RESISTANCE TO DOWNY MILDEW IN SINGLE- AND DOUBLE-LOW OILSEED RAPE AND NEW SOURCES OF RESISTANCE IN A RANGE OF GENOTYPES

N.I. Nashaat, C.J. Rawlinson

AFRC, Institute of Arable Crops Research,
Rothamsted Experimental Station, Harpenden, Herts, UK AL5 2JQ

SUMMARY

The expression of resistance to three isolates of Peronospora parasitica at different seedling growth stages was investigated in a range of single- and double-low cultivars of winter oilseed rape. There was no consistently significant difference in resistance between the two cultivar types, none of which had a known major gene for resistance to downy mildew. Screening for resistance to four isolates among a much wider range of winter and spring types of Brassica napus ssp. oleifera has identified two groups carrying different race specific resistance factors. Most of the genotypes in these groups were selected from the population of accessions exhibiting a heterogeneous reaction to some isolates. The specificity of resistance in one group differs from that expressed by cv. Cresor, the only oilseed rape cultivar reported to have a major gene for resistance to downy mildew.

INTRODUCTION

Downy mildew is the most frequently recorded disease of winter oilseed rape in the United Kingdom (Gladders, 1987). Some cultivars are very susceptible at the seedling stage, but the disease is not thought to limit yield except, rarely, when seedlings are killed (Davies, 1986). Consequently, selection and breeding for resistance is not given a high priority at present. By contrast, elsewhere in northern Europe (Sadowski, 1989) and Asia (Kolte, 1985) severe infection can cause regular, significant yield loss. The status of downy mildew in much of Europe may change with the recent introduction and widespread cultivation of new cultivars with a low content of glucosinolates in seed. Some glucosinolate breakdown products are toxic to P. parasitica (Greenhalgh & Mitchell, 1976), so smaller amounts or changed types of glucosinolates in vegetative tissue may favour infection (Rawlinson, et al., 1989). Moreover, predictions of climate warming (Parry, 1989) and the possibility of milder, wetter winters in Europe may also favour downy mildew. These considerations indicate a need for work on this disease to be given higher priority, to establish whether the new double-low cultivars are inherently more prone to infection than the single-low cultivars grown hitherto, and to seek sources of resistance for use by plant breeders. Lucas et al., (1988) identified the only reported major gene for resistance to downy mildew in the spring rape cv. Cresor. This paper describes a screening method requiring minimum facilities, which has been used to identify new sources of resistance to downy mildew.

MATERIALS AND METHODS

Provenance and maintenance of P. parasitica isolates

Four isolates of P. parasitica were used: R1 was a single spore isolate collected from winter oilseed rape cv. Cobra at Rothamsted Experimental Station; P033, P004 and P003 (virulent on cv. Cresor) were obtained from J.A. Lucas, University of Nottingham, UK. All isolates were maintained on cotyledons of cv. Ariana.

Cotyledons were obtained from 6-day old seedlings raised in soil-less compost in a modified plant propagator (35.5cm x 21.6cm x 18cm) sited in a glasshouse and supplied with continuous, filtered (spore-free) moist air at

18 ± 2°C through a central flue conducting air from beneath the propagator to exhaust at two adjustable ventilators on the cover, and the junction between the cover and the base (Jenkyn et al., 1973), and given supplementary light to maintain a photoperiod of 16h day^{-1}. Cotyledons and a short length of hypocotyl were detached and transferred to folded filter paper (Whatman 12.5cm, 113v) supports in glass jars (8cm diameter, 7cm depth) containing 20ml sterile distilled water. Cotyledons were then inoculated in a sterile air flow with 5µl of conidial suspension on each half-cotyledon using a micropipette. Suspensions were prepared by tapping infected cotyledons to dislodge conidia into sterile distilled water; this minimised bacterial contamination. After inoculating the cotyledons, the glass jars were covered with clear plastic lids, sealed with parafilm and incubated in Saxcil growth cabinets at 16°C with light at 70µe m^{-2} s^{-1}, 16h day^{-1} for 7 days after which peak sporulation occurred. Using this method, the reculturing interval was normally 7 days, but this could be extended to 21 days at *c.*10°C.

