STRATEGIES FOR MANAGING RESISTANCE TO FUNGICIDES IN PRACTICE IN VITICULTURE

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ABSTRACT

Strategies for managing resistance concern *Botrytis cinerea* (benzimidazoles, dicarboximides, phenylcarbamates), *Plasmopara viticola* (phenylamides) and *Uncinula necator* (DMI). Present measures, proposed and in practice, for the management of resistant pathogen populations are presented here and the difficulties encountered are stressed. In all circumstances, it is preferable to organize treatment programs which limit the use of fungicides at risk. It has been difficult to have this idea accepted even though the alternative products are more effective. Mixtures are generally used to guarantee efficacy (phenylamides – downy mildew) and are not always the best solution (DMI – powdery mildew).

INTRODUCTION

During the last 20 years, viticulture has been increasingly confronted with the problem of resistance to fungicides. There are three diseases of principal economic concern, grey mold, downy mildew, and powdery mildew. Each year, these diseases require a considerable number of preventive treatments as a form of insurance (7 to 9 for powdery mildew, 2 to 4 for grey mold, on average each year in France). These diseases are caused by airborne pathogens which possess a remarkable sporulation capacity rendering them extremely destructive to the grape harvest and its quality. For this reason, the selective pressure exerted by the fungicide treatment on the pathogen population is considerable. This pressure is even greater in regions where the risk of epidemics is higher (i.e. *Plasmopara viticola* in the Atlantic vineyards, and *Botrytis cinerea* in Champagne) because growers in these regions favor fungicides whose most effective active ingredients generally have a unisite mode of action. This has lead to the rapid development of resistant strains 2 years, after benzimidazole was put on the market, then the dicarboximides and the phenylcarbamates (diethofencarb) for *Botrytis* or the phenylamides for *Plasmopara*. On the other hand, several years (5 to 7) of intensive use of DMIs have been necessary to see the first signs of the development of resistant strains of powdery mildew (*Uncinula necator*) in the vineyard. These effects are attributed to whether the nature of the resistance is disruptive or progressive (monogenic for the former, polygenic for the latter) (Clerjeau, 1991).

In response to the problem of the decrease in efficacy of these fungicide treatments, it has been necessary to instruct growers as to how to minimize risk. This has required close cooperation between research, offical and professional organizations and agrochemical firms (monitoring of pathogen populations, experiments, information operations and communication) leading to a code for "living with resistance".

Anti–resistance strategies : probably an ambitious expression

Even though there are good intentions on the part of private companies and professional and offical services to cooperate in the face of the problem of resistance, it must be admitted that the actual putting into practice of anti–resistance measures has lacked total consensus. There has sometimes been disagreement as to the evaluation of risk and measures to be taken. In this context, where problems of individual interests are far from minor, actions taken by the partners are not always coordinated or the objectives are not necessarily a veritable anti–resistance strategy.

To date, in France, the diverse problems of fungicide resistance in the vineyard have led to certain lines of action which can be summarized as : 1) Detection of the first resistant strain by researchers. 2) Establishment of monitoring operations thoughout the country with the aid of private firms. 3) Recommendations from the official services in order to reduce the number of fungicide treatments. 4) Multiyear, multisite experimentation to evaluate the use of mixtures of fungicides, the effect of fungicide rotation programs, and the effect of the applied dose. 5) Adjustment of recommendations in light of acquired information. In as far as possible, the cooperation of private companies is sought in order to harmonize information given to the growers.

Although, until relatively recently, anti-resistance measure were rarely of a preventive nature, today, new fungicides coming on to the market have been developed with relative prudence. This is the case notable for pyrimethanil against *B. cinerea* in spite of the fact that the risks have not yet been assessed.

Principal recommended anti-resistance measures

The principal recommendations given in France will be summarized here.

Botrytis cinerea

Because of a lack of effective methods to predict the risk of disease, measures against grey mold have traditionally relied on a series of four preventive treatments as a form of insurance : A) at the end of flowering ; B) at bunch closing ; C) at veraison ; D) 3 weeks before harvest. Treatment D is rarely justified.

Few conventional fungicides (thiram, dichlofluanid, chlorothalonil) are as effective as growers would wish. This explains the success of benzimidazoles in the 1970's, dicarboximides in the 1980's and the combination of carbendazim and diethofencarb at the end of the 1980's which rapidly led to the selection of pathogen strains resistant to these products. The development of strains such as Rb2, doubly resistant to carbendazim and diethofencarb, showed that the idea of negative cross-resistance as a management strategy was rather short lived for these particular compounds.

TABLE 1. Anti-*Botrytis* strategies recommended in France.

