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Plasmodiophora brassicae: aspects of pathogenesis and resistance in *Brassica oleracea*

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Summary

Clubroot is one of the most damaging diseases in *Brassica oleracea* crops world-wide. The pathogenicity of *Plasmodiophora brassicae* is highly variable between as well as within field populations. Several sources of resistance to clubroot have been identified in *B. oleracea*. Generally, resistance tends to inherit partly as a recessive, partly as an additive trait, and appears to be controlled by few major genes. Progress in the understanding of the inheritance of resistance is being made through the use of single-spore isolates of the pathogen, and the use of molecular markers for resistance genes.

Abbreviations: cv – cultivar, DH – doubled haploid, ECD – European Clubroot Differential set, RFLP – Restriction Fragment Length Polymorphism

Introduction

Clubroot, caused by the fungus *Plasmodiophora brassicae* Wor., is probably the most damaging disease of cole crops (*Brassica oleracea* L.) and other cruciferous crops worldwide. The pathogen causes swelling of parts of the roots and sometimes of the stem base into characteristic clubs. These clubs inhibit nutrient and water transport, stunt the growth of the plant and increase the susceptibility to wilting. After some weeks the clubbed roots decay, weakening the support of the plant.

The incidence of clubroot was discussed by Crête (1981). He estimated that in Northwestern Europe, Japan, North America and Australia, about 10% of a total of 660.000 ha of *B. oleracea* crops was infested with clubroot. For all cruciferous crops, the infested area in those regions amounted to 196.000 ha. The spores can remain infectious for at least 15 years (Mattusch, 1977). Cultural practices, especially the application of calcium and boron, and liming to decrease the pH of the soil, may reduce disease pressure, but are often not sufficient to keep the crop healthy. The effects of chemical control are limited, since most treatments are either banned due to environmental regulations, or are too expensive.

The introduction of cultivars with resistance or tolerance to clubroot would be desirable. Nevertheless, only a very small number of resistant cultivars have been released. Breeding programs aimed at the introduction of resistance to clubroot have been few, and the results of these programs were often disappointing. This general lack of success is due to the relatively small number of sources of resistance, the recessive and often apparently complex inheritance of resistance, and the genetic variability of the pathogen.

In this paper the literature on resistance to clubroot in *Brassica oleracea* is reviewed. In addition, relevant studies of the biology of *P. brassicae* and results obtained in host species other than *B. oleracea* are discussed. Earlier reviews of Colhoun (1958), Karling (1968) and Crute et al. (1980) have been complemented with more recent publications.

Terminology

Some terms have been employed ambiguously in clubroot literature. To prevent misinterpretation, they are briefly introduced here.

A field isolate is a population of the pathogen obtained either directly from the soil of an infested field, or from clubs of susceptible plants grown in the field, and maintained in isolation on plants not carrying any known resistance. A single-spore isolate is a population derived from clubs of a plant inoculated with one single resting spore and maintained in isolation.

Pathogenicity is the ability of a pathogen population to cause clubroot symptoms on host plants. Differential pathogenicity (*sensu* Crute et al., 1980) indicates different ranking of pathogenicity of populations in dependence of the host genotype. Conversely, the relative ranking of populations exhibiting non-differential pathogenicity does not depend on the host genotype.

Resistance to clubroot indicates the ability of a plant genotype to limit the development of clubs on the roots, when infected by *P. brassicae*. Partial resistance and complete resistance refer to the level of reduction of club formation in comparison with a completely susceptible genotype. Differential and non-differential resistance are defined analogous to differential and non-differential pathogenicity.

Pathogenesis

The pathogenesis of clubroot is well described by Ingram & Tommerup (1972). The disease cycle can be roughly divided into two stages, the first occurring in the root hairs and the second in the root cortex.

The first stage starts with the germination of a primary zoospore from a haploid resting spore in the soil. The zoospore attaches to a root hair and injects its cell contents into the host cell (Aist & Williams, 1971). In the infected root hair, repeated nuclear divisions of the pathogen lead to a multinucleate plasmodium, which later develops into tens or hundreds of uninucleate zoosporangia. In contrast to the second stage, the development of *P. brassicae* in root hairs into zoosporangia has also been observed in non-crucifers, including some monocotyledons (Webb, 1949; MacFarlane, 1952; Kole & Philipsen, 1956). From the zoosporangia, haploid secondary zoospores are released. Naiki et al. (1984) showed that secondary zoospores again can infect root hairs, which results in a rapid, asexual propagation of the pathogen.

