# Virulence Factors of *Bremia lactucae* in New York

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## ABSTRACT

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Field collections of *Bremia lactucae* from the lettuce cultivars Ithaca, Minetto, and A-1 were determined to have virulence factors 2 and 7 when tested on 5-day-old cotyledons of 21 differential lettuce cultivars grown on vermiculite in a controlled environment chamber. A field experiment consisting of 11 of the 21 differential cultivars was conducted in Oswego County, New York. Plants possessing resistance factor 7 became infected first. Eventually all plants of cultivars possessing the resistance factors 5, 6, 7, 8, and 10 became infected. *B. lactucae* isolates collected from the planting were tested on 5-day-old cotyledons of the 21 differential cultivars grown in the controlled environment chamber, which confirmed the presence of virulence factors 5, 6, 7, 8, and 10 and also revealed the presence of virulence factors 1, 2, and 4. Virulence factors 9 and 11 may have been present, since 12–54% of the plants possessing the corresponding resistance factors became infected.

*Additional key words:* downy mildew, gene-for-gene, genetics, *Lactuca sativa,* resistance.

*Bremia lactucae* Regel, the cause of downy mildew of lettuce (*Lactuca sativa* L.), can develop on lettuce grown commercially on organic soils in New York during the entire growing season. During summer and early fall, mildew can substantially damage lettuce plants if cool, wet conditions occur. Cultivars of lettuce resistant to downy mildew have been developed since the early stages of the crisphead lettuce breeding program in the United States (2). These and other downy mildew-resistant cultivars have enabled pathologists to identify different races of the pathogen. Races of *B. lactucae* are distinguished by their ability to overcome one or more of the known factors for resistance. The present study was undertaken in order to identify the race or races of *B. lactucae* present in New York. Knowledge of which races are present would identify the factors for resistance that the population of *B. lactucae* could not overcome, and these factors then could be utilized in resistant cultivars.

Four races of *B. lactucae* were described by Jagger and Chandler (11). In their study, nine cultivars of lettuce were planted at different locations. All nine were resistant when grown at Chula Vista, California, and at Sanford, Florida, but some were susceptible when grown in England and in the Salinas and the Imperial Valleys in California, indicating that there were at least four races of *B. lactucae.* Which cultivars were susceptible and resistant at the different locations were not mentioned; thus, it has not been possible to further characterize these earlier races. One lettuce cultivar (called Romaine blonde lente a monter) from France was resistant at all locations and was used as a source of resistance (10). Some of the early cultivars of lettuce that were developed with resistance from that source included Imperial D, Imperial F, and Imperial 847 (12). A fifth race, distinguished by its ability to overcome this resistance, appeared in California (12), but PI 91532 (*Lactuca serriola* L.) and a French butterhead cultivar, Bourguignonne, were resistant to this fifth race. Crosses made with PI 91532 eventually led to the release of the cultivars Valverde (19) and Calmar (32). Another wild species of *L. serriola*, PI 167150, collected by J. R. Harlan in Turkey, also was found to be resistant to race 5, and R. C. Thompson incorporated this resistance into a romaine-type cultivar, Valmaine (20). In Texas, a sixth race of *B. lactucae* was described that overcame the resistance from either PI 91532 or PI 167150 (28). The cultivars of lettuce used to determine races 1–6 of *B. lactucae* are listed in Table 1.

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In 1976, a "gene-for-gene" system of race designation correlating specific virulence factors in *B. lactucae* with specific resistance factors in lettuce was published by Crute and Johnson (4). A virulence factor is assigned to a collection of *B. lactucae* when it is able to overcome the corresponding resistance factor. Thus, in order to identify the virulence factors present in a *B. lactucae* collection, a set of differential cultivars possessing different resistance factors can be inoculated, the resistant and susceptible cultivars noted, and the virulence factors present identified. Crute and Johnson (4) assigned numbers to each of the known resistance factors, numbering them 1 through 11 (4). Although the term "resistance factor" is used in this paper to avoid any implication concerning the inheritance of these factors in *L. sativa,* a number of genetic studies (9,10,12,14,15,27,31,35) have revealed that many of the resistance factors behave as though controlled by single genes. Virulence factors corresponding to the ability of the fungus to overcome each of the resistance factors also were proposed, and can only remain defined as factors until studies of their inheritance in *B. lactucae* have been conducted. Thus, if a collection of *B. lactucae* is tested on cultivars with all known resistance factors, but is only virulent on cultivars with the resistance factor found in PI 91532 (numbered R-8 by Crute and Johnson [4]), that collection possesses virulence factor 8 (V-8). The Crute and Johnson (4) system carries more information about a particular *B. lactucae* collection than the traditional system of numbering each new race as it appears. It identifies which resistance factors have been

