BREEDING FOR DURABLE RESISTANCE TO THE MAIN DISEASES OF SUNFLOWER

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Abstract

The sunflower diseases for which publications are most frequent are Sclerotinia, downy mildew, broomrape and Phomopsis. Resistance breeding has so far been concerned with either major gene resistance (downy mildew, broomrape) or quantitative resistance (Sclerotinia, Phomopsis). With major genes, resistance has been complete but not durable. Although new genes are regularly found, they are quite rapidly overcome. For quantitative resistance, although progress has been quite rapid in the case of Phomopsis, it has been slow for Sclerotinia and some research has concerned the possibility of genetic transformation. For efficient disease control in the future, it is proposed that, when available, vertical and horizontal resistances should be combined and if this is not the case, marker-assisted selection should be used to combine QTLs with different and additive defence mechanisms.

Discussion

Main Diseases of Sunflower. Taking the number of publications as a measure of the amount of research done and thus the importance of a disease, at the last three International Sunflower Conferences (ISC) in 1992, 1996 and 2000, white rot, *Sclerotinia sclerotiorum* (Lib.) de Bary was the most important disease of sunflower worldwide (Table 1). In 1992, the second disease was Phomopsis (*Diaporthe helianthi* Munt-Cvet. et al.), followed by charcoal rot (*Macrophomina phaseolina* [Tassi] Goid.), downy mildew (*Plasmopara halstedii* [Farl.] Berl. and de Toni) and broomrape (*Orobanche cumana* Wallr.). However, in 1996, downy mildew overtook Phomopsis and in 2000 there was a relative increase in papers on broomrape and blackstem (*Phoma macdonaldii* Boerma) (all of the last from France). The other main diseases reported were Alternaria leaf blight (*Alternaria helianthi* [Hansf.] Tubaki and Nishihara), white rust (*Albugo tragopogonis* [Pers.] S.F. Gray) and rust (*Puccinia helianthi* Schwein.). At this International Sunflower Conference (2004), *Sclerotinia* is definitely still the most studied disease, followed by downy mildew and broomrape (Table 1). Of course, this measurement of the importance of diseases depends on the scientists attending the ISC with an underestimation of diseases in tropical areas. For example, recent bibliography includes many papers on *Alternaria* from scientists in India who do not appear to have the funding to come to the International Sunflower Conference (ISC).

Recent bibliography confirms that *Sclerotinia* and downy mildew are still the diseases on which most work is done. Some research concerns use of the sunflower/downy mildew model to study fundamental aspects of host/pathogen interactions, but it appears reasonable to suggest that these are the two diseases that preoccupy the sunflower profession most at present. They are followed by papers on the increase of broomrape, with interest in research

on herbicide control, and papers on Phomopsis, where, in contrast, it may be suggested that the success of genetic and chemical control has somewhat reduced the number of studies in progress. It may be also, as reported in a paper by Vukojevic et al (2001), that truly pathogenic *D. helianthi* isolates occur only in Europe, and elsewhere it has been decided that Phomopsis is not a problem even if it occurs on sunflower.

Table 1. Numbers of papers concerning sunflower diseases presented at the last four International Sunflower Conferences (in brackets, several papers from one country).

Year	1992	1996	2000	2004
Location Disease	Pisa (Italy)	Beijing (China)	Toulouse (France)	Fargo (U.S.A.)
Sclerotinia	12 (Fra 5, Ser 2, Rum 2)	12 (Fra 5, Chi 2, Usa 2)	15 (Fra 5, Usa 3, Arg 3)	9 (Fra 2, Ser 2)
Downy mildew	4	12 (Fra 4, Chi 6)	10 (Fra 7)	5 (Fra 4)
Phomopsis	12 (Ser 4, Fra 4)	9 (Fra 4, Ser 3)	6 (Fra 4)	2 (Fra 2)
Broomrape	3	4 (Chi 3)	5 (Fra 2)	3 (Esp 3)
Macrophomina	7 (Pak 2, Rum 2)	1	0	0
Alternaria	3 (Ser 2)	2	2	2 (Aus 2)
Rust	1	5 (Aus 2)	1	1
Phoma	0	2	5 (Fra 5)	0
Albugo	0	4 (Saf 3)	1	0
Fusarium	0	0	0	2 (Bul 2)
Verticillium	0	1	1	1
Botrytis	1	1	0	0

Arg=Argentina; Aus=Australia; Bul=Bulgaria; Chi=China; Esp=Spain; Fra=France; Pak=Pakistan; Saf=South Africa; Ser=Serbia; Rum=Rumania; and Usa=U.S.A.