The second stage in the pathogenesis starts with the secondary zoospores. Two zoospores can fuse, resulting in a dikaryotic zoospore, as reviewed by Ingram & Tommerup (1972). It is not known whether fusion is necessary for infection of the root cortex to occur, nor whether different mating types of *P. brassicae* exist. However, genetically uniform single-spore isolates can complete the disease cycle, implying that either fusion of zoospores is not necessary, or that homothallic genotypes of *P. brassicae* exist.

After infection of the root cortex, the pathogen exists as intra-cellular, multinucleate plasmodia. Dekhuijzen (1975, 1981) observed that, at least in infected callus tissue, isolated plasmodia did not penetrate the host cell wall. He postulated that the spread of the pathogen occurs mainly by the stimulated division of infected cells, a process which results in the formation of the clubs. The enhanced cell division is thought to be stimulated by the elevated concentrations of cytokinins (Dekhuijzen & Overeem, 1971; Dekhuijzen, 1980) and auxins. The auxins are presumably derived from indole glucosinolates, normally present in roots of crucifers, due to the presence of intracellular *P. brassicae* plasmodia (Butcher et al., 1974, 1976).

Later in development, the haploid nuclei in multinucleate plasmodia fuse in pairs (Tommerup & Ingram, 1971). After meiosis the newly formed diploid nuclei develop into haploid resting spores which are released into the soil when the clubbed roots decay. During this second stage in the pathogenesis as many as 10^{11} resting spores per plant can be produced (Voorrips, unpublished results).

Mechanisms and components of resistance

At any stage in the life cycle, host resistance could conceivably block or hamper pathogen development. Investigations have focussed on two stages: the infection of root hairs by primary zoospores, and the production of auxins in the root cortex.

Voorrips (1992) demonstrated large differences in the levels of root hair infection among 13 host genotypes with varying levels of resistance, but observed no correlation between resistance to root hair infection and resistance to clubroot development. Conceivably, even a very low survival of *P. brassicae* during the root hair stage allowed infection of the root cortex. Only complete resistance to root hair infection, which was

not observed in this study, would confer resistance to clubroot.

If the enhanced auxin levels present in infected roots are indeed processed from glucobrassicin and other indole glucosinolates (Butcher et al., 1974, 1976), then host genotypes producing no indole glucosinolates might be (non-differentially) resistant to clubroot. However, the evidence for a correlation between indole glucosinolate content and clubroot susceptibility is conflicting. Butcher et al. (1976) and Ockendon & Buczacki (1979) found clear correlations between resistance and low indole glucosinolate content among cruciferous species. Chong et al. (1981, 1984) obtained similar results with cabbage breeding lines. In contrast, Mullin et al. (1980) found no such correlation in 43 rutabaga and turnip cultivars. Ludwig-Müller et al. (1993) found no difference in indole glucosinolate content between control plants of resistant and susceptible Chinese cabbage, but found an enhanced level in infected plants of the susceptible genotype. Rausch et al. (1983) proposed that only very small amounts of auxins, and therefore of indole glucosinolates are needed for club formation. So, although presumably the auxin production from indole glucosinolates is an important factor in the development of clubroot, no general relation between indole glucosinolate content and clubroot resistance has been found.

Thus, although processes occurring during pathogenesis are known in some detail, the mechanisms responsible for resistance have not yet been elucidated.

Pathogenicity of *P. brassicae*

Field populations of *P. brassicae* exhibit clear differences for pathogenicity. Several test series of host genotypes carrying resistance from different sources have been employed to classify populations with respect to differential pathogenicity (reviewed by Crute et al., 1980). Currently the differential series of Williams (1966) and the European Clubroot Differential set (ECD, Buczacki et al., 1975) are commonly used.

