What a geneticist/breeder can do when faced with a new disease or disease race.

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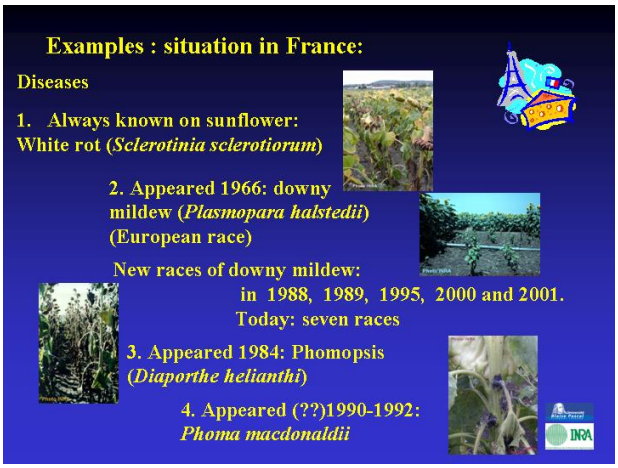
Summary: When confronted with a new disease, there are five main steps in developing resistant varieties : 1. Identify the disease; 2: Identify first sources of resistance; 3: Develop resistance observations or tests; 4: Determine variations in pathogenicity and the genetics of resistance; 5: define the most efficient breeding programmes. At all stages, correct observations of disease are of utmost importance.

Introduction

When a crop such as sunflowers is being developed in a country where it was not grown over large areas, it is very likely that disease problems will appear. There are a number of basic steps in the work of a breeder to overcome these problems.

In France, sunflowers have been grown on a large scale since the 1960, so we have quite wide experience of new disease problems. For example, white rot and wilt, caused by *Sclerotinia sclerotiorum* has always been known, whereas downy mildew (*Plasmopara halstedii*) was first observed in 1966 and new races appear quite regularly (there are at present 7). Phomopsis stem canker (*Diaporthe helianthi)* was first observed in France in 1984 whilst the most recent disease of importance Phoma black stem (*Phoma macdonaldii*) was first observed in 1990-92. These diseases will serve as examples in the work necessary to obtain resistant varieties.

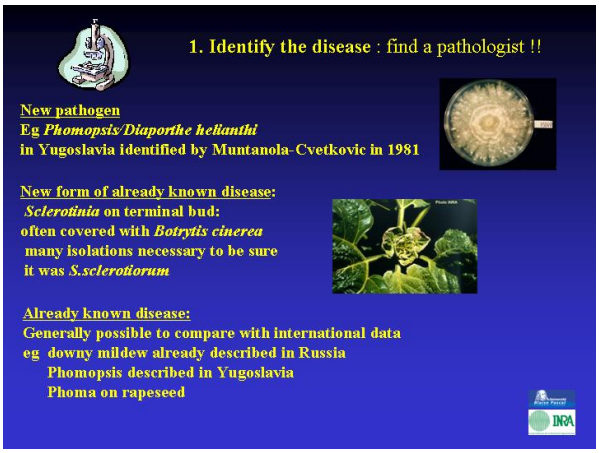


1. Identify the disease

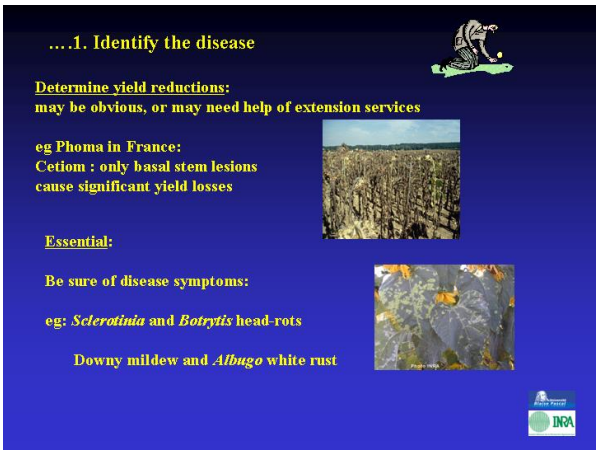
It is of great help if collaboration with a pathologist is possible: they are used to making fungal isolations and to microscopic examination of spores which help identification. However, it is very rare that a quite new pathogen is identified. The only recent example is Phomopsis in Yugoslavia in 1981 (Munanola-Cvetkovic et al, 1981). In France, we had the case of a new form of an already known pathogen, when there were attacks of white rot on terminal buds in the 1980. In the field, the problem looked very like the *Sclerotinia* symptoms known on capitula, but when isolations were first made, it was always *Botrytis cinerea* spores that were observed. It was necessary to make isolations from the very first symptoms, and

then to grow the mycelium on agar to obtain sclerotia to be sure that the disease was caused by *S. sclerotiorum* and that *B. cinerea* was only there as a saprophyte.