TABLE 1. Cultivars of lettuce used to differentiate races 1–6 of *Bremia lactucae* in the United States

| | Race of *B. lactucae* | | |
|---|---|---|---|
| | 1–4 | 5 | 6 |
| Susceptible cultivar of lettuce | New York | Imperial 847, Great Lakes | Calmar, Valverde, Valmaine |
| Resistant cultivar of lettuce | Imperial 847, Great Lakes | Calmar, Valverde | .....[b] |
| Source of resistance | Romaine blonde lente a monter | PI 91532[a] | .....[c] |
| First described | Jagger (1926) | Jagger (1940) | Jones and Leeper (1971) |

[a] Bourguignonne Grosse Blonde d'Hiver also was reported as resistant by Jagger (1940), but was not utilized in his breeding program.
[b] Lettuce cultivars resistant to race 6 have not been released for commercial use.
[c] Jones and Leeper (1971) found several plant introductions that were resistant to race 6.

overcome, thus indicating the cultivars that would be susceptible to that particular collection, and also identifies which resistance factors have not been overcome, thus indicating the cultivars that would be resistant. To a plant breeder, it identifies the resistance factors that might be worth incorporating into a lettuce cultivar (those that have not been overcome) and the resistance factors that are not usable (those that have been overcome). If the American system of race designation (races 1–6) is used to identify collections, information concerning virulence on only a few resistance factors is available. If the American system of classification is compared with the Crute and Johnson (4) scheme, races 1–4 cover any that lack V-7. Race 5 is any that has V-7, but lacks V-5, V-8, and possibly V-9. Race 6 includes any of those that have at least V-5, V-7, and V-8.

The Crute and Johnson (4) set of differential cultivars was utilized in the present study because its use supplies the maximum amount of information concerning pathogenic specialization of *B. lactucae*. Collections of *B. lactucae* could be identified as those either having or lacking virulence factors (as defined by Crute and Johnson [4]) or they could be characterized as race 1–4, race 5, or race 6.

## MATERIALS AND METHODS

**Establishment of *B. lactucae* collections.** The sporangia of *B. lactucae* used in the present study were obtained from commercial lettuce fields in Oswego County, New York. Lettuce leaves exhibiting downy mildew symptoms were collected in plastic bags and transported to the laboratory the same day. Leaves of the same cultivar from the same field were rinsed briefly in tap water and placed in a glass casserole with 1 cm of water in the bottom. These casseroles were placed in an illuminated incubator (14 C, 3,000 lux, and a 12-hr photoperiod). After 3 days, several lesions from the same cultivar were excised, placed in a 250-ml Erlenmeyer flask containing 50 ml of water, and shaken to obtain a suspension of sporangia. The concentration of sporangia was adjusted to $10^5$ per milliliter and inoculated onto lettuce seedlings. The resulting cultures (each representing a single *B. lactucae* collection) were maintained by using the technique described by Yuen and Lorbeer (34). These collections were grown for three to four generations on seedlings of lettuce (cultivar Ithaca; Joseph Harris Seed Company, Rochester, NY 14642) and tested for virulence. Collections not tested immediately were frozen as suspensions of sporangia in distilled water, as in the method described by Jones and Leeper (16).

TABLE 2. Seed sources and postulated resistance factors of lettuce cultivars used to test for virulence factors in *Bremia lactucae* isolates