It was first shown by Haji Tinggal (1980, cited in Haji Tinggal & Webster, 1981) and Jones et al. (1982a) that field isolates were not genetically uniform, as they found differences between inocula obtained from different slices of the same club or from clubs of different plants from the same field. The variation within field isolates was even more clearly demonstrated by using single-spore isolates (Buczacki, 1977; Haji Tinggal & Webster, 1981; Jones et al., 1982b; Scott, 1985; Schoeller & Grunewaldt, 1986). Since resting spores are haploid (Tommerup & Ingram, 1971), single-spore isolates are genetically uniform except for mutations. Frequently single-spore isolates were obtained which were less pathogenic than the original field isolate. This could be indicative of segregating pathogenicity genes in the pathogen population. However, Jones et al. (1982b) also obtained single-spore isolates pathogenic on host genotypes that were resistant to the original field isolate. Possibly genotypes with rare pathogenicity genes were extracted from the population, perhaps even selected for by the isolation procedure. Jones et al. (1982b) showed that a pathogenic isolate was inhibited by a non-pathogenic isolate, suggesting competition for infection sites or host resources. Another explanation may be a rapidly induced resistance, as described in other host-pathogen interactions (e.g. Ward, 1983).

The existence of genetic variation for pathogenicity within field populations is also demonstrated by the response of field isolates to selection pressure. Crute & Pink (1989) showed that five successive passages of a field isolate through a partially resistant line of Böhmerwaldkohl yielded an isolate with a higher pathogenicity on the resistant line as well as on a susceptible control. Also, the erosion of the resistance of cabbage cv Badger Shipper within a few years after its release was probably due to selection of pathogenic genotypes (Seaman et al., 1963). In absence of the resistant host, the pathogenicity of the population in the field towards Badger Shipper diminished again (Seaman et al., 1963), supporting the hypothesis of reduced fitness of genotypes carrying pathogenicity genes.

This genetic variation implies that the characterization of a field isolate using a differential test series is in fact only valid for the inoculum used in the test. Other clubs, and especially clubs obtained after further propagation of the isolate may contain a population with different pathogenicity. It is therefore essential that relevant control host genotypes are included in every clubroot test. The concept of races is not very effective in the case of field isolates of clubroot, since neither the pathogen populations nor the differential hosts fulfill the necessary conditions of genetic uniformity and stability (Parlevliet, 1985). Rather, a system is required based on identified pathogenicity and resistance genes.

Progress in this area may become possible by the use of host plants carrying well characterized resistance

genes, and by studying genetically uniform single-spore isolates. The advantages of single-spore isolates are the stability of the genotype of the isolate over propagation cycles, and the absence of interaction between different pathogen genotypes. Meanwhile, it must be recognized that single-spore isolates may represent atypical pathogen genotypes. It will therefore be necessary to compare the results obtained with single-spore isolates, with those obtained with the original field isolates and mixtures of single-spore isolates.

Sources of resistance to clubroot in *B. oleracea*

Contrary to the situation in *B. rapa* and *B. napus*, completely resistant accessions have only rarely been described in *B. oleracea*. This has stimulated much screening work in the cole crops, uncovering several accessions partially resistant to field isolates (Table 1). Most of these accessions were cabbage, kale or curly kale types. In other cole crops, only low levels of resistance were found (e.g. Dixon & Robinson, 1986; Crisp et al., 1989; Voorrips & Visser, 1993). Several highly resistant lines were bred from some of the resistant accessions. However, the cultivars carrying clubroot resistance have found only limited application, because of an insufficient level of resistance, a quick erosion of resistance, or because of insufficient quality.

Voorrips & Visser (1990) produced doubled haploid lines (DH-lines) from partially resistant cabbage and kale accessions. Some of these DH-lines were almost completely resistant. This suggested that some accessions described as partially resistant did in fact segregate for resistance. As *B. oleracea* is a cross-fertilizing species many accessions are likely to be heterogeneous. Since the level of resistance of an accession is expressed as the average disease severity of several plants, a partially resistant accession may still harbour genes for complete resistance. It is perhaps unfortunate that research and breeding for clubroot resistance have concentrated on the few sources with high levels of resistance, and ignored the potential of accessions with low partial resistance. Other sources of resistance could possibly have been identified in this way.

