rainy season 1979

Distance from field (m)	Post-rainy season 1978 Plant nos.	DM incidence (%)	Rainy season 1979 Plant nos.	DM incidence (%)
0	48	58.3	8	100
20	62	3.2	27	100
40	60	3.3	20	100
60	52	1.9	24	79.2
80	33	6.1	39	94.9
100	60	0	...	...
120	...[a]	...	31	90.3
160	...	...	16	37.5
190	...	...	16	68.7
250	...	...	5	40.0
310	...	...	21	33.3
340	...	...	17	17.6
Check[b]	69	0	33	0

[a] No plant maintained at this distance.
[b] Plants kept in a screenhouse 1 km from the field of DM-infected pearl millet.

DISCUSSION

The results of this study indicate clearly that sporangia can play a major role in the epidemiology of pearl millet DM. Wind direction affected sporangial spread, and spread was considerably further during the rainy season than during the dry post-rainy season. In all three sections of the study, considerable infection in young seedlings resulted from the movement of sporangia from older infected adjacent crops or groups of plants. This has important implications both for research workers and for farmers. In experiments on the effect of planting date on pearl millet DM incidence, interplot movement of sporangia from earlier planted plots could result in later plantings getting more DM. In some seed transmission studies the possibility of primary or secondary infection from sporangia is not considered, and data on "the amount of seed transmission" were taken when plants had reached the heading stage (1,10,11).

Prior to 1975, screening for resistance to pearl millet DM had been conducted with reliance on soilborne oospores for primary infection in plots with a history of pearl millet DM and into which infected crop debris had been incorporated. In our study early-planted infector rows, strategically planted in alternate rows of the screening nursery, allowed more efficient and uniform resistance screening.

In the isolation plot study, the plots with no initial central sporangial source had only a few single isolated infected plants at the first scoring; we believe these were infected from soilborne oospores. By final scoring the disease had spread throughout these plots, although it was less severe than in plots in which the initial sporangial source had been placed. Pearl millet is a highly tillering crop; new tillers develop until crop maturity. Thus, unlike sorghum and maize, the period of susceptibility of pearl millet to DM extends throughout the life of the plant, because the young tillers are susceptible until inflorescence differentiation is completed in the developing tiller buds.

It is significant that pearl millet DM reached epidemic proportions in India only when large scale production of F_1 hybrids was practiced. The movement from highly heterogeneous cultivars to much more homogeneous and highly tillering F_1 hybrids gave the sporangia more epidemiological significance, particularly because the early male-sterile lines used to make the F_1 hybrids were highly susceptible to DM. In addition, the practice in some areas of northern India of growing an off-season forage crop of pearl millet which overlaps with the seeding and seedling growth of the subsequent millet grain crop allows earlier infection of the latter through spread of sporangia from the former.

LITERATURE CITED

1. ARYA, H. C., and R. SHARMA. 1962. On the perpetuation and recurrence of the green ear disease of bajra (*Pennisetum typhoides*) caused by *Sclerospora graminicola* (Sacc.) Schroet.). Indian

Phytopathol. 15:166-172.
2. BHAT, S. S., and K. M. SAFEEULLA. 1974. Investigations on the biology and control of *Sclerospora graminicola* on bajra. J. Mysore Univ., Sect. B. 26:118-128.
3. GIRARD, J. C. 1977. Downy mildew of pearl millet in Senegal. Pages 59-73 in: R. J. Williams, ed. Proceedings of the Consultants' Group Meetings on Downy Mildew and Ergot of Pearl Millet, 1–3 October 1975. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. 148 pp.
4. KING, S. B. 1977. Downy mildew and ergot of pearl millet. Pages 97-101 in: R. J. Williams, ed. Proceedings of the Consultants' Group Meetings on Downy Mildew and Ergot of Pearl Millet, 1–3 October 1975. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. 148 pp.
5. NENE, Y. L., and S. D. SINGH. 1976. Downy mildew and ergot of pearl millet. PANS (Pest Artic. News Summ.) 22:366-385.
6. SAFEEULLA, K. M. 1976. Biology and Control of the Downy Mildews of Pearl Millet, Sorghum and Finger Millet. Downy Mildew Research Laboratory, Manasagangothri, Mysore University, Mysore, India. 304 pp.
7. SAFEEULLA, K. M. 1977. Genetic vulnerability: the basis of recent epidemics in India. Pages 72-85, in: P. R. Day, ed. Genetic Basis of Epidemics in Agriculture. Ann. N.Y. Acad. Sci. Vol. 287.
8. SAFEEULLA, K. M. 1977. Downy mildew of pearl millet. Pages 85-95 in: R. J. Williams, ed. Proceedings of the Consultants' Group Meetings on Downy Mildew and Ergot of Pearl Millet, 1–3 October 1975 International Crops Research Institute for the Semi-Arid Tropics. (ICRISAT), Hyderabad, India. 148 pp.
9. SINGH, S. D. 1974. Studies on downy mildew disease (*Sclerospora graminicola* (Sacc.) Schroet.) of bajra (*Pennisetum typhoides* (Brum. f) Stapf and C. E. Hubb.). Ph.D. Thesis, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. 126 pp.
10. SUNDARAM, N. V., D. V. RAMASASTRY, and S. K. NAYAR. 1973. Note on the seed-borne infection of downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) of pearl millet. Indian J. Agric. Sci. 43:215-217.
11. TIWARI, M. M., and H. C. ARYA. 1966. Studies on green ear disease of bajra (*Pennisetum typhoides* Stapf. and Hubb.) caused by *Sclerospora graminicola* (Sacc.) Schroet. Indian Phytopathol. 19:125.