Most often, it is possible to compare with published data. For example, when downy mildew was first observed in France, there were already publications that described the disease (Leppik, 1962); when Phomopsis first appeared, comparisons were made with the reports from Yugoslavia (Regnault, 1984). When black stem was observed, comparisons were made with phoma on rapeseed (although this is not the same species).



Once the disease has been identified, it is necessary to decide whether it causes yield reductions sufficient to warrant a resistance breeding programme. This may be evident, as when downy mildew attacks appear, causing dwarfing, sterility and almost no yield. In contrast, leaf lesions of several diseases do not appear to cause yield loss. In the case of Phoma black stem, the CETIOM in France showed that the only significant yield losses are caused by basal stem attacks (Peres et al, 2000).

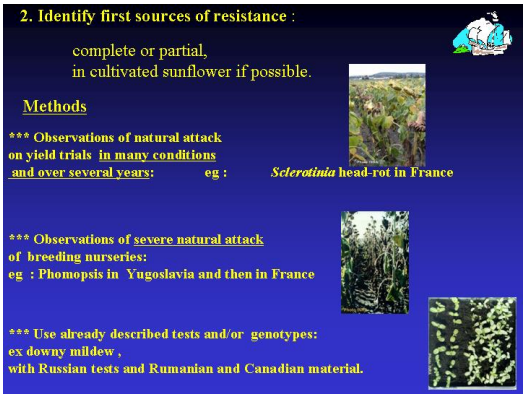


In conclusion on identification, this is the most important part of any disease work. It is necessary to be absolutely sure of disease symptoms. In France, it is necessary to be quite sure whether rotted spots on capitula are due to *Sclerotinia* or to *Botrytis*. If you do not know downy mildew very well, it may be confused with *Albugo* white rust.

2. Identification of first sources of resistance

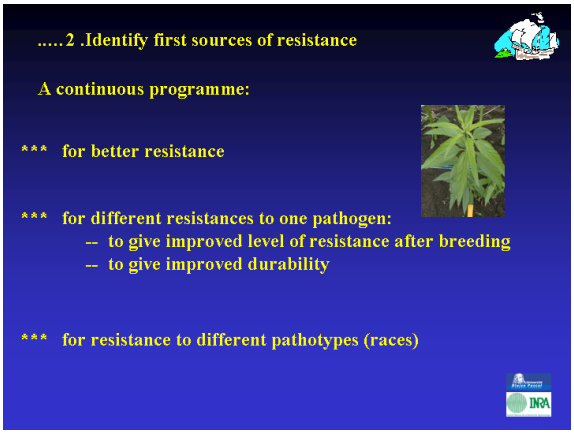
To develop resistance tests, and to have some differences in level of resistance or susceptibility useable in breeding, the first requisite is to find genotypes that react differently to the disease. In the first case, it is best to have cultivated sunflower genotypes, something that is comparable with the varieties grown in the region. There are three possible methods to find such different reactions:

a) Observation of natural attack in yield trials over several years. This was used in France for *S. sclerotiorum* head rot, for which we made counts of percentage attack in yield trials each time that there were sufficient symptoms (at least 10%). Although the results varied between locations and years, after several years, some genotypes appeared much more susceptible than others.



b) If attacks are sufficient, the same observations may be possible directly on breeding nurseries. This was the case for Phomopsis in Yugoslavia, where the first sources of resistance were observed in the breeding nursery at the IFVC, Novi-Sad (Skoric, 1985).

c) When tests and examples of resistant and susceptible genotypes have already been published, these can be used as controls in search for new forms of resistance. This was the case for downy mildew in France, using Russian tests and Rumanian and Canadian resistant genotypes (Vranceanu and Stoenescu, 1970).