Also of interest is the range of pathogen isolates to which a host genotype is resistant. Toxopeus et al. (1986) reported a summary of ECD tests (Buczacki et al., 1975) involving 299 field isolates, carried out by 18 researchers in different countries. None of the 15 ECD host genotypes was resistant to all field populations tested. The five *B. oleracea* ECD hosts were susceptible to the majority of the isolates. Crute et al. (1983) and Crute (1986) demonstrated that cvs Böhmerwaldkohl, Bindsachsener and Verheul interacted non-differentially with a range of isolates, whereas cv Badger Shipper interacted differentially. Tests of Voorrips & Thomas (unpublished) also indicated that cv Böhmerwaldkohl and, to a lesser extent, Bindsachsener were partially resistant against different clubroot isolates in Europe. Badger Shipper and related accessions, as well as the OSU broccoli lines (Baggett, 1976; Baggett & Kean, 1985) were highly resistant or highly susceptible, depending on the clubroot isolate. Nevertheless, some field isolates were highly pathogenic to Böhmerwaldkohl and Bindsachsener (Voorrips & Thomas, unpublished). The distinction between differential and non-differential resistance is therefore gradual rather than absolute, and is dependent on the isolates used. The suggestion of Crisp et al. (1989) that genes for differential resistance should be excluded in order to breed for non-differential resistance is therefore likely to be counter-productive. What is really needed is information on the interaction of resistance genes with pathogenicity genes, and information regarding the distribution of pathogenicity genes in pathogen populations. Studies in this field would greatly benefit from the use of well-defined single-spore isolates of *P. brassicae*.

Classical genetic studies of resistance and tolerance

Several classical genetic studies of clubroot resistance in *B. oleracea* have been published, using either diallel crossing schemes, or segregating progenies from crosses between two lines (Table 2). Generally, symptoms were assessed visually and rated on a symptom scale. For genetic analysis, either a qualitative approach was used by classifying plants with low symptom grades as resistant and the others as susceptible, or a quantitative approach based on the symptom grades. Hansen (1989) studied tolerance to clubroot, defined in this case as the yield on infested relative to uninfested test plots in the field. This measure of tolerance has a practical foundation, but it is seriously confounded with resistance.

Most of these studies pointed in the same general direction: the various types of resistance and tolerance inherit as partly or completely recessive traits and are

Table 1. Original sources of resistance to clubroot and derived lines and cultivars in *Brassica oleracea*

Original source	Lines and cultivars	References
Böhmerwaldkohl and Bindsachsener	Respla, Resista	Gante, 1951; Weisaeth, 1977
Irish fodder cabbage		Crisp et al., 1989; Crute & Pink, 1989
Shetland fodder cabbage		Dennis & Gray, 1954
Russian cabbage	cabbage cultivars	Giessmann & Bauch, 1974
unknown cabbage[a]	cabbage lines Oregon-100, -123, -140, -142	Baggett, 1983
unknown kale[b]	cvs Badger Shipper, Richelain, cabbage line 8–41[e]	Walker & Larson, 1960; Chiang & Crête, 1989
curly kale	curly kale cultivars	Nieuwhof & Wiering, 1962; Voorrips & Visser, 1993
B. napus cv Wilhelmsburger	cv Richelain, cabbage line 8–41[e]	Karling, 1968; Chiang & Crête, 1989
cauliflower[c]		Catovic-Catani & Rich, 1964
unknown	MSU 134 broccoli	Vriesenga & Honma, 1971
unknown[d]	broccoli lines OSU CR-1 to -8	Baggett, 1976; Baggett & Kean, 1985

[a] Baggett (1983) used an accession obtained from the Institute for Horticultural Plant Breeding, the Netherlands (now CPRO-DLO) as source of resistance. This accession was most likely derived from Bindsachsener, Böhmerwaldkohl or from the breeding programme leading to cv Badger Shipper, since those were the only clubroot-resistant cabbage accessions available at the institute at that time.
[b] Cabbage cv Badger Shipper was derived from a chance cabbage × kale hybrid.
[c] The results of Catovic-Catani & Rich (1964) were based on only five plants per accession and were therefore questionable.
[d] The clubroot resistant parent used by Baggett (1976) was a leafy annual with small, loose flower heads of unknown origin.
[e] Cabbage line 8–41 and the derived cv Richelain were descended from both *B. napus* cv Wilhelmsburger and, via cv Badger Shipper, from an unknown kale accession.

Table 2. Summary of classical genetic studies of clubroot resistance and tolerance in *Brassica oleracea*

Resistant parents	Populations	Test	Analysis	Conclusions	Reference
cabbage line 8–41	F_2, BC	field	qualitative	2 recessive genes	Chiang & Crête, 1970
cabbage line 8–41, cv Badger Shipper	diallel	glasshouse	quantitative	mainly additive, 1 recessive gene group	Chiang & Crête, 1976
broccoli MSU 134	F_2	glasshouse	qualitative	1[a] or 2 recessive genes	Vriesenga & Honma, 1971
Böhmerwaldkohl	F_2, BC	glasshouse	quantitative	4 genes[b]	Yoshikawa, 1983
Böhmerwaldkohl	F_2, BC	glasshouse	quantitative	Additive and recessive	Crute & Pink, 1989
kale K269	F_2, BC	glasshouse	qualitative	1 recessive gene	Yoshikawa, 1983
several cabbage accessions	diallel	field	quantitative	additive and recessive	Hansen, 1989
several kale accessions	diallel	glasshouse field	quantitative	many genes, mostly dominant	Laurens & Thomas, 1993
several kale and cabbage accessions	F_1	glasshouse	qualitative	all recessive	Voorrips & Visser, 1993

