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The genetics of blackleg [*Leptosphaeria maculans* (Desm.) Ces. et De Not.] resistance in rapeseed (*Brassica napus* L.).

II. Seedling and adult-plant resistance as quantitative traits

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Abstract Inheritance studies of seedling and adult-plant resistance to blackleg [*Leptosphaeria maculans* (Desm.) Ces. et De Not.] in rapeseed (*Brassica napus* L.) were conducted using 49 families derived by intercrosses between 14 randomly chosen F2 plants from the cross cv "Maluka"(resistant) cv "Niklas" (susceptible), conforming to the North Carolina mating design II (NCM-II). Four concurrent experiments were performed, where plants from each family were: (I) Spray inoculated with a 10^5 pycnidiospores/ml suspension 10 days after germination and assessed 2-weeks later for cotyledon-lesion development, (II) As for (I), but assessed 12 weeks after inoculation for crown-canker development, (III) Wound-inoculated on the stems at growth stage 2.4–2.5 on the Harper and Berkenkamp scale and assessed for crown-canker development 5 weeks after inoculation, and (IV) Spray inoculated at growth stage 2.3–2.4 with a 10^5 pycnidiospores/ml suspension and assessed for crown-canker development nine weeks after inoculation. A *L. maculans* isolate possessing high levels of host specificity (MB2) was used in all inoculations. Seedling resistance was evaluated using a 0–5 cotyledon-lesion severity scale. Adult-plant resistance/susceptibility was evaluated using three separate measures of crown-canker size, i.e. the percentage of crown girdled (%G), external lesion length (E) and internal lesion area (%I). Quantitative genetic analysis of blackleg resistance using the NCM-II design revealed significant non-additive genetic variances for all measures of disease severity, in all four experiments, indicating the presence of strong dominance/epistasis at loci controlling blackleg resistance. The resistance to crown-canker development, after wound-inoculation of the stem, was found to possess the highest ratio of additive to non-additive genetic variance. Crown-canker development in mature plants of the NCM-II population was not related to the degree of cotyledon-lesion development at the seedling stage, indicating the limited value of the cotyledon test in screening for adult-plant blackleg resistance. The implications of these findings to breeding for resistance to blackleg in rapeseed are discussed.

Key words Quantitative genetics · North Carolina mating design II · Adult plant · Blackleg resistance · *Brassica napus* · *Leptosphaeria maculans*

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Introduction

Numerous workers (Barbetti 1975; Gladders and Musa 1980; Hammond and Lewis 1987) have indicated that the crown-canker phase of blackleg is largely responsible for the observed yield loss in the field. However, little is known of the genetic control of blackleg resistance in rapeseed. Indirect evidence, from the inoculation of a number of *B. napus* cultivars with ascospores and pycnidiospores of *L. maculans* (Thurling and Venn 1977; Cargeeg and Thurling 1979), indicated that resistance could be determined by the combined effects of major and minor genes. However, the evidence derived from these experiments may be inconclusive, as many were conducted using genetically heterogeneous (ascospore) inocula. Many of the cultivars used may also have been genetically heterogeneous for blackleg resistance, because *B. napus* is partially outpollinated (Downey et al. 1980). The variation arising from the use of genetically heterogeneous hosts and inocula may have obscured any real differences (arising from major-gene effects) between cultivars. To these workers, variation in cultivar response thus appeared to be continuous, giving rise to the present widely accepted view that blackleg resistance is polygenically controlled.

Studies by Delwiche (1980) indicated that resistance to cotyledon-lesion development in *B. napus* was under the control of two dominant genes. At present, it is difficult to determine the siginificance of these findings, because the

relationship between the degree of cotyledon-lesion development and that of subsequent crown-canker development has not been firmly established. Further, the validity of the cotyledon test as an accurate measure of field (adult-plant) blackleg resistance has not been proven. Recent studies of the genetic basis of blackleg resistance in rapeseed, using three methods for assessing resistance (Pang and Halloran 1996), revealed the likelihood of quantitative genetic control. The present study aimed to further examine this likelihood and to elucidate the genetic basis and heritability of resistance.

Materials and methods

Experimental design

The North Carolina mating design II (NCM-II) proposed by Cockerham (1963) was used in the present study. This design allows the estimation of additive and dominance effects in the absence of epistasis. When epistasis is present, the variance attributable to this effect is included in the estimated dominance component, thus providing an estimate of total non-additive variance (Kearsey 1965). Other designs, such as the North Carolina mating design III (NCM-III) (Cockerham 1963) and the Cavalli joint scaling test (Mather and Jinks 1971), may be considered as superior to NCM-II because they allow the separate estimation of dominance and epistatic effects. However, due to their reliance on homozygous or inbred lines, these designs were considered to be inappropriate for the present experiment, as both parental cultivars were possibly genetically heterogeneous for blackleg resistance (Salisbury, personal communication).