According to disease, research programmes have different priorities. It appears safe to say that symptoms, environmental effects and testing methods are quite well known for *Sclerotinia*, downy mildew and Phomopsis and most papers concern genetic resistance. In contrast, for broomrape there have been several reports of environmental effects, especially temperature, on sunflower reaction to this parasite (Eizenberg et al., 2003). For *Phoma*, there is still debate on which symptoms are prejudicial to yield (Gulya, 1996, Peres and Poisson, 2000) and for *Alternaria* also, most papers concern descriptions and screening methods (for example Amaresh and Nargund, 2000).

Types of Resistance Available. Control of diseases can be by cultural, chemical, biological and genetic methods. Cultural methods, such as rotations or ploughing in of crop residues, may help reduce the incidence of a disease but are generally insufficient for diseases which can cause economic losses. Chemical control can be effective, but may cause environmental problems, and may be overcome by pathogen changes. This has been particularly evident for seed treatment with metalaxyl to protect against downy mildew. For several years now, and in different countries, *P. halstedii* isolates which are not controlled by this compound have been observed (Albourie et al., 1998, Molinero Ruiz et al., 2000). Chemical control also depends on the possible market for a compound and sunflower diseases often do not provide the acreage necessary to recover the costs of development of new products. Biological methods have been proposed, for example to limit *Sclerotinia* attacks on sunflower roots (Trutman and Keane, 1990), but so far none has been applied on a large scale.

Disease control generally means genetic resistance of the host plant. Resistance to most sunflower diseases can be placed, quite easily, in one of the two categories. One: Complete, race-specific, vertical, major gene; this has so far mainly concerned resistance to downy mildew, broomrape and rust. Two: Partial, non-race-specific, horizontal, polygenic; this type of resistance has so far concerned mainly *Sclerotinia*, Phomopsis, *Botrytis* and *Alternaria*.

There are a few diseases where resistance appears, at present, more difficult to classify, perhaps because I have not worked with them. *Verticillium* wilt (*Verticillium dahliae* Kieb.) resistance used to be classed as race-specific (Fick and Zimmer, 1974) but Sackston (1992) reported that there were also some additive genes, and in Toulouse in 2000, Escande et al. (2000) presented a paper on *Verticillium* resistance in the "horizontal resistance" workshop. No complete resistance to *Phoma* is known, only QTLs concerning differences in seedling reactions have been identified (Al-Chaarani et al., 2002), so this disease can probably be classed in the second category. However, some quite striking isolate/sunflower genotype interactions have been reported (Larfel et al., 2002) in reactions of seedlings to disease tests. *Albugo* is another disease where the category of resistance can be questioned. It has been suggested (Gulya et al., 2000) that relative differences in reaction compared with results in other countries were due to a specific pathotype present in South Africa.

Research and Breeding for Resistance in Recent Years. In more detail, I will deal mainly with downy mildew and *Sclerotinia* resistance, the subjects I know best, but with some reference to other diseases such as broomrape and Phomopsis.

Resistance to downy mildew is probably one of the best examples of use of major gene resistance. Except in the period 1990-1995, when chemical seed treatment was needed to protect against the disease, major resistance genes have given farmers satisfactory sunflower crops since about 1980.

For the pathogen, the centre of origin appears to be North America, but from about 1960, downy mildew was reported in Russia, with a race (100) never reported in the USA where the first race was 300. While new races appeared quite regularly in the USA (Gulya et al., 1991), in Europe, there was stability until 1988 and 1989 when races 710 and 703 were reported (Tourvieille de Labrouhe et al., 1991). It now appears that race 100 is extremely homogeneous and probably homothallic and that races 710 and 703 are more closely related to North American isolates (Roeckel-Drevet et al., 2003). It was concluded that they were introduced. However, since then other new races have appeared specifically in France, in particular race 304, which is now observed each year (Tourvieille de Labrouhe et al., 2000). It must be supposed that this race developed from a mutation or some similar event; according to its molecular profile, probably from race 710. Since then, occasional samples of several other

races have been found (300, 307, 314, 700, 704 and 714; Penaud et al., 2003). From a geneticist's point of view, when you study their virulence pattern they look like an F2 between races 710 and 304. Whether this is the case remains to be seen but certainly there is now more variability among European populations of *P. halstedii,* raising questions as to the durability of major resistance genes.

