PROGRESS IN RESEARCH ON DISEASES OF OILSEED BRASSICAS

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Introduction

It is indeed an honour to be invited to summarize the conference reports in the area of crop protection. The organizing committee indicated that the task of the rapporteur should be to focus on directions and objectives for future research and to try to be provocative and stimulating in presenting these ideas. The task is rather difficult, not least in summarizing unseen research reports. Thus in many cases, since I have only a brief idea concerning work that is to be presented, I will discuss fairly new work in the same area that I am familiar with. My ideas of what is important in pest control problems and what should be priorities for future studies are coloured by my Canadian perspective and my own research interests but I will try to take a broad view.

It is my intention to limit my discussion here to research concerning the diseases of the oilseed brassicas. My rationale for this is based on two reasons. Firstly, my expertise, such as it is, resides solely in the area of plant disease and not with other pest control areas such as entomology, and, secondly, the prior information I received concerning the presentations to the congress was almost entirely devoted to disease problems in oilseed rape.

From the numbers of presentations, it seems that major concerns for disease control in oilseed rape are stem rot, caused by *Sclerotinia sclerotiorum*, and stem canker or blackleg, caused by *Leptosphaeria maculans*. I will devote a substantial part of my time and begin my discussion with these two disease problems. A number of other diseases, including *Verticillium dahliae* and *Alternaria* black spot are also of concern and these will also be discussed but in less detail.

Predicting the severity of Sclerotinia stem rot

It seems clear that this disease continues to threaten stable production in many areas of the world, e.g. France, Germany, China, Canada. Since unnecessary fungicide applications are environmentally and economically costly, many researchers have strived to develop methods to predict the severity of stem rot in rape in order to improve the capacity for farmers to make appropriate management decisions.

Considerable interest and significant progress has been made in the development of these systems in recent years and some of this work has been presented at the congress. As part of an overall management approach, more detailed information concerning the infection pathway of sclerotinia stem rot and the environmental requirements for infection and disease progress has been presented by Penaud et al. and Pierre and Regnault. Also, evaluation of new fungicides and studies into fungicide applications have been presented. The work of Morrall's group in Saskatoon, which has been summarized at the congress, is of particular note in its broad approach to management of stem rot under western Canadian environmental conditions. The development of petal-testing procedures to estimate inoculum loads and the consequential potential disease severity is perhaps applicable for sclerotinia prediction in other parts of the world.

Developing resistance to Sclerotinia stem rot

I would like to see more emphasis placed on resistance of brassicas to sclerotinia stem rot. This would be of considerable economic value in those areas where sclerotinia is a continuous threat, for example in some parts of China, where weather conditions almost always favour the disease. Of course, this is a difficult problem, in large part due to inconsistencies in obtaining reliable and repeatable interaction phenotypes on host materials.

The development of reliable greenhouse infection techniques and procedures as reported by Regnault's group is a step towards alleviating these problems. The ability to handle the large population sizes needed in plant breeding programs is still a limiting factor. The availability of sources of resistance in Brassica spp. is now well documented. Buzza has presented some of my data on the dramatic reduction in sclerotinia severity associated with an apetalous line of *B. napus*. This form of resistance, of course, should more properly be described as disease avoidance. However, its effectiveness is real and should be exploited. Resistance to stem rot in normal flowered cultivars of *B. napus* has been reported from China (Liu 1990) and examination of some of this material in my laboratory confirms their results. Wu and Liu report on the use of oxalic acid, a toxic metabolite produced by pathogenic strains of *S. sclerotiorum*, for screening and selection of germplasm and breeding materials and the relationship between susceptibility to the pathogen and sensitivity to oxalic acid.

Because of the unreliability of field assessment and other difficulties associated with reliable selection within large populations of breeding materials for stem rot resistance, it seems to me that stem rot resistance is an ideal trait for selection using RFLP markers tightly linked to the resistant phenotype. In the near future I expect there will be considerable development in this area.

Is *Leptosphaeria maculans* two species?

A number of important agronomic and scientific questions concerning the genetics of the Brassica-*Leptosphaeria maculans* interaction are currently being addressed. These questions include the variation for pathogenicity among isolates of *L. maculans*, the genetic basis for this variation and whether the race concept can be applied to this pathogen. The question whether or not aggressive and non-aggressive isolates of *L. maculans* even belong to the same species can now be raised. Evidence for this comes from the molecular genetic studies of Taylor et al. Large genetic differences based on restriction fragment hybridisations were found between the two groups of isolates but little variation within aggressive isolates occurred. This is consistent with the findings of Koch et al. 1991. Taylor et al. have also shown for the first time large differences between isolates from the two groups in the numbers of 'chromosomes' separated by pulse field electrophoresis in agarose gels.

