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Inheritance of virulence in Bremia lactucae to match several resistance factors in lettuce

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Abstract

A 1:1 mixture of spores of the physiologic races NL5 and NL6 of *Bremia lactucae* was sprayed on leaf discs of the lettuce cultivar Olof. The resulting oospores were allowed to germinate on seedlings after a two-month ripening period. Monospore lines of 49 of the 79 recovered isolates were tested on cotyledons and leaf discs of a differential series for virulence to 11 resistance factors. All monospore lines were also tested for the mating type factor. A backcross of a progeny line, line H, with NL5 was made and 41 isolates tested on the differential series.

Results indicated complex inheritance based on more than one locus each for virulence to R2 and R11. Vilulence to match the resistance factor R4 seems to be based on a dominant factor or on a combination of a dominant and a recessive factor. Virulence to factors R5, R8 and R10 are recessive and possibly interchangeable. Inheritance of virulence to factors R3, R6 and R9 must be studied further before definite conclusions can be drawn. Virulence to R7 resulted in many incomplete reactions, but all showed significantly less sporulation than the virulent parent.

Variations in necrosis in intermediate reactions indicate the presence of minor genes affecting these symptoms. Many of the avirulent loci in the parent lines proved to be heterozygous.

Mating type segregation fits a 1:1 ratio, indicating that this factor is controlled by a single gene.

Selfing, mutation and somatic crossing-over can affect the ratios of recovered progeny and so our conclusions concerning the inheritance of virulence.

Additional keywords: Lactuca sativa; oospores.

Introduction

Downy mildew *(Bremia lactucae* Regel) in lettuce *(Lactuca sativa* L.) has long been controlled by the use of resistant cultivars with monogenic resistance factors. The protection these offer is often reduced within a few years by the appearance of new physiologic races of the pathogen (Johnson and Crute, 1975; Groenwold, 1978). Genetic studies by Crute and Johnson (1976) and Johnson et al. (1977, 1978) have shown that there are at least 11 specific genes for resistance present in the known cultivars; most of these are inherited by dominant alleles at one or in some cases two loci.

The possibility of studying the genetic background of the complementary virulence factors in the pathogen was opened when Michelmore and Ingram (1980)

demonstrated the heterothallic nature of most isolates. The combination of isolates of the complementary B_1 and B_2 mating types resulted in the production of large numbers of the previously rare oospores. Preliminary tests showed that the oospores resulted from sexual recombination of the two races (Michelmore and Ingram, 1981). This paper reports on results of a progeny analysis of isolates from a cross between two Dutch races and a backcross of a progeny isolate from this cross to one parent. These crosses gave further insight into the genetic background of the virulence factors of *B. lactucae.*

Materials and methods

Production of oospore progeny. B. lactucae races NL5 and NL6 were maintained on 2 cm diameter leaf discs taken from mature, fully expanded lettuce leaves of the cultivar Solito, respectively a Hilde × *L. serriola* selection indicated as H×B. The leaf discs were placed on moist filter paper in closed glass or plastic petri dishes and sprayed with a suspension containing c. 5×10^4 conidiospores per ml, made by shaking leaf discs with a well sporulating *B. lactucae* infection in distilled water. The closed petri dishes were placed in 45×30×8 cm plastic boxes with transparent lids and incubated in a conditioned (15-20 °C) glasshouse. The isolates were transferred to fresh material 10 days after inoculation.

A cross between NL5 and NL6, which differ for virulence to eight of the eleven resistance factors, was made by spraying a 1:1 mixture of conidiospores of NL5 and NL6 on leaf discs. The inoculated discs were stored according to the method described by Blok (1981). Oospores were found in dense clusters in sectors of the tissue within ten days after inoculation. Two months later 2×2 cm squares of the filter paper containing the remains of a leaf disc were cut out and placed on a 2 cm deep layer of sterilized potting soil in 5 cm high covered glass boxes. Five to six seeds of the cultivar Olof were placed on this paper and lightly moistened with distilled water. The boxes were stored in a cool (15 °C) glasshouse and the germinated seedlings were examined daily for infection. The first sporulation could be expected after c. 10 days, when the cotyledons were nearly expanded. Each seedling with a sporulating colony was removed from the paper and the spores were transferred in a few drops of distilled water to plastic petri dishes with expanded detached cotyledons of 'Olof' on moist filter paper for multiplication of the isolate. Five days after the appearance of the first conidiospores all remaining non-infected seedlings in the glass box were discarded, as any following infections could have originated from secondary infection. The filter paper with the oospore-containing tissue was resown and the cycle repeated until no new infections were obtained.