A	B	C	D
1. carbendazim + diethofencarb	pyrimethanil	dicarboximide (alone or with thiram)	–
2. carbendazim + diethofencarb	dicarboximide + thiram	dicarboximide (alone or combined)	–
3. carbendazim + diethofencarb	multisite	dicarboximide + thiram	dicarboximide (if need be)
4. pyrimethanil	dicarboximide + thiram	dicarboximide (alone or combined)	–
5. pyrimethanil	multisite	dicarboximide + thirame	dicarboximide (if need be)

The analysis of 10 years of monitoring done by Leroux & Moncomble (1993) showed that the only way to maintain the number of resistant strains below a critical level is to limit the number of treatments. This number can be under no circumstances greater than one treatment for each type of product. The only exception is for dicarboximides where two treatments are allowed in vineyards where Rd strains have not been detected or are not very frequent. Under these conditions, dicarboximides should be used in mixture with thiram at its full concentration (3.2 Kg ai/ha) (Leroux & Moncomble, 1993 ; Bugaret & Lafon, 1990). Nevertheless, in certain vineyards where the number of Rd and Rb2 strains is high (i.e. Champagne), the struggle is at an impass. One must fall back on strict preventive cultivation techniques and the use of conventional fungicides. Recently, the fungicide, pyrimethanil, has been registered for use especially in these vineyard, but its use should be strictly limited.

Several strategies for alternating fungicides have been recommended for other vineyards. These are presented in Table 1.

It is recommended to improve the efficacy of these programs by using an anti-downy mildew fungicide (7 to 8 treatments per year on average) with a secondary action against *Botrytis* (i.e. folpel, fosetyl-Al, methirame-Zn). The program should be chosen taking into account rather the nature of the pathogen population present in the plot (related to past treatment history) than the nature of the pathogen population present on average in the region. *Botrytis* of grapevine, contrary to popular ideas, has, in fact, a short dissemination range.

Plasmopara viticola

Resistance of downy mildew to phenylamides developed rapidly in France in the beginning of the 1980's, and then later in other European countries (Leroux & Clerjeau, 1985). It is not certain that these resistant strains appeared later in countries where these fungicides were used mixed with conventional products than in those where they were used alone. Monitoring activities conducted over several years in France have shown a clear relationship between the frequency of resistant strains isolated in diverse vineyard regions and the frequency of use of these products and the pathogen pressure (Moreau et al., 1984). However, it seems that the mixture of pathogen populations due to their wide dissemination has lead to population structures in the plots which are generally independent of their treatment history. It is in this context that in 1982, the following recommendations were established which are still valid today :

- Temporarily stop treatment with phenylamides in the regions most affected by resistance (i.e. the Cognac region between 1982 and 1985).
- No curative treatments : inactivity of conventional fungicides when combined with phenylamides.
- No treatments in the nursery : these products do not protect well during active vegetative growth. Newly formed organs are not protected when treatments are spaced at 14 days.
- A maximum of three treatments based on phenylamides per year, alternating with fungicides from other families.

The recommendations have been, on the whole, well followed because of the availability of effective alternative products whether penetrating (cymoxanil and dimetomorphe) or systemic (fosetyl-Al). In that the market share of phenylamides among the anti-mildew products is rather low (less than 10%), management of the resistant populations appears to be satisfactory. Even though resistant populations are frequent at certain sites, alternation programs provide growers with an adequate level of protection. This is also due to a synergistic effect between certain phenylamides and the other fungicides with which they are mixed in the commercial formulations. This synergistic effect has been observed for oxadixyl (Grabski & Gisi, 1987), and ofurace (Bugaret et al., 1989). In addition, benalaxyl has been shown to have an original, preventive action on contact with susceptible or resistant strains of downy mildew (Bugaret & Clerjeau, 1991).

It should be noted that, in 1993, insufficient protection was observed in high risk situations because of repeated rainfall during the period of optimal mildew sensitivity. In this situation, the non systemic associated fungicides were not able to provide sufficient protection when applied every 14 days.

Uncinula necator

Although resistance to DMI's has been observed for several years now in most European countries, first in Portugal, then in France (Steva et al., 1989), it is probably the principal current problem in vineyard protection. Many difficulties have been encountered in developing and putting into practice the anti-resistance measures that are summarized here (Anonymous, 1992) :

- The maximum number of treatments with DMI's : two or three per year. This means an alternation with less effective conventional fungicides (sulfur, dinocap, dichlofluanid). Continuous use of 2 or 3 DMI's is considered to be preferable to alternation of DMI's with conventional products when rotation poses practical problems such as a break in the rythm of treatment (10 or 14 days).

- Use of products at their maximum registered dose and with good spraying quality to avoid attack by strains with a weak resistance factor.

- No recommendations encouraging the use of mixtures of DMI with protective fungicides rather than a DMI alone. Due to under-dosage of active ingredients in the mixtures, a more rapid selection of resistant strains has been observed (Steva, 1992) ; in addition, an interaction between DMI (triadimenol) and sulfur has proved to be antagonistic (Steva, 1992).

