EXPERIENCE FROM PRACTICE

Mixing or alternating fungicides

A long-term field trial has been conducted by BASF with different spray regimes (mixtures and alternations) of fenpropimorph and triadimenol, in order to obtain information for possible resistance strategies (Lorenz et al., 1992). "Data .... indicate that triazole/morpholine combinations and alternations are equally well suited to avoid a shift in fenpropimorph sensitivity."

In the year 1988 field trials were performed in 'Caribo' winter wheat with triadimenol, tridemorph and a mixture of triadimenol&tridemorph. The 'triadimenol&tridemorph' mixture was much more effective in controlling disease than the alternation 'tridemorph/triadimenol/tridemorph', which in turn was more effective than triadimenol. There was hardly any difference between the sensitivity to triadimenol of mildew isolates from the mixture plot and mildew isolates from the triadimenol treated plot. Mildew from the alternating spray sequence tended to be more sensitive to triadimenol, as the last treatment was performed with tridemorph. However, the individual plots showed different levels of residual infection.

In 1989, field trials were performed in 'Caribo' winter wheat with tebuconazole and a mixture of tebuconazole&tridemorph. After 2 treatments, the efficacy of the mixture was far superior to that of tebuconazole. Mildew isolates from all plots, including the control, showed only minimal differences in their sensitivity to tebuconazole.

In 1992, field trials were carried out in 'Kanzler' and 'Apollo' winter wheat with tebuconazole, fenpropimorph and a mixture of tebuconazole+fenpropimorph (tank mix).
In Kanzler, no clear differences in efficacy were detectable on account of heavy infection pressure although disease control of the mixture was slightly better. At the end of the season, sensitivity to fenpropimorph was greater in isolates from the mixture plot than in isolates from the fenpropimorph treated plot.
In Apollo, the mixture was much more effective in disease control than the single compounds. Sensitivity to tebuconazole was greater in isolates from the mixture plot than in isolates from the tebuconazole treated plot.

In 1993, field trials were performed in 'Kanzler' winter wheat with tebuconazole, fenpropimorph and a mixture of tebuconazole+ fenpropimorph. The mixture was slightly more effective in disease control than tebuconazole and fenpropimorph. At the end of the season, and with similar performance of the different fungicides, mildew isolates from the mixture plot were as sensitive to tebuconazole and much more sensitive to fenpropimorph than isolates from the tebuconazole and fenpropimorph treated plots respectively.

Our conclusions from the studies mentioned above are that mixtures of azoles and morpholines can positively influence both field performance and changes in sensitivity with respect to the individual mixture components.

Splitting and reducing application rates

A proposed control strategy is to reduce the rate at which a fungicide is applied and to apply it at more frequent intervals (splitting doses).

The next step would then be to dispense with one or more of the applications in order to reduce the amount of fungicide used. In Denmark, spray models of this kind are sought and supported by both the public and politicians. Jørgensen and Nielsen (1992) have examined this topic thoroughly, and pointed out both the benefits of such programmes and the problems and risks of incorrect use. Spray programmes using lower application rates were also advocated in Germany (Bosse et al., 1991; Schönberger et al., 1993).

In 1992, field trials were carried out in 'Kanzler' and 'Apollo' winter wheat. A strict spraying regime using 2x1/1, 4x1/2 and 8x1/4 of the application rates of tebuconazole+ fenpropimorph, tebuconazole and fenpropimorph was demanded. The quantities of fungicide applied were thus split, but not reduced.
The available data demonstrate no negative influence of a split application rate on the fungicide performance. The crucial point is the correct timing of the sprays.
No general trend to reduced sensitivities could be proven when reduced rates were applied. The frequency distributions of the sensitivities seemed to have been mainly influenced by the fungicide performance. Good performance left a small amount of less sensitive mildew isolates surviving. Poor performance left a mixture of sensitive and less sensitive isolates.

