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Genetics of virulence in *Leptosphaeria maculans* (Desm.) Ces. et De Not., the cause of blackleg in rapeseed (*Brassica napus* L.)

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Abstract The genetic basis of virulence of 24 isolates of *L. maculans* collected from various sites throughout south-eastern and south-western Australia were studied using five clone-lines of *B. napus*. The experimental design allowed the estimation of the environmental and genetic components of variance using a standard analysis of variance. Virulence of these isolates (as measured by the percentage of stem girdling, %G) on the clone-lines NCII and Tap was found to be most likely controlled by a small number of genes; the broad-sense heritabilities were 79.7% and 67.5% for virulence on NCII and Tap, respectively. The significance of these results in relation to the potential of *L. maculans* in adapting to new resistant *B. napus* cultivars is discussed.

Key words *Leptosphaeria maculans* · *Brassica napus* · Blackleg · Genetics · Virulence

Introduction

Leptosphaeria maculans, the pathogen known as blackleg in rapeseed (*Brassica napus* L.), possesses a high degree of variability in its capacity to cause severe disease on a range of *B. napus* genotypes (McGee and Petrie 1978; Humpherson-Jones 1986). In the past, its isolates have been classed as 'virulent' or 'avirulent', according to their capacity to either attack certain *B. napus* cultivars or to produce a brown pigment in liquid Czapek-Dox medium (Bonman et al. 1981; Humpherson-Jones 1983). Restriction fragment length polymorphism (RFLP) analyses have shown that there are major genetic differences between the 'virulent' and 'avirulent' groups, indicating that the two groups may represent different species (Johnson and Lewis 1990; Koch et al. 1991). Ballinger and Salisbury's (1989) study of Australian field isolates showed that at that time they were all from the 'virulent' group. Recently, however, Plummer et al. (1994) showed, by means of molecular techniques, that "avirulent" strains are also present in Australia.

Genetic variablity in Australian field populations of *L. maculans* has been detected by Cargeeg and Thurling (1980) and by Ballinger et al. (1991). While these studies were able to demonstrate the existence of significant host × pathogen interaction, they did not provide evidence for either the genetic basis of virulence or host specificity of different isolates of *L. maculans*.

Estimates of heritabilities, effective factors and genetic coefficients of variation for virulence in *L. maculans* may provide a means of gauging the potential of the pathogen in adapting to new resistant *B. napus* cultivars. To estimate the variability in *L. maculas* accurately, however, genetically uniform host genotypes must be used, as *B. napus* is partially out-pollinated (Downey et al. 1980), and many cultivars are genetically heterogenous for blackleg resistance (Cargeeg and Thurling 1980). The use of clone-lines allows the partitioning of total variance for resistance/susceptibility into that due to the environment and that arising from genetic differences between isolates of *L. maculans*. The form of the analysis is a modification of that used by Libby (1962) to estimate the environmental and total genetic components of variance and broad-sense heritability of internode length of the yellow-monkey flower, *Mimulus guttatus*, and that by Comstock et al. (1958), who used clonal replicates to estimate the genetic variation for yield in strawberry (*Fragaria ovalis*). In both studies analysis of variance was used to derive estimates of the environmental and genetic components of variance. The advantage of using clonal replicates is that the variance between clone plants (ramets), within a clone-line (ortet), grown in a common environment, may be regarded as environmental variance (V_E), as all plants within an ortet are, theoretically, genetically identical. Thus, the environmental variance of a trait may be subtracted from total variance; the residual variance may then be as-

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cribed to that arising solely from genetic differences between the clone-lines. This variance is known as the genotypic variance (V_E).

The present study was designed to determine the genetics of virulence in 24 field isolates of the blackleg fungus (*L. maculans*) to five clone-lines of *B. napus*. The estimation of genetic components for virulence was conducted according to the procedures of Libby (1962) and Comstock et al. (1958).