Production of NCM-II families

The NCM-II design involves a systematic crossing program in which n_1 males and n_2 females are chosen at random from a population (usually the F_2) and each male is crossed to each of the females in turn to yield $n_1 n_2$ progenies (Mather and Jinks 1971). In the present study, the reference population from which the males and females were chosen was the F_2 from the cross between "Maluka" "Niklas", used in a preceding study (Pang and Halloran 1996). Since *B. napus* plants are hermaphroditic, the assignation of "male" and "female" plants was done randomly. The present NCM-II design involved the systematic mating of seven males, in turn, with seven female plants, which yielded a total of 49 families. Each family was in a half-sib relationship with the other families, both through a common mother and a common father (Mather and Jinks 1971).

Fourteen seeds were randomly selected from the F_2 population for the production of the NCM-II families. The four experiments conducted using these families (to be described later) required that at least 45–50 progeny (seeds) be produced for each family. To allow for possible seedling death and poor germination, at least 80 seeds/family were required. Due to the limitations imposed by the number of flowers which may be successfully pollinated without contamination by pollen from another male parent, it was necessary to firstly vegetatively propagate the female parents, such that 3–4 clones of each female parent were available for crossing. The procedure for producing clone plants from each female parent were as described in a previous study (Pang and Halloran 1995). The clone-line derived from the female plant designated NCM-II(7F) was used in the experiment described in the above study (Pang and Halloran 1995), where it was designated as "NCII". The male plants were also clonally propagated to ensure that they flowered at the same time as the female clones. Clonal propagation of the male plants further ensured an abundance of pollen for the crossings.

Clone-lines derived from the 14 parents were transferred to the glasshouse and grown in 17-cm diameter pots containing a soil-peat-sand mix, with two plants per pot. The glasshouse in which the clone-lines were grown and crossed was maintained at 25°C (day) and 15°C (night), with natural daylength. At flowering, each male was crossed with each female parent, to yield 49 families.

Experiments, cultural conditions, infection procedure and disease severity assessments

Four concurrent experiments employing the NCM-II families were conducted, during April–June 1991, in a glasshouse at the University of Melbourne Field Station, Mt. Derrimut, under natural daylength. The glasshouse was maintained at 25°C (day) and 15°C (night) throughout the experimental period. Seeds from each NCM-II family were prepared and planted for each experiment using the procedure described in the preceding study (Pang and Halloran 1996). The *L. maculans* isolate used in all inoculations was MB2, the same isolate used in the preceding inheritance study (Pang and Halloran 1996). The four experiments were:

Experiment I -Blackleg resistance at the cotyledon stage

The aim of this experiment was to investigate the genetic basis of blackleg resistance at the cotyledon stage in the NCM-II families. The experiment was laid out as a randomised complete block design with two replicates. Each NCM-II family was represented by 20 plants, with ten seedlings per pot.

The seedlings were infected by spray-inoculation 10 days after germination. The preparation of inoculum was as described previously (Pang and Halloran 1995). The spore concentration was adjusted to 10^5 pycnidiospores/ml with the aid of a haemocytometer. Two drops of a wetting agent (Tween 80) were added to the spore suspension. Seedlings were sprayed to run-off with the aid of a hand-operated pressurised garden sprayer. To encourage infection, transparent polythene hoods were placed over benches containing inoculated plants for 3 days to maintain high humidity.

Assessment of cotyledon-lesion development was conducted 14 days after inoculation. A 0-5 severity scale, based on the percentage of cotyledon area affected, was used. The individual plant scores were calculated as the average of the scores obtained on each of its cotyledons. The severity scale used was as follows:

0=no lesion
1=1–5% of cotyledon area affected
2=10–20% of cotyledon area affected
3=40–60% of cotyledon area affected
4=70–80% of cotyledon area affected
5=100%, cotyledon dead

Experiment II – Blackleg resistance to crown-canker development, following inoculation at the cotyledon stage, assessed 12 weeks after inoculation

The aims of this experiment were: (1) to determine the genetics of resistance to crown-canker development in the NCM-II families, following the cotyledon infection procedure, and (2) to evaluate the correlation between cotyledon-lesion scores, assessed in Experiment I with crown-canker scores on the same plants 10 weeks later.