Research on these genes has shown that there are at least three clusters of genes plus several others which segregate independently from these clusters but which have not been mapped. The cluster on linkage group 1 of the map of Gentzbittel et al. (1995), including *Pl1, Pl2, Pl6* and *Pl7*, has been shown to cover a large area, with about 0.5cM between resistance to races 100 and 300 on one hand and 700, 703 and 710 on the other (Bouzidi et al., 2001), so that occasional segregants are susceptible to race 100 and 300 but resistant to race 710 (Vear et al, 1997). It should be noted that this paper uses the linkage group nomenclature of Gentzbittel et al. (1995, 1999) concerning mapping of disease resistance).

All the downy mildew resistance gene clusters appear to be able to give resistance to all known races, but different origins of resistance, perhaps with different blocks or structures of genes, may give the same or different race resistance patterns. For example, on group 1, *Pl6* (from *H. annuus* L.) and *Pl7* (from *H. praecox* Engelm. and A. Gray) (Miller and Gulya, 1991) both give resistance to races 710 but susceptibility to race 304, whereas on group 6, Pl5 (from *H. tuberosus* L.) is in the same cluster as *Pl8* (from *H. argophyllus* T. and G.)) but while the latter is resistant to all known races, *Pl5* does not give resistance to a US isolate of race 330 (Bert et al., 2001). At present the gene-for-gene hypothesis appears reasonable, but has not proven since no segregants having lost resistance to some races have been reported for the cluster on group 6. It is still possible that some genes give resistance to several races. This organisation in several independent clusters is quite comparable with resistance to downy mildew in lettuce, studied by Michelmore and Meyers (1998). Figure 1 presents a summary of the downy mildew races which have been studied in France and the efficiency of resistance genes.

Resistance to *Orobanche* (broomrape) appears to follow a similar pattern to that of downy mildew, but with the centre of origin around the Black Sea, and more recently introduced into Spain. The first races (A and B) appeared in Russia and Ukraine about 1935, races C to E appeared in Eastern Europe and then in Spain in the 1980s and race F in Spain in 1996. It is not certain whether these six races are strictly the same in eastern Europe and in Spain, since the differential lines proposed by Vranceanu et al. (1980) do not give the expected reaction to all broomrape isolates (Melero-Vara et al., 2000). The resistance genes, denoted *Or1* to *Or5*, generally appear dominant (Dozet et al., 2000). Gagne (2000) mapped resistance to 3 isolates that were probably three races to one linkage group (16), but since the different origins of resistance were not crossed with each other, it is not possible to say whether these genes form a strict cluster with little recombination or whether they are situated in different areas on the same linkage group. Each source of resistance to a new race appears to give resistance to all the less virulent races (Melero-Vara et al., 2000), so it is not yet possible to know whether the gene-for-gene hypothesis applies. A paper by Venkov and Shindrova (2000) suggested that the efficiency of some of these genes was more durable than that of others.