Germplasm screening and evaluation

Seedlings were grown in propagators, as described for culture maintenance, except that 5cm 'Jiffy-pots' x 2 for each accession (cultivar or line) were used as pots. The pots were placed on capillary matting to ensure uniform water supply. Each propagator contained up to 13 accessions arranged as two randomised blocks (propagators) with each line occurring only once in each propagator. Initially, 9-15 seedlings per accession were grown in each propagator, but were thinned to 6-10 six days after sowing to reduce growth variability. Sowing dates were staggered to produce seedlings at the required growth stage for inoculation at the same time. The average times required under these conditions to reach fully expanded cotyledons, first and second true leaves were 7, 16 and 22 days respectively.

Seedlings were inoculated by spraying to run-off with a suspension of conidia (*c.*2.5 x 10^5 conidia ml^{-1}). Seedlings at first and second true leaf stage were then transferred with their original trays to a larger plant propagator (57cm x 29cm x 23.5cm). This was not necessary for seedlings at the cotyledon stage. The propagators were sealed after inoculation, the ventilators adjusted to allow the relative humidity to rise to *c.*100%, then incubated in growth cabinets under the conditions described for culture maintenance. Infection phenotypes (IP) were observed 7 or 9 days after inoculation (on cotyledons and leaves respectively) using a 0-9 scale (Williams, 1985).

A disease index (DI) was calculated from the following formula:

$$DI = \frac{\sum_{i=0}^{9} (ixj)}{n}$$

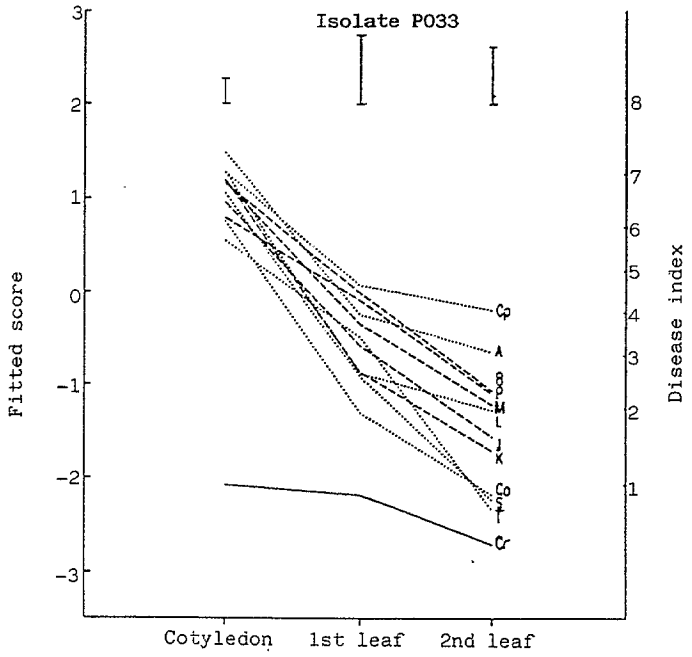
where n = total plants, i = IP class and j = number of plants/class.

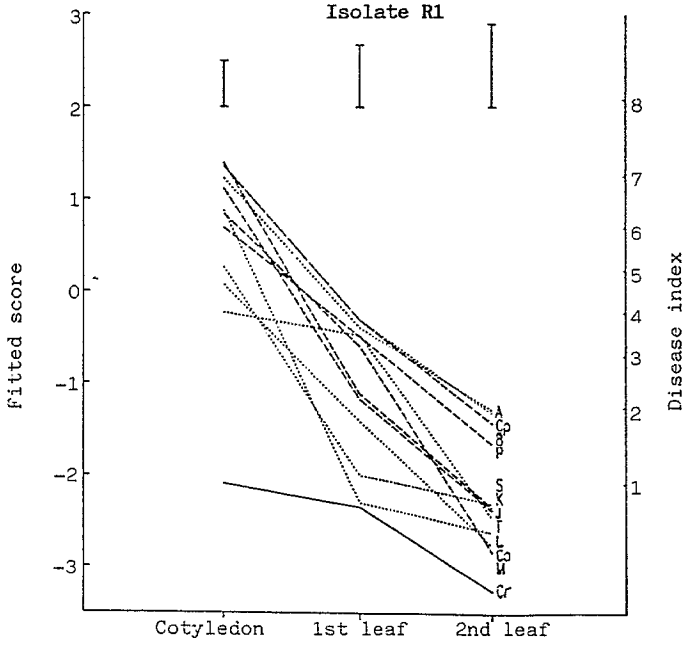
Additionally, all IP scores on the 0-9 scale (where 9 represented 100% susceptibility) were transformed to logits, to give similar variance for all cultivars, before being subject to analysis of variance. These fitted scores and the disease indices are given in Fig.1 together with Least Significant Differences derived from the analysis of logits. Kendall's coefficient of concordance over growth stage was also calculated for all three isolates to assess the consistency of the score over the three growth stages. Host material was classified into three selection categories: susceptible (IP = 7 to 9), partial resistance (IP = 3 to 5) and resistant (IP = 0 to 1).