In 1993, field trials were performed in 'Kanzler' winter wheat. No strict spraying regime was followed. The fungicides tebuconazole+fenpropimorph, tebuconazole or fenpropimorph at rates of 1/1, 1/2, 1/4 and 1/8 were applied whenever the field evaluation found 3-5% living mildew. The application rates were thus split and at the same time reduced, if by the end of the spraying period the total amount of active ingredient used did not reach the amount used at the full application rate.

Reduced rates, applied with adequate timing, were able to control the powdery mildew as well as full rates. This holds for all the three fungicides: tebuconazole+fenpropimorph, tebuconazole, fenpropimorph. However, there is a limit for the splitting and reduction of rates. Below this limit, sprays are not feasible due to 'no acceptable efficacy'.

In general, shifts in sensitivity to tebuconazole and fenpropimorph were surprisingly limited. The performance of a fungicide programme, independent of the amount of AI applied, seems to be the critical factor determining sensitivity changes in a population. The better the performance the higher the proportion of less sensitive strains which survived. If performance decreased after long spray intervals, even with high dose rates, populations could have a higher proportion of more sensitive strains at the end of the programme. The mixture of azole and morpholine influenced the sensitivity to both compounds positively, i.e. the mildew populations became more sensitive.

In conclusion, where frequent application of reduced rates leads to good performance, a high and continuous selection pressure is maintained, which may negatively influence the sensitivty of mildew to the fungicide in question.

Choosing and mixing varieties

It has been known for quite some time that not only the choice of the mildew fungicide but also the choice of the crop variety contributes decisively to the preservation of crop health (Wolfe, 1984; Schaffner et al., 1992). The idea that the efficacy of the mildew fungicides can and should be supported by the aid of cereal varieties with effective resistance genes has been discussed in various countries, and proposals for its practical realization have been made (Wolfe, 1985; Frahm, 1986).

One should expect neither "permanently effective" fungicides nor "permanently effective" varietal resistances. A mildew population reacts to each and every selection pressure to which it is exposed. Fungicides and crop varieties select from the population those strains which withstand the selection pressure. It is therefore likely that a small fraction of the population will always survive.

For agricultural practice, then, it is important to be able to choose relatively resistant crop varieties which, together with the necessary fungicidal applications, would give optimal yields (Schulz and Lein, 1993). This would also be in line with the Integrated Crop Management model, which takes account of various cultivation parameters, combining them in an appropriate way.

CONCLUSIONS

All experience from research and practice with mildew fungicides in cereals can be summarized in the following recommendations, formulated by FRAC's SBI Working group in 1993 (FRAC, 1994):

"Repeated applications of DMI or 'morpholine' fungicides alone should not be used on the same crop in one season against a high risk pathogen (eg. cereal powdery mildew) in areas of high disease pressure for that particular pathogen. Split/reduced rate programmes using repeated applications which provide continuous selection pressure should be avoided."
"For control of cereal powdery mildews, mixtures or alternation of a DMI with a 'morpholine' fungicide represent the best currently available none cross-resistant combination."
"Fungicide input is only one part of crop management. Fungicide use does not replace the need for resistant crop varieties, good agronomic practice, plant hygiene/sanitation, etc..."

Breeders and manufacturers of crop protection products are here jointly called upon to provide concepts for agricultural practice which take account of all the important parameters and combine them in an appropriate way within the context of Integrated Crop Management. The aim is to put a system at the grower's disposal which is as stable as possible, and the more complex its composition the more stable it will be.

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EVALUATING ANTI-RESISTANCE STRATEGIES FOR CONTROL OF *UNCINULA NECATOR*

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ABSTRACT

Since 1989, different strategies of treatment (mixtures, alternations) have been compared to cope with *Uncinula necator* resistance to sterol demethylation inhibitors in French vineyards. After treatments with DMI fungicides, populations show at first a progressive evolution with an increase in the proportion of less sensitive phenotypes, followed eventually by the appearance of resistant phenotypes. Low application rate of triadimenol (18,75 g/ha) results in rapid reduction in the population sensitivity compared to the recommended rate (37,5 g/ha). Mixtures containing triadimenol and sulphur do not slow down the evolution of resistant spores in natural populations. Reduction of the number of treatments, and use of sulphur are the only strategies helpful in slowing down the evolution of resistant phenotypes and keeping the disease under control.