Results which bear directly on the question concerning the genetic variation among isolates of *L. maculans* is presented by Gall et al. They considered sirodesmin production, and pathogenicity on cotyledons of *B. napus* cv. Tapidor and *B. juncea* cv. Aurea, along with cultural characteristics, as a basis for grouping. They also report on genetic studies of crosses among isolates for pathogenicity, toxin production and disease reaction. Variation for many characteristics is now well established. Koch et al. (1989) showed that sirodesmin production was confined to aggressive isolates of the pathogen and recently Koch et al. (1991) and Mengistu et al. (1989) have demonstrated that four pathogenicity groups can be differentiated in *L. maculans* using *B. napus*

cv. Westar, Quinta and Glacier or Bienvenu. Almost continuous variation was reported by Ballinger et al. who could differentiate 60 races among Australian isolates of *L. maculans* using 12 Brassica cultivars. Genetic studies involving crosses between two pathogenicity groups indicated that a two gene model for avirulence explains well the segregation for pathogenicity in aggressive isolates on the three differentials *B. napus* cv. Westar, Quinta and Glacier (Rimmer at al. unpublished).

Genetics of resistance to *L. maculans*

Considering the importance of this disease in many areas of the world, and the reliance on resistance for disease control, it is very surprising that so little information has been presented in this area. So it is good to see more work presented on the inheritance of resistance of *B. napus* to *L. maculans*. Work by Sippell et al. and Hill are useful contributions to our understanding of the genetics of resistance to this disease. It is important that we know what resistance genes are available in the *B. napus*, also in *B. rapa* and other oilseed Brassica spp. and how they interact with one another. In my laboratory, McNabb showed that resistance to western Canadian isolates of *L. maculans* was conditioned by two dominant unlinked genes with additive effects in spring rape germplasm from France, while cotyledon resistance was conditioned by a recessive, single locus (Rimmer et al. in press).

There is significant interest in many breeding programs in transferring resistance from *B. nigra* or *B. juncea* into *B. napus* and *B. rapa*. The rationale for this is that species with the B genome are reportedly immune even at the seedling stages to this disease (Roy 1984), thus yield loss due to seedling infections may be reduced in *B. napus* if it contained this resistance. Some plant breeders have even suggested to me that they expect this type of resistance will be very durable because blackleg is not an important disease on *B. juncea*.

In light of this we have studied the inheritance of resistance in *B. juncea*. Resistance in this species appears to be conditioned by two unlinked epistatic genes with dominant recessive interaction (Keri et al. 1990). This is a very unusual genetic system for inheritance of resistance in any plant-pathogen interaction. How this will influence the transfer and expression of resistance in different Brassicas is unclear.

Observations by Gugel et al. 1990 that rapid asymptomless infection occurs commonly in *B. juncea* by both aggressive and non-aggressive isolates is also of considerably importance. In the course of our studies with *B. juncea*, we have also observed that asymptomless infections occur on cotyledons and young seedlings in many accessions of *B. juncea*. Subsequently, considerable root-infection occurred. The implications of these observations for the epidemiology of stem canker need to be explored if 'resistance' from the B genome species is to be successfully employed in other Brassicas. Another problem in conventional breeding programs is that napus resistance would be masked in populations containing juncea resistance and probably lost, the vertifolia effect as described by van der Plank. Perhaps, the only way to combine both resistances is with F_1 hybrids where juncea resistance is incorporated into the 'A' line and napus resistance is in the 'R' line. However, in *B. rapa* where almost no resistance to *L. maculans* seems to occur, then the transfer of resistance from *B. nigra* or *B. juncea* may be the only hope to develop resistant cultivars.

Phytotoxins and *in vitro* selection.

A topic which has received considerable attention in recent years is *in vitro* selection for resistance in Brassicas to *L. maculans* and

Alternaria brassicicola. Often, crude culture filtrates have been employed for this purpose and I would like to emphasize that, in my view, the efficacy for *in vitro* selection work of these undefined concoctions is dubious. The chemistry, physiology and molecular biology of the *Alternaria* host specific toxins (AM-toxin, AK-toxin) is a model which should be very useful to us. These toxins have been extensively investigated, primarily by Japanese workers (see Kohmoto and Durbin, 1989). Of considerable interest here is that the toxins are produced at active concentrations in spore germination fluids. If cell biologists do not have a pure toxin to work with, spore germination fluids are probably a much better source of phytotoxins than culture filtrates or other sources.