Materials and methods

Brassica clone-lines

A total of five clone-lines was used. These lines were generated from seedlings randomly selected from various cultivars and breeding lines of rapeseed (Table 1). To obtain seedlings for cloning, we placed seeds from the five cultivars/lines in small muslin bags in which they were surface-sterilised by a 3-min immersion in 80% ethonol; this was followed by gentle agitation for 12 min in a 15% v/v solution of sodium hypochlorite. The seeds were then rinsed three times with sterile distilled water and transferred aseptically to tissue culture vials containing solidified (1% Difco-Bacto agar) half-strength Murashige and Skoog (1962) (MS) medium (Gibco Organics) without hormones. Three weeks after germination, the seedlings were harvested and epicotyl pieces, each possessing a node (nodal explant), were excised and grown on full-strength MS medium without hormones. The clone-lines were propagated entirely from nodal explants. Care was taken to ensure that propagants did not arise from callus because of the likelihood of their possessing somaclonal variation (Larkin and Scowcroft 1981). The tissue-culture room where all clonal-material was grown was maintained at 25 °C with a 16-h photoperiod. Light to the plants (350 μE insteins mol^{-1}) was supplied by Phillips cool white fluorescent tubes.

To obtain adequate numbers of plantlets of uniform size for glasshouse trials, we first made nodal explants from stock plants and then grew them for 2 weeks on MS medium supplemented with 0.5 mg/l BAP and 0.2 mg/l IBA. This medium induced the formation of multiple shoots from the nodes. At the end of this period the shoots were removed and subdivided into nodal explants, which were subsequently cultured on the same medium for a further 2 weeks. This process was repeated until sufficient numbers of shoots were obtained. The pre-transplant phase involved the excision of the top 5 mm of the shoots containing the apical meristem, which was subsequently transferred onto solidified MS medium, without hormones. The plantlets were grown in sterile culture for 3 weeks. This ensured their uniformity of size and physiological age and the promotion of abundant root development. Plantlets were then transferred to 18 × 17-cm plastic pots containing a steam-sterilised soil-sand mix, in a glasshouse, and were left for a further 3 weeks to acclimatise before being inoculated. The level of humidity during the acclimatisation period was controlled by enclosing the pots containing the plantlets in clear polythene bags (78 × 40 cm), which were sealed at the top. Humidity was reduced by progressively unsealing the bags over a period of 8–9 days.

The glasshouse in which all of the trials were conducted was maintained at between 15 °C (night) and 25 °C (day) with a 10-h photoperiod during June–July, 1991.

Fungal cultures

The cultures of *Phoma lingam* (imperfect state of *L. maculans*) used in this trial were provided by Dr. P. A. Salisbury of the Victorian Institute for Dryland Agriculture (VIDA), Horsham, Victoria. The 24 isolates used were from 12 sites around Australia (Table 2). All isolates were derived from single ascospores from pseudothecia found on infected rapeseed stubble, and they were subsequently found to belong to the "virulent" group of Ballinger and Salisbury (1989). Cultures were preserved in sterile paraffin at 4 °C, or in the short term, in sterile distilled water. Prior to trials, the various isolates were passaged through seedlings of the susceptible rapeseed cultivar 'Niklas' to maintain pathogenicity.

Infection of clone-lines

Inoculum for infection was obtained by growing the *P. lingam* isolates on V8-juice agar for 4 weeks under continuous blacklight (Phillips 'TL' near-ultra-violet) to attain good sporulation. Pieces of agar supporting pycnidia were then excised from the plates in bottles of sterile distilled water. After 15 min the bottles were shaken to disperse the discharged pycnidiospores. The inoculum suspensions were then filtered through four layers of fine muslin (to remove mycelial fragments), and the number of spores determined with a haemocytometer. The concentration of the suspensions was then adjusted to give a spore concentration of 1×10^6 spores/ml.