INTRODUCTION

Resistance of grape powdery mildew to fungicides that inhibit the C-14 demethylation of sterols (DMI) was discovered for the first time in 1988 near Lisbon, Portugal (Steva et al, 1988) after reduction in efficacy of these fungicides in the vineyard. Since then, resistant strains have been identified in France (Steva et al, 1989) and in Italy (Aloi et al, 1990). In spite of this fact, DMI fungicides were still effective in the majority of situations in recent years. This normal performance should not lead one to believe there was no risk that resistant strains would develop in the field. To define this risk and develop treatment strategies that will limit the emergence and multiplication of resistant strains, it was necessary to compare different programs of treatment.

The purpose of our research was to observe any changes within a mildew population (*Uncinula necator* (Schw.) Burr.) as a result of different fungicide pressures during four years (1989 to 1992). Exclusive sprayings of DMI fungicide during the season were compared to an alternation or a mixture of DMI and sulphur and DMI and dinocap.

METHOD TO ASSESS THE SENSITIVITY OF *U. NECATOR* TO DMI FUNGICIDES

Since *U. necator* is an obligate fungus, it has to be grown on plant material. Our method is based on the use of leaf discs maintained on agar medium and observation of an individual hyphal growth .

Leaf disc test

The sensitivity of conidia of *U. necator* to triadimenol was evaluated using a test on leaf discs kept alive on a water agar medium (20 g/l) amended with benzimidazole (30 mg/l). The discs (18 mm diameter) were punched out from grape leaves (cv. Cinsaut) and disinfected for 10 minutes in a solution of calcium hypochoride (50 g/l). The upper surface of the leaf disks was placed in contact with filter paper imbibed with 3 ml of triadimenol (Baytan 5, 50 g/l provided by Bayer France, Paris). The range of fungicide concentrations was as follows : 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mg/l. After 24 hours of incubation at 20°C, the discs were transferred onto water agar medium. The upper surfaces of the leaf discs were powdered with spores by placing the Petri dishes at the base of a settling tower (0.09 m^2 and 0.6 m high). The Petri dishes were placed in a culture chamber at 21±1°C with 16 hours light per day at an intensity of 25 $\mu E.m^{-2}.s^{-1}$.

Observation of hyphal growth

Since *U. necator* is an ectoparasite, hyphae were removed after 3 days of incubation by touching the upper surface of the inoculated leaf disc with Scotch tape. The tape was then stuck to a microscope slide on a drop of cotton blue stain. Hyphae were observed and measured using a light microscope at 100 magnification with a binocular micrometer.

Determination of population sensitivity

Preliminary tests with sensitive and resistant strains and different concentrations of triadimenol showed that measurement of hyphal length allows discrimination between sensitive and resistant spores at a given concentration of fungicide. Therefore, under our experimental conditions we determine that:

- Conidia are sensitive to the concentration of triadimenol when hyphal length is less than 250 µm (hyphae will not form conidiophores and secondary conidia);
- Conidia are resistant to the same concentration when hyphal length is more than 250 µm.

Using this simple measurement, it is possible to calculate, for a given population of spores exposed to a range of concentrations of triadimenol, the percentage of sensitive and resistant spores at each concentration.