[a] Crute et al. (1980) re-interpreted the data of Vriesenga & Honma (1971) and concluded to 1 rather than 2 genes.
[b] Yoshikawa (1983) apparently neglected the fact that his accession of Böhmerwaldkohl was not a pure line, which invalidates his analysis.

under control of a limited number of major genes. Laurens & Thomas (1993) obtained different results: they found evidence for many dominant alleles for resistance in kale accessions. These kales were selected for resistance to field isolates from Brittany, which are rather different from most European and American isolates (Voorrips & Thomas, unpublished results).

Quantitative genetic theory as applied in the studies mentioned in Table 2 is only applicable to continuous, normally distributed data. With the exception of Hansen (1989) however, all the quantitative genetic studies were based on ordinal data, the symptom grades. It would be interesting to verify the conclusions by analyzing the original data using more appropriate statistical methods (e.g. Jansen, 1991).

Molecular markers for resistance genes

Landry et al. (1990, 1992) were the first to report linkage studies of clubroot resistance genes with genetic markers. They studied an F_2-population from a cross between a cabbage breeding line resistant to race 2 (Williams, 1966) and a susceptible rapid-cycling *B. oleracea* line. The parentage of the resistant line included *B. napus* cv Wilhelmsburger, resistant to races 2, 3, 6 and 7 as well as cv Badger Shipper, resistant to races 1, 3 and 6. The resistance tests were performed with a field isolate designated as race 2, using the test series of Williams (1966). For the QTL analysis, the symptom ratings were transformed to obtain normally distributed data. Two QTL's for clubroot resistance were mapped, located in two separate linkage groups. Together they explained 61% of the variance in the F_2. Presumably these QTL's originated from *B. napus*, but this was not verified.

Figdore et al. (1993) studied the segregation of RFLP markers and clubroot resistance to race 7 (Williams, 1966) in an F_2 from a cross between a susceptible cauliflower cv and broccoli line OSU CR-7 (Baggett & Kean, 1985), which was resistant to race 7. Symptoms were rated in three grades. Linkage of resistance with RFLP markers was determined using contingency Chi-square tests, appropriate for ordinal data. They found strong evidence for one resistance gene on chromosome 1C, and possibly spurious indications for two other resistance genes. The resistance gene on chromosome 1C was expressed in a semi-dominant way. Treating the symptom ratings as quantitative data, they estimated that this gene accounted for 12.6% of the variance in the F_2.

Breeding perspectives

Selection for clubroot resistance might in some cases be expected to be reasonably efficient in view of the relatively large heritabilities and the small number of major genes involved (Chiang & Crête, 1976; Table 2). However, for practical breeding the partially recessive nature of resistance implies that both parental lines of a resistant F_1-hybrid cultivar need to carry the same resistance genes. Backcrossing recessive alleles into lines involves alternating generations of selfing or of doubled haploids in order to reselect for resistance. The need for those generations could be eliminated by the use of molecular markers for resistance genes. Introgression could be accelerated even further by selecting for several markers from the recurrent parent.

The main problems in breeding resistant cultivars are the large variation for pathogenicity in *P. brassicae*, which may require the incorporation of several resistances in cultivars intended for use in a large area, and the lack of information on the interaction of resistance genes with pathogen populations. These problems may in the near future be addressed more effectively, using techniques such as production of single-spore isolates, microspore culture and molecular mapping of resistance genes. Pathogen isolates can be more precisely characterized by their interaction with plants carrying known resistance genes. For genetic studies, populations of DH-lines derived from resistant × resistant or resistant × susceptible crosses will be more useful than F_2 or backcross populations, as such lines can be tested repeatedly to reduce environmental variation. In addition, resistance to several pathogen isolates, preferably of monospore origin, can be mapped in the same population of DH-lines. In this way the level and specificity of resistance conferred by any resistance gene can be determined. In the future, it will be worthwhile to combine efforts to characterize genes for clubroot resistance obtained from various sources and to find molecular markers to trace those genes in successive generations, in order that they can be rationally employed in breeding for resistance.