Monospore lines. Infection in three of the five 'bait' seedlings per paper square per sowing were no exception. The chance of a double infection in one seedling was considered to be large enough to warrant the making of monospore lines. These were made after one or two multiplications on cotyledons of 'Olof', using the technique indicated by Michelmore and Ingram (1981). A cotyledon with a well sporulating colony was shaken lightly above a 1% water agar plate. At 250× magnification single conidiospores could be removed from the surface with a micropipette connected via rubber tubing with a mouthpiece. Twenty spores from one isolate were each transferred

to an 'Olof' cotyledon on moist filter paper in a 9 cm diameter plastic petri dish. To enhance germination the petri dishes were kept for 24 h in darkness immediately upon completion of the transfer and then brought to a growth chamber for incubation under 16/14 °C and 16 h fluorescent light. All petri dishes were stored in 45×30×8 cm plastic boxes with transparent lids.

Determination of virulence phenotype. Monospore lines were tested for virulence by spraying a spore suspension onto the differential series of lettuce cultivars shown in Table 1. This differential series is a slightly modified version of that described by Michelmore and Crute (1982). One or two monospore lines from each isolate were tested on cotyledons of the series, using two or three cotyledons per cultivar per line. One line of each isolate was also tested on leaf discs from mature plants of each cultivar using three or six discs per cultivar per line (Table 1). Tests were carried out in 26×20×3 cm plastic boxes with 15 subdivisions and with transparent lids, one or two cultivars per compartment.

Cotyledons were examined 7 days, and leaf discs 7 and 14 days after inoculation for sporulation and necrosis. Necrosis, sometimes coupled with extremely sparse sporulation, was scored as avirulence, as there was a visible, although incomplete defense response by the plant. Only sporulation without necrosis, usually abundant, was scored as a positive indication of virulence. Where the results of seedling and leaf disc tests for virulence conflicted for one or more cultivars, the test for the pertinent

Table 1. The differential series of lettuce cultivars and the number of leaf discs used per cultivar for determination of virulence phenotype of *Bremia lactucae* lines.

Cultivar	R genes	Number of leaf discs
Olof	0	6
Blondine	1	6
Mildura	3[1]	3
T57/E	4	6
Valmaine	5	6
Sabine	6	6
Mesa	7	3
Valverde	8	6
Bourguignonne (GBH)	9	6
Sucrine	10	6
Hilde × L. serriola	11	6
Noran	2,4	6
Brioso	2,7	3
Kwiek	3,4	3
Solito	3,7	6
Avondefiance	6,8	3
Calmar	7,8	3
Diana	3,7,8	6

[1] R genes 1,3 according to I.R. Crute (personal communication).

cultivar was repeated on six extra leaf discs and two extra cotyledons per cultivar on moist filter paper in a plastic petri dish.

Mating type determination. All monospore lines of all isolates were tested for mating type. Five 1 cm diameter leaf discs of the cultivar Olof were inoculated with a drop of spore suspension of the test line and a drop of a suspension of a B_1 mating type race (NL6). Five similarly treated discs were inoculated with a B_2 mating type race (NL5 or NL7). The leaf discs were incubated on moist filter paper in closed petri dishes at 16/14 °C and 16 h fluorescent light. After one week the discs were cleared by boiling in a 1:3 mixture of 99% acetic acid and 96% ethanol, cooled for 2-3 min and examined for oospores at 250× magnification. Tests for self-fertility were not carried out.

Backcross. Line H, a B_1 type virulent only to resistance factor R1, was selected from the F_1 progeny of the NL5 × NL6 cross. A backcross of line H to NL5 was made following the procedure described for the F_1 cross. Monospore lines of the progeny isolates were not made. The virulence patterns of the backcross progeny were tested on leaf discs of the cultivars of the differential series shown in Table 1 plus the cultivar Lucia (R3,11). The mating type of each isolate was determined. Leaf discs inoculated only with the pertinent isolate were also observed for possible secondary homothallism. Fourteen days after inoculation the discs were examined under the binocular microscope for the presence of oospores.