It now seems probable that there are no true host-specific toxins produced by any rapeseed fungal pathogen. The toxins, destruxin B and other destruxins, have been purified from *Alternaria brassicae* (Bains and Tewari, 1987; Ayer and Pena-Rodrigues, 1987; Buchwaldt and Jensen, 1991). Although, Bains and Tewari reported that the toxin destruxin B was host-specific, this seemed very unlikely from a theoretical view as the pathogen itself shows no host-genotype specificity as occurs with all other host-specific toxin producing pathogens. Buchwaldt and Green (pers. comm.) report that the toxin is probably best described as a host-selective toxin, a term used to describe toxins involved in determining pathogenesis on species or genera but not on individual cultivars (Daly and Knoche 1982). It is probably a virulence factor (sensu Yoder 1980) in that it is a quantitative factor important for the aggressiveness of the isolate. The report by Kolte on distinct serological races of *A. brassicae* which possess pathogenic differences might be of crucial importance. It will be very interesting to see, if this work can be confirmed, whether or not the races vary in toxin production.

With *L. maculans*, only aggressive isolates produce sirodesmins (see Koch et al. 1989 for refs.). These toxins are non-host specific (Boudart 1978) and perhaps they should be considered to be virulence factors (Koch et al. 1989).

The question remains as to whether or not any useful resistance can be obtained by exposing microspores, protoplasts, secondary embryoids or other plant materials, directly or after mutagenesis treatments, to phytotoxins which are not host-specific. McDonald and Ingram (1986) and Ingram and McDonald (1988) have investigated this with semi-purified toxins from *A. brassicicola* and with crude culture filtrates from *L. maculans*. With both systems they had little problem in obtaining selections of secondary embryoids with a high tolerance to toxic solutions. However, regenerated plant materials when increased and tested against the pathogen in the field were no different in resistance or susceptibility than the parental material from which they were derived or, in the case of selection for tolerance to toxins from *A. brassicicola*, were actually more susceptible than the parental material.

Verticillium dahliae, a potential threat?

Reports concerning attacks of rapeseed in Europe by *Verticillium dahliae* are becoming more frequent. A few years ago it seemed that this disease was largely confined to northern areas, Sweden and Germany, but now it appears more widespread. The work presented by Paul and Gunzelmann suggests that this pathogen can be very damaging on rapeseed. It seems unlikely that the isolates of *V. dahliae* which attack rapeseed are host-specific as very severe attacks occurred in a field which had not been cropped to rapeseed in the past 9 years. This is of interest to some of us in Canada who had been concerned that the introduction of rapeseed specific isolates of *V. dahliae* might occur as microsclerotial

contaminants with seed. I am very interested in obtaining any information you may have concerning the vegetative compatibility grouping, host range and specificity of rapeseed isolates of *V. dahliae*.

Are glucosinolates involved in pest control?

A few papers report on the role of glucosinolates in disease and insect resistance of rapeseed. Rawlinson et al. report that though double low cultivars were not noticeably more susceptible to diseases or infested by pests than single low cultivars, they were apparently less tolerant to damage. Consequently, insecticides and fungicides increased yield more on double low than on single low cultivars. Doughty et al. report on the use of isothiocyanates as natural pesticides for the control of pest problems in rapeseed.

Van den Berg and Rimmer investigated the phenology of glucosinolates throughout the growing season in spring rape which varied in susceptibility to *L. maculans*. These included single and double low cultivars. Indole glucosinolates were present in leaves of all cultivars at higher concentrations than those reported to be fungitoxic to *L. maculans* (Mithen et al. 1986). This supports the suggestion that glucosinolates may be involved in disease resistance. Towards this end, Mithen and Herron discuss the cosegregation of glucosinolates and disease resistance in allotetraploids derived from wild *B. oleracea* types crossed with *B. rapa*. It is interesting that the indolyl glucosinolates are structurally very similar to the phytoalexins which are elicited when exposed to pathogens (Dahiya and Rimmer 1989). The relationship between the biosynthesis of the constitutive glucosinolates in rapeseed tissues and the *de novo* biosynthesis of phytoalexins needs to be more fully investigated.

Conclusion

In summary, it is very clear that exciting information for our understanding of host-pathogen interactions between rapeseed and its pathogens has been presented. Some of the research on these pathogens is at the forefront of plant pathological research. Indeed, I think that *B. napus/L. maculans* and also Brassica spp./*Albugo candida* have considerable potential to become model systems for studies in host-pathogen interactions. I would like to compliment all the participants for the high quality of work they presented. I thank the organizing committee for the opportunity to present this summary paper today.

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