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References

Aist, J.R. & P.H. Williams, 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. 49: 2023–2034.

Baggett, J.R., 1976. 'Oregon CR-1' broccoli. HortScience 11: 622–623.

Baggett, J.R., 1983. Clubroot-resistant cabbage breeding lines Oregon 100, 123, 140, and 142. HortScience 18: 112–114.

Baggett, J.R. & D. Kean, 1985. Clubroot-resistant broccoli breeding lines OSU CR-2 to OSU CR-8. HortScience 20: 784–785.

Buczacki, S.T., H. Toxopeus, P. Mattusch, T.D. Johnston, G.R. Dixon & L.A. Hobolth, 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. Trans. Br. mycol. Soc. 65: 295–303.

Buczacki, S.T., 1977. Root infections from single resting spores of *Plasmodiophora brassicae*. Trans. Br. mycol. Soc. 69: 328–329.

Butcher, D.N., S. El-Tigani & D.S. Ingram, 1974. The rôle of indole glucosinolates in the club root disease of the Cruciferae. Physiol. Plant Pathol. 4: 127–140.

Butcher, D.N., L.M. Searle & D.M.A. Mousdale, 1976. The role of glucosinolates in the club root disease of the Cruciferae. Med. Fac. Landbouww. Rijksuniv. Gent 41: 525–532.

Catovic-Catani, S. & A.E. Rich, 1964. Testing crucifers for resistance to clubroot in New Hampshire. Plant Dis. Rep. 48: 47–50.

Chiang, M.S. & R. Crête, 1970. Inheritance of clubroot resistance in cabbage (*Brassica oleracea* L. var. *capitata* L.). Can. J. Genet. Cytol. 12: 253–256.

Chiang, M.S. & R. Crête, 1976. Diallel analysis of the inheritance of resistance to race 6 of *Plasmodiophora brassicae* in cabbage. Can. J. Plant Sci. 56: 865–868.

Chiang, M.S. & R. Crête, 1989. Richelain: a clubroot-resistant cabbage cultivar. Can. J. Plant Sci. 69: 337–340.

Chong, C., M.S. Chiang & R. Crête, 1981. Thiocyanate ion content in relation to clubroot disease severity in cabbages. HortScience 16: 663–664.

Chong, C., M.S. Chiang & R. Crête, 1984. Studies on glucosinolates in clubroot resistant selections and susceptible commercial cultivars of cabbages. Euphytica 34: 65–73.

Colhoun, J., 1958. Clubroot disease of crucifers caused by *Plasmodiophora brassicae* Woron. A monograph. Phytopathological Paper No. 3, Commonwealth Mycological Institute, Kew, Surrey. 108 pp.

Crête, R., 1981. Worldwide importance of clubroot. Clubroot Newsl. 11: 6–7.

Crisp, P., I.R. Crute, R.A. Sutherland, S.M. Angell, K. Bloor, H. Burgess & P.L. Gordon, 1989. The exploitation of genetic resources of *Brassica oleracea* in breeding for resistance to clubroot (*Plasmodiophora brassicae*). Euphytica 42: 215–226.

Crute, I.R., 1986. The relationship between *Plasmodiophora brassicae* and its hosts: the application of concepts relating to variation in inter-organismal associations. Adv. Plant Pathol. 5: 1–52.

Crute, I.R., A.R. Gray, P. Crisp & S.T. Buczacki, 1980. Variation in *Plasmodiophora brassicae* and resistance to clubroot in Brassicas and allied crops – a critical review. Pl. Breed. Abstr. 50: 91–104.

Crute, I.R., K. Phelps, A. Barnes, S.T. Buczacki & P. Crisp, 1983. The relationship between genotypes of three *Brassica* species and collections of *Plasmodiophora brassicae*. Plant Pathol. 32: 405–420.

Crute, I.R. & D.A.C. Pink, 1989. The characteristics and inheritance of resistance to clubroot in *Brassica oleracea*. Asp. applied Biol. 23: 57–60.

Dekhuijzen, H.M., 1975. The enzymatic isolation of secondary vegetative plasmodia of *Plasmodiophora brassicae* from callus tissue of *Brassica campestris*. Physiol. Plant Pathol. 6: 187–192.

