Heterothallism in *Sclerospora graminicola*

R. W. Michelmore, M. N. Pawar, and R. J. Williams

Visiting scientist, research technician, and principal pathologist, respectively, Millet Improvement Program, International Crops Research Institute for the Semi-Arid Tropics, ICRISAT, P.O., Patancheru, 502 324, Andhra Pradesh, India. Present address of the first author is Department of Vegetable Crops, University of California, Davis 95616.

The first author gratefully acknowledges financial support from the Royal Society-Indian National Science Academy exchange of scientists programme, and thanks K. M. Safeeulla and colleagues at the Downy Mildew Research Laboratory, University of Mysore, for helpful discussions while many of the ideas in this paper were developed.

Journal Series Article 201 of the International Crops Research Institute for the Semi-Arid Tropics.

Accepted for publication 25 February 1982.

ABSTRACT

Michelmore, R. W., Pawar, M. N., and Williams, R. J. 1982. Heterothallism in *Sclerospora graminicola*. Phytopathology 72:1368-1372.

Six isolates of the pearl millet downy mildew (DM) pathogen, *Sclerospora graminicola*, were established and maintained in plants of a susceptible pearl millet (*Pennisetum americanum*) cultivar. Sporangia were originally collected from field grown plants that showed no evidence of sexual reproduction. The isolates were used singly and in all paired combinations to inoculate seedlings of a highly DM-susceptible pearl millet cultivar. Mature oospores were observed in seedlings inoculated with certain specific pairs of isolates. Inoculation with single isolates usually did not result in oospore production. The abundance of oospores varied, but large numbers of oospores were found in seedlings that had shown little or no asexual sporulation, nor extensive chlorosis. The six isolates were subcultured individually for four asexual generations, and no oospores were detected in any of the plants infected with five of the isolates. A few oospores were found infrequently in plants infected with one of the isolates. These results demonstrate the existence of heterothallism in *S. graminicola*. The isolates could be assigned to one of two sexual compatibility types that have been designated G_1 and G_2. Scanning electron micrographs showed hyphae of two morphological types, similar to those observed preceding the formation of gametangia in the heterothallic *Bremia lactucae*. The patterns of asexual and sexual sporulation of *S. graminicola* can be explained in terms of differential colonization of the apices of young host plants. The consequences of heterothallism to pathogenic variability and to breeding for resistance are discussed.

Additional key words: isolation plant propagator, *Phytophthora*.

The importance of pearl millet (*Pennisetum americanum* [L.] Leeke) as a staple food crop in the semi-arid tropics, and the role of diseases in preventing sustained yield increases with improved cultivars of this crop, have been reviewed recently (15,18,19,21,22). Of the diseases affecting pearl millet, downy mildew (DM), caused by *Sclerospora graminicola* (Sacc.) Schroet., is the most destructive and widespread in Africa and Asia. Despite the recognized importance of this pathogen for many years, there are still several aspects of its biology that remain to be studied (15,22,23).

Oospores, produced during sexual reproduction, are thick-walled and are involved in the survival of *S. graminicola* during the long, hot, dry seasons of the semi-arid tropics. In pearl millet, DM oospores have been generally assumed to form in response to some nutritional trigger that develops when the infected host tissue has supported the production of many crops of sporangia for several days or weeks. There is apparently no information available on the sexual system of *S. graminicola*. Neither Nene and Singh (15) nor Safeeulla (18) refer to this important aspect of oospore production in their comprehensive reviews of pearl millet DM. Pathogenic variation has been reported between isolates of *S. graminicola* from different host species (8), and there are strong indications of such variation between isolates from pearl millet (1), but little is known of the nature and causes.

Heterothallism has been demonstrated in other plant pathogenic Oomycetes with oospore production requiring the coincidence of the two sexual compatibility types (2,4,10,20).