Storage. B. lactucae isolates remain viable for up to six months when stored on leaf discs at −20 °C. Samples of all isolates and lines are routinely preserved in this way. Due to peaks in the work load and as a result of accidental loss of a line, some tests were carried out with spores grown from the material stored in the deep freezer. An extra multiplication on 'Olof' was thus necessary before the material was further tested for virulence phenotype.

Results

Seventy-nine isolates were obtained from the F_1 oospore culture during a two month period. From 65 isolates one or more monospore lines were obtained and tested on cotyledons of the differential series; 49 isolates were also tested on leaf discs from mature plants. During storage in the deep freezer 16 isolates were lost and could not be retested on leaf discs. Only the results of the 49 isolates tested on both cotyledons and leaf discs will be reported, as the results of the cotyledon test only, with few cotyledons per cultivar, were often inconclusive.

The obtained isolates varied in such visible traits as growth rate, latent period, mycelium thickness and colour, and in spore density on 'Olof'. While no attempts were made to classify these differences, a minimum level of sporulation was necessary to subculture the line and to supply sufficient conidiospores for determination of virulence phenotypes. Four isolates were lost and two monospore lines sporulated insufficiently to be tested on the differential series. This or other uncontrolled selection during recovery and purification of the F_1 progeny could have had an effect on the results, where there was epistasis or close coupling between fitness on 'Olof' and virulence sensu Van der Plank (1975).

Sparse sporulation, usually with necrosis, was often found on cultivars Avondefiance (R6,8), Calmar (R7,8), Mesa (R7) and Sabine (R6), and occasionally on some of the other cultivars. For cultivars with a relatively high level of background (partial) resistance, and for isolates unable to give a dense spore production, even on 'Olof', the density of spore production necessary for a positive score was difficult to determine. This has possibly resulted in an overestimation of the number of virulent isolates, although the use of both seedling and leaf disc tests, and the checks provided by the cultivars with two resistance factors helped to keep this, hopefully, to a minimum. Larger numbers of leaf discs would have better eliminated uncertainty.

The expected extra susceptibility of cotyledons was more often seen as an increase in spore density and in a decreased latent period in compatible reactions than as an increase in the number of compatible reactions. Still, 20 cases of sporulation on cotyledons without sporulation on the leaf discs and 19 cases with the reverse reaction were found. Eighteen of these cases occurred on 'Sabine' and 'Mesa', two cultivars which consistently gave necrosis without or with sparse sporulation.

The parent isolates differed in virulence to eight resistance factors; both were virulent to R1 and R4 and avirulent to R6; however, NL6 sometimes caused necrosis on 'Sabine'. The ratios virulent/avirulent found among the 49 tested isolates are shown in Table 2.

Most isolates had multiple virulences. Two isolates were found with virulence only to R1; one isolate was found with nine of the eleven virulence factors present. Tests on 'Blondine' revealed that all isolates were virulent to the R1 factor.

Table 2. Segregation for virulence and mating type among F_1 progeny isolates.

R factor	NL5	NL6	Progeny		χ^2 1:1
			+	−	
1	+	+	49	0	
2	−	+	6	36	21.43[1]
3	+	−	3	46	37.33[1]
4	+	+	44	5	31.04[1]
5	−	+	35	14	9.00
6	−	(−)	6[2]	43[2]	22.22[1]
7	+	−	0	49	
8	−	+	29	20	1.65
9	−	+	3	46	37.73[1]
10	−	+	35	14	9.00
11	−	+	12	37	12.76[1]
Mating factor	B_2	B_1	Progeny		χ^2 1:1
			B_1	B_2	
			26	30	0.29

[1] $P < 0.01$

[2] On cotyledons 16 + and 33 −.

Because no cultivar is available with only the R2 gene, virulence to R2 had to be tested with cultivars Noran (R2,4) and Brioso (R2,7). The chance of coupling between genes for virulence to R2 and R4 or R7 remains a problem when analysing the results. Six of the 42 isolates were virulent to R2, indicating that more than one gene is involved in the virulence.

NL5 is virulent to the R3 gene. Three of the progeny lines sporulated on 'Mildura' (R3). Two of these also sporulated on 'Kwiek' (R3,4) and on 'T57/E' (R4). These results may indicate a complex inheritance for virulence to R3.