Several factors play a role in slowing down a significant reduction in the use of DMI treatments :

- The absence of alternative products which offer good control at the same price. As a result, growers only look into their use after the first signs of declining efficacy of DMI's.

- The absence of systematic cross-resistance between the DMI's (Steva and Clerjeau, 1990) : the pathogen populations of powdery mildew preferentially acquire resistance to DMI's used in the plot. As a result, growers alternate different DMI's without reducing the total number of applications. This practice must, of course, be condemned because it leads to the development of strains of the pathogen with multiple resistance.

- The progressive nature of resistance (appearance of strains with higher and higher factors of resistance) : due to a reduction in efficacy observed firstly for weakly resistant strains which have developed under inadequate control conditions (effects of under-dosage of fungicides), growers are encouraged to improve their treatment practices rather than change their program of treatment.

CONCLUSION

If an effective anti-resistance strategy is to consist above all of a good level of prevention, it is necessary to recognize that, in practice, measures are not actually taken until monitoring activities indicate widespread dissemination of resistant pathogen strains and cases of reduced treatment efficacy. The reason for reluctance to adopt alternation programs is that no-risk products are rarely as effective as fungicides at risk. Indeed, recommendation of these programs is often seen as a means to restrict usage of these at risk fungicides. Nevertheless, it is very clear that restriction is the only effective way to prevent development of resistance. Unfortunately, it usually takes several years of experience in the field to evaluate the maximum number of treatments acceptable and this number is generally low (1 to 2 for *Botrytis cinerea* ; 2 to 3 for anti-downy mildew treatments).

And what about the use of mixtures of fungicides to deal with the problem of resistance? Examples found in viticulture do not provide universal answers. For *Botrytis*, the improvement of protection using mixtures is small but appears to be real. For *Plasmopara*, the advantages are more evident. On the other hand, for powdery mildew, the under-dosage of DMI's in the mixtures has contributed to an accentuation of the selection of weakly resistant strains.

Over the last ten years, studies on the development of resistant pathogen populations in relation to the selective pressure of treatment has provided significant progress in our knowledge of the conditions of dissemination of grapevine pathogens (dissemination limited to the parcel for *B. cinerea* and *U. necator*; widespread development for *P. viticola*). It can be said, however, that only real progress in the epidemiology of these fungi allowing better management will give us the upper hand on the problem of resistance.

REFERENCES

Anonymous (1992). Oïdium de la Vigne : Résistance aux IBS et recommandation d'emploi des fongicides. *Phytoma*, **435**, 56.

Bugaret, Y. ; Clerjeau, M. ; Lafon, R. (1989). Résistance du Mildiou aux phénylamides, une donnée nouvelle : la synergie ofurace-folpel. *Phytoma*, **412**, 34-40.

Bugaret, Y. ; Lafon, R. (1990). Iprodione + thirame : une association efficace. *Viti*, **148**, 109-112.

Bugaret, Y. ; Clerjeau, M. (1991). Le bénalaxyl : de surprenantes possibilités. *Phytoma*, **427**, 29-34.

Clerjeau, M. (1991). Questions d'actualité sur la résistance des champignons parasites aux fongicides. *Troisième conférence internationale sur les maladies des plantes, Bordeaux 3-5 Déc. 91, ANPP*, **4 (1)**, 97-104.

Grabski, C ; Gisi, U. (1987). Quantification of synergistic interactions of fungicides against *Plasmopara* and *Phytophthora*. *Crop Protection*, **6**, 64-71.

Leroux, P. ; Clerjeau, M. (1985). Resistance of *Botrytis cinerea* Pers. and *Plasmopara viticola* to fungicides in the french vineyards. *Crop Protection*, **4 (2)**, 137-160.

Leroux, P. ; Moncomble, D. (1993). Lutte chimique contre la Pourriture grise, passé, présent, futur (2ème partie). *Phytoma - La Défense des Végétaux*, **451**, 23-27.

Moreau., C. ; Clerjeau, M. ; Malato, G. (1984). La résistance du Mildiou aux anilides lors de la campagne 1983. *Phytoma*, **357**, 25-29.

Steva, H. ; Cartolaro, P. ; Clerjeau, M. ; Lafon, R. (1989). Premier cas de résistance de l'Oïdium à un traitement fongicide. *Viti*, **137**, 124-125.

Steva, H. ; Clerjeau, M. (1990). Cross resistance to sterol biosynthesis inhibitor fungicides in strains of *Uncinula necator* isolated in France and Portugal. *Medelingen van de Faculteit Landbouwetenschappen Rijksuniversiteit Gent*, **55**, 983-988.

Steva, H. (1992). Résistance de l'Oïdium de la Vigne (*Uncinula necator*) aux fongicides inhibiteurs de la biosynthèse des stérols. *Thèse de doctorat Université Bordeaux II*, 248 p.