Results are presented in this paper which suggest that the sexual system of *S. graminicola* is similar to those of the heterothallic members of the Peronosporaceae. The importance of these findings to the reinterpretation of DM symptoms and to resistance breeding programs is discussed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/10136805/$03.00/0

MATERIALS AND METHODS

Collection and maintenance of isolates. Single infected leaves, supporting profuse sporangial production of *S. graminicola*, were collected from 20 DM-infected plants that did not show extensive chlorosis in the ICRISAT pearl millet DM screening nursery (24). Each leaf was washed and gently rubbed with moist cotton wool to remove old sporangia and sporangiophores, cut into 2-cm segments, and then placed into a separate moist box. The leaves in the boxes were incubated in the dark for 7.5 hr at 20 C and were then kept at 8 C until the leaf segments were used ~9 hr later. The six leaves with the most profuse sporulation were selected; a spore suspension was made from each by vigorously shaking the leaf segments in distilled water and used as described below as separate isolates 1–6. The leaf segments from which the sporangial suspensions were prepared were fixed and cleared in 95% ethanol followed by 5% NaOH and then inspected microscopically for the presence of oospores.

The isolates were maintained on plants of the highly DM-susceptible pearl millet cultivar 7042 grown from surface-sterilized seed (treated with 2% Clorox for 30 min and then washed in sterile distilled water). The plants were grown in sterilized soil (autoclaved for 1 hr at 206.8 kPa [30 psi] on three consecutive days) in an isolation plant propagator (Burkard Manufacturing Company Ltd., Rickmansworth, U.K.), in which the seedlings were in covered pots maintained at slightly above atmospheric pressure so that airborne spores were not able to enter the pots. Seedlings were inoculated at the two- or three-leaf stage by injecting the sporangial suspensions into the bases of the seedlings in the vicinity of the growing points. This mode of inoculation resulted in less rapid death of infected seedlings than the method used to test for heterothallism and was therefore more useful for maintaining the isolates. Two 12.5-cm-diameter pots, each with 10 plants, were maintained per isolate at each subculture. Isolates were subcultured at 3–4 wk intervals. The use of the isolation plant propagator for the maintenance of isolates and a laminar-flow clean-air bench for the subculture operations minimized the possibilities of isolate contamination.

Tests for heterothallism. Two tests for heterothallism were made, the first with the sporangial suspensions of the six isolates when first collected, and the second 17 weeks (and four subcultures) later, with sporangial suspensions of the same six isolates prepared from sporangia produced on intact leaves of the plants used for the maintenance of the isolates.

Seeds of pearl millet, cultivar 7042, were surface sterilized and then incubated on moist blotting paper in 10-cm-diameter petri plates at 30 C for 24 hr; germinated seeds were selected and placed on the centers of 24 10-cm-diameter petri dishes, 20 seedlings per dish in experiment 1 and 30 in experiment 2. The seedlings were inoculated by immersion in 2 ml of sporangial suspension ($\sim 9 \times 10^5$ sporangia per milliliter) dispensed from a syringe. A clean syringe was used for each isolate. Isolates were used singly (2 ml per isolate per dish) and in all paired combinations (1 ml per isolate per dish). The seedlings in three dishes were treated with 2 ml of sterile distilled water to serve as checks. Inoculated and check seedlings were incubated in the dark at 22 C for 9 hr, planted in sterilized soil (10 seedlings per 12.5-cm-diameter pot in experiment 1 and 15 seedlings per pot in experiment 2, two pots per isolate and isolate combination), and incubated in an isolation plant propagator in a glasshouse.

Seedlings were observed daily and the occurrence of asexual sporulation was recorded. Periodically leaves or whole seedlings that had become necrotic were removed from the pots on a laminar-flow clean-air bench. The necrotic tissues were rolled flat in a drop of sterile distilled water on a microscope slide with the aid of a glass rod and were carefully inspected microscopically for the presence and abundance of oospores (Table 1). Necrotic leaves from the plants used for maintaining the isolates were also similarly examined.

Scanning electron microscopy. Chlorotic leaves and malformed inflorescences ("green ears") from systemically infected pearl millet plants were collected from the ICRISAT pearl millet DM nursery. This material was teased apart in distilled water, squashed, and examined under the light microscope for the presence of oogonia. When oogonia were observed, adjacent leaf segments were fixed for scanning electron microscopy (SEM). The tissues were fixed in 2.5% gluturaldehyde in 0.1 M phosphate buffer (pH 6.8) at room temperature for 4 hr and then at 5 C for 22 hr. After two washes in buffer, the tissues were treated with 1% osmium tetroxide overnight; the tissues were then dehydrated through an alcohol series to 70% ethanol (11). The samples were stored and transported in 70% ethanol.