The clone plants were wound-inoculated with 10 μl of inoculum, (containing approximately 10^4 spores) delivered into the axil of the first leaf with a micropipette (Eppendorf Varipette 4710; 2–10 μl). The stem adjacent to the droplet was pierced with a syringe needle through the droplet whereupon it was absorbed by the plant within 5–10 s. To encourage infection, we maintained high humidity conditions for 3 days by enclosing each pot in a polythene bag.

Plants were assessed for resistance/susceptibility 5 weeks after infection using (1) external lesion size (length, width) and (2) internal lesion diameter. No scoring procedure was used. The following formulae were used to calculate stem canker severity:

(A) Percentage of stem girdling (%G)

$$= \frac{\text{crown canker circumference}}{\text{circumference of the crown}} \times 100$$

(B) Percentage of internal infection (%II)

$$= \frac{\text{internal lesion area}}{\text{transverse area of the crown}} \times 100$$

Table 1 Source of clone-lines, and the origin and level of blackleg resistance of thir respective source cultivar/line

Clone-line designation	Source cultivar/line	Origin	Blackleg resistance
Midas	Midas	Canada	Highly susceptible
NCII	F_2 progeny from:		
	Maluka	Australia	Resistant
	Niklas	Sweden	Susceptible
R12	582N129-5-6789	Australia	Intermediate
Tap	Taparoo	Australia	Resistant
Wes	Wesroona	Australia	Resitant

Table 2 Origin of *L maculans* isolates used in this study

Collection site	Isolate prefix code	Number of isolates used
Dooen(Vic.)	D	2
Gerogery(NSW)	G	2
Galong(NSW)	Ga	2
Mt. Barker(WA)	MB	2
Millicent(SA)	Mc	4
Mundulla(SA)	Md	1
Numurkah(Vic.)	N	1
Old Junee(NSW)	OJ	3
Penshurst(Vic.)	P	2
Rutherglen(Vic.)	R	1
Streatham(Vic.)	S	2
Wagga Wagga(NSW)	WW	2
Total		24

%G was subsequently found to be correlated with %II. This is in agreement with the findings by Newman (1984a,b), who combined the two measurements into a overall score. In the present study, however, it was decided that for the statistical analyses these two components should be treated as separate measures of disease severity.

Preliminary analyses showed that the residuals from the model were normally and independently distributed. As a Bartlett's test (Snedecor and Cochran 1989) showed that the variances for the treatments (isolates) were homogeneous, no transformation was performed on the data.

Estimation of genetic components

A split-plot design with three replicates was used, with the clone-lines as the main plots and the isolates as subplots. The layout of the design allowed, in addition to a general analysis of variance, separate analyses of variance for each clone-line. The separate analyses allowed the partitioning of the components of genetic and environmental variance for virulence of the 24 isolates on each clone-line. The expectation of the mean squares for these analyses are as described by Cooper (1959) (Table 3). If the estimated components of variance to causal components are to be correctly translated, the following assumptions must apply to the isolates (Comstock et al. 1958; Foster and Shaw 1988). There must be:

1) Regular diploid behaviour at meiosis.
2) No cytoplasmic or maternal effects.
3) No linkage among genes affecting virulence, or where linkage existed, the distribution of genotypes was as expected in the absence of linkage.
4) The distribution of genotypes in the parents (which gave rise to the single-ascospore isolates used in this study) was a random sample from a random breeding population.
5) No epistasis.

Another assumption is necessary when vegetatively propagated plants are used, name, that there are no 'C effects' (Libby and Jund 1962). 'C effects' arise as common environmental effects associated with specific clones. In this experiment, significant 'C effects' would cause the inflation of the estimates of between-isolate variance, leading to over-estimates of broad-sense heritabilities and genetic coefficients of variation.

With recognition of these assumptions, the broad-sense heritability for virulence/aggresiveness may be estimated for each clone-line (Table 4). A common problem associated with the interpretation of genetic variances is that they are associated with the mean of the trait. For this reason, workers such as Burton and DeVane (1953) and Comstock et al. (1958) advocated the use of the genetic coefficient of variation (GCV) (Table 4). In this instance, the GCV represents an index of the potential for increased virulence latent within the population of *L. maculans*. Estimates of the number of effective factors for virulence were made according to the formulae of Burnett (1975) (Table 4).