This experiment was an extension of Experiment I. After the assessment of cotyledon-lesion severity in Experiment I, half the plants in each pot were randomly removed without damaging the cotyledons on the remaining five plants. The remaining plants were individually tagged with small (jeweller's) tags. The final family size for the experiment was ten. Ten weeks after the initial assessment, measurements of (1) the percentage of crown girdling (%G), (2) external lesion length (E) and (3) the percentage of internal infection (%I) were made.

Experiment III – Blackleg resistance to crown-canker development, following wound inoculation.

The aim of this experiment was to determine the quantitative nature of the genetics of resistance to crown-canker development, following wound inoculation. In particular, this experiment was conducted to verify some of the conclusions made in the preceding study (Pang and Halloran 1996), on the genetic control of blackleg resistance as evaluated by the %G, E and the %II. The experiment was laid out as a randomised complete block design with two replicates. The family size for the experiment was 16, with four plants per pot.

Plants in each family were infected via the wound-inoculation technique described previously (Pang and Halloran 1995), at growth stage 2.4–2.5 of the Harper and Berkenkamp scale (1975). Plants were assessed for crown-canker development (using the three measurements given above) 5 weeks after inoculation.

Experiment IV – Blackleg resistance to crown-canker development, following spray inoculation (leaves)

The aim of this experiment was to determine the genetic control of possible resistance mechanisms to invasion by the blackleg fungus at the leaf surface, within the leaf, petiole and stem. The experiment was laid out as a randomised complete block design with two replicates. The family size for the experiment was eight, with four plants per pot. The plants were spray-inoculated with a pynidiospore suspension (10^5 spores/ml) at growth stage 2.3–2.4 of the Harper and Berkenkamp (1975) scale, using the procedure described for "Experiment I".

Plants were assessed for crown-canker development, using the %G, E and %II 9 weeks after inoculation. Assessment of leaf lesions were not conducted, due to the risk of accidental defoliation. As the sequence of events leading to successful crown-canker formation involves the growth of mycelium down the petiole into the stem (Hammond et al. 1985) accidental defoliation during the assessment of leaf lesions would affect the subsequent assessment of crown-canker formation on the same plants.

Genetic analysis

The first stage in the genetic analysis, for all experiments, involved the partitioning of the total variance into that due to crosses, blocks and error. Subsequently, the variance due to crosses was further partitioned into that contributed by the male parents, the female parents, and their interaction (males females). To maintain consistency and for ease of comparison, no transformations were performed on the data from all experiments. All analyses involving ANOVA procedures were conducted using the statistical package MINITAB (version 7.2; Minitab, Inc. 1989). The mean squares for the males, females, interaction and error were used to calculate the components of variation due to males (σ^2_m), females (σ^2_f) and male female interaction (σ^2_{mf}), using the expectations of mean squares (Table 1) according to Kearsey (1965).

Estimates of genetic variances were then derived from these components, based on the formulae by Kearsey (1965):

$$\sigma^2_m = \sigma^2_f = \text{cov.(H.S.)} = \frac{1}{8} D_R$$

$$\sigma^2_{mf} = (\text{cov F.S.}) - 2\,\text{cov.(H.S.)} = \frac{1}{6} H_R$$

$$\sigma^2_w = V_P - \text{cov.(F.S.)} = \frac{1}{4} D_R + \frac{3}{16} H_R + nE_2$$

where,

cov. =covariance
H.S. =half sibs
F.S. =full sibs
D_R =additive component
H_R =dominance component
V_P =phenotypic variance
nE_2 =environmental component.

The broad- and narrow-sense heritabilities for blackleg resistance were calculated from the estimates of D_R and H_R, according to the formulae of Kearsey (1965):

$$h^2_{ns} = \frac{V_A}{V_P} = \frac{\frac{1}{2} D_R}{\frac{1}{2} D_R + \frac{1}{4} H_R + nE_2}$$

$$h^2_{bs} = \frac{V_G}{V_P} = \frac{\frac{1}{2} D_R + \frac{1}{4} H_R}{\frac{1}{2} D_R + \frac{1}{4} H_R + nE_2}$$

where,

h^2_{ns}=narrow-sense heritability
h^2_{bs}=broad-sense heritability
V_G=genotypic variance
V_A=additive variance.