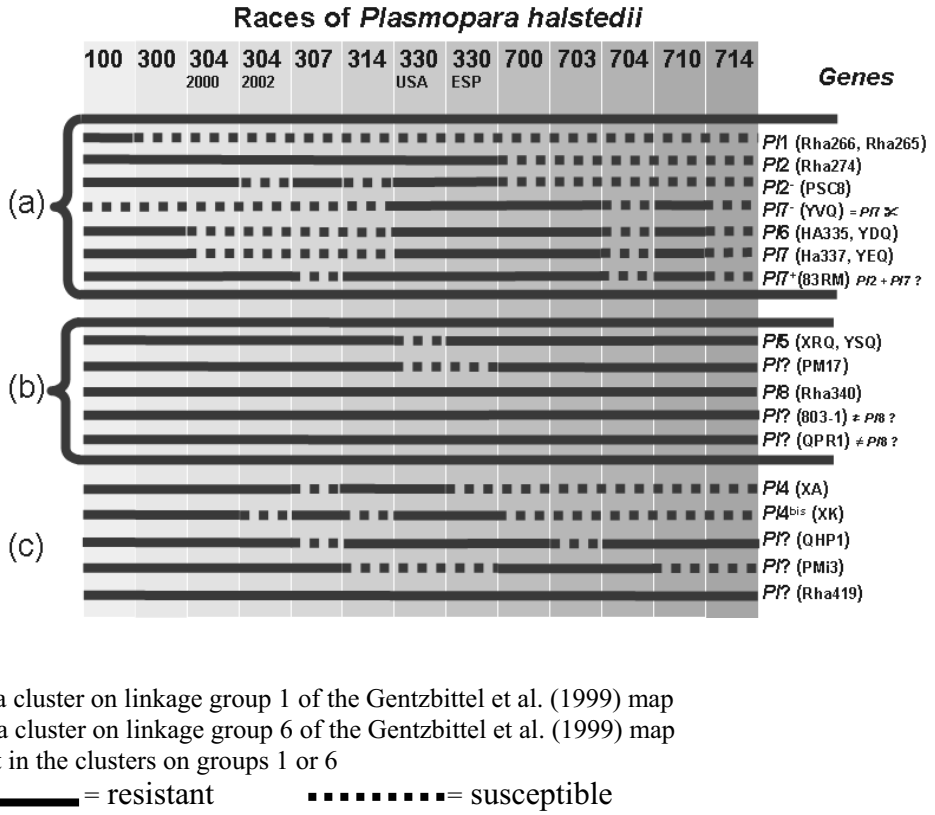


Figure 1. Efficiencies of *Pl* genes against *Plasmopara halstedii* races studied in France.

For both downy mildew and broomrape resistance, new dominant major genes are found quite regularly in wild *Helianthus* species (Miller et al., 2002, Jan and Fernandez-Martinez, 2002). Their use in breeding is relatively simple, but they do require an effort of backcrossing into different lines, an effort which is taken from other breeding programmes such as improvements in yield. The extent to which this is done depends on the economic importance of the disease. For example, in Spain, until quite recently, new varieties had to be resistant to all Spanish races of broomrape, so parental inbred lines were backcrossed to introduce new genes. In France, where rainy sowing seasons mean that downy mildew would be a real threat if susceptible varieties were grown, since 1995 many conversions for downy mildew resistance have been made. For example, for varieties originally carrying *Pl1* or *Pl2*, one of the parents was backcrossed to introduce *Pl6* or *Pl7*, to give resistance to races 703 and 710; then, in order that both parents should be resistant, and with the appearance of race 304, either *Pl5* or *Pl8* has been added to the parent already carrying *Pl1* or *Pl2*. The genes available must not be wasted and the question may be asked as to whether adding one new efficient gene each time a new race appears is the best solution. I will come back to ideas on their future use in the section on future use in breeding.

For quantitative, apparently horizontal resistance, there is no table of genes and races, although lists of quantitative trait loci (QTL) are becoming available (Bert et al., 2004). I will start with Phomopsis resistance, which is quite a success story. Resistance sources found in naturally attacked breeding nurseries (Skoric, 1985) gave good levels of resistance, and to start with it appeared that they might contain oligogenic resistance (Vranceanu et al., 1994). However, with experience and observations of modern sunflower hybrids, it soon appeared that parental lines which were not particularly resistant could give hybrids with good levels of resistance (Vear et al., 1996). Studies of factorial crosses showed that when measured over several years or several locations, resistance was quantitative and strictly additive, with no interactions between different parental lines (Viguié et al., 2000). It could thus be concluded that combinations of inbred lines with the best levels of resistance will give the best hybrids and that it should be possible to obtain increased resistance by selecting combinations from the different sources.

Interactions between effects of parental lines have been reported, but these all come from observations of Phomopsis reactions in single locations (Deglène et al., 1999). It has been suggested that this fungal species is still evolving quite fast and results in France agree with this, there being most molecular variation in areas newly colonised by *D. helianthi* (Says-Lesage et al., 2002). In addition, when sunflower lines are infected with mycelium of different Phomopsis isolates, some small host/pathogen interactions appear, although these do not affect high levels of resistance (Viguié et al., 1999). It was concluded that observations of natural attack in several locations was the best method to assure that a given genotype would generally show a low level of susceptibility.

It might be thought that with some sunflower genotypes showing significant levels of resistance, there would be a few strong QTLs. However, Bert et al. (2002), in a cross between two lines both with good resistance but with different origins, found three QTLs (on groups 3, 10 and 11) each explaining up to 20% of the variability for reaction to natural attack, and only one of these (on group 10) was in common with those (on groups 4, 8 and 10) found for reaction to mycelium extension rates. Langar et al. (2004) found two QTLs explaining up to 30% of variability in reaction to natural attack in one recombinant inbred line population, but, in all, there were 10 significant QTLs which explained a total of 50% of the variability. For different measurements following artificial infections on leaves and stems, they found from 1 to five QTLs. Quite logically, the individual measurements following artificial infections on leaves or stems appear to be controlled by fewer QTLs than the overall response to natural attack (% of plants showing girdling stem lesions), but so far, even for genotypes with almost complete resistance, knowledge of the genetic basis is quite incomplete.