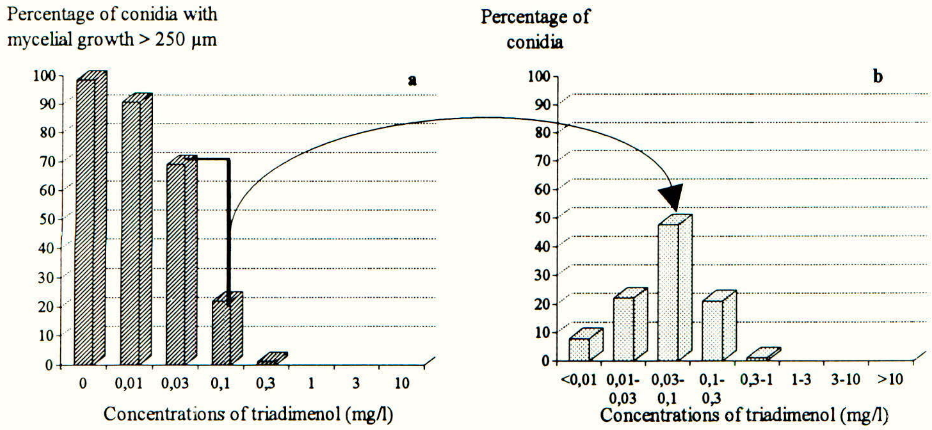

Figure 1. Representation in the case of *Uncinula necator* resistance to DMI fungicides of population sensitivity. **a** : percentage of conidia in each dose witch mycelial growth is superior to 250 µm; **b** : percentage of conidia witch sensitivity is comprise between two concentrations.

Figure 1 illustrates the process used to evaluate the population sensitivity. This population was isolated in 1989 in the south of France, from a DMI-untreated vineyard plot. Figure 1a shows for each concentration the percentage of conidia with hyphal length greater than 250 µm. For example, this value is 70% for the 0.03 mg/l concentration and 20% for the 0.1mg/l concentration. The difference between the percentages obtained for two consecutive concentrations of triadimenol is showed in figure 1b. Each bar represents the percentage of spores that grows on the lower concentration but are killed on the upper dose.

This example illustrates the variation in sensitivity that exists among a wild population of conidia of *U. necator*.

FIELD TRIALS

Triadimenol sensitivity evolution of sulphur (10 000 g AI/ha) or DMI (triadimenol, 37.5 g AI/ha) sprayed populations was studied using different experimental designs. Our goal was to define the optimal plot size to avoid natural external contamination. These results are described as follows.

Experimental design

Experiments were conducted on cv. Carignane vines planted with 1.5 m between plants in the row and 2 m between rows. Sulphur and triadimenol were each sprayed 6 times per year for 4 years on a 500 m² plot of 5 rows, each with 40 vine plants. There was no replication and rows were planted in the prevailing wind direction.

Sampling

Infected material (30 leaves or bunches) was harvested only on the central row. This material was wrapped in healthy leaves and stored at 20-25°C. When received at the laboratory, samples were directly inoculated onto leaf discs treated with a range of triadimenol concentrations. Population sensitivity was then determined as described previously.

Population sensitivity evolution

Figure 2 shows evolution of the population sensitivity in 1992 on plots located in Narbonne (France), and sprayed since 1989 with sulphur and DMI. Three samplings were conducted during the season (1 per month starting in May).

On the sulphur sprayed plot, the population was still sensitive and no evolution during the season was observed (figure 2a). At the last sampling, only a slight increase was observed in the percentage of spores with sensitivities between 0.1 and 0.3 mg/l of triadimenol.

On the DMI sprayed plot, two less-sensitive phenotypes were identified at the first date of sampling (figure 2b). These grew on leaf discs treated with 0.3 mg/l of triadimenol but were killed by 3 mg/l. At the last observation, a resistant sub-population appeared. Sixty percent of spores had a sensitivity within the 0.1 and 0.3 mg/l range. Few spores (< 2%) survived at the 10 mg/l concentration.