Both parent races are virulent to R4. Forty-four of the progeny were virulent and 5 avirulent when tested on 'T57/E', indicating that either multiple genes determined this factor or that a single dominant gene was present. Little or no necrosis was found.

NL6 is virulent to the R5 factor in 'Valmaine'. Fourteen of the 49 lines were avirulent and 35 virulent, indicating a complex inheritance which will be discussed in more detail elsewhere in this paper.

Expression of virulence in the F_1 progeny to R6 was complicated by the incomplete expression of this resistance. Both parent races were avirulent to R6, although NL6 occasionally caused necrosis on 'Sabine'. Johnson et al. (1978) reported that expression of R6 is sub-optimal at 15 °C, the test temperature. Differences were found between cotyledons and leaf discs, as well as a range in reaction type, from complete absence of necrosis to reasonably normal sporulation. The majority of the avirulent progeny caused no necrosis in the test material. These resultes indicate that, despite the avirulence in both parents, some progeny lines were able to infect the leaf material to some degree. More tests are needed to better determine the virulence to R6. Difficulties in determining virulence to this factor were also found by Michelmore et al. (1984) and Norwood et al. (1983).

An incomplete resistance reaction is also common in cultivars protected by the R7 factor (Crute and Norwood, 1978). The resistance of the cultivar Mesa has been shown (Johnson et al., 1977) to be based on two complementary genes; the presence of one of these, R7/1, is already sufficient to impart resistance of the cultivar to certain *B. lactucae* races. NL5 is virulent to 'Mesa'. Most tested progeny lines were avirulent, although a number caused abundant necrosis and occasional sporulation. No isolates sporulated at the level of the parent race NL5 itself. Virulence to R7 would seem to be recessive to avirulence, although the modifying effect of minor genes probably specific for this factor can evidently influence the degree of necrosis and sporulation.

The segregation among the progeny for virulence to R8 fits well by a 1:1 ratio, indicating a heterozygote condition for one of the parents and a homozygote recessive condition for the other. This factor will be discussed in more detail later in the paper.

Three progeny lines were found virulent to R9. The results should be confirmed before any conclusions can be drawn.

Thirty-five of the 49 lines were virulent to R10. The figures fit a 3:1 ratio, indicating that two loci are involved in this virulence. More will be said on this virulence later in the paper, in combination with that for virulence to R5 and R8.

Segregation was also found among the progeny isolates for virulence to R11. The figures do not fit a 1:1 ratio, indicating that two or more loci were segregating.

The mating type was determined for 56 isolates. Of these, 26 were of the B_1 and 30 of the B_2type, indicating that the factor is inherited by one locus. This is in agreement with the results of preliminary tests by Michelmore and Ingram (1981) and the results

of Norwood et al. (1983) with other isolates of *B. lactucae*. Tests for linkage between the mating type and each virulence factor were not carried out.

Backcross results. Forty-one isolates from the backcross of line H from NL5×NL6, virulent to only R1, with NL5 were tested for virulence with the differential series. A summary of the results is shown in Table 3. Three isolates of the NL5 type and one isolate of the H type were among the 21 different genotypes found. The most complex genotype found was virulent to eight of the 11 factors, including four virulences not present in either of the parent isolates. Four isolates of this phenotype were recovered.

All isolates were virulent to R1, and all were avirulent to R2, R6 and R9. Virulence to R2 could only be tested in 34 of the 39 isolates, i.e. those with virulence to R4 or R7, as no cultivar with only R2 was available in the differential series.

Segregation was found for virulence to R3, R4, R5, R7, R8, R10 and R11. All ratios fit a 1:1 and a 3:1 segregation, with the exception of virulence to R11, which fits a 1:1 and a 1:3 segregation. In contrast to the F_1 results, virulence to R11 would seem to be determined by a single recessive allele present in both heterozygous avirulent parents.

Virulence to R5, R8 and R10 were always found to be present or absent in a block of three, indicating strong coupling or epistasis. If all three virulences were determined by a single recessive locus, then 1/4 of the progeny would be expected to be virulent to each R factor, rather than the 24 of the 41 tested progeny, as found. A model where virulence to all three resistance factors is imparted by a homozygous, recessive condition in any one of the three loci fits well with the experimental results, if one assumes that both parent isolates were heterozygous for each of the three loci. The F_1 results

Table 3. Segregation for virulence and mating type among backcross progeny isolates.