| Cultivar | Postulated resistance factors | Source of seed |
|---|---|---|
| Hilde | 0 | A. L. Tozer[a] |
| Premier | 1 | Sutton's Seeds Ltd.[b] |
| Mildura | 3 | A. L. Tozer[a] |
| Valmaine | 5 | Harris Seed[c] |
| Mesa 659 | 7 | Harris Seed[c] |
| Valverde | 8 | Asgrow Seed[d] |
| Grosse Blonde d'Hiver (GBH) | 9 | Tezier Freres[e] |
| Sucrine | 10 | Vilmorin-Andrieux[f] |
| Capitan | 11 | C. W. Panneveis[g] |
| Amanda Plus | 2, 4 | D. van der Ploeg's Elite Zaden[h] |
| Kordaat | 3, 4 | C. W. Panneveis[g] |
| Larganda | 2, 7 | Rijk Zwaan[i] |
| Solito | 3, 7 | Rijk Zwaan[i] |
| Bremex | 1, 7 | C. W. Panneveis[g] |
| Fila | 2, 11 | C. W. Panneveis[g] |
| Avondefiance | 6, 8 | A. L. Tozer[a] |
| Calmar | 7, 8 | Keystone Seed[j] |
| Edgar | 2, 3, 7 | Samuel Yates[k] |
| Ardente | 1, 6, 7 | Rijk Zwaan[i] |
| Avoncrisp | 6, 7, 8 | A. L. Tozer[a] |
| Diana | 3, 7, 8 | A. L. Tozer[a] |

[a] A. L. Tozer, Cobham, Surrey, England.
[b] Sutton's Seeds Ltd., Reading, England.
[c] Harris Seed Company, Rochester, New York, USA.
[d] Asgrow Seed Company, Florida, New York, USA.
[e] Tezier Freres, Valence sur Rhone, France.
[f] Vilmorin-Andrieux, Paris, France.
[g] C. W. Panneveis, Enkhuizen, The Netherlands.
[h] D. van der Ploeg's Elite Zaden, Barendrecht, The Netherlands.
[i] Rijk Zwaan, de Lier, The Netherlands.
[j] Keystone Seed Company, Hollister, CA, USA.
[k] Samuel Yates, Ltd., Macclesfield, Cheshire, England.

**Origin of lettuce cultivars utilized.** The differential cultivars of lettuce used in the present study were Hilde, Premier, Mildura, Valmaine, Mesa 659, Valverde, Grosse Blonde d'Hiver (GBH), Sucrine, Capitan, Amanda Plus, Kordaat, Larganda, Solito, Bremex, Fila, Avondefiance, Calmar, Edgar, Ardente, Avoncrisp, and Diana. Seed was obtained from a number of different seed firms in the United States and Europe. The cultivars used, their postulated resistance factors, and the names and addresses of the seed firms are summarized in Table 2.

**Laboratory assay for virulence factors in *B. lactucae* collections.** Virulence factors present in different collections of *B. lactucae* were assayed by first growing a large number of sporangia on seedlings of lettuce of the cultivar Ithaca (source plants), harvesting the sporangia, and then inoculating seedlings of the differential cultivars with the sporangia.

Seed of the lettuce cultivar Ithaca was sown on sterile vermiculite in 10-cm-diameter plastic pots to produce the source plants. Pots with seed were placed in glass casserole lids containing 1–2 cm of water and covered with the bottom of the casserole. These inverted glass casseroles were incubated in a controlled environment chamber (14 C, 10,000 lux, 14-hr photoperiod) for 5 days. The glass cover then was removed and the seedlings were sprayed with a suspension of sporangia of *B. lactucae* ($1–5 \times 10^5$ sporangia per milliliter). The casserole was incubated for an additional day in the controlled environment chamber with the cover on, and then the cover was removed for 4 days so that the fungus would grow within the lettuce, but would not sporulate. The cover was replaced, and sporulation was induced the following day as a result of exposure to the high humidity. Sporangia then were collected by shaking the cotyledons in distilled water and straining the mixture of sporangia and lettuce cotyledons through a single layer of cheesecloth to remove the cotyledons. The concentration of sporangia in the filtered suspension then was computed using a hemacytometer. The suspension was adjusted to $10^5$ sporangia per milliliter by first pelleting the sporangia in a centrifuge at 10,500 *g* for 10 min and then resuspending the pellet in the appropriate amount of water. If the concentration of the initial suspension was greater than $10^5$ sporangia per milliliter, the pelleting step was omitted and the suspension diluted to achieve a concentration of $10^5$ sporangia per milliliter.

Seed of the differential cultivars was sown on sterilized vermiculite in 5-cm-diameter plastic pots. These were arranged on the lids of glass casseroles with about 2 cm of water, covered with the bottom of the casserole, and incubated in the same controlled environment chamber for 5 days. The seedlings were sprayed with the suspension of sporangia of *B. lactucae* from the source plants and covered with the bottom of the casserole for 24 hr. The cover then was removed, and the seedlings were incubated in the controlled environment chamber for an additional 5 days. Then the cover was replaced to allow for sporulation. The cultivars were classified as resistant if there was not sporulation of *B. lactucae* on any of the seedlings or susceptible if there was abundant sporulation on more than 90% of the seedlings. If the sporulation was not as intense as on the nonresistant cultivar (Hilde), or if only a few of the seedlings exhibited sporulation, an intermediate reaction was recorded for that cultivar.