Prior to observation the samples were dehydrated further in an alcohol series to 100% ethanol, and were critical-point dried from liquid CO_2. Fracture planes inside the leaves were obtained by tearing back the vascular tissue, or by dry-fracture using a sandwich of double-sided "Scotch" tape (3M Co., St. Paul, MN 55101) (11). Repeated peels with the "Scotch" tape were necessary to provide suitable fracture planes. Specimens were splutter-coated with a layer of gold 20 nm (200 Å) at 5 C and were observed with a Stereoscan S4 scanning electron microscope (Cambridge Instruments Co., Cambridge, U.K.).

RESULTS

Asexual sporulation of *S. graminicola* was evident on many seedlings within 6–7 days after inoculation (DAI). Some seedlings became completely necrotic by 12–14 DAI and their first leaves never unfolded. The necrosis of some seedlings was associated with little or no asexual sporulation. No necrosis or sporulation was observed in any of the uninoculated check seedlings. In the first experiment necrotic tissue was removed from the pots and examined 13, 21, 35, and 56 DAI and in the second experiment at 14, 28, and 43 DAI. The total numbers of infected seedlings, the total numbers of seedlings with oospores, and the relative abundance of oospores are presented in Table 1.

In both experiments isolates 1–5 did not produce oospores when inoculated singly. Isolate 6, when inoculated singly, produced oospores sparsely in one seedling in each experiment. Inoculations with isolates 1, 4, and 6 in all paired combinations, and inoculations with isolates 2, 3, and 5 in all paired combinations did not result in the production of oospores. Oospores were produced when any one of isolates 1, 4, and 6 was paired with any one of isolates 2, 3, and 5. Table 2 summarizes the production of oospores within and between these two groups. The abundance of oospores varied, and large numbers of oospores were found in the seedlings that had shown little or no asexual sporulation. Abundant production of oospores was also associated with extensive necrosis. Oospores with a mature morphology were observed 13 DAI in seedlings that had produced sporangia, and therefore sexual sporulation commenced at approximately the same time as asexual sporulation. Figure 1 shows patterns of colonization that may result in sequential

TABLE 1. The numbers of downy mildew infected seedlings of pearl millet cultivar 7042, the number of infected seedlings containing oospores, and the relative abundance of oospores after inoculation with six isolates of *Sclerospora graminicola* singly and in all paired combinations, in two experiments

Isolate combination	Experiment 1			Experiment 2		
	Plants infected (no.)	Plants with oospores (no.)	Oospore abundance[a]	Plants infected (no.)	Plants with oospores (no.)	Oospore abundance[a]
1	11	0	−	24	0	−
2	12	0	−	18	0	−
3	14	0	−	9	0	−
4	11	0	−	16	0	−
5	12	0	−	24	0	−
6	13	1	+	22	1	+
1 × 4	8	0	−	4	0	−
4 × 6	17	0	−	9	0	−
1 × 6	10	0	−	15	0	−
2 × 3	12	0	−	20	0	−
2 × 5	12	0	−	11	0	−
3 × 5	13	0	−	15	0	−
1 × 2	13	2	+++	19	2	+++
1 × 3	11	2	++	15	2	+++
1 × 5	14	4	+++	10	1	++
2 × 4	10	4	+++	18	3	+++
2 × 6	15	1	+	12	4	+++
3 × 4	13	3	++	21	3	+++
3 × 6	18	5	+++	18	4	+++
4 × 5	17	8	+++	20	10	+++
5 × 6	16	8	+++	19	9	+++

[a] Abundance of oospores in seedlings where sexual reproduction occurred: + = sparse—a few scattered oospores; ++ = intermediate—oospores encountered in several microscope fields, but in easily countable numbers; +++ = abundant—oospores encountered throughout tissue, too numerous to count easily.

production of asexual, then sexual, spores in field-grown plants. No oospores were observed in the roots of seedlings or in the segments of the leaves that were used to provide the initial inoculum of the six isolates.