R factor	NL5	H	Progeny		χ^2 1:1
			+	−	
1	+	+	41	0	
2	−	−	0	38	
3	+	−	27	14	4.12
4	+	−	24	17	1.20
5	−	−	24	17	1.20
6	−	−	0	41	
7	+	−	24	17	1.20
8	−	−	24	17	1.20
9	−	−	0	41	
10	−	−	24	17	1.20
11	−	−	16	25	1.98
Mating factor	B_2	B_1	Progeny		χ^2 1:1
			B_1	B_2	
			21_1	15	1.00

[1] Five isolates not included in the ratio due to inconclusive test results.

showed a 3:1 segregation for virulence to R5 and R10, and a 1:1 segregation for R8, which would indicate that the virulent parent, NL6, was homozygous recessive for virulence for one or, more probably two of the three loci.

It is not clear whether the three loci are completely interchangeable with each other. In the F_1 6 of the 49 lines were found with only two, and two with only one of the three virulences. Similar isolates have been found by Michelmore and Ingram (1980) and Dixon and Wright (1978). Other factors or modifying genes may be able to alter the expression of the virulence of one or more of the three loci. We have found that very accurate testing is needed to determine virulence or avirulence in these cases. All Dutch races possess either all three virulences or none of the three.

Of the 40 backcross isolates tested, 21 were B_1 and 15 were B_2 types; four isolates were either self-fertile or a mixture of B_1 and B_2 types. As no monospore lines were made, no conclusions can be drawn about these four isolates.

Discussion

The diploid nature of *B. lactucae* is clearly shown in the F_1 and backcross progeny results. The recovery of F_1 lines avirulent to R4, and backcross lines virulent to R5, R8 and R10 demonstrate the presence of recessive alleles in heterozygous conditions. This was indicated earlier by Blok (1981) and Norwood et al. (1983).

Evidence that true crossing between the parents has occurred can be found in the many new virulence phenotypes recovered. When selfing should occur in fairly high percentages one will expect to recover more parental types in the progeny, especially if the parent field isolates themselves are a result of a selfed line. The parent phenotypes were not recovered at all among the 49 tested F_1 progeny lines; 4 parental types were found among the 41 tested backcross isolates. Selfing can also be expected to result in more homozygous loci. Both the cross and backcross tested here showed, rather, that many of the avirulent loci in the parents and the F_1 were heterozygous for this condition. Similar results were found by Norwood et al. (1983) and Michelmore et al. (1984) with other crosses between *B. lactucae* isolates. Especially all crosses have shown a 1:1 ratio among progeny for mating type, indicating crossing between a heterozygous and a homozygous recessive parent. Selfing would result in a predominance of the homozygous recessive type and the presence of a new homozygous dominant genotype, possibly recognizable.

Proof of sexual recombination between the two isolates, as was possible for *Phytophthora infestans* (Galindo and Gallegly, 1960) remains difficult to obtain for *B. lactucae,* an obligate parasite. Our attempts to follow indidividual mycelial strands or to observe the reaction of a colony when it comes into contact with heat-killed mycelium of another colony both proved ineffective. The mating type can thus not be further characterized as a compatibility or a sex type.

The role of sexual recombination in nature has not been proven for *B. lactucae* and, in fact, would appear to be slight. The great majority of isolates found in the Netherlands are B_2 types; the chance that the proper combination and ratio of isolates occur in one leaf is therefore slight. Oospores of *B. lactucae* are rarely found in nature (Ingram et al., 1975), and outside the laboratory there are no reports known to the authors of systemic infections of *B. lactucae* from the soil.

Table 4. The virulence patterns of eight successive Dutch races.

Race	Virulence factors present										
NL1	1	2	·	4	·	·	·	·	·	·	·
NL2	1	2	3	4	5	6	·	8	9	10	·
NL3	·	·	·	·	5	6	7	8	·	10	·
NL4	1	2	·	4	5	·	7	8	9	10	·
NL5	1	·	3	4	·	·	7	·	·	·	·
NL6	1	2	·	4	5	·	·	8	9	10	11
NL7	1	2	3	4	·	6	7	·	·	·	·
NL10	1	2	3	4	5	6	7	8	9	10	·

This would imply that the high levels of heterozygosity found in the parents result from other causes, such as mutation. However, the phenotypes of eight successive Dutch races, which have been numbered in order of discovery (Table 4) show little evidence that new types result from mutations in older types.