Diaporthe helianthi is specific to sunflowers, whereas *S. sclerotiorum* can attack everything except grasses and this is perhaps the reason why it is more difficult to find the same level of resistance to *Sclerotinia* as to Phomopsis. Concerning *S. sclerotiorum,* we made studies in 2003 on different isolates and found that there were differences in molecular characteristics and in aggressiveness (growth rate on sunflower). However, when 16 sunflower lines were infected with ascospores of 10 isolates there were no interactions, for either percentage attack or latency index, so the resistance to *Sclerotinia* presently available can be considered as strictly horizontal (Vear et al., 2004). This means that they should be durable.

Table 2. Significant QTLs for resistance to *Sclerotinia sclerotiorum* measured by the ascospore test on six F3 populations (from Bert et al., 2004).

Linkage group [a]	Marker	Position [b] (cM)	LOD	PVE [c]	Trait	Cross [d]	Authors [e]
1	S124E2H1	36.0	9.3	58.9%	% attack	CP73x**PAC1**	II
		31.0	6.6	43.0%	Latency index	CP73x**PAC1**	II
	KinaseH3E1	0.0	4.1	13.0%	Latency index	SDx**PAC1**	I & II
		7.4	3.2	11.1%	% attack	SDx**PAC1**	I & II
2	S067S131	11.0	4.3	19.6%	% attack	CP73x**SD**	III
	S086E1H3	0.0	4.1	14.0%	Latency index	CP73x**SD**	III
4	S008E1H3	0.0	4.0	46.7%	Latency index	GHx**PAC2**	I
5	S050E1	15.0	3.3	19.9%	% attack	XRQx**PSC8**	IV
6	S069E1-1	13.0	3.8	35.0%	% attack	FUx**PAZ2**	V
		6.0	2.4	29.3%	Latency index	FUx**PAZ2**	V
	S144H3-1	22.9	2.7	9.2%	Latency index	**SD**xPAC1	I & III
	S094H3	20.0	3.6	12.7%	% attack	XRQx**PSC8**	IV
7	Branching	60.3	4.2	10.0%	% attack	GHx**PAC2**	I
		2.0	10.1	28.6%	% attack	FUx**PAZ2**	V
		1.0	7.3	14.8%	% attack	XRQx**PSC8**	IV
		6.0	8.1	27.1%	Latency index	CP73x**PAC1**	III
		3.8	16.2	39.0%	Latency index	GHx**PAC2**	I
		2.0	11.9	32.2%	Latency index	FUx**PAZ2**	V
	S005E1-1	3.0	5.8	16.9%	% attack	CP73x**PAC1**	III
	S070H3-1	5.0	3.7	9.9%	Latency index	XRQx**PSC8**	IV
8	S005E1-3	15.0	3.1	9.0%	% attack	XRQx**PSC8**	IV
9	S062E1	0.0	3.4	11.4%	% attack	SDx**PAC1**	I & III
	S300H3	2.0	4.2	15.2%	Latency index	CP73x**SD**	III
		5.0	3.0	12.9%	% attack	CP73x**SD**	III
	S008E1-2	5.0	3.3	11.3%	Latency index	**FU**xPAZ2	V
		1.0	3.6	12.1%	% attack	**FU**xPAZ2	V
10	S081-E1	18.6	4.4	57.7%	% attack	GHx**PAC2**	I
	S080H3-3	0.0	3.1	9.3%	% attack	CP73x**PAC1**	III
13	S122H3	40.0	4.2	11.2%	% attack	**XRQ**xPSC8	IV
		7.7	3.6	11.7%	Latency index	SDx**PAC1**	I
	S051E1	7.0	4.5	31.5%	Latency index	CP73x**SD**	III
14	S053H3-1	28.4	3.4	29.6%	Latency index	**SD** xPAC1	I & III
15	S109H3-3	0.0	8.8	63.5%	% attack	**GH**xPAC2	I
		0.0	3.9	44.9%	Latency index	**GH**xPAC2	I
16	S141H3	4.0	2.7	8.9%	Latency index	CP73x**PAC1**	III
17	S012E1-1	3.0	3.0	15.5%	Latency index	**FU**xPAZ2	V
	S121E1	0.0	-	38.1%	Latency index	CP73x**SD**	III

[a] linkage groups from Gentzbittel et al., 1999 [b] Indicates distance below the marker, from top to bottom of the linkage group [c] Percentage of phenotypic variation explained by each QTL, as given by mapmaker/QTL (SIM)
[d] Parent providing the best resistance is in bold type [e] I: Mestries (1996); II: Gentzbittel et al. (1998); III: Gentzbittel et al. (unpublished); IV: Bert et al. (2002); V: Bert et al. (2004).