Results

Analysis of variance and estimation of the components of variation

Experiment I – Blackleg resistance at the cotyledon stage

Highly significant mean squares were obtained for the females (F), males (M) and F×M items in the analysis of variance of cotyledon-lesion severity data for the 49 NCM-II families (Table 2). With the exception of the M×B inter-

Table 1 Expectation of mean squares for the North Carolina mating design II

Source	*df*	Expectation of mean squares
Females (F)	(n_1-1)	$\sigma^2_w + mn_3\sigma^2_{mf} + mn_3n_2\sigma^2_f$
Males (M)	(n_2-1)	$\sigma^2_w + mn_3\sigma^2_{mf} + mn_3n_1\sigma^2_m$
Blocks (B)	(n_3-1)	
F×M	$(n_1-1)(n_2-1)$	$\sigma^2_w + mn_3\sigma^2_{mf}$
F×B	$(n_1-1)(n_3-1)$	
M×B	$(n_2-1)(n_3-1)$	
F×M×B	$(n_1-1)(n_2-1)(n_3-1)$	
Error	$n_1n_2n_3(m-1)$	σ^2_w

Table 2 Analysis of variance for data derived from plants of 49 NCM-II families inoculated with pycnidiospores of *L. maculans* (by spray inoculation) at 10 days after germination (cotyledon stage). Plants were assessed for cotyledon-lesion development 14 days after inoculation

Source	*df*	MS	Expectation of mean squares
Females (F)	6	5.7705***	$\sigma_w^2+20\sigma_{mf}^2+140\sigma_f^2$
Males (M)	6	5.2342***	$\sigma_w^2+20\sigma_{mf}^2+140\sigma_m^2$
Blocks (B)	1	3.0309*	
F×M	36	1.8636***	$\sigma_w^2+20\sigma_{mf}^2$
F×B	6	1.7360**	
M×B	6	0.8684*ns*	
F×M×B	36	3.2415***	
Error	882	0.6160	σ_w^2

*** Significant at $P\leq0.001$
** Significant at $0.01>P>0.001$
* Significant at $0.05>P>0.01$
ns not significant at $P=0.05$

action item, all block interactions were significant. This precluded the inclusion of the block-interaction items with the Error for the estimation of the pooled within-family variance. Estimates of the components of variation (see Table 6) showed that the female item (σ_f^2) was not significantly greater than the male item (σ_m^2), indicating the absence of maternal effects.

Experiment II – Blackleg resistance to crown-canker development, following inoculation at the cotyledon stage, assessed at 12 weeks post-inoculation

With the exception of the females (F) item for %G, the items M, F and M F were significant for %G, E and %II (Table 3). The non-significant females (F) item for %G was bordering on significance ($0.10>P>0.05$), and hence for the purposes of estimation it has been treated as a real effect. None of the variation due to blocks alone was significant for %G, E and %II. However, since the majority of interaction items involving blocks were significant, they were not included in the Error.

Estimates of the components of variation (see Table 6) revealed a large disparity between the values obtained for the female (σ_f^2) and male (σ_m^2) items, for %G. Differences of lesser magnitude were also observed between these two items for E and %II. In all instances, σ_m^2 had larger values than σ_f^2This result was not interpreted as evidence of maternal influences on the expression of these three traits, for the reason that maternal factors affect the progeny by inducing its phenotype to more closely resemble that of the maternal, rather than the paternal, parent (Mather and Jinks 1971). Thus, estimates of the female component (σ_f^2), in the presence of maternal effects, would be higher than that of the male component (σ_m^2). However, in the present experiment, the reverse relationship was found between the values for these two components (see Table 6). Therefore, the disparities between the values of these two components were probably not the result of maternal influences, but possibly the consequence of a sampling error of the F_2 population.

Experiment III – Blackleg resistance to crown-canker development, following wound-inoculation of the stem

With the exception of the females (F) and F×M items for%II, the items M, F, and M×F were significant, for all measures of resistance (Table 4). Of the block interactions, only the F×B and F×M×B items were significant. However, to maintain consistency between experiments, the non-significant block items were not included in the Error.

Estimates of the components of variation (Table 6) showed that the female component (σ_f^2) was not significantly greater than the male item (σ_m^2), for %G and E, indicating the absence of maternal effects. A large disparity was observed, however, between the values obtained for the male and female components for %II (see Table 6). However, for the same reasons as proposed above (for Experiment II), this was not interpreted as evidence for the presence of maternal effects.