Selfing is known to occur in the secondary homothallic lines, which form oospores in isolated culture (Michelmore and Ingram, 1981). Germinated oospores of a secondary homothallic race (NL1) showed that this isolate was heterozygous for several loci. This would indicate that NL1 itself was not a result of selfing, as this would lead to homozygosity in lines with the self-compatible trait.

Virulence was expected to be caused by a single recessive gene corresponding to a single gene for resistance in the plant, commonly referred to as the 'gene-for-gene' system (Flor, 1956). Surprisingly, segregations in the F_1 indicated that more loci were involved in causing virulence to nine of the ten R factors which could be tested, while in the backcross three of the seven possible virulences seemed to have a complex inheritance. The possibility that the ratios for virulence to R3 and R9 were due to improper scoring, contamination, or to mixtures in the test plants used (especially 'Bourguignonne') must be explored before the conclusions of a complex inheritance for these loci is accepted; more extensive tests of the same cross are now underway.

Virulence to R4 seems to be based on more than one locus, a conclusion also found by Michelmore et al. (1984) with other *B. lactucae* lines. They discussed the possibility of a dominant inhibitor (I) of avirulence for this virulence factor. If it is this dominant inhibitor which is segregating in this case, both NL5 and NL6 would seem to be heterozygous at this locus, and at least one parent carries one or more dominant alleles for avirulence as well (see Table 5). The number of progeny tested is too small to distinguish between the possibilities of a single dominant virulence allele or a system as suggested by Michelmore et al. (1984). Norwood et al. (1983) reported a segregation with many incomplete reactions for this virulence. The data did not fit any conceivable segregation ratio. The clear presence of a dominant gene affecting virulence, possibly alone, but more probably in connection with the action of another recessive gene, as has been found for *Melampsora lini* on flax (Lawrence et al., 1981), shows that a variety of models are possible in the host-pathogen relations. It can be postulated that the various combinations of inhibitors of avirulence and virulence alleles themselves can lead to varying symptoms in a plant. This could, for example, account for the presence or absence of necrosis.

Table 5. Proposed genotypes for the tested isolates.

Isolate	R factors										
	1	2	3	4	5	6	7	8	9	10	11
NL5	aa	AaBb	aa[1]	iiaa or IiAa[2]	AaBbCc	AA[1]	aa[1]	AaBbCc	AA[1]	AaBbCc	AaBb
NL6	aa	aabb	AA[1]	iiaa or IiAa[2]	aaBbCc	AA[1]	AA[1]	aaBbCc	aa[1]	aaBbCc	aabb
H	aa	A-B-	Aa	iiAa	AaBbCc	A-	Aa	AaBbCc	A-	AaBbCc	aaBb or Aabb

[1] Modifying factors probably present.
[2] If NL5 = iiaa and NL6 = IiAa, $\chi^2_{F_1} = 5.72$ and $\chi^2_{BC} = 1.20$;
If NL5 = IiAa and NL6 = iiaa, $\chi^2_{F_1} = 5.72$ and $\chi^2_{BC} = 0.28$;
If NL5 = IiAa and NL6 = IiAa, $\chi^2_{F_1} = 2.51$ and $\chi^2_{BC} = 0.28$.

The proposed relationship between virulences to R5, R8 and R10 better fits the ratios found than allelism or tight coupling, as has been suggested for these three virulence factors by Michelmore et al. (1981). Repeated testing of lines and isolates reported to have only one or two of the three virulences present is necessary to better describe the reaction. The possibility of minor genes affecting the expression of virulence for these factors is not ruled out.

Inheritance of virulence to other loci, especially R6 and R7, was marked by a great number of lines with incomplete reactions, varying from light necrosis to heavy necrosis with sporulation. Since no isolates were found which sporulated well on the cultivar Mesa (R7), it is likely that this virulence factor is caused by a single recessive major gene plus a number of minor genes causing necrosis, a conclusion supported by data from the backcross.