Heredity of resistance to *Sclerotinia* has been shown to be quantitative, generally under mainly additive control, but with some interactions between parental lines (Vear and Tourvieille, 1988). The QTLs for resistance published so far have mostly concerned capitulum reaction to the ascospore test, and in six crosses, QTLs have been found on 14 of the 17 sunflower linkage groups, in general each explaining less than 20% of phenotypic variance (Table 2, from Bert et al., 2004). One particularly strong QTL was reported on linkage group 1, linked to a protein-kinase gene (Gentzbittel et al., 1998), but while it explained 50% of the variability in one cross, in other crosses it explained only 15% or was not present. The linkage group which most often carries a QTL for resistance is group 7, which also carries the recessive branching gene b1 (Putt, 1964), and although when tests are made branches are removed, resistance to the ascospore test may be a pleiotropic effect of this phenotype. Analyses of QTLs for natural attack of hybrids (all unbranched) are in progress and it will be very interesting to compare these QTLs with those for reaction to ascospore tests on the same parental genotypes. It may be noted that favourable alleles on group 9 and 17 come from female lines. QTLs for reaction to mycelium tests on leaves and capitula and for natural attack on terminal buds have also been reported. These QTLs often appear to co-localise with the QTLs for resistance to the ascospore test, but fine mapping will be necessary to check this. So far, there do not appear to be any reports of QTLs for root resistance.

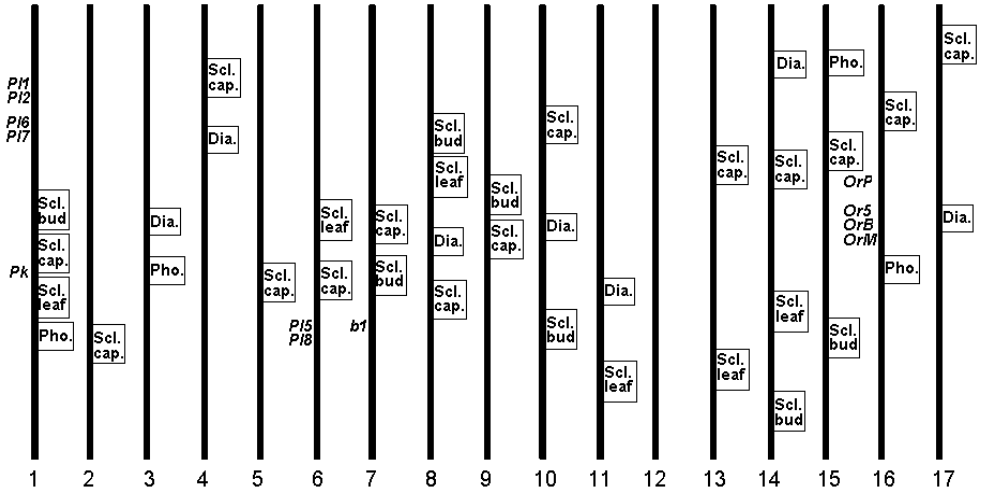
The other type of work published on *Sclerotinia* resistance has concerned genetic modification, with an oxalate oxidase gene introduced from wheat (Bazzalo et al., 2000; Scelonge et al., 2000). This gene did increase resistance compared with the original line, but overall, the level was not better than lines obtained by conventional breeding.

Until the present, breeding for resistance to both *Sclerotinia* and Phomopsis has concentrated on combining resistances from different sources. Concerning *Sclerotinia*, recurrent selection has certainly been successful in increasing the level of resistance (Vear et al., 1992) and pedigree selection also appears to give the possibility of obtaining genotypes with increased levels of resistance (Vear et al., 2000). Progress by such conventional breeding has given a 60% reduction in attack in modern varieties grown in France, compared with those grown in 1960-70 (Vear et al., 2003). Nevertheless, in extremely favourable conditions, most sunflower genotypes may show high levels of attack (Serre et al., 2004) and further work is certainly necessary.

Proposals for Future Research and Breeding for Durable Resistance. There have now been 40 years of research on sunflower genetics, but compared with wheat, barley or potato, use of genetic disease resistance is still "young," and it has been a question of using what was most easily available. At this point, it would appear useful to reflect on strategies for long term control, perhaps requiring effort in the next few years, but then allowing research to concentrate on more rapid improvements in yield or quality.