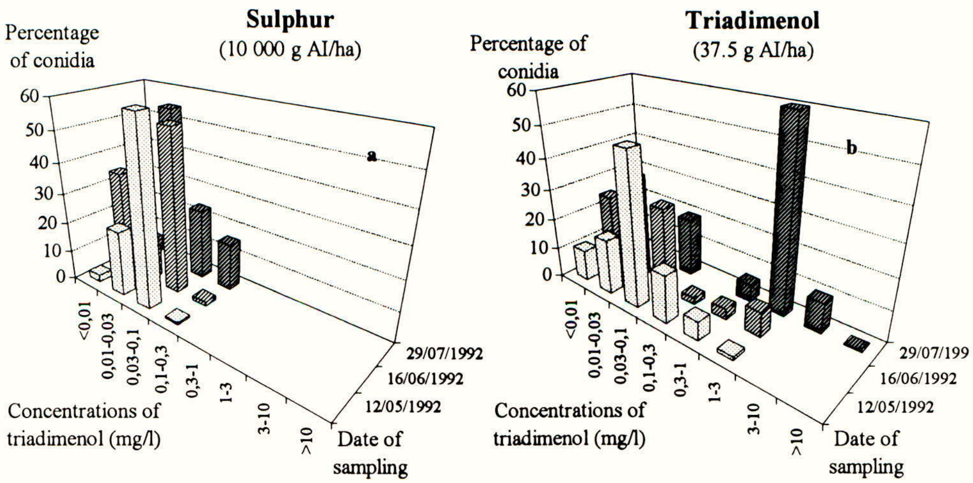

Figure 2. Evolution of *Uncinula necator* population sensitivity to triadimenol during the 1992 season in a vineyard located in France (Narbonne) after 4 years of treatments (6 per year) with : **a**. sulphur (10 000 g AI/ha); **b**. triadimenol (37.5 g AI/ha)

This example demonstrates that such a design allows the precise survey of the selective effect of a DMI fungicide at the vineyard level. Large scale field trials and determination of population sensitivity as described comprise an accurate approach for evaluating the selective effect of different anti-resistance strategies for the control of *U. necator*.

EVALUATION OF ANTI-RESISTANCE STRATEGIES

There are two main rules when applying an anti-resistance strategy :

- the selective effect should be as low as possible;
- the efficacy should be as high as possible, even on the resistant populations.

To define such optimal conditions, the effects of dose, mixtures and reduction of the number of DMI application have been studied in different field trials.

Selective effects

Programs of treatment

Various programs were compared in practice:
- standard non selective program: sulphur (10 000 g AI/ha)
- DMI program: triadimenol (37.5 g AI/ha);
- half rate DMI: triadimenol (18.75 g AI/ha);
- mixtures: triadimenol+sulphur (25+4000 g AI/ha) or triadimenol+dinocap (25+105 g AI/ha);
- alternation of DMI with non selective compound: triadimenol (37.5 gAI/ha) / sulphur (10000 g AI/ha) or triadimenol (37.5 g AI/ha) / dinocap (210 g AI/ha).

Field experiments

Two experiments have been carried out in the south of France (Narbonne and Perpignan) on Carignane vineyard. Each year, a high frequency of flag shoots was observed after bud burst. Programs were compared according to the previously described experimental design (500 m² plot, sampling on central row). A total of 6 sprayis was applied each year for four consecutive years on the same plot. Disease pressure was assessed each year at the end of the season and 3 samples were taken : first before spraying, second at the flowering stage, third after the last spray and before véraison.