FINANCIAL IMPLICATIONS OF FUNGICIDE RESISTANCE

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ABSTRACT

TRANSAGRA Advice and Development Service works with 2000 farmers who till 174000 ha, 70000 ha of which are cereals.

The use of fungicides on cereals represents a third of the total operating costs. Even though new problems of fungicide resistance may appear in the country, farmers still have to maintain good protection for their crops. The use of diagnostic and forecasting models, new varieties and active materials will all help the farmer to make the correct decisions.

FARMING IN CHER

The Department of Cher is located in the center of France, 240 km south of Paris. It is a large Department of open fields with crops of cereals and oil producing plants : rape seed and sunflower. Maize is grown with spray irrigation.

Climate and soils

In summer, sometimes from April, the climate is dry and warm. The soils (rendzinas) are very thin with limestone. Crops need rain or irrigation, but there is not enough water. (The annual rainfall is 650-750 mm). Seven natural areas constitute the Cher : Champagne Berrichonne covers half of the area.

Production and yields

Cher has 480000 ha of arable surface including 20000 ha of winter wheat and barley and 90000 ha of oil plants. 25000 ha of maize are irrigated. 4000 ha of set-aside are grown with rape-seed and flax as industrial crops. Cattle decreases : Charolais for meat, goats for cheese (Crottin de Chavignol) and poultry (turkeys, chickens) for CODIVOL.

The average yields are around 6.5 - 8.5 t/ha of wheat, 10t - 12t of maize, 3 - 4 t/ha of rape-seed and 2 - 2.5 t/ha of sunflower.

Vineyards cover 3000 ha of Sancerre, Menetou and Quincy, while apple orchards occupy 2000 ha.

Men and machines

4000 farmers cultivate an average 120 ha per man, working with a high degree of mechanization. The individual farm area has increased over the past two years and 150 ha per man will soon be reached.

TRANSAGRA : SURFACE AND ROTATION OF CROPS

Transagra is a cooperative which produces 650000 t of grain per annum and sells seeds, pesticides and fertilizers. The areas grown for harvest 1993 are shown in Table 1 :

TABLE 1. Transagra crop areas 1993

	Hectares		Hectares
Wheat	60 000	Rape seed	23 000
Winter barley	9 000	Sunflower	27 000
Winter oat	900	Peas	5 500
Hard wheat	700	Corn (Maize)	15 000
Spring barley	2 700	Set aside	30 500
		with rape seed	3 000
TOTAL CEREAL	73 300	Flax	1 000
		TOTAL	101 000

Rotation

On loamy soils, a two year rotation is used, rape seed-wheat. On rendzina soils rotation was traditionally on a three yearly cycle of rape seed or sunflower, wheat, barley or second wheat. Now the second wheats are only sown on 5000 ha.

CEREAL DISEASES AND LOSS OF YIELDS

Septoria is the main disease every year, brown rust is also present but less severe.

Eyespot covers a third of the area, mainly in loamy, wet and clay soils. Powdery mildew increases each year, even on the cultivar "SOISSONS".

Field tests

In the past 12 years, we have conducted and harvested 37 fungicide tests on wheat in the different areas, using random block trials with four replicates and plots 3 x 12m.

The 37 trials included 15 for eyespot located in clay and loamy soils, often with flints. The fields are drained but wet in winter. Losses from eyespot were between 0.2 t to 1.5 t/ha. Overall gains due to control of foot and leaf diseases were estimated at:

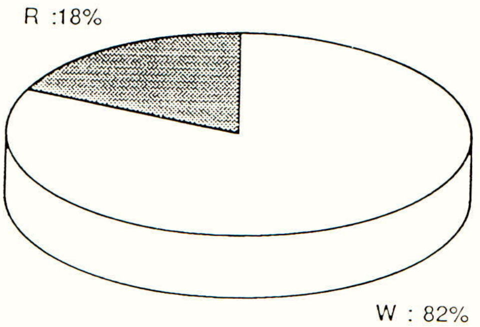
$<$ 0.6 t/ha : 11 trials
$>$ 0.6 t/ha : 26 trials

The mean yield gain was 1.43 t/ha

Cartography of eyespot in Cher

The DuPont eyespot kit was used by our laboratory to assess eyespot in 200 fields. For two years a third of the fields produced results > 10 UA. In 1993, SPV Orleans tested 900 stems from 21 fields not treated with fungicide. Samples were taken from the fields between 10-15 June and showed all fields to be > 30 UA.