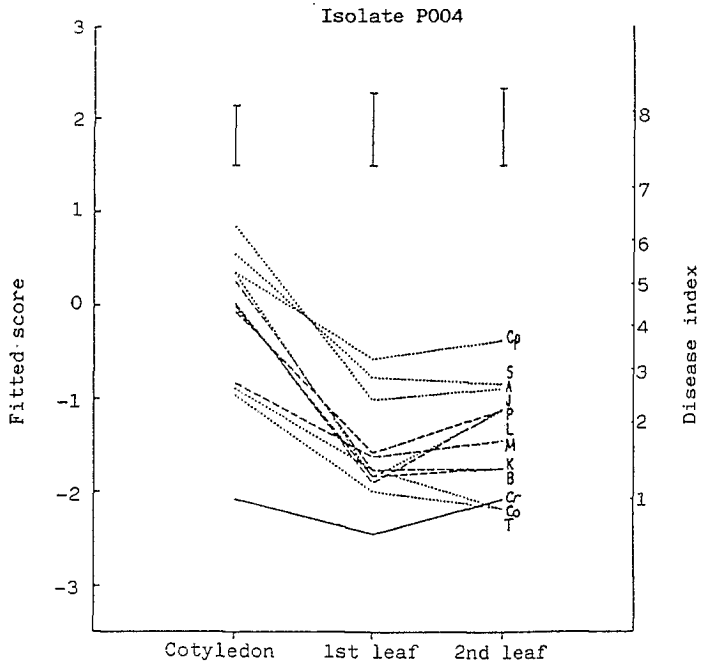
RESULTS AND DISCUSSION

Comparison of cultivar types and tissues

Fig. 1 compares the response to infection among the single- and double-low cultivars; cv. Cresor was included in each test, for comparison, as an


Isolate P033
Fitted score
Disease index
3
2
1
0
-1
-2
-3
8
7
6
5
4
3
2
1
Cotyledon
1st leaf
2nd leaf


Isolate R1
Fitted score
Disease index
3
2
1
0
-1
-2
-3
8
7
6
5
4
3
2
1
Cotyledon
1st leaf
2nd leaf



Key

Cr = Cresor	A = Ariana
B = Bienvenu	Cp = Capricorn
J = Jet Neuf	Co = Cobra
K = Korina	L = Lecor
M = Mikado	S = Score
P = Primor	T = Tapidor

--- single-low varieties

... double-low varieties

Bars indicate LSD at P=0.05 (n=12)

Figure 1. The expression of resistance in 12 cultivars challenged with three isolates of P. parasitica.

example of a host with a known major gene for resistance. There was no consistent significant difference between the two cultivar types when measured at the three seedling growth stages. These tests should fairly reflect large differences in amounts of glucosinolates in the tissues of the two cultivar types because concentrations in seedling tissues are highly correlated with those in the seed (Glen et al., 1989), unlike the concentrations in older vegetative tissue (Milford et al., 1989). The absence of a clear distinction between the two cultivar types indicates that total glucosinolate concentration is unlikely to be related to the level of resistance to downy mildew. This observation is compatible with other work with naturally infected field crops (Rawlinson et al., 1989).

The conclusion of Greenhalgh & Mitchell (1976) that resistance to downy mildew in cabbage (B. oleracea) may be related to concentration of the glucosinolate hydrolysis product, allyl isothiocyanate, may be explained by the much greater concentration of its precursor, sinigrin, in cabbage tissues than in winter oilseed rape.

Cotyledons of all cultivars, except Cresor, were markedly less resistant than first or second true leaves. This was related to the absence of surface wax rodlets (revealed by scanning electron microscopy of fresh tissue), which resulted in increased wettability and spore retention on cotyledons, and to the greater efficiency of the necrotic reaction to infection on leaves. However, the relative differences in resistance between cultivars remained largely unchanged, regardless of which tissue was inoculated. The differences in the level of resistance expressed in cultivars to each of the three isolates is likely to be attributable mainly to the effect on host tissue and development of fluctuations in ambient temperature and light intensity, since each isolate was tested at a different time of year. The three experiments using isolates P004, P033 and RI were done in July, September and October 1989 respectively.