**Field assay for virulence factors present in naturally occurring *B. lactucae* populations.** A field experiment consisting of 11 of the differential cultivars was planted in a grower's field in 1978. The 11 cultivars used were Hilde, Premier, Mildura, Valmaine, Mesa 659,

Valverde, GBH, Sucrine, Capitan, Kordaat, and Avondefiance. The experiment was a randomized block design with four replicates. Plots of the differential cultivars in each replicate consisted of single 12-m rows planted with a Planet Junior cone seeder (S. L. Allen and Company, Philadelphia, PA 19140). Thinning and weeding, along with routine application of herbicides and insecticides, was performed by the grower cooperator. On several dates the total number of plants and the number of plants showing downy mildew symptoms in each of the plots were counted. *B. lactucae* was collected from the infected differential plants as previously described, and these collections were tested for virulence on the differential cultivars in the growth chamber.

## RESULTS

**Virulence factors in *B. lactucae* collections from commercial lettuce fields.** Four collections of *B. lactucae* from commercial lettuce fields in Oswego County were made during 1977 and 1978 for testing for virulence factors. Collection 1-77-22 was made from Ithaca lettuce in 1977. Collections 2-78-6, 4-78-6, and 5-78-8 were made in 1978 from cultivars Minetto, Ithaca, and A-1, respectively.

Collection 1-77-22 was virulent on Hilde and Mesa 659 (Table 3). Since Mesa 659 possesses R-7, this collection has V-7. Of the collections made in 1978 (Table 3), 2-78-6 displayed the same pattern of virulence on Hilde and Mesa 659, indicating that V-7 was present. Collection 4-78-6 was virulent on Hilde, Mesa 659, and Larganda, indicating that V-2 and V-7 were present, since Larganda has resistance factors 2 and 7. Collection 5-78-8 was virulent on Hilde and Mesa 659, indicating that V-7 was present.

**Virulence factors detected in field assay of the natural *B. lactucae* population.** Although lettuce downy mildew was relatively rare in 1978, *B. lactucae* was present in the field planting of the differential cultivars. This field experiment had been planted on 19 July and by 24 August *B. lactucae* had infected Hilde and Mesa 659, indicating that V-7 was present. Four weeks later, on 18 September, *B. lactucae* had infected all the plants of Hilde (no resistance), Valmaine (R-5), Mesa 659 (R-7), Valverde (R-8), Sucrine (R-10), and Avondefiance (R-6 and R-8), which indicated that virulence factors 5, 6, 7, 8, and 10 were present. Fifty-four percent of the plants of Premier (R-1), 12% of the plants of GBH (R-9), and 40% of the plants of Capitan (R-11) also were infected, indicating that V-1, V-9, and V-11 were present.

**Virulence factors in *B. lactucae* collections from the differential cultivars planted in commercial lettuce fields.** Collections of *B. lactucae* taken from infected plants of cultivars Valverde, GBH, and Capitan were tested for virulence factors (Table 4). The collection from Valverde, 9-78-6, had virulence factors 5, 6, 7, and 8 and intermediate reactions for virulence factors 2, 4, 9, and 10. The collection from GBH, 14-78-6, had virulence factors 2, 4, 5, 6, 7, 8, and 10, and intermediate reaction for V-9. The collection from Capitan, 16-78-6, had virulence factors 1, 2, 4, 5, 6, 7, 8, and 10, and an intermediate reaction for V-9.

## DISCUSSION

The most commonly occurring virulence factor in *B. lactucae* from Oswego County during this investigation was V-7. Under the traditional system of denoting races of the pathogen, these collections would be considered race 5, since they are able to overcome the resistance found in the lettuce cultivar Romaine blonde lente a monter. Cultivars with this resistance factor have been extensively grown in Oswego County, starting with the release of Imperial 44 in 1938 (13) and continuing to the release of Oswego in 1961 and Fulton in 1962 (26). The two most recent releases from New York were Ithaca in 1969 (1) and Minetto in 1962 (26). These two cultivars do not have R-7 (I. R. Crute, *personal communication*). Resistance factor 7 has been so common in cultivars grown in Oswego County that it is possible that the corresponding gene or genes for virulence in the pathogen have become fixed (all individuals have this gene or genes).