When resistance is incomplete, breeding programmes search to improve levels of resistance, whereas when resistance is complete, durability is the main objective. Probably, a combination of these two objectives, to get a high level of durable resistance, should be the goal in the future. Figure 2 presents a simplified molecular map with many of the resistance genes and QTLs so far identified. The question is how best to use this knowledge. Parleviet (1977) suggested that horizontal resistance could include some race-specific resistance genes with small effects such that interactions between host and pathogen do not have significant effects on the overall resistance level. The idea of "not putting all one's eggs in one basket," of combining race-specific resistance genes, of varying importance according to what is

available, with strictly non-race-specific resistances would seem to be the best guarantee of long-term success.



Or= resistance to broomrape isolates (P = Pipa blanca, Spain; B = Bulgarian; M = Mencia, Spain)
b1 = branching
Pl= resistance to downy mildew isolates
Pk= protein kinase
Scl.= *Sclerotinia sclerotiorum*; cap. = capitula; Pho. = *Phoma macdonaldii*; Dia. = Phomopsis (*Diaporthe helianthi)*

Figure 2. Simplified linkage group map of sunflower from Gentzbittel et al. (1999) showing disease resistance genes (on left) and QTLs (on right).

For resistances which have so far been basically non-race-specific, such as *Sclerotinia* and Phomopsis, the first important step will be to use marker-assisted selection (MAS) to combine the different QTLs for resistance of any one plant part. Lack of success in improving levels of resistances from some crosses between two quite good lines may well have been due to the lines having the same favourable alleles at the main QTL. A first project would be to add one or two additional QTLs to already interesting genotypes to determine the increase in level of resistance that is obtained. Knowledge of the resistance mechanisms underlying the different QTLs would help to decide those which are most likely to have additive effects. This programme will be long and complex and it may appear of interest to see if more rapid progress could be obtained by genetic transformation. I would like to suggest that, alone, transformation with a single resistance source would not be durable.

Any genetically modified organism (GMO) with significantly increased resistance would necessarily contain something resembling a major gene. There might or might not be recognition between the host and the pathogen, but this gene would be under considerable pressure from pathogen mutations or other genetic exchanges. It would seem better to me that any transformation should concern introduction of "foreign" genes into sunflower genotypes

with the best possible combination of horizontal, partial resistance, such that overall, resistance depends on many different factors that are unlikely to be overcome by pathogen changes.

In addition, since each time a new segregating population is studied, some new QTLs for resistance have been identified; search for new QTLs should be continued, in particular in wild *Helianthus* species. They could quite well add to the partial resistances already identified, especially if they depend on mechanisms different from those already available. Introduction of such resistances may be, as in the past from wild *H. annuus*, by conventional techniques, or from more difficult crosses, by using improved embryo rescue techniques. If genes are identified, sequenced and isolated, transfer would become equivalent to that of genes from different plant genera and the same precautions appear necessary. In the case of resistance to Phomopsis, since the pathogen appears to be still evolving quite rapidly, it will be necessary, in addition, to check from time to time that the quantitative resistances used are not race-specific.

For resistances which have been so far basically monogenic, the problem is the opposite: to make durable the complete resistance available. This has become of considerable urgency in recent years, both for downy mildew and broomrape. Figure 3 from Tourvieille de Labrouhe (2003) shows that for downy mildew resistance, the gene *Pl2* gave complete control in France for about 10 years, from 1978 to 1988. The genes *Pl6* and *Pl7* were efficient also for about 10 years (1991-2001) and it may be suggested that *Pl5* and Pl8 are likely to have a similar life span if they are used in the same way.