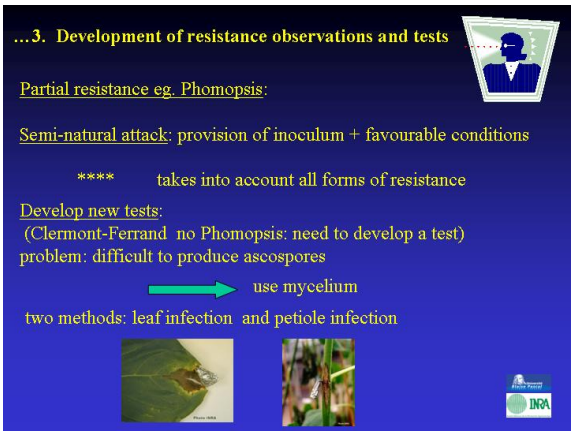
First sources of resistance will make it possible to develop tests and to start breeding satisfactory genotypes, but, of course, research for resistance sources is a continual necessity, to get better levels of resistance either directly or after crossing between different sources, in the case of partial resistance, and to get improved durability in the case of total, race-specific resistance.

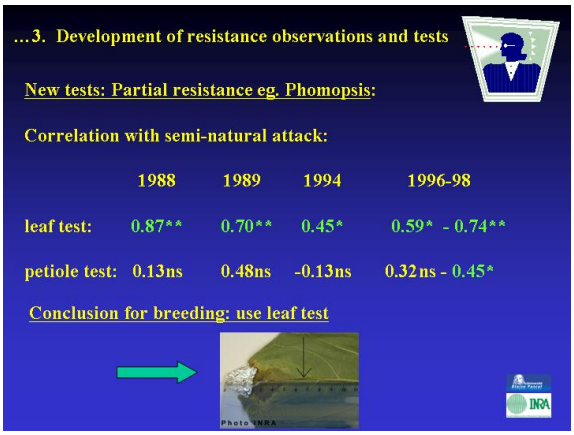
3. Development of resistance observations and tests

It may be possible to copy or adapt what is already known. For example, in France, we used the seedling resistance test for downy mildew described by Pantchenko (1965).
However, improvements are still necessary : several of the resistance genes now used show cotyledon limited infection, with sporulation on the cotyledons but not on the true leaves (Vear, 1978), and we are still trying to find the best conditions to make it easy to distinguish resistant and susceptible plants. (In the field, the resistant plants show no sporulation at all).

Another example of using what is already known is the observation of semi-natural Phomopsis attacks, where infected stems are placed through trials and then favourable conditions are provided by irrigation (Tourvieille, 1994).

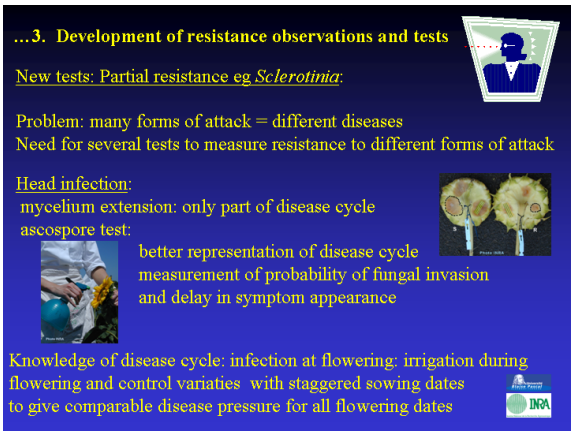
It may, however, be necessary to develop new tests. For example, at Clermont-Ferrand, Phomopsis is quite rare, so we do not want to introduce it, by using natural-type infections. In addition, it is very difficult to produce ascopores, the usual infecting agent of the pathogen. Thus, we tried two methods infecting leaves and petioles with mycelium explants. Since, in both cases, we were able to infect sunflower plants with Phomopsis, to determine which test to use on a large scale, we compared with natural attack (Viguié et al, 1999). The results were very clear; the leaf test was much more closely correlated with natural attack, so it is now used in breeding.





For resistance to *Sclerotinia*, we were obliged to develop several tests, since each plant part attacked may show a different level of resistance. The first tests, started when ascospores were not available, measured used mycelium growth on the back of the head (Vear and Guillaumin, 1977), but this is only a limited part of the disease cycle. When we found how to produce ascospores easily, these were used to spray the head during flowering, which is a much better reproduction of the normal disease cycle. This test also has the advantage that both the probability of successful attack (% attack) and the delay in symptom appearance can be measured (Vear and Tourvieille, 1988).