Table 3 Analysis of variance for data derived from NCII lines inoculated with pycnidiospores of *L. maculans* (by spray inoculation) at 10 days after germination (cotyledon stage). Plants were assessed for crown-canker development 12 weeks after inoculation

Source	*df*	Mean square for measurement			Expectation of mean squares
		% G	E	% II	
Females (F)	6	1287.0 *ns*	1005 2*	3457.0*	$\sigma_w^2+10\sigma_{mf}^2+70\sigma_f^2$
Males (M)	6	3647.0***	1299.1*	6253.0**	$\sigma_w^2+10\sigma_{mf}^2+70\sigma_m^2$
Blocks (B)	1	3034.3 *ns*	0.8 *ns*	1373.0 *ns*	
F×M	36	2040.2***	770.5*	2404.0*	$\sigma_w^2+10\sigma_{mf}^2$
F×B	6	2616.7*	1527.8**	4699.0**	
M×B	6	2448.5*	756 6 *ns*	3018.0 *ns*	
F×M×B	36	1707.6**	1032.7***	1793 0 *ns*	
Error	392	978.3	468.8	1567.0	σ_w^2
Total	489				

*** Significant at $P\leq0.001$
** Significant at $0.01>P>0.001$
* Significant at $0.05>P>0.01$
ns not significant at $P=0.05$

Table 4 Analysis of variance for data derived from NCII lines inoculated with pycnidiospores of *L. maculans* (by wound inoculation) at growth stage 2.4–2.5. Plants were assessed for crown-canker development 5 weeks after inoculation

Source	*df*	Mean square for measurement			Expectation of mean squares
		% G	E	% II	
Females (F)	6	2544.8***	4445.8***	720.8 *ns*	$\sigma_w^2+16\sigma_{mf}^2+112\sigma_f^2$
Males (M)	6	2690.9***	4250.0***	3693.4***	$\sigma_w^2+16\sigma_{mf}^2+112\sigma_m^2$
Blocks (B)	1	3885.8**	181.3 *ns*	811.0 *ns*	
F×M	36	723.6*	978.1*	684.4 *ns*	$\sigma_w^2+16\sigma_{mf}^2$
F×B	6	292.7 *ns*	1647.6*	527 9 *ns*	
M×B	6	454.0 *ns*	484.3 *ns*	952.3 *ns*	
F×M×B	36	696.2 *ns*	995.5*	712.8 *ns*	
Error	686	501.6	666.2	584.3	σ_w^2
Total	783				

*** Significant at $P \leq 0.001$
** Significant at $0.01 > P > 0.001$
* Significant at $0.05 > P > 0.01$
ns not significant at $P = 0.05$

Table 5 Analysis of variance for data derived from NCII lines inoculated with pycnidiospores of *L. maculans* (by spray inoculation) at growth stage 2.3–2.4. Plants were assessed for crown-canker development 9 weeks after inoculation

Source	*df*	Mean square for measurement			Expectation of mean squares
		% G	E	% II	
Females (F)	6	1907 8*	1401.8*	2784.0 *ns*	$\sigma_w^2+8\sigma_{mf}^2+56\sigma_f^2$
Males (M)	6	1838.0*	1619.6**	4819.0**	$\sigma_w^2+8\sigma_{mf}^2+56\sigma_m^2$
Blocks (B)	1	613.0 *ns*	3332.8*	994.0 *ns*	
F×M	36	1033.1 *ns*	1186.8***	2363.0*	$\sigma_w^2+8\sigma_{mf}^2$
F×B	6	1053.8 *ns*	935.6 *ns*	1704.0 *ns*	
M×B	6	1318.8 *ns*	672.3 *ns*	2995.0 *ns*	
F×M×B	36	799.4 *ns*	720.0 *ns*	1491.0 *ns*	
Error	294	723 7	528.4	1472.0	σ_w^2
Total	391				

*** Significant at $P \leq 0.001$
** Significant at $0.01 > P > 0.001$
* Significant at $0.05 > P > 0.01$
ns not significant at $P = 0.05$

Experiment IV – Blackleg resistance to crown-canker development, following spray inoculation (leaves)

With the exception of the F×M item within %G, and the females (F) item within %II, the items F, M, and F×M were significant for all measures of resistance (Table 5). None of the block interactions was significant. For reasons discussed above, no integration of the non-significant block items with the Error was performed.

Disparities between the values of the male and female components of variation (Table 6), similar to those observed in Experiments II and III, were found for E and %II. As with the previous two experiments, these disparities were possibly not indications of maternal influences on the expression of these characters.

Estimation of genetic variances and heritabilities

The estimates of genetic variances for Experiment I (Table 7) indicated that resistance to cotyledon-lesion development in the 49 NCM-II families was significantly affected by non-additive genetic effects. The dominance variation (V_D), estimated as $\frac{1}{4}H_R$ (assuming no epistasis), was over twice as large as the additive genetic variance (V_A). The moderate broad-sense heritability (48.3%) reflected the considerable effect of the environment on the expression of this trait. Due to the large contribution of dominance to the genetic variance, the narrow-sense heritability for this trait was low (Table 7).