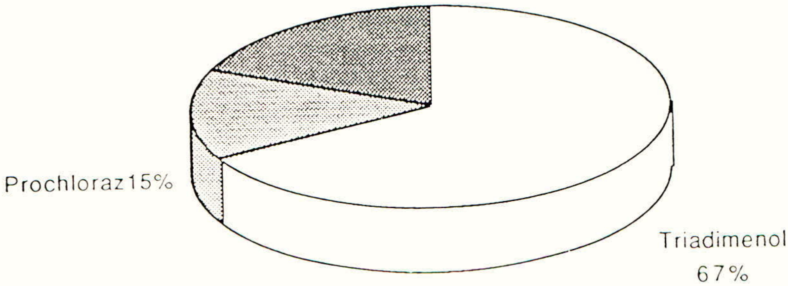
Analysis of the populations showed that the W type was dominant in these 21 fields:



Distribution of W/R types in the population

Isolates from these fields were tested for sensitivity to fungicides. The tests with prochloraz (0,5 and 2 mg/l) and triadimenol (30 mg/l) showed that 70% of isolates were resistant to triadimenol.

At 5 locations, 15% of isolates showed resistance to prochloraz, but only in the laboratory, not in the field.



An assessment of the effect of disease on cereals

Winter wheat variety SIDERAL was sown on October 20 1992 at Laverdines, and followed wheat after rape seed.

At the end of March 1993, the "DUPONT" eyespot kit was used to show 125 UA without eyespot visible on plants. A 4 replicate trial was established with treatments as shown in Table 2, applied at GS 31.

TABLE 2. Control of eyespot at Laverdines

Treatment	g AI/ha	Frequency %	Necrosis section %	Efficiency %	Lodging %
Check 0 treatment		100	100	-	100
1 - Prochloraz + Fenbuconazole + Fenpropimorph + Fenpropidine	450 67 280 94	60	32	68	25
2 - Prochloraz + Fenpropimorph + Fenpropidine	450 280 94	76	43	57	50
3 - Fluzilazole + Fenpropimorph + Fenpropidine + BMC	250 280 94 125	92	77	23	100
4 - Epoxyconazole	125	100	94	6	100

The field of the farmer was only treated with Flusilazol + BMC on April 8 at GS 31. This resulted in 100% lodging and 5% efficiency.

As SIDERAL is very sensitive to powdery-mildew, on May 17th, at ear emergence stage, propiconazole + fenpropidine was sprayed over all plots.

The trial was harvested in July and yield data are presented in Table 3. Yield losses due to disease were estimated as:

- for eyespot : 1, 5 t/ha
- for foliar diseases : powdery mildew and <u>Septoria</u> = 1, 6 t/ha
- the best treatment : 1 -> + 3, 52 t/ha

TABLE 3. Laverdines yields

Treatment	g AI/ha	Yield (t/ha)	Gain (t/ha)	Test	Weight of 1000 grains
Check 0 treatment		4, 97	-	c	27, 3 g
1 - Prochloraz + Fenbuconazole + Fenpropimorph + Fenpropidine	450 67 280 94	8, 49	+ 3, 52	a	36, 8
2 - Prochloraz + Fenpropimorph + Fenpropidine	450 280 94	7, 72	+ 2, 75	ab	38, 1
3 - Fluzilazole + Fenpropimorph + Fenpropidine + BMC	250 280 94 125	7, 12	+ 2, 15	bc	34, 2
4 - Epoxyconazole	125	6, 85	+ 1, 88	c	35, 0

CV = 6, 1% ETR : 0, 426 t/ha

A second trial was conducted north of Cher at Aubigny on a loamy wet soil with many stones (flints).

For two years, the area had shown many eyespot R types, but in 1993 only W types were found. Analysis of the trial yield data showed a different picture to Laverdines :

- for eyespot : + 0, 34 t/ha
- for powdery-mildew : + 0, 28 t/ha
- for Septoria : + 1, 49 t/ha

There were no differences between treatments

FINANCIAL IMPLICATIONS OF FUNGICIDE RESISTANCE

With the new Common Agricultural Policy, farmers are seeking to decrease their inputs. Table 4 shows the average operating costs to produce wheat.

Table 4. Average operating costs to produce wheat in Cher

Input	Cost F	
	Minimum	Maximum
Seeds : 350 grains/m2	270	370
P.K. : 50 - 50	100	300
N : 160 - 210	350	460
Herbicides black grass + wild oat + broadleaves	100	300
Fungicides : Eyespot Powdery mildew Septoria - Brown rust	400	600
Insecticides (leafhopper)	50	100
TOTAL	1270 f/ha	2130 f/ha

The cost of fungicides is thus around a third of the operating-costs. Fungicide resistance for eyespot and powdery mildew will have the effect of increasing these costs.

For eyespot control, BMC has been ineffective for 10 years and triazoles (flusilazol, bromuconazole, epoxyconazole) have presented resistance for two years Prochloraz remains effective at present but for how long?

To fight powdery mildew, we have to use morpholines on susceptible cultivars, sometimes at the first node stage in the north area.

For a farmer, the cost of resistance is around 60 F/ha. For the total cereal crops of our farmers, the cost is around 4 000 000 F.