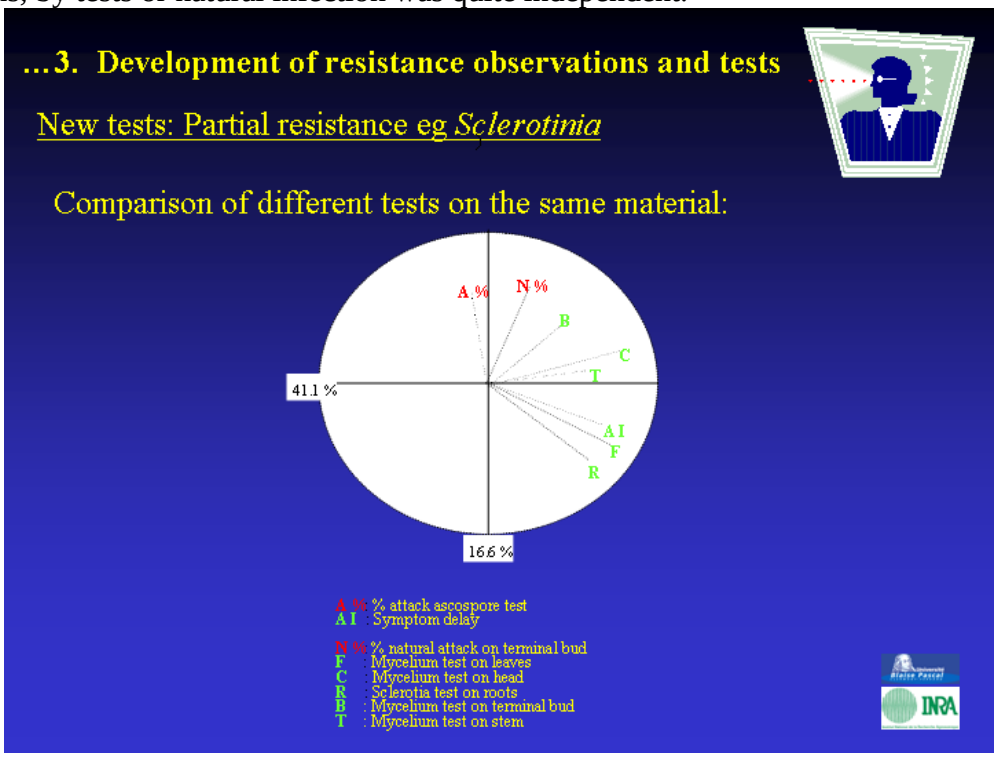
The work done to develop the ascopore test gave us improved knowledge of the *Sclerotinia* disease cycle, which made it possible to improve semi-natural attack observations (Vear and Tourvieille, 1987). We now know that the florets must be maintained with 100% RH when they produce pollen to have good conditions for *Sclerotinia* infection and that controls with staggered sowing dates are necessary to take into account variations in other factors of the environment, such as temperature.



Other forms of *Sclerotinia* attack have required other tests. We developed a test for root and basal stem resistance, by placing sclerotia in contact with roots (Tourvieille and Vear, 1990), but other methods have also been developed (Grezes-Besset et al, 1994). For leaf

infections, we use exactly the same test as for Phomopsis (Castaño et al, 1992.). For terminal bud infections, although some techniques have given symptoms (Peres, 2000), there is still no test that is satisfactory for breeding, probably because the plants are small when infected and, although high humidity is necessary for infection, too much irrigation washes the ascospores off the young leaves. We still depend on observations of natural infection to eliminate susceptible plants from breeding programmes (Achbani et al, 1994).

Having developed all these tests to cover all the different types of *Sclerotinia* attack, we made a study to determine whether there was any relation between them (Castaño et al, 1993). By using all the tests on a series of inbred lines, we were able to show that, although there were some similarities between mycelium-based tests, the reaction to ascospore infections, by tests or natural infection was quite independent.

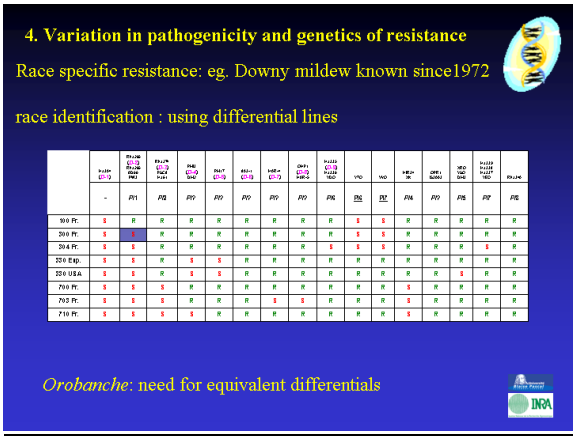


4. Variation in pathogenicity and genetics of resistance

To be able to breed for resistance efficiently, it is essential to know something of the genetics both of fungal virulence patterns and the genetics of resistance in sunflowers.