The estimates of additive- and non-additive genetic variances, for %G, E and %II in Experiment II, indicated the existence of considerable dominance for blackleg resistance in the NCM-II families (Table 7). The ratios of V_D to V_A, for %G, E and %II, were 9.3, 5.5 and 2.4 respectively. The high proportion of non-additive to additive genetic variance indicated the possibility of discrete inheritance of resistance to crown-canker development, following inoculation at the cotyledon stage. However, the moderate to low estimates of broad-sense heritabilities, for all three measures of resistance (Table 7), did not support this possibility. The low broad-sense heritabilities for the three

Table 6 Estimated components of variation for blackleg resistance in lines derived from a North Carolina II mating scheme, using three different infection procedures

Infection procedure	Estimated components	Disease severity measure			Cotyledon lesion[a]
		%G	E	%II	
Spray inoc. (cotyledons) *Assessed 14 days after inoculation*	σ_f^2				0.0279
	σ_m^2				0.0241
	σ_{mf}^2				0.0624
	σ_w^2				0.6160
Spray inoc. (cotyledons) *Assessed 12 weeks after inoculation*	σ_f^2	0.010	3.353	15.043	
	σ_m^2	22.954	7.551	54.988	
	σ_{mf}^2	106.190	30.170	83.700	
	σ_w^2	978.300	468.800	1567.000	
Wound inoc. (G.S. 2.4–2.5) *Assessed 5 weeks after inoculation*	σ_f^2	16.261	30.962	0.280	
	σ_m^2	17.565	29.213	26.821	
	σ_{mf}^2	13.406	19.494	6.019	
	σ_w^2	509.100	666.200	593.100	
Spray inoc. (G.S. 2.3–2.4) *Assessed 9 weeks after inoculation*	σ_f^2	15.620	3.839	7.518	
	σ_m^2	14.373	7.730	43.857	
	σ_{mf}^2	38.675	82.300	111.375	
	σ_w^2	723.700	528.400	1472.000	

[a] Cotyledon-lesion. disease severity based on a 0–5 severity scale

measures indicated the possible sensitivity of these traits to environmental factors. Narrow-sense heritability estimates were very low for the three measures (Table 7), which reflected the small contribution of the additive component to the genotypic variance.

The wound-inoculation experiment (Experiment III) was found to give the smallest ratios of non-additive to additive genetic variance (Table 7). The ratios of V_D to V_A, for %G, E and %II were 0.79, 0.65 and 0.44 respectively. The low broad-sense heritabilities for %G, E and %II (Table 7) indicated the possible sensitivity of these traits to environmental factors. These broad-sense values were not consistent with the values estimated in the preceding study (Pang and Halloran 1996), using the same inoculation technique, possible reasons for which will be discussed later.

The estimates of additive- and non-additive genetic variances, for %G, E and %II in Experiment IV, indicated, in agreement with Experiments I, II and III, the existence of significant dominance for blackleg resistance in the NCM-II families. The ratios of V_D to V_A, for the three components were 2.6, 14.2 and 4.3 respectively. The high ratio of V_D/V_A for E, together with a moderately high broad-sense heritability (56.6 %) for this trait, indicated the likelihood of oligogenic control.

Correlations between seedling (Experiment I) and adult-plant scores (Experiment II)

Regression of crown-canker severity scores, as measured by %G, E and %II, against the corresponding cotyledon-lesion scores (Fig. 1a,b,c), revealed no correlation (R^2=0.00) between cotyledon scores, assessed at 2-weeks post inoculation, and subsequent crown-canker development on the same plants. This indicated that the severity of cotyledon infection by blackleg had no bearing on subsequent crown-canker infection. The implications of this finding, for screening for blackleg resistance, will be discussed later.

Discussion

The broad- and narrow-sense heritability estimates for blackleg resistance in the NCM-II population were lower than those derived from the analysis of the F_2 and first-backcross populations in a preceding study (Pang and Halloran 1996). This indicated that the random sample of 14 parents used to generate the NCM-II families may not have represented all possible resistant/susceptible genotypes in the F_2 population. Kearsey (1965) demonstrated that heritability estimates for flowering time in poppy (*Papaver dubium*) were significantly reduced through the use of a small sample of parents, which were possibly unrepresentative of the population as a whole. An unrepresentative sample of parents from a segregating population, such as the F_2, may possibly affect the heritability estimates of oligogenically-controlled, more than polygenically controlled, traits. This is because the inclusion or exclusion of any individual polygene from the new population would be unlikely to seriously affect heritability estimates, since

Table 7 Genetic variances and heritabilities for blackleg resistance in 49 NCM-II families, using three different infection procedures