During subculture of the isolates for four generations, a few oospores were detected in two of 34 infected plants inoculated with isolate 6. No oospores were detected in any of the plants used to maintain isolates 1–5.

Examination of chlorotic, infected pearl millet plant material from the field revealed that many leaves and malformed inflorescences contained no oogonia. Sparse production of oogonia was observed only in phyllodes of a few malformed inflorescences, and small segments of these were fixed and prepared for subsequent examination with the scanning electron microscope.

Two hyphal morphologies were shown by SEM (Figs. 2–5). Many hyphae had a fairly constant diameter and were as straight as the irregularities of the host tissue allowed. In places, however, there were extensive associates of highly branched, irregularly shaped hyphae. In one case an interaction between such hyphae appeared to have been initiated at the point of contact between two thicker, straighter hyphae (Fig. 4). Associations of hyphae were often observed in substomatal cavities, which probably explains their regular spacing in Fig. 5.

TABLE 2. Summary[a] of sexual reproduction occurring within and between sexual compatibility types of *Sclerospora graminicola*

Compatibility type	Compatibility type[b]	
	G_1	G_2
G_1	0/172	75/279
G_2		2/160

[a]Number of plants containing oospores/number of infected pearl millet plants.
[b]G_1 = isolates 2, 3, and 5; G_2 = isolates 1, 4, and 6.

DISCUSSION

The basic assumptions that determined the experimental design were that *S. graminicola* was predominantly heterothallic, the frequency of the sexual compatibility types in the population was approximately equal, and high levels of asexual sporulation and the lack of severe chlorosis were indicative of the absence of sexual reproduction and therefore the presence in such host tissue of only one sexual compatibility type. The results obtained clearly demonstrate that *S. graminicola* exhibits heterothallism. The isolates can be grouped into two distinct sexual compatibility types; crosses within each group were sterile, whereas those between them were fertile. A study of a large number of isolates of *S. graminicola* from diverse locations, including many from West Africa, which is the center of origin of pearl millet (3) and probably also of *S. graminicola,* is needed to determine whether more than two compatibility types exist. Also the relative frequencies of the compatibility types in natural populations of the pathogen and the extent of variation between different populations should be determined. In other heterothallic Oomycetes only two sexual compatibility types have been identified (2,4,10,16,20).

Further experimentation is required to determine whether the sparse and infrequent production of oospores by isolate 6 is due to a form of self-fertility or due to the isolate being a mixture of isolates of the two compatibility types, with one at a low frequency. Self-fertility due to secondary homothallism has been observed in *B. lactucae* (13) and several predominantly heterothallic *Phytophthora* species (14). The low level of sexual reproduction in isolate 6 of *S. graminicola* over several asexual generations may also be a form of secondary homothallism as it is similar to that