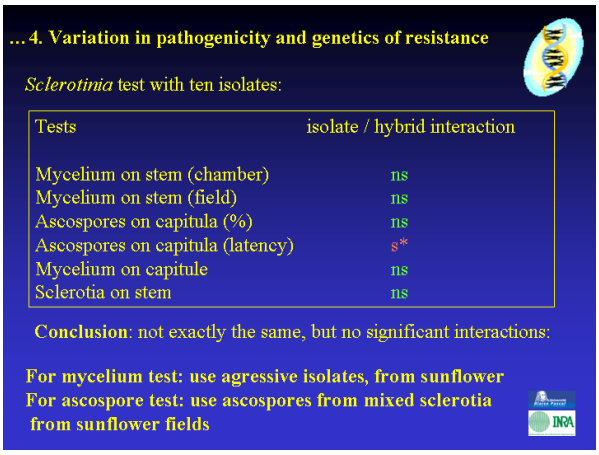
4.1 Variations in pathogenicity

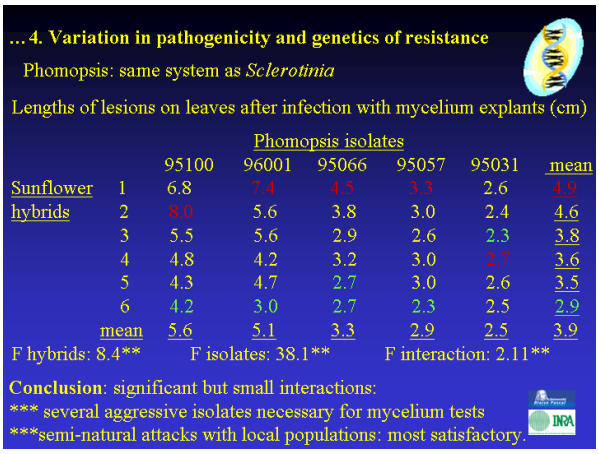
a) Race specific resistance: This has been known since 1972 (Zimmer and Kinman, 1972) when it was found that genotypes resistant in Europe were not resistant in the USA. Since then many differences in reaction have been observed and new races appear quite regularly. Gulya et al (1998) proposed a race nomenclature that has been adopted internationally, with a series of 9 differential inbreds which make it possible to define races. However, some sunflower genotypes show differences within what were considered as the same *P. halstedii* race, so the differentials will have to be updated regularly (at each International Sunflower Conference, for example).



Race specific resistance also occurs for rust (*Puccinia helianthi*) and for broomrape (*Orobanche cumana*), which is very important in the Mediterranean region. Vranceanu et al (1986) published a list of differentials concerning races of this parasitic plant, but these are not all available, and several new races have appeared since, so an update of this list is also extremely necessary, for breeders to know what race they find in the field or what race they are using in resistance breeding tests (Gagne et al, 1998)

b) Quantitative resistance: We have worked on *Sclerotinia* and Phomopsis isolates to determine whether there is any interaction between these and sunflower genotypes. For *Sclerotinia*, infections of 10 sunflower genotypes with 8 isolates showed no significant interaction, except in the case of ascospore tests (Thuault and Tourvieille 1988). It was concluded that mycelium tests could be made with any aggressive isolate and ascospore tests were best with mixtures of ascospores obtained from sclerotia collected in infected sunflower fields.