Zink and Duffus (36) characterized a collection of *B. lactucae* from California as race 5 because of its ability to overcome the resistance factor originally found in Romaine blonde lente a monter. Their tests indicated that V-1 also was present since their collection was virulent on the cultivar Blondine that has R-1, but V-2 was absent since their collection was not virulent on cultivars May King or Meikoningen. While our Oswego County collections from commercial lettuce fields shared V-7 (and hence the designation as race 5) with this California collection, they were different in that they lacked V-1 and sometimes had V-2.

TABLE 3. Virulence factors present in *Bremia lactucae* collections obtained from commercial lettuce fields in Oswego County, New York, during 1977 and 1978

| Cultivar | Postulated resistance factors | Identity and source of *B. lactucae* isolates[a]: 1-77-22 (from Ithaca) | 2-78-6 (from Minetto) | 4-78-6 (from Ithaca) | 5-78-8 (from A-1) |
|---|---|---|---|---|---|
| Hilde | 0 | + | + | + | + |
| Premier | 1 | − | − | − | − |
| Mildura | 3 | − | − | − | − |
| Valmaine | 5 | − | − | − | − |
| Mesa 659 | 7 | + | + | + | + |
| Valverde | 8 | − | − | − | − |
| GBH[b] | 9 | − | − | − | − |
| Sucrine | 10 | − | − | − | − |
| Capitan | 11 | − | − | − | − |
| Amanda Plus | 2, 4 | − | − | − | − |
| Kordaat | 3, 4 | − | − | − | − |
| Larganda | 2, 7 | − | − | + | − |
| Solito | 3, 7 | − | − | − | − |
| Bremex | 1, 7 | − | − | − | − |
| Fila | 2, 11 | − | − | − | − |
| Avondefiance | 6, 8 | − | − | − | − |
| Calmar | 7, 8 | − | − | − | − |
| Edgar | 2, 3, 7 | − | − | − | − |
| Ardente | 1, 6, 7 | − | − | − | − |
| Avoncrisp | 6, 7, 8 | − | − | − | − |
| Diana | 3, 7, 8 | − | − | − | − |

[a] + indicates susceptible lettuce cultivar; − indicates resistant lettuce cultivar.
[b] GBH indicates cultivar Grosse Blonde d'Hiver.

TABLE 4. Virulence factors present in *Bremia lactucae* collections obtained from differential lettuce cultivars grown in commercial lettuce fields in Oswego County, New York, during 1978

| Cultivar | Postulated resistance factor | Identity and source of *B. lactucae* isolates[a]: 9-78-6 (from Valverde) | 14-78-6 (from GBH) | 16-78-6 (from Capitan) |
|---|---|---|---|---|
| Hilde | 0 | + | + | + |
| Premier | 1 | − | − | + |
| Mildura | 3 | − | − | − |
| Valmaine | 5 | + | + | + |
| Mesa 659 | 7 | + | + | + |
| Valverde | 8 | + | + | + |
| GBH[b] | 9 | ± | ± | ± |
| Sucrine | 10 | ± | + | + |
| Capitan | 11 | − | − | + |
| Amanda Plus | 2, 4 | ± | + | + |
| Kordaat | 3, 4 | − | − | − |
| Larganda | 2, 7 | ± | + | + |
| Solito | 3, 7 | − | − | − |
| Bremex | 1, 7 | − | − | + |
| Fila | 2, 11 | − | − | − |
| Avondefiance | 6, 8 | + | + | + |
| Calmar | 7, 8 | + | + | − |
| Edgar | 2, 3, 7 | − | − | − |
| Ardente | 1, 6, 7 | − | − | + |
| Avoncrisp | 6, 7, 8 | ± | + | + |
| Diana | 3, 7, 8 | − | − | − |

[a] + indicates susceptible lettuce cultivar; − indicates resistant lettuce cultivar; ± indicates intermediate reaction.
[b] GBH indicates cultivar Grosse Blonde d'Hiver.