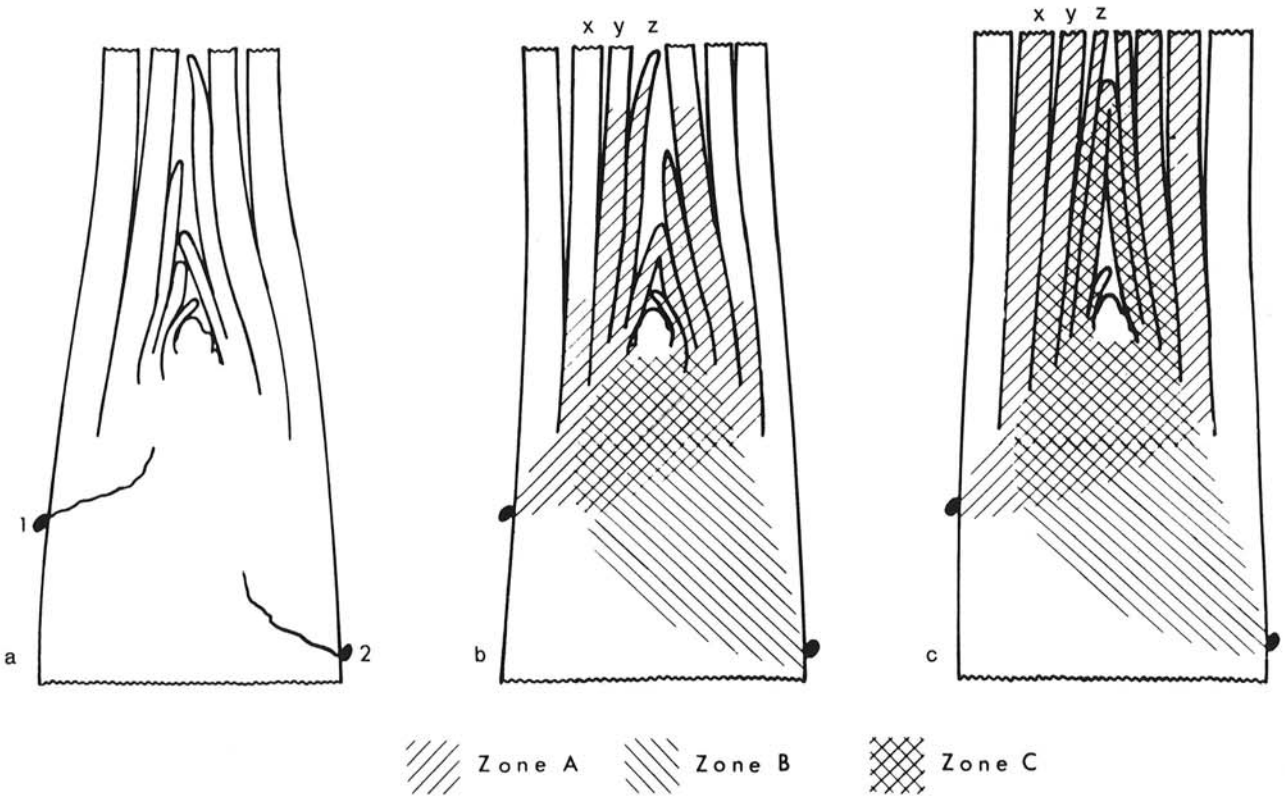

Fig. 1. Scheme to illustrate the possible patterns of colonization and sporulation in stem apices and leaves of pearl millet following multiple infections by heterothallic strains of *Sclerospora graminicola*: a, b, and c show stages in progressive colonization of the tissues. Zone A is the host tissue colonized from infection 1, which will show asexual sporulation only. Zone B is the host tissue colonized from infection 2. Zone C is the tissue colonized from both infections and in which, if the mycelia are of opposite sexual compatibility types, there will be sexual reproduction. Leaves x, y, and z will show ¼, ½, and ¾ leaf symptoms, respectively, when expanded. Leaves y and z will contain oospores only in their basal portions. Leaves developing subsequently will contain oospores throughout their lengths.

observed with self-fertile isolates of *B. lactucae*. The lack of oospores in crosses involving isolate 6 with isolates 1 or 4 probably reflects the infrequent nature of self-fertility of isolate 6. In *B. lactucae* the self-fertile isolates act predominantly as compatibility type B_2. Therefore, isolates 1, 4, and 6 of *S. graminicola* are tentatively designated compatibility type G_2, and isolates 2, 3, and 5, compatibility type G_1 (Table 2). Because sexual reproduction is dependent upon mycelia of both compatibility types becoming established in the same seedling, higher frequencies of sexual reproduction were not expected when there is not 100% infection of seedlings. The nomenclature G_1 and G_2 is proposed as there are characteristics peculiar to each of the compatibility groups within *B. lactucae* and *Phytophthora* species. Also, heterothallism may have evolved several times in the Peronosporales and at least minor differences are to be expected, as have already been found between *B. lactucae* and the heterothallic *Phytophthora* species (13). The locus (or loci) determining sexual compatibility type is (or are) likely to be complex and the G_1/G_2 nomemclature does not imply that compatibility type is necessarily determined by two alleles at a single locus.