In the future, if problems develop with prochloraz, we can use cyprodinil, but today, it's expensive at 300 F/ha and does not have activity on Septoria and rusts. Farmers will not buy this new fungicide in 1994.

DISCUSSION

What advice can we give to the farmers today? We have to preserve the crop potential from diseases and to make each operating cost profitable.

Resistance is a problem, but we can sow different varieties with different host resistance. Last year, we studied mixtures with SOISSONS + SIDERAL and hybrid MESNIL. There was no difference for the cost of fungicides. For 1994, three hybrids and SOISSONS with two different fungicide protections will be tested.

Today, farmers can choose between several wheat varieties to decrease the cost of fungicides as shown in Table 5.

TABLE 5. The susceptibility of wheat varieties to foliar pathogens

Cultivar	Powdery mildew	Septoria	Brown rust	Yellow rust
EUREKA	+	+	-	-
SOISSONS	(-)	+	+ + +	+ +
ORQUAL	+	+	+ +	+ + +
GASCOGNE	-	+	+ +	+
GASPARD	+ +	+	+ +	-
SIDERAL	+ + +	+	+ +	+ +
THESEE	+ +	+ +	+ + +	+ + +
TEXEL	+ +	+ +	+ +	+ + +
TREMIE	+ +	+ +	+ +	+ +
QUALITAL	+ + +	+	+ + +	+ + +

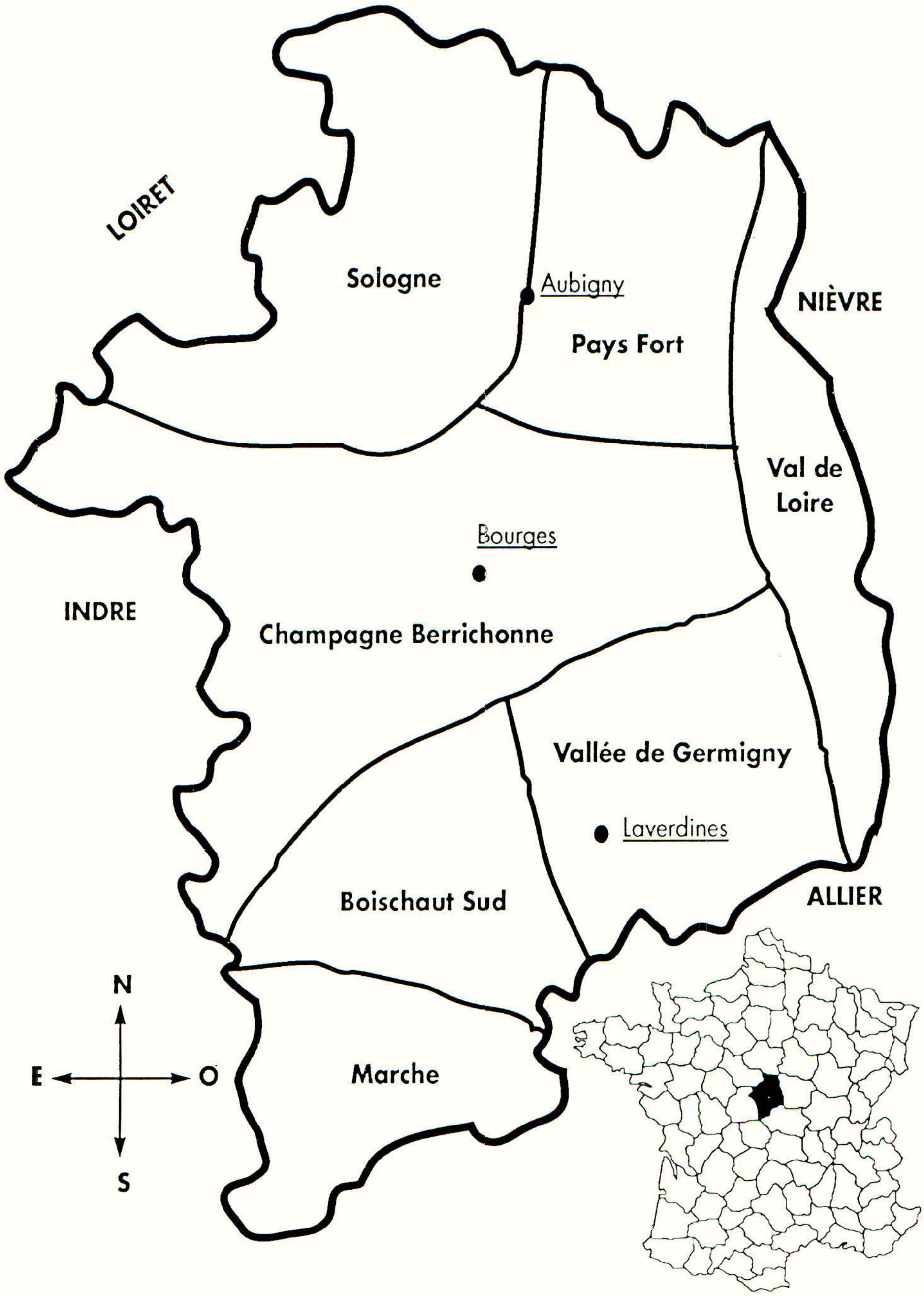
Our advice to farmers is to examine each field weekly in order to detect disease bulid up.