Variations in the degree of necrosis between avirulent isolates can be ascribed to the action of modifying factors in each isolate. These can be factors specific to the virulence system of that isolate, but can also be related to the general fitness of the isolate (Michelmore et al., 1984). However, even when one is sure of the genetic uniformity of the plant material (not always to be assumed without testing) the reaction of a single isolate often varies significantly between test objects, even between leaf discs from a single plant or between cotyledons. These variations in the degree of necrosis and in sporulation on such similar material indicate that the source of some incomplete reactions cannot be sought only in the incomplete action or 'leakage' of the R genes, as proposed by Crute and Norwood (1978). Difference in the leaf discs due to leaf position, nutrition, or short or long term growth conditions should, however, not completely be ruled out as contributing to the final effect. The increased susceptibility of older, senescent lettuce leaves is an indication that the general condition of the plant plays a role in expression of resistance.

Crute and Norwood (1980) suggested that the cause of the often found 'escapes' in seemingly uniform, heavily inoculated cotyledons should be sought in the inoculum itself. They suggested that the races of *B. lactucae* are heterocaryotic, containing a mixture of nuclei, each with a different genetic make-up. This genetic mixture can result in varying reactions of a plant, depending on the chance proportion of nuclei and on the sensitivity of the plant to a greater or lesser proportion of the types. A second consequence of the possible heterocaryotic nature of *B. lactucae* races would be that, strictly seen, the race is then a collection of genotypes, each of which can participate in oospore formation. Segregation ratios would then be skewed, depending on the ratios of the two or more genotypes per race.

The possibility of somatic crossing-over has already been suggested (Sansome, 1980; Michelmore et al., 1984). Both Norwood et al. (1983) and Michelmore et al. (1984) have mentioned observations of somatic selection for abundant sporulation and increased virulence in field isolates cultured for long periods in the laboratory. Results of more genetic analyses are needed to better determine whether the segregation ratios are indeed subject to fluctuations due to heterocaryosis, and to measure the degree of heterozygosity of a maximum number of loci. This information can help determine the degree to which somatic crossing-over occurs in *B. lactucae*. Until this is known it seems desirable to analyse the genetics of virulence using isolates of *B. lactucae* newly germinated from oospores and, where possible, purified by monospore culture. The chance of variations in such lines due to somatic crossing-over can be expected to be smaller than in field isolates.

Samenvatting

Overerving van verschillende virulentiegenen in Bremia lactucae corresponderend met de overeenkomstige resistentiefactoren in sla

Een sporesuspensie van de fysio's NL5 en NL6 van *Bremia lactucae* werd in een 1:1-verhouding verspoten op bladschijfjes van het slaras Olof. De oösporen die hierdoor in het bladweefsel gevormd werden, werden na een rijpingsperiode van twee maanden tot kieming gebracht met behulp van kiemend slazaad. Op de zaailingen werden zo 79 isolaten van *B. lactucae* verkregen. Van de meeste isolaten werden monosporelijnen gemaakt en 49 van de 79 werden getoetst op zowel zaailingen als bladschijfjes van een toetssortiment van slarassen. Hiermee werd de aan- of afwezigheid van virulentie voor 11 resistentiefactoren bepaald. Van alle lijnen werd bovendien het compatibiliteitstype bepaald. Een terugkruising van een van de verkregen lijnen, H, met NL5 werd uitgevoerd en 41 isolaten werden getoetst op het toetssortiment.

De resultaten duiden op een complexe overerving van virulentie voor de factoren R2 en R11, die gebaseerd is op meer dan één locus. Virulentie tegen R4 is ofwel gebaseerd op één dominante factor ofwel op een combinatie van een dominante en een recessieve factor. Virulenties tegen de factoren R5, R8 en R10 zijn recessief en mogelijk onderling verwisselbaar. Over de virulentie tegen de factoren R3, R6 en R9 kon geen uitspraak gedaan worden. Virulentie tegen R7 resulteerde in veel onvolledige reacties, maar steeds werd beduidend minder sporulatie verkregen dan met de virulente ouder.

Variaties in necrose in intermediaire reacties (onvolledige resistentie) duiden op de

aanwezigheid van 'minor genes' die een invloed op dit verschijnsel hebben. Veel loci voor avirulentie in de kruisingsouders blijken heterozygoot te zijn.