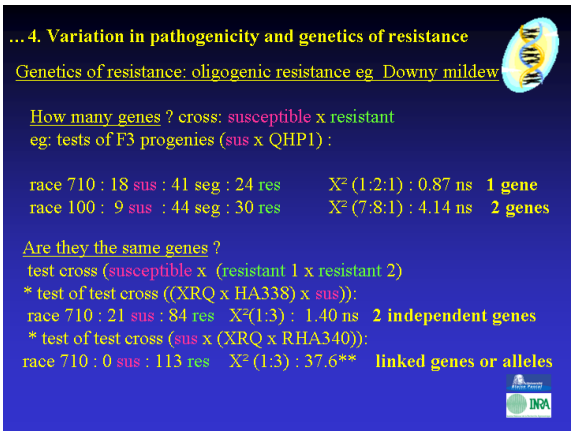


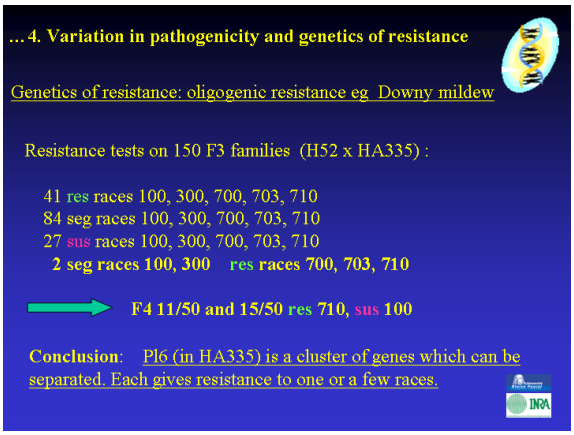


For Phomopsis, Viguié et al (1999) made mycelium infection on leaves with isolates (5 are presented in the slide) and showed that there are small but significant interactions between isolates and sunflower genotypes. Extremes of resistance and susceptibility do not change, but intermediate reaction may differ. It was concluded that several aggressive isolates are necessary to make mycelium infections and that observations of semi-natural attack in several locations, from spores of mixed and variable populations, are the most satisfactory.

4.2 Genetics of resistance in sunflowers

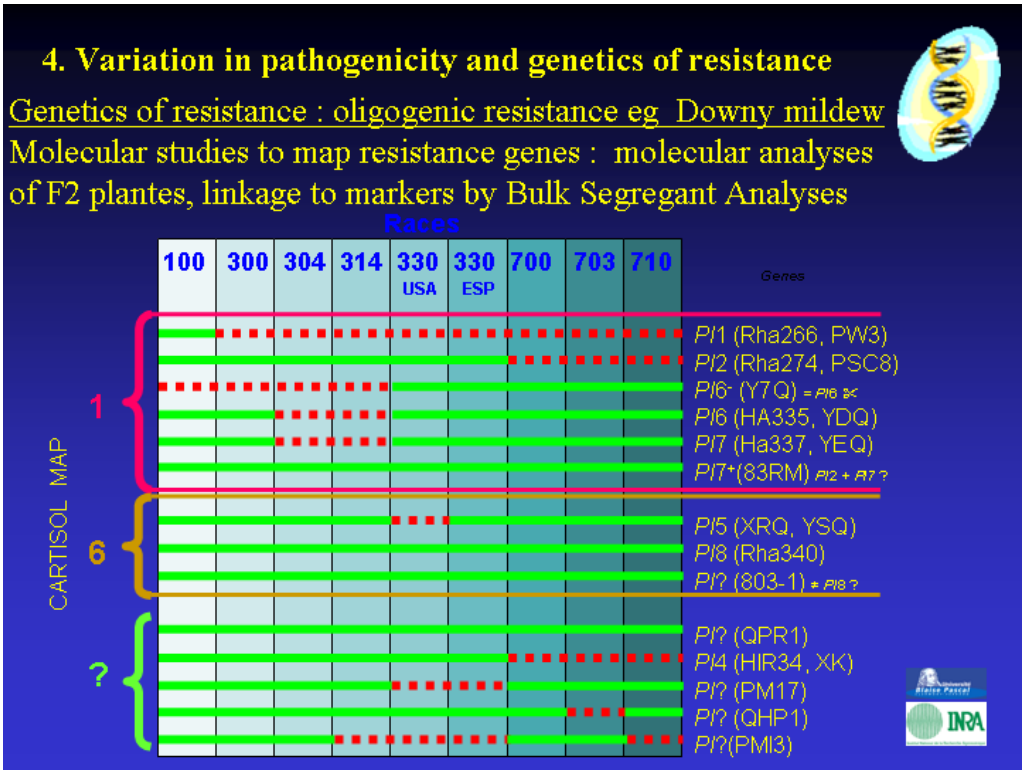
a) Oligogenic resistance: example of downy mildew. For this studies are of basic Mendelian genetic ratios. For each new source of resistance, you need to know how many genes control resistance and whether they are the same as those in already known lines. The slide presents an example of a cross to determine the number of genes in an inbred line QHP1. It should be noted that one genotype may have different numbers of genes giving resistance to different races.