The 1978 field trial using the differential cultivars revealed potential virulence of *B. lactucae* populations in Oswego County. Collections of the pathogen that were more virulent (here used in the sense of a collection that contains more virulence factors) than those from commercial lettuce fields were detected in this field experiment. These more virulent collections of *B. lactucae* showed considerable variation in the additional virulence factors contained. All of the more virulent collections had, in addition to V-7, at least V-5 and V-8. Race 6 was defined by Sleeth and Leeper (28) as "the cause of the recent outbreaks of downy mildew on Valverde, Calmar and Valmaine lettuce," implying that a single race had the necessary virulence factors to overcome the resistance from both PI 91532 and PI 167150. While the term race 6 could be used to describe the more virulent collections of *B. lactucae* detected in Oswego County, these collections can be characterized more accurately by describing the specific virulence factors they contain. While V-5 and V-8 are associated both in Oswego County collections of *B. lactucae* and Texas collections, these are separate virulence factors (4,17).

When the collections 14-78-6 and 9-78-6 were tested on 21 differential cultivars, virulence factors 2, 4, 5, 6, 7, and 8 were detected. However, these collections were not virulent on the cultivars Bremex or Ardente. The nature of the resistance factors in these two cultivars is uncertain. Originally, they were thought to have had R-4, and on that basis, they should have been susceptible to these collections (3). However, Osara and Crute (24) suspected that these two cultivars did not contain R-4, but instead had either R-1 or a previously undiscovered resistance factor. The lack of virulence of 14-78-6 and 9-78-6 on Bremex and Ardente indicates that these two cultivars do not possess R-4.

Some differential cultivars displayed intermediate resistance to some collections. Such intermediate interactions can be interpreted in three possible ways (5): (i) If part of the *B. lactucae* inoculum lacked the necessary virulence factors to successfully colonize a cultivar, the effective inoculum would have been less than $10^5$ sporangia per milliliter, and sporulation might have been reduced, since the latent period is inversely related to inoculum density (7). (ii) The differential cultivar might have been heterogenous for resistance. This was most likely for GBH, since the supplier warned that the responses of plants grown from this particular lot of seed was not uniform regarding its response to lettuce mildew. When tested with collections with only V-7 or V-2 and V-7, GBH was resistant, indicating that all the individuals in this seed lot must contain at least one resistance factor different from either R-2 or R-7. However, it is possible that some individuals within the seed lot contain yet another resistance factor which would then impart additional resistance. Without knowing which resistance factor is universally present and which one only occurs in some individuals within this lot of GBH, it is not possible to tell whether V-9 is present or not. (iii) The resistance factors are subject to environmental influences. Crute and Norwood (6) found that resistance factors 6 and 7 were not effective at higher temperatures.

Collection 16-78-6 was derived from the cultivar Capitan growing in the field experiment, but it was not virulent on Capitan in the growth chamber. Repeated attempts to infect Capitan with this collection were not successful. It is possible that the resistance factor in Capitan is not completely expressed in the field. Collection 14-78-6 was derived from the cultivar GBH, but produced intermediate reactions in the growth chamber. Attempts to subculture 14-78-6 on GBH did not increase the proportion of susceptible plants indicating that this intermediate reaction is due to heterogeneity of this particular cultivar.

Virulence factor 3 was not detected in either the field planting or in the growth chamber. While use of the corresponding resistance factor may have been effective during the 1978 growing season, it would be premature to suggest that this particular resistance factor be incorporated into a lettuce cultivar without further testing.

More complex races of *B. lactucae* were detected only in field plantings of the differential cultivars. The mere existence of these more virulent races indicated that the population of *B. lactucae* has been able to maintain a wide range of virulence factors despite the lack of the corresponding resistance factors in the cultivated lettuce. Only R-7, and to a much lesser extent R-5, have been used in commercial lettuce cultivars grown in New York. If the pathogen population is this diverse without selection for particular virulence factors, breeding for resistant lettuce by using genes for which corresponding virulence already exists in *B. lactucae* would not be a practical means of control. These more virulent races of *B. lactucae* are probably present in lower numbers in the pathogen population. This would explain why they were only detected in the field plantings of the differential cultivars. Additional support for this hypothesis is the observation that the lettuce cultivars with several genes for resistance developed mildew much later in the growing season than those with single genes. This could be due to either a lower gene frequency in a randomly mating *B. lactucae* population or to stabilizing selection.