Evolution of the sensitivity of the populations in different plots

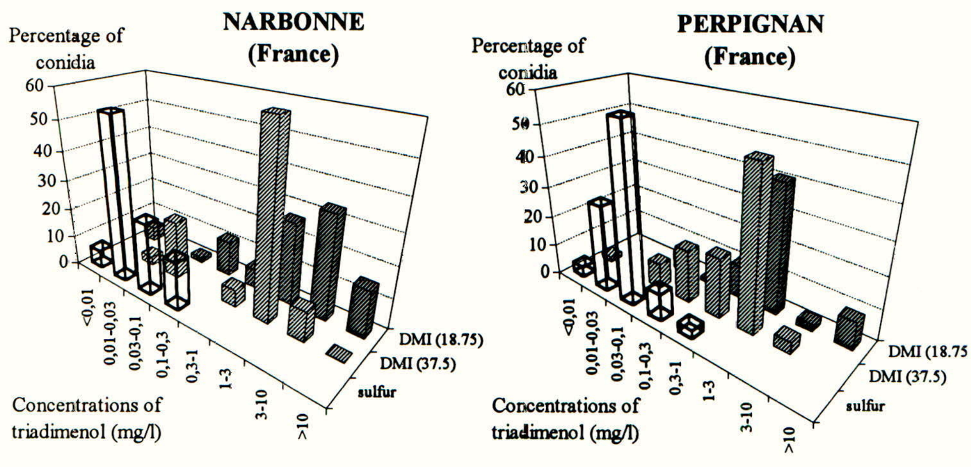

Figure 3. Sensitivity to triadimenol of *Uncinula necator* populations treated for 4 years (6 treatments per year) with sulfur (10 000 g AI/ha); a registered rate of triadimenol (37.5 g AI/ha) and a half rate of triadimenol (18.75 g AI/ha) in two french vineyards in Narbonne and Perpignan, populations sampled after the last spray

On sulphur treated plots, the population was still sensitive (figure 3). There was a slight difference between Perpignan and Narbonne locations. In Narbonne, spore growth was inhibited by 0.3 mg/l, in Perpignan this dose is higher: 1 mg/l of fungicide. There was no significant shift in comparison with the population from the beginning of the experiment before treatements wee applied.

On DMI sprayed plots (triadimenol, 37.5 g AI/ha), the most sensitive phenotypes disappeared. The populations showed a shift towards increasing levels of resistance. The MIC value in Perpignan is higher than 3 mg/l and 10 mg/l in Narbonne (figure 3).

On *half rate DMI* sprayed plots (triadimenol 18.75 g AI/ha), shift of sensitivity was greater than that observed with the population sprayed with the full concentration of triadimenol. In Narbonne (figure 3), the percentage of very resistant (> 10 mg/l) and resistant (3-10 mg/l) phenotypes reached 20% and 34% of the total respectively. In Perpignan (figure 3), phenotypes resistant to 10 mg/l were also detected but at lower frequencies.

Mixtures. Sulphur and dinocap, two surface fungicides were mixed with triadimenol at 25 g AI/ha.

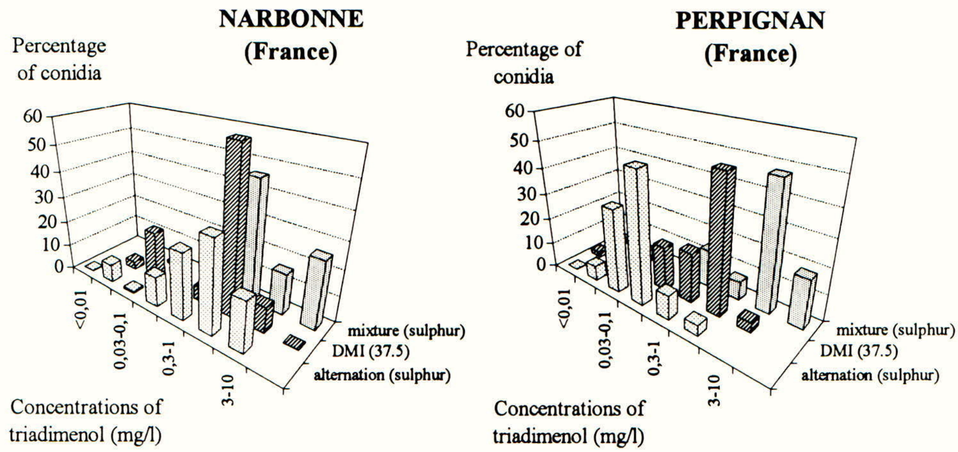

Figure 4. Sensitivity to triadimenol of *Uncinula necator* populations treated during 4 years (6 treatments per year) with triadimenol (37.5 g AI/ha), an alternation of triadimenol (37.5 g AI/ha) and sulphur (10000 g AI/ha) and a mixture of triadimenol+sulphur (25+4000 g AI/ha) in two french vineyards in Narbonne and Perpignan.

With sulphur (figure 4), we notice a strong selective effect of the mixture. The shift in sensitivity was greater than with triadimenol alone at its registered dose (37.5 g AI/ha).

The percentage of spores growing on leaf discs treated with 3 mg/l was 50% in Narbonne and 70% in Perpignan. In both cases, the most sensitive part of the population disappeared.