Splitsing van compatibiliteitstype vindt plaats in een verhouding 1:1. Dit duidt er op dat deze factor bepaald wordt door één gen.

Zelfbevruchting, mutatie en somatische overkruising kunnen van invloed zijn op de verhoudingen in de nakomelingen en daarmee ook op onze conclusies betreffende de overerving van virulentie.

References

Blok, I., 1981. A procedure to infect lettuce seedlings with oospores of *Bremia lactucae.* Neth. J. Pl. Path. 87: 159-162.

Crute, I.R. & Johnson, A.G., 1976. The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactua sativa.* Ann. appl. Biol. 83: 125-137.

Crute, I.R. & Norwood, J.M., 1978. Incomplete specific resistance to *Bremia lactucae* in lettuce. Ann. appl. Biol. 89: 467-474.

Crute, I.R. & Norwood, J.M., 1980. Inter-isolate variation for virulence in *Bremia lactucae.* Ann. appl. Biol. 94: 273-310.

Dixon, G.R. & Wright, I., 1978. Frequency and geographical distribution of specific virulence factors in *Bremia lactucae* popultions in England from 1973-1975. Ann. appl. Biol. 88: 287-294.

Flor, H.H., 1956. The complementary systems in flax and flax rust. Adv. Genet. 8: 29-54.

Galindo, J.A. & Gallegly, M.E., 1960. The nature of sexuality in *Phytophthora infestans.* Phytopathology 50: 123-128.

Groenwold, R., 1978. Het *Bremia* probleem in sla. Zaadbelangen 32: 132-136.

Ingram, D.S., Tommerup, I.C. & Dixon, G.R., 1975. The occurrence of oospores in lettuce cultivars infected with *Bremia lactucae* Regel. Trans. Br. mycol. Soc. 64: 149-153.

Johnson, A.G. & Crute, I.R., 1975. *Bremia* and the way it affects lettuce varieties which are resistant. The Grower, Oct. 25, 1975: 742-743.

Johnson, A.G., Crute, I.R. & Gordon, P.L., 1977. The genetics of race specific resistance in lettuce *(Lactuca sativa)* to downy mildew *(Bremia lactucae).* Ann. appl. Biol. 86: 87-103.

Johnson, A.G., Laxton, S.A., Crute, I.R., Gordon, P.L. & Norwood, J.M., 1978. Further work on the genetics of race specific resistance in lettuce *(Lactuca sativa)* to downy mildew *(Bremia lactucae).* Ann. appl. Biol. 89: 257-264.

Lawrence, G.J., Mayo, G.M.E. & Shepherd, K.W., 1981. Interactions between genes controlling pathogenicity in the flax rust fungus. Phytopathology 71: 12-19.

Michelmore, R.W. & Crute, I.R., 1982. A method for determining the virulence phenotype of isolates of *Bremia lactucae.* Trans. Br. mycol. Soc. 79: 542-546.

Michelmore, R.W. & Ingram, D.S., 1980. Heterothallism in *Bremia lactucae.* Trans. Br. mycol. Soc. 75: 47-56.

Michelmore, R.W. & Ingram, D.S., 1981. Recovery of progeny following sexual reproduction of *Bremia lactucae.* Trans. Br. mycol. Soc. 77: 131-137.

Michelmore, R.W., Norwood, J.M., Ingram, D.S., Crute, I.R. & Nicholson, P., 1984. Further studies on the inheritance of virulence in *Bremia lactucae:* Specific virulence to match resistance factors 3,4,5,6,8,9,10 and 11 in lettuce *(Lactuca sativa).* Pl. Path. 33: 301-315.

Norwood, J.M., Michelmore, R.W., Crute, I.R. & Ingram, D.S., 1983. The inheritance of specific virulence in *Bremia lactucae* (downy mildew) to match R-factors 1,2,4,6 and 11 in lettuce *(Lactuca sativa).* Pl. Path. 32: 177-186.

Sansome, E.R., 1980. Reciprocal translocation heterozygosity in heterothallic species of *Phytophthora* and its significance. Trans. Br. mycol. Soc. 74: 175-185.

Van der Plank, R.E., 1975. Principles of plant infection. Academic Press, New York, London, 216 pp.