Table 3 Analysis of variance and expectation of mean squares for a randomised complete-block design involving clonal replicates

Source	*df*	MS	Expected MS
Isolates	$(n_1 - 1)$		$\sigma_w^2 + m\sigma_{ib}^2 + mn_2\sigma_i^2$
Blocks	$(n_2 - 1)$		$\sigma_w^2 + m\sigma_{ib}^2 + mn_1\sigma_b^2$
Isolates × blocks	$(n_1 - 1)(n_2 - 1)$		$\sigma_w^2 + m\sigma_{ib}^2$
Error	$n_1 n_2 (m - 1)$		σ_w^2

$\sigma_i^2 = V_G$ = genotypic variance
$\sigma_w^2 = V_E$ = environmental variance

Table 4 Formulae used to calculate broad-sense heritabilities (h_{bs}^2), genetic coefficients of variation (GCV) and effective factors (EF) for the virulence of 24 isolates of *L. maculans* on five clone-line of *B. napus*

Broad-sense heritability h_{bs}^2	$\frac{V_G}{V_G + V_E} = \frac{\sigma_i^2}{\sigma_i^2 + \sigma_w^2}$
Genetic coefficient of variation (GCV)	$\frac{\sqrt{V_G}}{\text{clone-line mean}} = \frac{\sqrt{\sigma_i^2}}{\text{clone-line mean}}$
Number of effective factors (EF)	$\frac{(\text{highest} - \text{lowest})^2}{4V_G} = \frac{(\text{Max}_{LS} - \text{Min}_{LS})^{2a}}{4V_G}$

[a] Max_{LS} = maximum lesion score for isolate on clone-line; Min_{LS} = minimum lesion score for isolate on clone-line

Results

Analysis of variance

The analyses of variance on the two virulence measurements, girdling (%G) and internal infection (%II), revealed highly significant differences in disease reaction between both host and isolate genotypes (Table 5). Host × isolate (C × I) interaction was also significant for both measures of virulence. The magnitude of the C × I interaction, relative to random error, was greater for %G than for %II (Table 5). However, the level of this interaction accounted for only 2.0% of the total variance for %G and for %G and 2.2% for %II.

The isolate for %G ranged from 16.1% (S4) to 55.6% (G1) (Table 6). Generally, highly or weakly virulent isolates were not associated with particular geographical sites. This random distribution of isolate genotypes was also apparent from the mean values for %II (Table 7), whose means ranged from 2.9% (S4) to 13.7% (MB2). Further, there was a moderate, positive correlation between the means of the two virulence components ($R^2 = 049$; $P < 0.001$) for the isolates. Due to the small sample of isolates from each site, however, the observed

Table 5 Analysis of variance (split-plot) for the virulence of 24 isolates of *L. maculans* on five clone-lines of oilseed rape (*B. napus* L.)

Source	*df*	%G	%II
		MS_1	MS_2
Main-plot			
Clone-lines (C)	4	40484.1**	1853.4**
Blocks	2	781.2 *ns*	582.1 *ns*
Main-plot error	8	448.0	148.1
Subplots			
Isolates (I)	23	3661.0**	218.5**
C × I	92	919.9**	65.5*
Subplot error	590	133.7	45.2
Total	719		

* Significant at $0.05 > P > 0.01$; ** Significant at $P \leq 0.01$; ns, not significant at $P = 0.05$

Table 6 Mean lesion size (%G) caused by isolates od *L maculans* each on five clone-lines of oilseed rape (*b. napus* L.)

