Natural and Experimental Production of Oospores of *Bremia lactucae* in Lettuce in New York

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ABSTRACT

Yuen, J. E., and Lorbeer, J. W. 1987. Natural and experimental production of oospores of *Bremia lactucae* in lettuce in New York. Plant Disease 71:63-64.

Oospores of *Bremia lactucae* were observed in leaves of infected lettuce plants from commercial lettuce fields in New York. Oospores formed in cotyledons of the cultivar Ithaca after dual inoculation with isolates of opposite compatibility type. Two compatibility types were detected in New York. With one exception, each field sampled contained only one compatibility type.

Additional key words: downy mildew, *Lactuca sativa*

Bremia lactucae Regel, causal agent of downy mildew of lettuce (*Lactuca sativa* L.), can be a common pathogen of lettuce in New York. The manner by which the pathogen overwinters is not known, but the disease usually occurs regularly every growing season in certain fields. Oospores of *B. lactucae* were reported in cultivated lettuce in England by Humphrey-Jones in 1971 (2) and Tommerup et al (9) in 1974. Their development was detailed by Sargent et al (8) in 1977. In 1980, Michelmore and Ingram (4) reported that *B. lactucae* usually is heterothallic and that two compatibility types are required for formation of oospores in most isolates. Some isolates, however, are homothallic (5). Our study was done to determine the presence of oospores of *B. lactucae* in New York and the factors leading to their production.

MATERIALS AND METHODS

Leaves of lettuce with downy mildew symptoms were obtained from commercial fields in New York during 1979–1980. Numbers of leaves from each field differed depending on the incidence of downy mildew, but at least 10 leaves were taken from each field. Lesions were excised and cleared using the method described by Marlatt et al (3) and examined with a light microscope to detect naturally formed oospores.

Isolates of *B. lactucae* used in this study came from single downy mildew lesions. Leaves with downy mildew were collected during 1981 in plastic bags and transported to the laboratory the same day. Leaves were rinsed briefly in tap water and placed in glass casserole dishes containing water to a depth of 1 cm. These dishes were placed in an illuminated incubator (14 C, 3,000-lux photoperiod of 12 hr). After 3 days, single lesions were excised, then each was placed in a 30-ml centrifuge tube with 2 ml of water and agitated in a Vortex-Genie (Scientific Industries, Bohemia, NY) for 30 sec to obtain a suspension of sporangia. Single 10-μl drops of this suspension were placed on 10 excised lettuce cotyledons on moist filter paper in glass petri dishes and incubated in the illuminated incubator. Isolates were maintained through periodic transfer of sporangia.

Single-spore isolates of *B. lactucae* also were made by first placing sporangia from individual single lesions on the surface of a sterile 2% water agar plate. The agar surface then was examined for single sporangia, which were removed from the surface of the agar by manipulating the mouth of a micropipette next to each sporangium and allowing the sporangium to be sucked up by capillary action. The sporangium then was deposited in a 10-μl drop of water previously placed on the abaxial surface of a lettuce cotyledon. Infection of the cotyledon resulted and progeny sporangia were produced. Cotyledons with water drops without sporangia were used to indicate that no contamination took place. These original single-spore isolates were subsequently maintained through periodic mass-transfer of progeny sporangia resulting from each serial transfer.

Lettuce seedlings were produced by sowing seed (cultivar Ithaca) on moist peat potting mix, covering the seed with a thin layer of vermiculite, and incubating the seed in a greenhouse at ±24 C. After 10–14 days, the two cotyledons of each seedling were removed and placed, abaxial side up, on moist filter paper in glass petri dishes with the lids intact. The number of cotyledons per dish ranged from one (for production of single-spore isolates) to 10 (for routine maintenance of *B. lactucae* isolates).

Cotyledons were inoculated during routine maintenance by placing a single 10-μl drop of a suspension of sporangia (concentration from 1 to 5×10^5/ml) on the abaxial surface of each of 10 cotyledons in a petri dish and placing the dish with the lid on in the illuminated incubator. Sporangia formed within 7–10 days.

Compatibility types were determined by first preparing the initial suspensions of sporangia from different isolates and mixing them in equal proportions. Four to six cotyledons were each inoculated with this mixture following the procedure described previously. Control groups of cotyledons were also inoculated at the same time with the unmixed isolates. Cotyledons inoculated in this manner were vacuum-infiltrated 8 days later with water (4) and observed for oospores with a light microscope.

RESULTS

In the field-collected leaves, oospores of *B. lactucae* were seen in leaves from two fields. Nine single-sporangial isolates of *B. lactucae* were crossed in all possible combinations. Oogonia, antheridia, and oospores formed in 20 of the 36 crosses (Table 1), indicating that two mating types were present among these nine isolates: four of one compatibility type (designated type A) and five of the other (designated type B). Two of these single-sporangial isolates, 21 (21-81-SS1 = type A) and 22 (22-81-SS1 = type B) were arbitrarily chosen as test isolates, and single-lesion isolates were crossed with them.