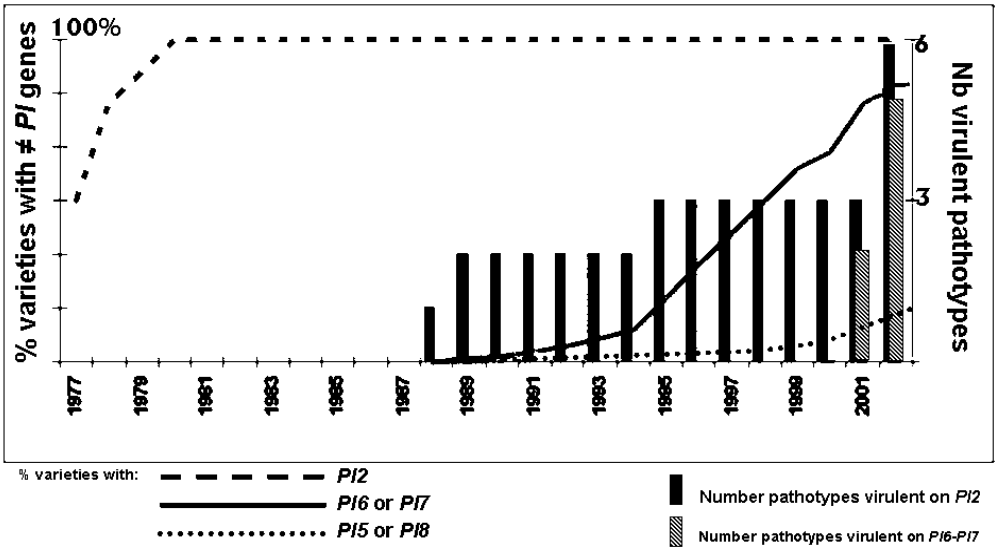


Figure 3. Coevolution of the percentage of varieties grown in France carrying certain *Pl* genes and number of *P. halstedii* pathotypes identified which overcome these genes (from Tourvieille de Labrouhe, 2003).

The first step in improving durability was thus to determine whether a system other than adding one new gene each time it was necessary could be more efficient. The first tendency of a plant breeder is to pyramid genes, with the idea of reducing the probability of the necessary mutation of the pathogen. However, Mundt (1991) reported for *Rhynchosporium secalis*

(Coudem) J. J. Davis on barley that the frequency of appearance of multiple virulences could be greater than expected from independent mutational events, due to peculiarities of fungal genetics and cytology. A poster by Tourvieille de Labrouhe et al. (2004a) reports a comparison between pyramiding, alternation of several forms of a hybrid with different *Pl* genes and mixtures of these different forms grown together. First results indicate that the last two systems give less pathogen change than pyramiding. Already, in the 1950s, Jenson (1952) and Borlaug (1954) suggested the use of multiline varieties of oats and wheat, so we use the term "multihybrids", but as multilines have never been widely developed, it may be questioned whether multihybrids could become a commercial reality.

To get the best use from these major genes, they need to be backed up by quantitative, non-race-specific resistance, which would mean that a form of *P. halstedii* with altered or mutated avirulence genes could only multiply with difficulty because it would be blocked by other resistance mechanisms. To this end, a programme supported by the whole French sunflower profession was initiated in 2003 and has given very encouraging first results. They are presented in a poster by Tourvieille de Labrouhe et al. (2004b). The main conclusions of the first year of multisite field trials with natural attack favoured by irrigation were that there are a wide range of reactions among modern cultivated sunflower lines (15-75% attack) and that these reactions were the same to both races 703 and 710, indicating a horizontal type of resistance. In addition, reactions were not related to the presence of ineffective *Pl* genes, contrary to many reports (e.g., Martin and Ellingboe, 1976), suggesting that different mechanisms are involved. Also, they were not related to resistance to *Sclerotinia* or Phomopsis, indicating that it should be possible to breed for quantitative resistance to downy mildew independently of these other characters. If these results are confirmed, it will then be essential to identify very good molecular markers of the QTLs involved, for use in MAS, since when combined with major resistance genes, it will not be possible to determine the presence of partial resistance QTLs phenotypically. Of course, if sufficient levels of quantitative resistance are identified, the major genes could be dispensed with, but I think this could only be in the long term.

For broomrape resistance, new races appear quite regularly, especially in Spain (Dominguez, 1996), but recent research on durable resistance has been in a rather different direction, with the use of herbicides. Imidazolinone tolerant sunflower varieties are now available and this herbicide gives good control of *O. cumana* (Dominguez et al., 2004). Will it last? Making a comparison with control of downy mildew by metalaxyl, it may be suggested that *O. cumana* isolates resistant to imidazolinone may appear if this compound is widely used on broomrape susceptible varieties. However, perhaps the combination of major gene resistance and herbicide treatment will give durable control. It is certainly an original model. I have not found any publications advocating research for quantitative resistance to broomrape, but from the outside, I would suggest that this could be useful in addition.

Conclusions

It seems to me that efficient control of diseases, both those at present most important on the sunflower crop and those which occur more rarely or are only beginning to be studied, depends on the integrated use of major genes available and the best combinations of quantitative resistance. Such an objective requires a good knowledge of the genetics of the different types of resistance, of the relative genomic locations of the genes and QTLs and of

the resistance mechanisms they control. I will finish by suggesting that research on sunflower disease resistance will continue to be essential in the foreseeable future.

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