Mean lesion size (%)						
Clone-line						
Isolate	Midas	NCII	R12	Tap	Wes	Isolate means
D1	76.5	11.0	48.3	7.3	10.8	30.8
D2	64.8	17.6	55.5	10.7	17.0	33.1
G1	83.2	78.5	51.8	47.9	16.6	55.6
G2	69.7	66.1	42.5	34.6	17.4	46.1
Ga1	40.9	13.0	35.8	7.4	14.0	22.2
Ga2	57.7	75.3	54.8	21.1	12.0	44.2
MB2	57.5	80.7	44.6	33.5	11.9	45.6
MB5	32.3	33.6	19.0	12.1	10.9	21.6
Mc2	77.7	51.2	39.2	25.6	10.3	40.8
Mc4	60.4	57.5	53.5	37.5	22.4	46.3
Mc5	54.3	46.8	48.6	20.3	24.4	38.9
Mc6a	66.1	73.5	55.4	39.2	13.3	49.5
Md2	60.4	53.7	51.3	33.7	19.5	43.7
N2	59.0	26.1	53.0	8.3	20.4	33.4
OJ2	47.8	51.8	46.3	34.1	17.1	39.4
OJ3	62.1	82.2	42.7	30.5	13.7	46.2
OJ4	27.9	13.8	18.7	13.7	11.3	17.1
P1	59.0	9.0	32.3	7.2	22.5	26.0
P2	62.3	11.6	52.0	16.8	23.1	33.2
R1	33.4	16.4	22.7	6.1	7.9	17.3
S2	54.3	56.5	39.7	23.8	11.1	37.1
S4	34.0	15.0	14.0	12.8	4.5	16.1
WW3a	64.6	19.0	45.1	9.5	25.6	32.7
WW4a	55.6	37.2	37.9	28.8	9.0	33.7
LSD[a]	18.6	16.3	10.5	9.0	8.4	
Clone-line means	56.7	41.5	41.9	21.8	15.3	35.4
LSD[b]						12.4

[a] Based on individual clone-line error MS values ($P = 0.05$)
[b] Based on pooled error MS ($P = 0.05$).

Table 7 Mean lesion size (%II) caused by 24 isolates of *L. maculans* each on five clone-lines of oilseed rape (*b. napus* L.)

Mean lesion size (%)						
Clone-line						
Isolate	Midas	NCII	R12	Tap	Wes	Isolate means
D1	14.6	3.8	12.8	3.1	5.0	7.8
D2	17.9	8.1	9.7	4.4	7.6	9.5
G1	21.4	22.1	11.6	5.8	7.0	13.6
G2	9.8	9.1	10.0	3.7	5.6	7.7
Ga1	7.6	3.5	8.4	0.4	4.8	4.9
Ga2	14.1	12.9	11.0	10.2	5.2	10.7
MB2	14.0	18.8	19.6	9.8	6.4	13.7
MB5	14.9	6.3	7.9	5.7	4.6	7.9
Mc2	12.0	6.6	9.6	5.4	4.0	7.5
Mc4	11.8	10.2	8.9	9.4	6.2	9.3
Mc5	7.2	5.5	12.0	5.2	13.6	8.7
Mc6a	13.1	11.3	18.1	7.4	11.2	12.2
Md2	11.0	9.7	8.6	6.0	12.1	9.5
N2	16.2	8.2	12.3	1.9	4.7	8.7
OJ2	15.4	14.2	12.5	11.5	3.8	11.5
OJ3	18.8	15.9	7.3	5.7	4.4	10.4
OJ4	15.1	8.7	9.0	5.7	4.9	8.7
P1	9.5	3.7	9.5	0.9	5.9	5.9
P2	9.7	4.4	9.9	2.0	5.1	6.3
R1	19.0	5.7	5.0	1.2	5.3	7.2
S2	24.8	11.0	13.6	6.6	6.6	12.5
S4	6.8	3.8	2.8	0.3	0.7	2.9
WW3a	17.2	9.2	20.8	1.2	10.1	11.7
WW4a	10.9	9.8	9.3	5.1	4.1	7.8
LSD[a]	9.9	7.9	7.6	4.9	3.4	
Clone-line means	13.9	9.3	10.8	4.9	6.2	6.7
LSD[b]						7.1

[a] Based on individual clone-line error MS values ($P = 0.05$)
[b] Based on pooled error MS ($P = 0.05$).

virulence reactions across sites may not accurately reflect the true distribution of *L. maculans* genotypes in the field.