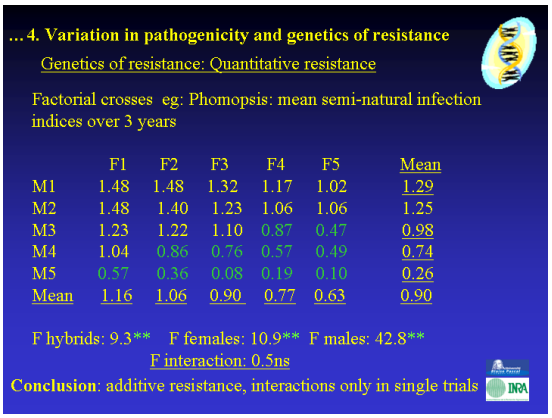


Test crosses between resistance lines are also presented in this slide to show the sorts of segregation that may occur (Vear et al, 2000a; Bert et al, 2001). Tests on large numbers of F3 progenies with different races have shown that some resistance genes are, in fact, clusters of several genes, each giving resistance to one or a few races (slide, Vear et al, 1997).

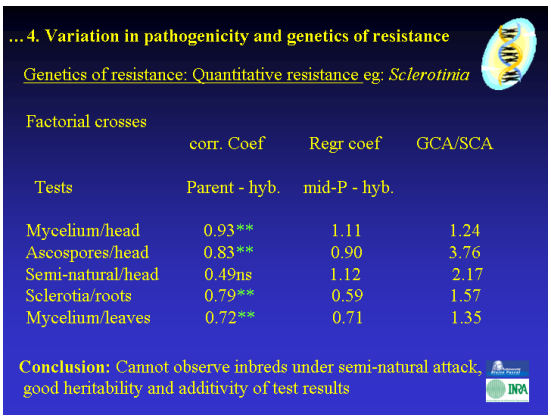
Most recent work makes use of molecular analyses to map these resistance genes or clusters of genes. Generally Bulk Segregant Analyses are used (Michelmore et al, 1991). At present for downy mildew resistance, our knowledge is summarised below. We know that there are at least two linkage groups with resistance genes, but it is almost certain that there are others, as some genes do not map in the 2 known regions.



b) Quantitative resistance. Again, the examples for this are Phomopsis and *Sclerotinia*. In both cases, studies are made of inbred lines and hybrids, and more particularly of factorial crosses between series of inbred lines with a range of resistance levels. The slide presents the results of observations semi-natural infection over 3 years of a 5F x 5M cross.



It shows that resistance is additive, without any interactions between female and male parents (Vear et al, 1996). Viguié et al (2000) showed that interactions occurred if the results of single trials were examined, but not means of multi-location or multi-year trials. This may be related to the slight interaction between isolate and sunflower genotype already mentioned.



For *Sclerotinia*, the same sort of factorial crosses were observed with the different resistance tests and results are summarised in this slide. Generally there is a good correlation between parents and their hybrids, the only exception being semi-natural attack, where inbred lines are difficult to observe. Heritability is generally quite high and in all cases resistance is additive.

5. Breeding Programmes

a) Oligogenic resistance

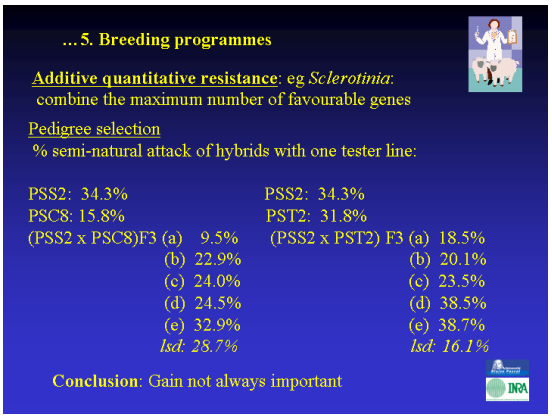
Taking the example of downy mildew, it is quite easy to breed for resistance by pedigree selection or to introduce genes by backcrossing. However, the important question is what combination of genes to aim for, to get the best durability of resistance in the face of possible pathogen change. Three possibilities are at present under study (Tourvieille de Labrouhe et al., 2002):

- Pyramiding a maximum number of genes on different linkage groups (for example *Pl6* or *Pl7* + *Pl5* or *Pl8*). This would require the fungus to mute at least two avirulence genes at the same time, which would be rare, but on the other hand, if it did occur, it would be a big problem for resistance breeders.
- Another possibility would be to use mixtures of genes. These would be "multihybrid varieties", after the idea of multiline varieties proposed at least 40 years ago, but very little used. This would mean that there would always be some susceptible plants, but that the pathogen would be under less pressure to mutate to be able to survive.
- The third possibility would be to alternate resistance genes over a period of time. Again there might often be some disease, but there would not be pressure for pathogen mutation.