Etiology

Effects of Benomyl and Ribavirin on the Lettuce Big Vein Agent and its Transmission

R. N. Campbell

Plant pathologist, Department of Plant Pathology, University of California, Davis 95616.
The author acknowledges the skilled technical assistance of K. L. Moon and F. V. Westerlund.
Accepted for publication 3 June 1980.

ABSTRACT

CAMPBELL, R. N. 1980. Effects of benomyl and ribavirin on the lettuce big vein agent and its transmission. Phytopathology 70:1190-1192.

Treatment of lettuce plants with benomyl up to 72 hr after inoculation with lettuce big vein agent (BVA)-transmitting *Olpidium brassicae* killed the fungal vector and reduced the frequency of BVA transmission. Benomyl applied to inoculated plants during the symptom expression period had no demonstrable effect on the BVA or symptom expression. Ribavirin applied to inoculated plants during the symptom expression period reduced the severity of symptom expression and the titer of BVA in the *O. brassicae* population in the roots. The BVA was not eradicated from the tops of the plants by ribavirin. The sensitivity of the BVA to ribavirin is evidence of a viral nature of the BVA.

The lettuce big vein agent (BVA) is an infectious, graft-transmissible entity that causes a distinctive vein-banding symptom on lettuce leaves. It is harbored in the soil within the resting spores of the chytrid fungus, *Olpidium brassicae* (Wor.) Dang., and is transmitted to lettuce plants by zoospores of the fungus. The BVA is not mechanically transmissible and no morphological or chemical entity has been associated with it (1).

O. brassicae zoospores mixed with the fungicide benomyl at 100 μg/ml remained motile and infective (2), but thalli in the roots were killed if the roots were treated with benomyl (100–500 μg/ml) applied either before or after inoculation (2,14). Fewer of these plants developed big-vein symptoms (2,14) and others had abnormal or mild symptoms (14) when benomyl treatments were applied within 72 hr of inoculation with BVA-transmitting *O. brassicae*. Symptom expression by lettuce or tobacco plants infected by beet western yellows virus or tobacco mosaic virus (TMV), respectively, was surpressed when the plants were treated with methyl benzimidazole-2-yl carbamate (MBC) (13). Benomyl is converted to MBC and this derivative accounts for the in vivo fungicidal activity observed in treated plants (4).

Antiviral agents, originally developed for animal viruses (8), have found no practical application for plant virus diseases (7). One of these chemicals, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (11) has been tested against some plant viruses. It inhibited local lesion formation by potato virus X in *Capsicum* sp. (6) and promoted the production of virus-free tobacco plants from infected, protoplast-derived calluses (10). Ribavirin also caused remission of symptoms of the rose ring pattern agent (9) and prevented multiplication of apple chlorotic leafspot virus in *Chenopodium quinoa* (5).

The present study was done to determine whether benomyl and ribavirin affected the BVA and symptom expression in lettuce plants or the transmission of BVA to lettuce plants.

MATERIALS AND METHODS

***O. brassicae* cultures and BVA assays.** *O. brassicae* was maintained in the roots of Climax lettuce in sand culture in 10-cm-diameter pots at 16–18 C and irrigated with weak nutrient solution as needed (14). The experimental pots consisted of about 50 lettuce seeds sown in plastic pots (100-ml capacity) with drainage holes. These pots contained about 50 ml of white quartz sand with a void volume of about 20 ml. The preparation of zoospore suspensions was done as previously described (14). The standard inoculum for each experimental pot was 10 ml of a zoospore suspension with

0031-949X/80/12119003/$03.00/0