Estimation of genetic components

Broad-sense heritabilities for virulence ranged from 31.4% (Wes) to 67.5% (Tap), and 4.7% (Midas) to 25.9% (NCII) for %G and %II, respectively (Table 8). However, comparisons between clone-lines using broad-sense heritabilities may not be appropriate because means for disease severity ratings varied widely between clone-lines. This is because genetic variances are associated with the mean of the trait (Foster and Shaw 1988). A better indicator of the potential for increased virulence, in this instance, is the genetic coefficient of variation (GCV). The GCV for virulence of *L. maculans* isolates on the five clone-lines ranged from 22.3%(Midas) to 60.3% (NCII), and 13.7% (Midas) to 47.1% (Tap) for %G and %II, respectively (Table 8). The low GCV obtained for the Midas clone-line was expected; the potential for increased pathogenicity on a highly susceptible genotype is likely to be low. The high

Table 8 Mean lesion sizes, broad-sense heritabilities (h^2_{bs}), genetic coefficients of variation (GCV) and effective factors (EF) for the virulence (based on %G and %II) of 24 isolates of *L. maculans* each on five clone-lines of *B. napus*

Clone-line	%G				%II			
	Mean (%)	h^2_{bs} (%)	GCV (%)	EF	Mean (%)	h^2_{bs} (%)	GCV (%)	EF
Midas	56.7	40.5	22.3	12.2	13.9	4.7	13.7	213
NCII	41.6	79.7	60.3	3.8	9.3	25.9	43.6	30
R12	41.9	62.1	27.9	10.3	10.8	12.8	23.4	100
Tap	21.8	67.5	55.7	5.2	4.9	22.9	47.1	37.5
Wes	15.3	31.4	31.1	23.5	6.2	17.3	30.3	50

GCV value obtained for NCII, with %G, seemed at first peculiar, given that this line was found to be, overall, relatively susceptible (Table 8). On scrutiny, however, it was found that, despite having a relatively high disease severity score, NCII was not uniformly susceptible to all the isolates used in the study (Table 6).

The specificity displayed by NCII implies that only a few genes for resistance are involved. Such specificity is probably due to the non-uniform distribution of the

corresponding virulence alleles within the 24 field isolates of *L. maculans*. There exists, therefore, the potential for all genotypes of *L. maculans* in the field to eventually become equally aggressive on NCII through the selection and mating of strains that possess the corresponding alleles for virulence. The GCV, essentially an index of the potential for increased virulence latent within the population of *L. maculans*, was consequently high for pathogenicity on NCII. The same explanation may account for the high GCV, based on %G, for pathogenicity on the clone-line Tap. However, the potential for increased virulence is realised only if one of the assumptions mentioned previously holds true; that linkage among the genes controlling virulence is absent. The presence of repulsion linkage (genes for virulence linked with those for avirulence) (Mather and Jinks 1971) among genes for virulence in *L. maculans* would slow the evolution of new strains possessing increased virulence on resistant cultivars of rapeseed.

The effective factor (EF) estimates provide another indicator of the number of genes segregating for virulence in the sample of isolates. With %G, the EF values ranged from 3.8 for NCII to 23.5 for Wes (Table 8). The small value obtained for NCII further confirms the oligogenic nature of the resistance/virulence interaction between this clone-line and the isolates used. The relatively large value obtained for Wes indicates the likelihood that virulence on this resistant line is polygenically controlled, which accounts for the low GCV obtained for the virulence isolates of *L. maculans* on it. The EF estimates for virulence, for %II, were generally an order of magnitude greater than those derived by using %G (Table 8). This implies that the capacity to cause internal infection on all clone-lines is polygenically controlled. This may be contrasted with the apparent oligogenic control of stem girdling (%G) for virulence on NCII and Tap. Caution, however, must be exercised when interpreting EF values. The underlying assumption that each gene affecting a particular trait must have an equal effect (Mather and Jinks 1971) may not hold in this instance. Further, EF estimates may close their validity if the isolates used in the study did not represent a truly random sample from a randomly breeding population. (Mather and Jinks 1971)