We will use the eyespot diagnostic kit, for the second year : an experimental Septoria kit and the use of forecasting models for Septoria and brown rust. Among the different fungicides, we prefer mixtures of triazoles + morpholines for foliar control and prochloraz 450 g ai/ha for eyespot.

In the future, I think we shall have to use some cyprodinil and maybe ethirimol to preserve some efficiency.

Plant breeders can also improve their varieties, if farmers use more "certified seeds" But it's not the trend today!

Department of Cher - Natural areas



INCIDENCE AND CONTROL OF ORGANO-MERCURY RESISTANT PYRENOPHORA GRAMINEA IN SCOTTISH SPRING BARLEY 1988-1992

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ABSTRACT

Organo-mercury seed treatments effectively controlled barley leaf stripe in Scotland until the mid-80s when the causal fungus P. graminea was reported to be resistant to organo-mercury. By 1990 leaf stripe was widespread in Scottish spring barley. 3454 Scottish spring barley seed samples were tested for P. graminea infection between 1988 and 1992. The percentage of samples infected with P. graminea increased significantly from 1988 to 1990. 26% of certified seed had more than 4% infection in 1990 compared to only 3% in 1988. A voluntary Code of Practice requiring all seed intended for sale to be tested for P. graminea infection was introduced in 1991. Seed with more than 4% infection was to be treated with a seed treatment effective against the disease or discarded. No sample of certified seed failed to meet the 4% standard in 1992. Resistance testing confirmed that all seed infection was caused by organo-mercury resistant strains of P. graminea.

INTRODUCTION

Organo-mercury seed treatments gave good control of barley leaf stripe (Pyrenophora graminea) from their introduction in the 1920s. As a result, the disease was considered to be of little practical importance in the Scottish spring barley crop and was only occasionally seen where untreated or inadequately treated farm-saved seed was sown (Richardson, 1986).

Resistance to organomercury in P. graminea was first reported in the West of Scotland during 1984 where infection was noticed in several fields of Mazurka spring barley grown from one certified seed stock and treated with a commercial organo-mercury fungicide containing phenyl-mercury acetate (PMA) (M R M Clarke, Pers. Comm.). Work by Jones et al. (1989) confirmed the wider occurrence of strains of P. graminea resistant to organo-mercury but concluded that there was no justification to change from the routine use of organo-mercury for leaf stripe control unless seed was being used for further multiplication.

During the summer of 1990 leaf stripe was widespread in the Scottish spring barley crop. Crops grown from both certified and farm-saved seed were apparently equally infected. A voluntary Code of Practice to contain the disease was introduced in January 1991. The Code required all seed intended for sale to be tested for P. graminea infection. Seed with more than 4% infection was required to be treated with a seed treatment effective against the disease or discarded. The

Code was aimed principally at certified seed, but a high proportion of farm-saved spring barley was also tested.

This paper reports the occurrence of seed-borne P. graminea in Scottish spring barley seed from 1988 to 1992 and preliminary work to assess fungicide resistance.

MATERIALS AND METHODS

Seed Testing

The incidence of P. graminea was determined in spring barley seed harvested in each of the 5 years 1988 to 1992. For the years 1988 and 1989, samples were taken at random from seed submitted to the Official Seed Testing Station for Scotland (OSTS) by merchants, for advisory germination testing prior to the processing of seed lots intended for certification, and by growers, who required advisory germination tests on farm-saved seed. Seed harvested between 1990 and 1992 was subject to the Code of Practice and tests were made to meet the requirements of the Code.

Working samples were drawn using a seed sample divider and either 100 seeds (1988 and 1989) or 200 seeds (1990 to 1992) were tested for P. graminea using a standard agar plate procedure (Rennie and Tomlin, 1984).

Seed testing results are reported separately for farm-saved seed and for seed intended for certification.

Resistance Testing

Isolates of P. graminea were obtained from UK barley seed harvested in 1990 and 1991 and from infected plants collected from certification control plots at the Scottish Agricultural Science Agency during the summer of 1993. Seeds were plated on agar as described previously and a small agar plug was taken from the actively growing margins of P. graminea colonies and transferred to fresh potato dextrose agar (PDA) plates. This procedure was repeated until the isolate was in pure culture. Diseased leaves were cut into 2cm sections and were surface sterilised by immersion in a solution of sodium hypochlorite (1-2% available chlorine) for 3 minutes. Leaf sections were drained and placed on PDA plates. Isolates were sub-cultured until a pure culture was produced. Cultures were maintained on PDA at 5°C in the dark. Isolates of P. graminea from Canada and Italy were included as controls.