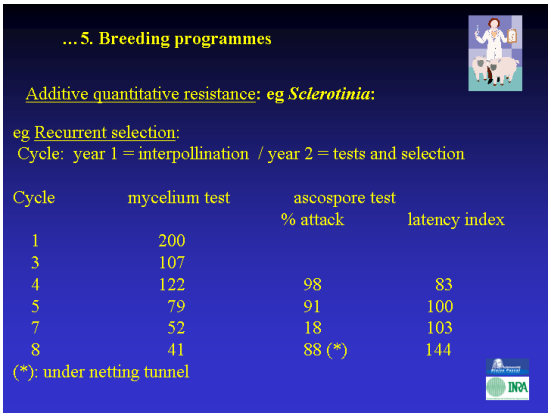
At present, we do not have a reply to this question, but it needs to be taken into account in breeding for all the race-specific resistances.

b) Quantitative resistance

Here we take the example of *Sclerotinia* resistance. The basic idea is to combine a maximum number of favourable genes to obtain the best possible level of resistance. Pedigree selection may be used, but it is not always evident whether a real increase in resistance level has been obtained (Vear et al, 2000b).



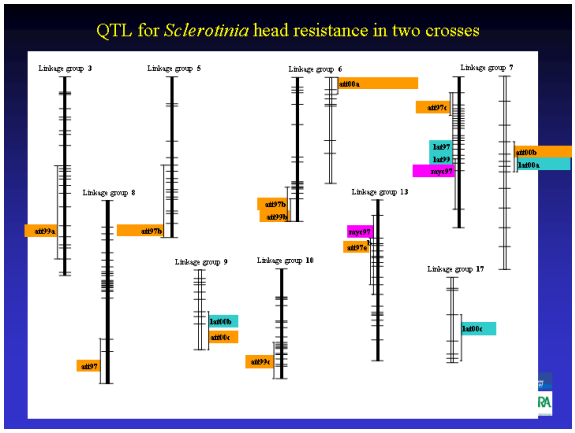
In this slide, a series of results of F3 hybrids obtained from two crosses are presented. Although some F3 appear better than the parents, the l.s.d. means that none are significant.



Recurrent selection can be efficient, but it is very necessary to adapt infection or testing procedures.

The slide presents results of recurrent selection for capitulum resistance over 8 cycles: improvement was quite regular for the mycelium test, but for the ascospore test improvement was very rapid to start with, but then the number of plants infected was too low to be able to select, so it was necessary to change conditions, to make the tests under netting tunnels with high humidity very favourable for the fungus. This was done at cycle 8, with an increase in percentage infection making it possible to select for latency index. (Vear et al, 1992)

To know more about which sources of genes will give significantly improved resistance when combined, we have carried out research on QTL for *Sclerotinia* resistance. This requires genotyping on F2 plants, disease tests on at least 150 F3 families, so it quite a costly programme. A simplified map is presented in the slide below, showing that a series of QTL have been found, each explaining a small part of total phenotypic variability, confirming that *Sclerotinia* resistance is truly multigenic (Bert et al, 2002). Now we have to determine the effects of different combinations of favourable alleles at these QTL to determine the best breeding strategies.



Conclusion

The development of varieties resistant to the important diseases of a region or country is a long term programme. Breeding can be carried out once tests and some differences in resistance have been discovered, it will be all the more efficient as the knowledge of genetics improves, but much useful work can be done in the field. The most important factor is good observation of disease. If you are sure of what you observe, the plants which do not have disease symptoms have a greater chance of being truly resistant, and any hypotheses concerning the genetics of resistance have to be based on correct disease observations.

Acknowledgements

I would like to thank all my colleagues of the sunflower team at Clermont-Ferrand whose results I have used, and Promosol and Cetiom for financial support for our programmes.

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