Vanderplank (30) suggested that more virulent races would be less fit merely because of their additional virulence. Because of this reduced fitness, they would be selected against during the reproduction of the pathogen on a nonresistant host, and this selection (stabilizing selection) would mean that these more virulent races would be present in lower numbers if the host plants did not possess the corresponding resistance factors. Leonard and Czochor (22) reviewed a number of models and concluded that stabilizing selection must exist if balanced polymorphisms (many different races of the pathogen and different resistant plants maintaining each other in equilibrium) are responsible for the differences observed in gene-for-gene systems. The opposite view was taken by Parlevliet (25) who felt that while stabilizing selection is operative in wild plant systems and in some crop pathosystems, it is not a general phenomenon that exists in all crop pathosystems. Parlevliet's view is that a pathogen will first acquire virulence, and then it will acquire additional fitness in a two-step process. In the terminology used by Leonard and Czochor (22), the polymorphisms that are seen are transient and the genes for virulence will eventually become fixed.

If stabilizing selection is operative as a universal phenomenon, then some mechanism must exist whereby races with unnecessary genes for virulence are maintained in the pathogen population. Unnecessary genes for virulence are present in a number of crop pathosystems, and several mechanisms whereby these genes can be retained in pathosystems with stabilizing selection have been presented by Leonard (21). Of the possibilities he mentioned, the role of wild *Lactuca* species may be the most important in Oswego County. Another possible mechanism that would be operative in *B. lactucae* involves ploidy, mating behavior, and overwintering. *B. lactucae* is self-incompatible, and two mating types are usually required for oospores to be formed (23). Furthermore, the vegetative thallus of the fungus, including the sporangia, is diploid (29). If *B. lactucae* overwinters as oospores, then it approximates a randomly mating, diploid population. If genes for unnecessary virulence are present in lower numbers in the pathogen population, then more virulent races should be present in reduced numbers, as was suggested by Wolf et al (33), regardless of the assumptions made regarding fitness of more virulent collections. Even if unnecessary virulence genes (or any gene) reduce fitness (even to the point of being lethal), they would still be retained if they are fully recessive and *B. lactucae* is a randomly mating diploid. In higher plants such as maize, lethal recessive genes are retained and become evident only after the plants have been selfed.

Stabilizing selection is probably not operating in the lettuce-*B. lactucae* system against some genes for virulence. Virulence factors 2 and 7 are commonly found in *B. lactucae* from commercial lettuce fields in New York, indicating that the genotype with only essential virulence is not more fit. The evidence from other studies is similar with universal presence of V-2 and V-4 from Czechoslovakia despite the widespread use of lettuce without this resistance factor (18), and universal occurrence of V-1 in England even though the planting of lettuce with this resistance factor is almost nil (8). Apparently, different virulence factors become fixed in different populations. Lebeda (18) felt that races of *B. lactucae* with V-7 were less fit, indicating that stabilizing selection acts against V-7 in Czechoslovakia, but the opposite is true in New York. Conversely, the most fit genotype in Czechoslovakia carries both V-2 and V-4,

but V-4 is present in reduced numbers in New York (being detectable only with field plantings of the differential cultivars), indicating that stabilizing selection acts against V-4 in New York, but not in Czechoslovakia. Depending on the particular system and location, genes for virulence factors can become fixed in the *B. lactucae* population or these same genes can be subject to stabilizing selection and reach equilibrium with the corresponding genes for resistance in the host plants. The polymorphisms that are seen in crop pathosystems are probably neither all transient nor all balanced. Depending on the particular instance, a polymorphism can be balanced in one case or eventually become fixed in another.

At this point, it would be difficult, if not impossible, to fit a simplified model to this disease situation. The outcrossing forced by different mating types, coupled with a diploid thallus, complicates the life cycle of the pathogen. While the ability of oospores of *B. lactucae* to survive has not been studied, other oomycetes possess oospores that are extremely long lived. Thus, the pathogen may have a "bank" of genotypes extending well back in time. A randomly mating diploid organism can carry recessive genes without much cost, and if the gene frequency is low enough, it would be difficult to eliminate these genes from the population.

It would be premature to suggest that race-specific resistance could be used in New York. The extreme variability for virulence in *B. lactucae* in the absence of corresponding resistance factors in the lettuce population indicates that any race-specific resistance incorporated into a commercial lettuce cultivar would only serve to uncover a more virulent race that may already exist. Presently, we are investigating other means of control, including nonspecific resistance and systemic fungicides specific for the lettuce downy mildew fungus. Further understanding of the life cycle of the pathogen and its relationship to its host may lead to control measures aimed at interrupting a different point in its life cycle.

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