The scanning electron micrographs show associations of hyphae very similar to the interactions which precede the formation of gametangia in *B. lactucae* (11). In *B. lactucae*, highly branched, irregularly shaped sexual hyphae are formed at the point of contact of two thicker vegetative hyphae presumed to be of opposite compatibility types; oogonia and antheridia arise from these interactions. The associations observed in *S. graminicola* were more extensive than those observed in *B. lactucae*; the host cells were very closely packed and no oogonia were observed in the fracture planes obtained. The distribution of hyphal interactions in

Figs. 2-5. Scanning electron micrographs of *Sclerospora graminicola* in pearl millet leaves: **2,** complex association among highly branched hyphae of irregular dimensions (i); **3,** another complex association (i) and a straighter hypha of more regular thickness (v); **4,** an association of irregularly shaped hyphae (i) at the junction of two regularly shaped hyphae (v); **5,** regular spacing of the groups of highly branched hyphae (i) that appear to occur in substomatal spaces. Scale bar = 5 μm.

the substomatal cavities may reflect the influence of the host anatomy on hyphal growth, but because the material was collected from the field, it is not possible to check that such hyphal associations do not occur when only one compatibility type is present and there is only asexual sporulation. Figure 4 strongly suggests, however, that hyphal interactions are initiated where two vegetative hyphae are in contact.

The inverse relationship observed between abundance of asexual and sexual sporulation is similar to that observed in *B. lactucae* and other members of the Peronosporaceae (9,10,18). In the experiments described in this paper, sexual and asexual sporulation commenced simultaneously, and there was no evidence for an obligatory developmental progression from asexual to sexual reproduction. The apparent progression from asexual to sexual sporulation observed in the field probably reflects the pattern of infection and colonization of the host plant by the two compatibility types. In pearl millet DM, local infection of unfolded leaves does not normally occur, and systemic colonization occurs only when mycelia become established in the growing points of young seedlings. Often the first leaves to show symptoms exhibit a characteristic partial-leaf symptom, with only the basal part of the leaf infected; leaves developing subsequently show greater proportions of infected tissue until the whole lamina is colonized and is chlorotic. The partially infected leaves bear asexual spores and the more chlorotic, fully colonized leaves often contain oospores (18). When young seedlings are infected from more than one spore, the infections may occur in separate zones of host tissue (Fig. 1a). The subsequent symptoms and patterns and intensities of sexual and asexual sporulation reflect the progressive colonization of the host apical region prior to leaf expansion (Fig. 1b and c). Jones (7) has shown that the colonization of the apical region of *Sorghum bicolor* by *Peronosclerospora sorghi* was variable and could be related to the patterns of systemic colonization and asexual sporulation. The axillary buds of *P. americanum* are additional sites for infection by *S. graminicola*, but again early infection is necessary for systemic colonization (21). The infrequency of oospore formation observed in the field at the time of the trial suggests that many of the plants had become systemically colonized by only one compatibility type. The distribution of chlorotic tissue probably reflects the degree of apical colonization prior to leaf expansion rather than whether sexual reproduction is occurring within the tissues. This is unlike the situation in downy mildews that form restricted lesions, and sexual reproduction results in enhanced chlorosis of green tissues.

S. graminicola seems to be predominantly heterothallic. Therefore, outbreeding and extensive variation within and between isolates from different geographical areas can be expected. The Oomycetes are almost certainly diploid (5) and the pathogen population may contain unexpressed variation that could allow it to respond rapidly to steep selection pressures, for example, produced by the introduction of highly resistant cultivars. The alleles for virulence in *B. lactucae* seem to be recessive and the fungus exhibits both sexual and asexual variation (12). Many lettuce cultivars resistant to *B. lactucae* have been developed and released since 1926, but none has remained resistant during commercial use (6). Heterothallism of *S. graminicola*, therefore, has important implications to disease control programs.

Pearl millet is predominantly an outcrossing species, and traditional cultivars are maintained as open-pollinated mixtures in which there is extensive variation in many characters, including resistance to DM. Some traditional cultivars have provided sources of high levels of resistance to DM (17), whereas several F_1 hybrids and inbred lines have been highly susceptible. To obtain long-term control of pearl millet DM with resistant hybrids, variability for resistance within hybrids should be maximized, so that the spread of the pathogen will be restricted. The potential for planting different cultivars in adjacent blocks or using cultivar mixtures (25) should be investigated so that the risks and consequences of changes of virulence in the pathogen population can be minimized.