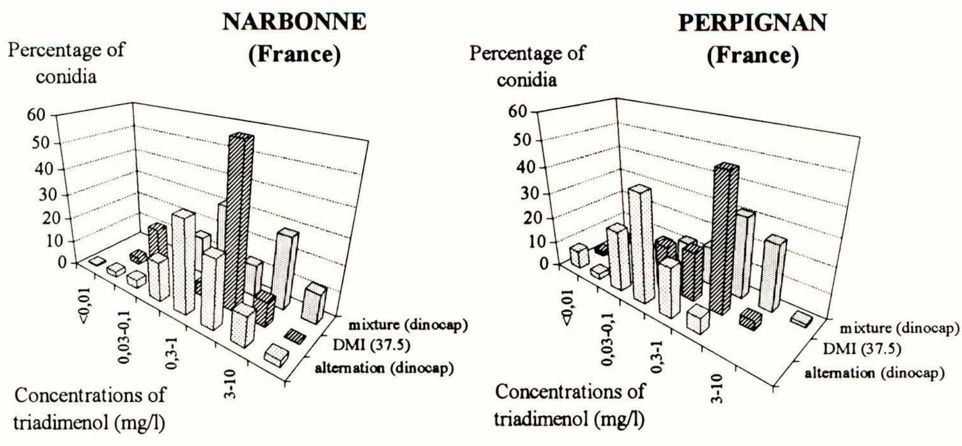

Figure 5. Sensitivity to triadimenol of *Uncinula necator* populations treated for 4 years (6 treatments per year) with triadimenol (37.5 g AI/ha), an alternation of triadimenol (37.5 g AI/ha) and dinocap (210 g AI/ha) and a mixture of triadimenol+dinocap (25+105 g AI/ha) in two french vineyards in Narbonne and Perpignan.

The selective effect of dinocap was stronger than triadimenol alone but remained lower than that of the sulphur mixture. In Narbonne (figure 5), there was a bimodal distribution of two (sensitive and resistant) subpopulations. In Perpignan, spores tolerant to 10 mg/l were detected at low frequency only in DMI+dinocap sprayed plots (figure 5).

Alternation. We tested an alternation spray progrmme where DMI and contact fungicides alternated at each application. The first spraying was a DMI and there were no more than 3 triadimenol treatments during the season. In this case, there was a generally slower population shift. However, we can notice slight differences within plots and compounds. With sulphur alternations (figure 4), in both locations the population shift is lower than with the DMI alone. In Narbonne, the MIC values for the alternation and exclusive DMI application were respectively lower and higher than 10 mg/l. With dinocap alternations (figure 5), when compared with DMI used alone, the population shift was less in Perpignan (figure 5) but the same in Narbonne (MIC > 10 mg/l).

Evaluation of efficacy

Our contention is that an anti-resistance strategy has to maintain constant efficacy, even when applied to resistant populations and after many years of application. We tried to highlight two specific and important points:

- efficacy should be as high as possible whatever the population sensitivity and disease pressure ;
- efficacy should be preserved over time.

To address these two points, two kinds of experiments have been carried out.

Similar trials were set up in different countries (France, Portugal, Italy) and on different sensitive vine cultivars (Cardinal, Carignane, Gros Manseing). The experimental design was the block type with three or four replicates and contiguous untreated control. Disease pressure on bunches was assessed after the last treatment.

Table 1 shows rates of diseases for three experiments in the south of France. In each location three application schedules were compared. High level of disease occured when triadimenol was exclusively used. This result illustrated the loss of efficacy of DMI fungicides in practice. Disease pressure was still strong when triadimenol was reduced to three consecutive sprays around the flowering stage. The most efficient results were obtained when two triadimenol applications were alternated with two applications of powdered sulphur. Statistical analysis between strategies was the same for all the locations. However, the efficacy level varies with location, probably because of different initial population sensitivity and disease pressure.