Dekhuijzen, H.M., 1980. The occurrence of free and bound cytokinins in clubroots and *Plasmodiophora brassicae* infected turnip tissue cultures. Physiol. Plantarum 49: 169–176.

Dekhuijzen, H.M., 1981. The occurrence of free and bound cytokinins in plasmodia of *Plasmodiophora brassicae* isolated from tissue cultures of clubroots. Plant Cell Rep. 1: 18–20.

Dekhuijzen, H.M. & J.C. Overeem, 1971. The role of cytokinins in clubroot formation. Physiol. Plant Pathol. 1: 151–161.

Dennis, R.W.G. & E.G. Gray, 1954. A first list of the fungi of Zetland (Shetland). Trans. Bot. Soc. Edinburgh 36: 215–223.

Dixon, G.R. & D.L. Robinson, 1986. The susceptibility of *Brassica oleracea* cultivars to *Plasmodiophora brassicae* (clubroot). Plant Pathol. 35: 101–107.

Figdore, S.S., M.E. Ferreira, M.K. Slocum & P.H. Williams, 1993. Association of RFLP markers with trait loci affecting clubroot resistance and morphological characters in *Brassica oleracea* L. Euphytica 69: 33–44.

Gante, Th., 1951. Hernieresistenz bei Weißkohl. Z. Pflanzenzüchtung 30: 188–197.

Giessmann, H.-J. & W. Bauch, 1974. Stand and Probleme der Kohlhernieresistenzzüchtung bei Kopfkohl in der DDR. Deutsche Gartenbau 21: 194–195.

Haji Tinggal, S., 1980. Physiologic populations of *Plasmodiophora brassicae* Woron. in Devon and Cornwall. Ph.D. Thesis, Department of Biological Sciences, University of Exeter.

Haji Tinggal, S. & J. Webster, 1981. Technique for single spore infection by *Plasmodiophora brassicae*. Trans. Br. mycol. Soc. 76: 187–190.

Hansen, M., 1989. Genetic variation and inheritance of tolerance to clubroot (*Plasmodiophora brassicae* Wor.) and other quantitative characters in cabbage (*Brassica oleracea* L.). Hereditas 110: 13–22.

Ingram, D.S. & I.C. Tommerup, 1972. The life history of *Plasmodiophora brassicae* Woron. Proc. R. Soc. Lond. B. 180: 103–112.

Jansen, J., 1991. Fitting regression models to ordinal data. Biometr. J. 33: 807–815.

Jones, D.R., D.S. Ingram & G.R. Dixon, 1982a. Factors affecting tests for differential pathogenicity in populations of *Plasmodiophora brassicae*. Plant Pathol. 31: 229–238.

Jones, D.R., D.S. Ingram & G.R. Dixon, 1982b. Characterization of isolates derived from single resting spores of *Plasmodiophora brassicae* and studies of their interaction. Plant Pathol. 31: 239–246.

Karling, J.S., 1968. The Plasmodiophorales. Hafner Publishing Co., New York. 239 pp.

Kole, A.P. & P.J.J. Philipsen, 1956. On the susceptibility of non-crucifers to the zoosporangial stage of *Plasmodiophora brassicae*. Tijdschrift over Plantenziekten 62: 167–170.

Landry, B.S., N. Hubert, R. Crête, M.S. Chiang, S.E. Lincoln & T. Etoh, 1992. A genetic map for *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). Genome 35: 409–420.

Landry, B.S., N. Hubert, T. Etoh, R. Crête, M.S. Chiang, J. Harada & S. Lincoln, 1990. Construction of a detailed RFLP-based genetic map and tagging of resistance genes to race 2 of clubroot disease

map and tagging of resistance genes to race 2 of clubroot disease in *Brassica oleracea*. In: J.R. McFerson, S. Kresovich & S.G. Dwyer (Eds): Proc. 6th Crucifer Genetics Workshop, Cornell University, Ithaca, New York, p. 29.

Laurens, F. & G. Thomas, 1993. Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *acephala*). Hereditas 119: 253–262.

Ludwig-Müller, J., U. Bendel, P. Thermann, M. Ruppel, E. Epstein & W. Hilgenberg, 1993. Concentrations of indole-3-acetic acid in plants of tolerant and susceptible varieties of Chinese cabbage infected with *Plasmodiophora brassicae* Woron. New Phytol. 125: 763–769.