Infection procedure	Genetic parameters	Disease severity measures			Cotyledon lesion[a]
		% G	E	% II	
Spray inoc. (cotyledons) *Assessed 14 days after inoculation*	V_P				0.73
	V_G				0.35
	V_A				0.10
	V_D				0.25
	V_E				0.49
	h^2_{ns} (%)				14.2
	h^2_{bs} (%)				48.3
Spray inoc. (cotyledons) *Assessed 12 weeks after inoculation*	V_P	1107.44	509.87	1720.73	
	V_G	470.67	142.49	474.86	
	V_A	45.91	21.81	140.06	
	V_D	424.76	120.68	334.80	
	V_E	636.78	367.39	1245.87	
	h^2_{ns} (%)	4.3	4.3	8.1	
	h^2_{bs} (%)	42.5	27.9	26.6	
Wound inoc. (G.S. 2.4–2.5) *Assessed 5 weeks after inoculation*	V_P	556.33	745.87	626.22	
	V_G	121.28	198.33	78.28	
	V_A	67.65	120.35	54.21	
	V_D	53.33	77.98	24.08	
	V_E	435.06	547.54	547.94	
	h^2_{ns} (%)	12.2	16.1	8.7	
	h^2_{bs} (%)	21.8	26.6	12.5	
Spray inoc. (G.S. 2.3–2.4) *Assessed 9 weeks after inoculation*	V_P	792.37	622.27	1634.75	
	V_G	214.69	352.34	548.25	
	V_A	59.97	23.14	102.75	
	V_D	154.70	329 20	445.50	
	V_E	577.68	269.93	1086.50	
	h^2_{ns} (%)	7.6	3.7	6.3	
	h^2_{bs} (%)	27.1	56.6	33.5	

[a] Cotyledon-lesion: disease severity based on a 0–5 severity scale
h^2_{ns}=narrow-sense heritability
h^2_{bs}=broad-sense heritability

polygenes, by definition (Mather and Jinks 1971), have small individual effects on the overall expression of a trait. The inclusion or exclusion of an oligogene, however, may be expected to have a significant effect on heritability estimates.

The overall resistance of *B. napus* to *L. maculans* has been shown to involve mechanisms preventing initial ascospore germination and leaf penetration (Carter 1982) and spread in the lamina and petiole (Hammond and Lewis 1987; Mithen et al. 1987). Wound-inoculation, by direct introduction of pycnidiospores into the crown region of the plant, bypasses possible resistance mechanisms to invasion by the blackleg fungus in the lamina and petiole. Therefore, differences in heritability estimates between Experiments III (wound-inoculation) and IV (spray-inoculation, leaves) were likely to reflect the genetic control of additional barriers to fungal invasion present in the lamina and petiole. It is possible that resistance mechanisms in the lamina and petiole could be bypassed if pycnidiospores which fell on the stems (after spray inoculation) were able to infect these stems directly. However, studies conducted by Hammond et al. (1985) indicated that, while pycnidiospores were able to germinate on intact stems, the mycelia formed were subsequently unable to penetrate these stems. Broad-sense heritabilites and V_D/V_A ratios, obtained for %G, E and %II were consistently higher in Experiment IV (spray-inoculation) compared with Experiment III (wound-inoculation). These results indicated the likely presence of resistance mechanisms in the leaf and petiole, whose expression were possibly under oligogenic control.

The high V_D/V_A ratios observed in all experiments indicated the existence of significant dominance/epistasis for blackleg resistance in the NCM-II families. This finding was in agreement with similar levels of dominance observed in the F_2 and first-backcross populations in the preceding study (Pang and Halloran 1996). These results support an earlier contention by Cargeeg and Thurling (1979) that resistance to blackleg in *B. napus* is governed through the combined action of major and minor genes. This mode of genetic control may pose serious problems for plant breeders wishing to "pyramid" blackleg resistance genes to produce cultivars with enhanced resistance, since ma-