TABLE 1. Number of isolates of P. graminea screened from 1990 to 1993 and concentrations of active ingredients.

Year	Number of isolates	Range of concentrations per fungicide screened (mg/l)	
		Organo-mercury	Imazalil
1990-91	195	2, 5, 10, 20, 40	None
1992-93	253	None	0.5, 2.5, 5.0

The sensitivity of the P. graminea isolates to organo-mercury and imazalil, was determined by transferring 1cm diameter discs of agar from the margins of actively growing colonies to Petri dishes containing PDA supplemented with concentrations of the fungicides. Concentrations of fungicides and numbers of isolates screened are given in Table 1.

RESULTS

Seed testing

Table 2 shows that P. graminea infection was highest in seed harvested in 1989 and 1990; in these years there was little difference in infection between seed intended for certification and farm-saved seed. Infection levels were considerably reduced in 1991 and 1992 after the introduction of the Code of Practice. No certified seed lot tested in 1992 failed to meet the 4% standard in the Code of Practice.

Table 2. Incidence of P. graminea in Scottish spring barley seed.

Year harvested	Number of samples tested		Percentage of samples infected		Percentage of samples with more than 4% infection	
	Certified	Farm-saved	Certified	Farm-saved	Certified	Farm-saved
1988	32	30	12	20	3	3
1989	53	45	74	64	35	37
1990	1052	953	80	85	26	44
1991	438	519	36	51	2	11
1992	98	234	4	16	0	1

Resistance Testing

All of the 195 P. graminea isolates collected from Scottish spring barley seed harvested in 1990 and 1991 were resistant to organo-mercury at a concentration of 10mg/l in agar. The mean colony growth at this concentration was 52% of the control. In contrast, isolates from Canada and Italy were sensitive to mercury; no growth occurred on agar containing 5mg/l MEMA (2-methoxyethylmercury acetate). Isolates of P. graminea from a single sample of Gaulois winter barley, grown in Lincolnshire in 1991, gave no growth at 10mg/l MEMA and their growth on agar containing 5mg/l MEMA was 20% of the control whereas for mercury resistant isolates 50-90% growth was recorded at this concentration.

None of the 253 isolates tested in 1993 was resistant to imazalil. No isolate grew on agar containing 5mg/l imazalil and in each case growth was inhibited by 90% or more in agar containing 2.5mg/l. The target dose for imazalil in commercial barley seed is approximately 40mg/l. There have been no records of leaf stripe in Scottish spring barley crops grown from imazalil treated seed.

DISCUSSION

Although P. graminea resistance to organo-mercury was reported during the 1980s, seed treatments based on organo-mercury continued to be used and in 1990 a high percentage of Scottish spring barley seed lots was infected. The incidence of infection was similar for certified and farm-saved seed harvested in 1989 and 1990. P. graminea infection increased from 1988 to 1990 and resistance testing confirmed that this seed infection was attributed to organo-mercury resistant strains of P. graminea.

The introduction of a voluntary Code of Practice, agreed between the seed trade and Scottish growers, required barley seed that was to be certified in 1991, and in subsequent years, to be tested for P. graminea infection. Where infection was greater than 4% seed stocks were required to be treated with a fungicide effective against P. graminea or discarded. The resultant seed crop in 1991 showed an increase in the percentage of certified and farm-saved samples that met the 4% standard. The continued requirement for testing under the Code and the increased use of effective treatments, especially those containing imazalil, resulted in a significant reduction in P. graminea infection from 1990 to 1992 when no stock of certified seed failed to meet the voluntary standard.

A number of commercially available cereal seed treatments include imazalil to control specifically P. graminea on barley. The results from resistance testing during 1993 showed that P. graminea isolates from Scottish spring barley were very sensitive to imazalil.

Seed-borne diseases such as leaf stripe, loose smut and bunt have the potential to multiply rapidly within a few seasons. Monitoring disease incidence and the sensitivity of pathogens to active ingredients in commercial fungicides will help to avoid the unacceptable levels of disease seen in the Scottish spring barley crop in 1990.

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REFERENCES

Jones, D. R.; Slade, M. D.; Briks, K. A. (1989) Resistance to organo-mercury in Pyrenophora graminea. Plant Pathology, **38**, 509-513.

Rennie, W. J.; Tomlin, M. M. (1984) Barley leaf stripe working sheet No 6 (2 Ed) ISTA Handbook on Seed Health Testing, Zurich.

Richardson, M. J. (1986) An assessment of the need for routine use of organo-mercurial cereal seed treatment fungicides. Field Crops Research, **13**, 3-24.