New sources of resistance identified

A range of 85 winter and 20 spring accessions of B. napus ssp. oleifera were also tested at the cotyledon stage for resistance to four isolates of downy mildew. These accessions came from diverse sources, including Canada, China, France, Germany, UK and USA. Two groups carrying different race specific resistance factors were identified. The first group had resistance equivalent to that in cv. Cresor (i.e. resistant to isolates R1, P033 and P004, and susceptible to isolate P003). This group represented 18 new sources of resistance and included double-low winter rape and single-low spring types. The second group was resistant to all four isolates and represented 7 new sources of resistance from single-low winter and spring types. Most of the genotypes were selected from the population of accessions exhibiting a heterogeneous reaction to the pathogen. The identity of these sources will be published elsewhere after confirmation of their stability. Work continues to breed truly homozygous lines to study the inheritance of resistance in these sources, and also to widen the base of material resistant to downy mildew. Our early work on screening for resistance to downy mildew indicated that, for speed and simplicity, initial screening could be done on cotyledons since this gave a good reflection of the resistance expressed in other seedling tissues (e.g. Fig.1). However, it is possible that resistance expressed at much later growth stages may be determined by a gene, or genes, different from those effective at the seedling stage, as noted by Hoser-Krauze, Lakowska-Ryk & Antosik (1987) for downy mildew of broccoli. Moreover, a gene, or genes, not fully expressed at the seedling stage may be expressed in a different fashion at later stages. The adult plant resistance of the new sources identified in this study has yet to be confirmed.

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REFERENCES

DAVIES, J.M.L. 1986. Diseases of oilseed rape. In Oilseed Rape. Eds. D.H.Scarisbrick and R.W. Daniels, pp. 195-236. William Collins Sons & Co Ltd. London.

GLADDERS, P. 1987. Current status of diseases and disease control in winter oilseed rape in England and Wales. International Organisation for Biological Control (WPRS) Bulletin X, 4: 7-12.

GLEN, D.M., JONES, J.K. and FIELDSEND, J.K. 1989. Effects of glucosinolates on slug damage to oilseed rape. Aspects of Applied Biology 23: 377-381.

GREENHALGH, J.R. and MITCHELL, N.D. 1976. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of Brassica oleracea. New Phytologist. 77: 391-398.

HOSER-KRAUZE, J., LAKOWSKA-RYK, E. and ANTOSIK, J. 1987. The inheritance of broccoli (Brassica oleracea var. Botrytis L.) leaf resistance to downy mildew - Peronospora parasitica (Pers.) Ex. Fr. Genetica Polonica 28 (4): 377-880.

JENKYN, J.F., HIRST, J.M. and KING, G. (1973). An apparatus for the isolated propagation of foliar pathogens and their hosts. Annals of Applied Biology 73: 9-13.

KOLTE, S.J. 1985. Diseases of Annual Edible Oilseed Crops. Vol. II Rapeseed -Mustard & Sesame Diseases. pp. 19-27. CRC Press, Inc. Boca Raton, Florida.

LUCAS, J.A., CRUTE, I.R., SHERRIFF, C. and GORDON, P.L. 1988. The identification of a gene for race specific resistance to Peronospora parasitica (downy mildew) in Brassica napus var. oleifera (oilseed rape). Plant Pathology 37: 538-545.

MILFORD, G.F.J., FIELDSEND, J.K., PORTER, A.J.R. and RAWLINSON C.J., 1989. Changes in glucosinolate concentrations during the vegetative growth of single- and double-low cultivars of winter oilseed rape. Aspects of Applied Biology 23: 83-90.

PARRY, M.L., CARTER, T.R. and PORTER, J.H. 1989. The greenhouse effect and the future of UK agriculture. Journal of the Royal Agricultural Society of England 150: 120-131.

RAWLINSON C.J., DOUGHTY, K.J., BOCK, C.H., CHURCH, V.J., MILFORD, G.F.J. and FIELDSEND, J.K. 1989. Diseases and responses to disease and pest control on single- and double-low cultivars of winter oilseed rape. Aspects of Applied Biology 23: 393-400.

SADOWSKI, C. 1989. Susceptibility of selected cultivars and lines of winter rapeseed to downy mildew. Proceedings of the 7th International Rapeseed Congress. Poznan, Poland, 11-14 May, 1987. Vol. 5: 1229-1234.

WILLIAMS, P.H. 1985. CrGC Resource Book. Dept of Plant Pathology, University of Wisconsin, Madison, USA.