Discussion

The main criticism often raised against the use of clonal replicates is that although all clone plants (ramets) within a clone-line (ortet) are genetically identical physiological differences may exist between them. Libby and Jund (1962) coined the term 'C effects' for common environmental effects associated with specific clones. 'C effects' are most pronounced when ramets are propagated and grown in batches under different environmental conditions. Significant 'C effects' in the present study would have led to inflated estimates of between-isolate mean squares and, consequently, over-estimates of broad-sense heritabilities for virulence. Since the magnitude of 'C effects' decreases with time in a uniform environment (Libby and Jund 1962), characters such as disease severity, measured at 8 weeks after transplanting, may be presumed to be free from this possible source of variation.

Analysis of the two measures of virulence, %G and %II, revealed that virulence of *L. maculans* on *B. napus* may not be polygenically controlled. Large GCV values and low effective factor estimates for virulence, based on %G, all indicated that virulence on clone-lines NCII and Tap was likely to be controlled by a small number of genes. The expression of %II, however, appeared to be under polygenic control. The significant clone-line × isolate interactions observed in the present study indicated that virulence, as measured by %G and %II, may be host-specific. As %G and %II are components of the overall expression of the stem canker phase, the number of effective factors estimated for one component, for example, %II, could include effective factors that also control the expression of the other component, %G. In this instance, the effective factors affecting %G could conceivably comprise a subset of the larger set of effective factors affecting %II.

Inherent in the estimates of GCV and effective factors, however, are that the assumptions derived from Comstock et al. (1958) and Foster and Shaw (1988) be affirmed. Because all of the isolates used in the study were, essentially, a sample of field genotypes of *L. maculans* and not derived from a single cross between two isolates it was impossible to gauge the effects of possible extra-chromosomal factors and linkage relationships on the expression of virulence in *L. maculans*. Burnett (1975) detailed several methods for studying the effects of extra-chromosomal factors and linkage on various traits in fungi. These methods rely on the analysis of progeny derived from the mating of strains with contrasting characteristics. While the effects of extra-chromosomal factors and linakge have been documented for certain traits in several fungal species (Croft and Simchen 1965; Simchen 1965), there is no information available in the literature on the effects of these factors on virulence of *L. maculans* on *B. napus*. Further studies in this area should therefore aim at elucidating the linkage relationships of these virulence genes as well as investigating the possibility of extra-chromosomal factors affecting virulence.

Genetic analysis of the virulence of *L. maculans* on *B. napus* has been greatly simplified of its occurrence in the haploid form (imperfect: *Phoma lingam*) during infection. In genetic analysis of haploid fungi, dominance of genes is no longer a concern, but linkage and gene interaction would still have to be considered (Burnett 1975). The presence of linkage among genes for virulence in *L. maculans* would complicate the interpretation of GCV values. The presence of repulsion linkage (Mather and Jinks 1971; Burnett 1975) of genes for virulence and avirulence would slow selection for increased virulence. Conversely, coupling linkage, which would result in

genes for virulence segregating in blocks, has the effects of (1) reducing the number of effective factors estimated for virulence and (2) increasing the rate of selection advance for virulence.

In the absence of linkage, the high GCV obtained for virulence, based on %G, on NCII and Tap, indicates that natural selection may be very effective in increasing the general level of virulence among field genotypes of this fungus toward these two host genotypes. This is a cause for concern, as the Tap clone-line was derived from the commercial cultivar, 'Taparoo' (Table 1). The clone-line Tap, which cannot be considered to represent the cultivar 'Taparoo' as a whole, may however reflect the underlying nature of the genetics of resistance in this cultivar.

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