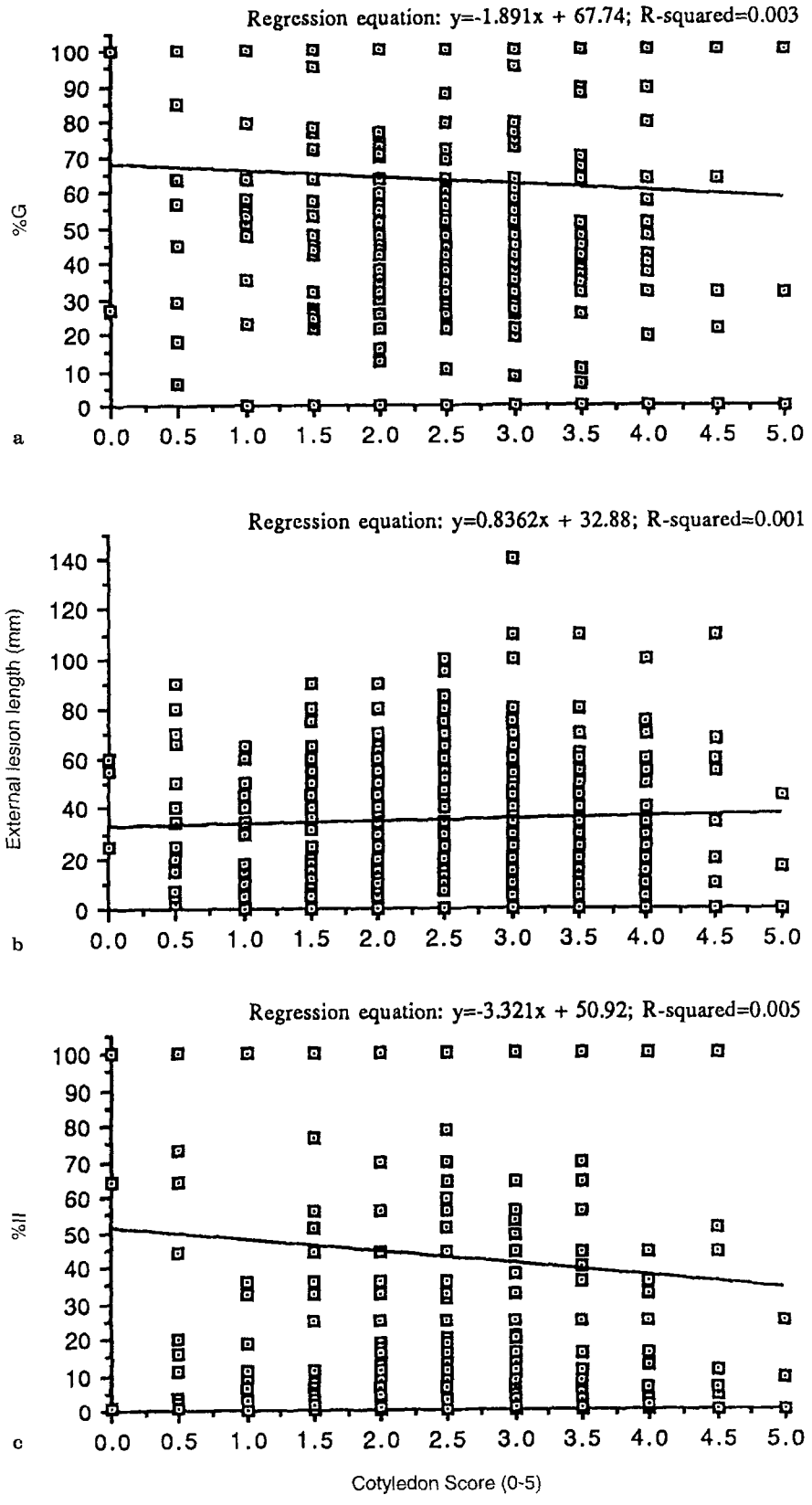

Fig. 1 Regression of blackleg crown-canker development (based on %G, E and %II) measured 12 weeks after inoculation against corresponding cotyledon scores (assessed 2 weeks after inoculation) in 49 NCM-II families

jor-gene effects are obscured (modified) by the polygenes present. Recently, Dion et al. (1994) used RFLP mapping to identify a quantitative trait locus (QTL) for blackleg resistance, corresponding to a single dominant major gene (or a number of tightly-linked genes ?) in doubled-haploid lines of *B. napus*. This gene(s) was associated with linkage group 5, and appeared to be effective against several pathotypes of *L. maculans*. As more QTLs for blackleg re-

sistance are identified, "pyramiding" of resistance genes may become more feasible in the future.

Delwiche (1980) showed that the resistance to cotyledon-lesion development in *B. napus*, following wound-inoculation, was controlled by two dominant genes. In the present study, the poor correlations found between cotyledon-lesion severity scores and subsequent crown-canker development indicated that resistance to cotyledon-lesion development was a poor indicator of adult-plant blackleg resistance. This may explain the failure of many workers (Cargeeg and Thurling 1979; Helms and Cruickshank 1979; Wittern and Kruger 1985) to relate seedling scores, based on cotyledon-lesion assessments, with blackleg resistance in the field.

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