MacFarlane, I., 1952. Factors affecting the survival of *Plasmodiophora brassicae* Wor. in the soil and its assessment by a host test. Ann. appl. Biol. 39: 239–256.

Mattusch, P., 1977. Epidemiology of clubroot of crucifers caused by *Plasmodiophora brassicae*. In: Buczacki, S.T. & P.H. Williams (Eds): Woronin +100 International Conference on Clubroot, Madison, Wisconsin, p. 24–28a.

Mullin, W.J., K.G. Proudfoot & M.J. Collins, 1980. Glucosinolate content and clubroot of rutabaga and turnip. Can. J. Plant Sci. 60: 605–612.

Naiki, T., C. Kawaguchi & H. Ikegami, 1984. Root hair reinfection in Chinese cabbage seedlings by the secondary zoospores of *Plasmodiophora brassicae* Woronin. Ann. Phytopath. Soc. Japan 50: 216–220.

Nieuwhof, M. & D. Wiering, 1962. Clubroot resistance in *Brassica oleracea* L. Euphytica 11: 233–239.

Ockendon, J.G. & S.T. Buczacki, 1979. Indole glucosinolate incidence and clubroot susceptibility of three cruciferous weeds. Trans. Br. mycol. Soc. 72: 156–157.

Parlevliet, J.E., 1985. Race and pathotype concepts in parasitic fungi. OEPP Bulletin 15: 145–150.

Rausch, T., D.N. Butcher & W. Hilgenberg, 1983. Indole-3-methylglucosinolate biosynthesis and metabolism in clubroot diseased plants. Physiol. Plantarum 58: 93–100.

Schoeller, M. & J. Grunewaldt, 1986. Production and characterization of single spore derived lines of *P. brassicae* Wor. Cruciferae Newsl. 11: 110–111.

Scott, E.S., 1985. Production and characterization of single-spore isolates of *Plasmodiophora brassicae*. Plant Pathol. 34: 287–292.

Seaman, W.L., J.C. Walker & R.H. Larson, 1963. A new race of *Plasmodiophora brassicae* affecting Badger Shipper cabbage. Phytopathology 53: 1426–1429.

Tommerup, I.C. & D.S. Ingram, 1971. The life-cycle of *Plasmodiophora brassicae* Woron. in *Brassica* tissue cultures and in intact roots. New Phytol. 70: 327–332.

Toxopeus, H., G.R. Dixon & P. Mattusch, 1986. Physiological specialization in *Plasmodiophora brassicae*: an analysis by international experimentation. Trans. Br. mycol. Soc. 87: 279–287.

Voorrips, R.E., 1992. Root hair infection by *Plasmodiophora brassicae* in clubroot-resistant and susceptible genotypes of *Brassica oleracea*, *B. rapa* and *B. napus*. Neth. J. Pl. Pathol. 98: 361–368.

Voorrips, R.E. & D.L. Visser, 1990. Doubled haploid lines with clubroot resistance in *Brassica oleracea*. In: J.R. McFerson, S. Kresovich & S.G. Dwyer (Eds): Proc. 6th Crucifer Genetics Workshop, Cornell University, Ithaca, New York, p. 40.

Voorrips, R.E. & D.L. Visser, 1993. Examination of resistance to clubroot in accessions of *Brassica oleracea* using a glasshouse seedling test. Neth. J. Pl. Pathol. 99: 269–276.

Vriesenga, J.D. & S. Honma, 1971. Inheritance of seedling resistance to clubroot in *Brassica oleracea* L. HortScience 6: 395–396.

Walker, J.C. & R.H. Larson, 1960. Development of the first clubroot-resistant cabbage variety. Bull. Wisconsin Agr. Exp. Stn. 547: 12–16.

Ward, E.W.B., 1983. Effects of mixed or consecutive inoculations on the interaction of soybeans with races of *Phytophthora megasperma* f. sp. *glycinea*. Physiol. Plant Pathol. 23: 281–294.

Webb, P.C.R., 1949. Zoospores, believed to be those of *Plasmodiophora brassicae*, in the root hairs of non- cruciferous plants. Nature 163: 608.

Weisaeth, G., 1977. Hodekålsortene 'Respla' og 'Resista' foredlinger med klumprotresistens. Gartneryrket 67: 937–939.

Williams, P.H., 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56: 624–626.

Yoshikawa, H., 1983. Breeding for clubroot resistance of crucifer crops in Japan. Jpn. Agr. Res. Quart. 17: 6–11.