LITERATURE CITED

1. Anonymous. 1980. Pearl millet pathology. Pages 71–79 in: 1978–79 Annual Report. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India.
2. Brasier, C. M. 1969. Formation of oospores in vivo by *Phytophthora palmivora*. Trans. Br. Mycol. Soc. 52:273-279.
3. Brunken, J., De Wet, J. M. J., and Harlan, J. R. 1977. The morphology and domestication of pearl millet. Econ. Bot. 31:163-174.
4. Bruyn, H. L. G. de. 1937. Heterothallism in *Peronospora parasitica*. Genetica 19:553-558.
5. Caten, C. E., and Day, A. W. 1977. Diploidy in plant pathogenic fungi. Annu. Rev. Phytopathol. 15:295-318.
6. Crute, I. R., and Dixon, G. R. 1981. Downy mildew disease caused by the genus *Bremia* Regel. Pages 421–460 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, New York.
7. Jones, B. L. 1978. The mode of systemic colonization of sorghum and Sudangrass by conidia of *Sclerospora sorghi*. Phytopathology 68:732-735.
8. Kenneth, R. G. 1976. The downy mildews of corn and other Graminaceae in Africa and Israel, and the present state of knowledge and research. Kasetsart J. 10:148-159.
9. McKay, R. 1939. Observations on the downy mildew caused by the fungus *Peronospora schleidiana*. J. R. Hortic. Soc. 64:272-285.
10. Michelmore, R. W., and Ingram, D. S. 1980. Heterothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 75:47-56.
11. Michelmore, R. W., and Ingram, D. S. 1981. Origin of gametangia in heterothallic isolates of *Bremia lactucae*. Trans. Br. Mycol. Soc. 76:425-432.
12. Michelmore, R. W., and Ingram, D. S. 1981. The recovery of sexual progeny following sexual reproduction of *Bremia lactucae* Regel. Trans. Br. Mycol. Soc. 77:131-137.
13. Michelmore, R. W., and Ingram, D. S. 1982. Secondary homothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 78:1-9.
14. Mortimer, A. M., Shaw, D. S., and Sansome, E. R. 1978. Genetical studies of secondary homothallism in *Phytophthora drechsleri*. Arch. Microbiol. 111:255-259.
15. Nene, Y. L., and Singh, S. D. 1976. Downy mildew and ergot of pearl millet. PANS (Pest Artic. News Summ.) 22:366-385.
16. Pratt, R. G., and Green, R. J. 1973. The sexuality and population structure of *Pythium sylvaticum*. Can. J. Bot. 51:429-436.
17. Rachie, K. O., and Majmudar, J. V. 1980. The ICRISAT Millet Improvement Program—Identification of stress resistances: Downy Mildew. Page 221 in: Pearl Millet. Pennsylvania State University Press, University Park and London. 307 pp.
18. Safeeulla, K. M. 1976. Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Downy Mildew Research Laboratory, Manasagangothri, Mysore, India. 304 pp.
19. Safeeulla, K. M. 1977. Genetic vulnerability: The basis of recent epidemics in India. Pages 72–85 in: Genetic Basis of Epidemics in Agriculture. P. R. Day, ed. Ann. N.Y. Acad. Sci. Vol. 287.
20. Savage, E. J., Clayton, C. W., Hunter, J. H., Brenneman, J. A., Laviola, C., and Gallegly, M. E. 1968. Homothallism, heterothallism, and interspecific hybridization in the genus *Phytophthora*. Phytopathology 58:1004-1021.
21. Singh, S. D., and Williams, R. J. 1980. The role of sporangia in the epidemiology of pearl millet downy mildew. Phytopathology 70:1187-1190.
22. Thakur, R. P., and Williams, R. J. 1980. Pollination effects on pearl millet ergot. Phytopathology 70:80-84.
23. Williams, R. J., Pawar, M. N., and Huibers-Govaert, I. 1980. Factors affecting staining of *Sclerospora graminicola* oospores with triphenyl tetrazolium chloride. Phytopathology 70:1092-1096.
24. Williams, R. J., Singh, S. D., and Pawar, M. N. 1981. An effective large-scale field resistance screening technique for downy mildew in pearl millet. Plant Dis. 65:239-241.
25. Wolfe, M. S., and Barrett, J. A. 1980. Can we lead the pathogen astray? Plant Dis. 64:148-155.