Table 1. Percentage of powdery mildew disease on bunches at the end of the season after treatments with different strategies in an experiment conducted by the CNPPA during three years at the same location in **Portugal** (Azambuja).

Programs of treatment									Percentages of disease in each year		
			*Flo***			*Bt*		*Vé*	**1989**	**1990**	**1991**
T*	T		T	T	T	T	T	T	85.4 d***	46.1 d	66.9 d
Sp			Sp			Sp			12.1 b	2.2 a	1.9 a
Sp	Sm	Sm	Sp	T	T	Sp	T	T	5.5 a	6.4 b	14.2 bc
D	D	D	D	T	T	D	T	T	22.2 c	17.8 c	21.3 c
Untreated									***100.0***	***83.5***	***74.2***

*Treatments : **T**. triadimenol (50 g AI/ha), **Sm**. wettable sulphur (10000 g AI/ha), **Sp**. powder sulfur (30000 g AI/ha), **D**. dinocap (210 g AI/ha).

Phenological stages : ***Flo* . flowering, ***Bt*** . berry touch, ***Vé*** . véraison.

***Means followed by the same letter are not significantly different according to the Newman and Keuls test after analysis of variance.

Table 2 illustrates the evolution of disease severity during three years at the same location (Azambuja, Portugal). When triadimenol alone was exclusively sprayed, disease severity was the same as the untreated control every year, except in 1990. When two sets of two consecutive triadimenol spraying are alternated with powder sulphur the damage increases between 1990 and 1991. Data from population sensitivity (STEVA, 1992) demonstrated that was the result of the selective effect of this strategy.

Table 2. Percentage of powdery mildew disease on bunches at the end of the season after treatments with different strategies in field trials conducted by VIVADOUR during two years (1991 and 1992) in three locations in the South West of **France**.

Programs of treatment							Percentages of disease in each year		
*Flo***			*Bt*			*Vé*	**Viella**	**Labarthète**	**Monpezat**
T*	T	T	T	T	T	T	59.9 c***	90.3 c	62.3 c
Sm	T	T	T	Sm	Sm	Sm	42.2 b	78.9 b	43.7 b
Sp	T	Sp	T	Sp	Sm	Sm	15.5 a	37.1 a	14.9 a
Untreated							*63.2*	*94.9*	*93.5*

*Treatments : **T**. triadimenol (37.5 g AI/ha), **Sm**. wettable sulphur (10000 g AI/ha), **Sp**. powdered sulphur (30000 g AI/ha).

Phenological stages : ***Flo* . flowering, ***Bt*** . berry touch, ***Vé*** . véraison.

*** Means followed by the same letter in the same column are not significantly different according to the Newman and Keuls test after analysis of variance.

CONCLUSION

The objectives of these studies were to describe the steps we took to assess the effect of different strategies of coping with DMI fungicide resistance in *U. necator*.

Considering the aerial dispersal characteristic of this obligate fungus, and the progressive nature of resistance, it was absolutely necessary to produce :

- a precise method for analyzing population sensitivity.
- an experimental design that takes into account the epidemiology of the grape powdery mildew.

Once such parameters have been defined, we could study the advantages of different strategies in various French vineyards. Four years of successive treatments led us to these conclusions.

On the vineyard scale, DMI fungicides had a strong selective effect on natural populations. As a first step, this does not necessarily lead to loss of field efficacy. Not only the number of sprayis but the DMI rate per hectare can induce a selective effect. Half-rates were more effective in selecting less sensitive populations than full registered doses.

Fungicide mixtures of DMIs with sulphur or dinocap were not an effective anti-resistance strategy in the case of *U. necator*. With sulphur, such results can be explained by an antagonism with DMI fungicides (STEVA, 1992).

When compared with exclusive DMI spraying, application schedules where DMI and classical compounds (sulphur and dinocap) alternate, showed good ability to slow down the resistance evolution process. This result can be easily explained by the reduction of DMI treatments.

One of the main questions that still needs to be answered concerns the acceptable maximum number of DMI applications and their period of spraying during the season, in relation to the existing sensistivity structure of the population to be treated.

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