The single-lesion isolates of *B. lactucae* were also grouped into the two compatibility types when crossed with either of the test isolates in a typical experiment (Table 2). In this experiment, seven of the isolates were type A and 13 were type B. During our investigation, only a single compatibility type was found in each field, except one, where both compatibility types were detected.

DISCUSSION

The capacity for oospore formation exists within the New York population of

Accepted for publication 31 May 1986.

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Table 1. Presence (+) or absence (−) of sexual reproduction in all possible crosses of nine single-sporangial isolates of *Bremia lactucae*[a]

Isolate	20	21	23	27	22	24	28	29	30
30	+	+	+	+	−	−	−	−	0
29	+	+	+	+	−	−	−	0	···
28	+	+	+	+	−	−	0	···	···
24	+	+	+	+	−	0	···	···	···
22	+	+	+	+	0	···	···	···	···
27	−	−	−	0	···	···	···	···	···
23	−	−	0	···	···	···	···	···	···
21	−	0	···	···	···	···	···	···	···
20	0	···	···	···	···	···	···	···	···

[a]Isolate numbers are abbreviations: 21 = 21-81-SS1, 22 = 22-82-SS1, etc., where SS1 indicates single-sporangial isolate of the corresponding single-lesion isolate (see Table 2). 0 = Crosses not made with same isolate. Isolates 22, 24, 28, 29, and 30 designated as compatibility type A; isolates 20, 21, 23, and 27 designated as compatibility type B.

Table 2. Single-lesion isolates of *Bremia lactucae* crossed with either 21-81-SS1 (compatibility type A) or 22-81-SS1 (compatibility type B), presence (+) or absence (−) of sexual reproduction, and fields of origin of isolates[a]

Isolate	Field of origin	Crossed with isolate: 21-81-SS1 type A	Crossed with isolate: 22-81-SS1 type B	Compatibility type
4-81	Crissafulli	−	+	A
5-81	Crissafulli	−	+	A
7-81	Jacobson	+	−	B
8-81	Jacobson	+	−	B
10-81	Desalvo	+	−	B
11-81	Desalvo	+	−	B
12-81	Desalvo	+	−	B
14-81	Arena	−	+	A
15-81	Sorbello	+	−	B
16-81	Marano	−	+	A
17-81	Desalvo	+	−	B
18-81	Desalvo	+	−	B
19-81	Desalvo	+	−	B
20-81	Arena	−	+	A
23-81	J. Ferlito	−	+	A
24-81	Sorbello	+	−	B
27-81	M. Ferlito	−	+	A
28-81	Marano	+	−	B
29-81	Marano	+	−	B
30-81	Marano	+	−	B

[a]Isolate 21-81-SS1 from Arena field; isolate 22-81-SS1 from J. Ferlito field.

B. lactucae. Oospores were detected in field-collected material, and both compatibility types were found in a collection of single-lesion and single-spore isolates. Sexual reproduction in *B. lactucae* had been suspected in a study that revealed a diversity of virulence factors in the fungus population in New York in the absence of selection for these virulence factors in commercial lettuce cultivars (10). The ability to reproduce sexually as now confirmed may help explain this diversity for specific virulence. In a study involving specific virulence in *Puccinia graminis* (7), sexually reproducing populations were more diverse than asexually reproducing ones.

Within the New York population of *B. lactucae*, two compatibility types were found. Usually one compatibility type was found in each field. This would explain why oospores in field-collected leaves were not found with more frequency. Sample sizes from individual fields were not large enough, however, to rule out the general existence of both compatibility types. In one instance, both compatibility types were detected in the same field, indicating that both can coexist within the same subpopulation.

The presence of oospores in the New York population of *B. lactucae* will have to be considered in devising disease control measures. If, as indicated by recent studies (1,6), germinating oospores commonly infect lettuce seedlings, which subsequently serve as a source of sporangia, then control of oospore infection, possibly through seed treatment or an in-furrow application of fungicide, might be an effective way to control the disease. We have reported (11) that an in-furrow application of metalaxyl controlled downy mildew of lettuce under field conditions in New York. Whether this treatment controlled oospore infection of seedlings in addition to infection by sporangia or only the latter is not known. However, elimination of a potential oospore initiation of the disease cycle each growing season by a seed or seed-furrow treatment could be an effective way to control the disease.

The presence of sexual reproduction in the New York population of *B. lactucae* probably contributes in some degree to the variability for specific virulence observed in this pathogen population (10). The oospores resulting from sexual reproduction also provide another means by which the fungus may overwinter. The existence of oospores indicates that new considerations need to be made concerning disease control strategies for *B. lactucae*.

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