# Grapevine breeding for resistance to powdery mildew: Bioassay system for evaluation of plant resistance and for characterization of different *Uncinula necator* strains

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Summary: Several isolates of *Uncinula necator* were separated and kept *in vitro*. The pathogenicity of these isolates was compared by a bioassay system using small leaves issued from *in vitro* plants; 2 $\mu$l of spore suspension was inoculated on these leaves.

Significative differences in sporulation time, aggressiveness, sporulation rate and resistance to fungicide triadimenol were observed between these isolates. Host plant variety also affects some of these characters of pathogenicity.

The isolates were classified into 2 mating types concerning the aspect of perithecia formation by paired combination between 2 isolates. Productivity of perithecia varied in response to the combination of isolates and to host plant variety.

Key words: oidium, variety of vine, resistance, biotype, fungicide resistance, perithecium, bioassay, breeding.

## Introduction

Powdery mildew, caused by *Uncinula necator*, is one of the most important diseases of grapevines. Susceptibility of cultivars to powdery mildew has been studied by various authors (BOUBALS 1961; POSPISILOVA 1978; DOSTER and SCHNATHORST 1985), however the levels of susceptibility reported for a given variety are not equivalent. These discrepancies could be ascribed to different causes namely the climatic conditions, the physiology of host plants and pathogens. DELP (1954), DOSTER and SCHNATHORST (1985) studied the effects of leaf maturity on development of powdery mildew and suggested that cultivar susceptibility should be compared on young leaves. BAVARESCO and EIBACH (1987) observed the influence of nitrogen fertilization on resistance to powdery mildew. Therefore studies on susceptibility should be done with stable physiological conditions of host plant under the controlled environments.

*U. necator* is an obligate parasite which requires a living host plant to be maintained, so that the studies on powdery mildew are difficult to be carried out in the laboratory. For this kind of obligate parasite, dual culture *in vitro* of the pathogen and its host can provide an excellent system for studying pathogen races and host-pathogen interaction. MOREL (1948) realized dual culture of downy mildew and callus tissue of grapevines and also tried for powdery mildew. Owing to successful procedures for propagation techniques of grapevines *in vitro* (GALZY 1969; BARLASS and SKENE 1978; HARRIS and STEVENSON 1982), sterile shoot culture provides a desirable system for inoculation.

Recently, LEE and WICKS (1982) applied dual culture for evaluation of systemic fungicide treatment against downy mildew and BARLASS *et al.* (1986) developed it for screening grapevines for resistance to downy mildew. ALDWINCKLE and BUTURAC (1980), KLEMPKA *et al.* (1984) established dual cultures of powdery mildew and several cultivars of grapevines. Thus dual culture would be useful for various investigations on obligate parasites under laboratory conditions.

Perithecia are observed in many viticultural regions but their formation is very erratic under natural conditions (BERNARD and MUR 1986; MAGAREY and WICKS 1986; DIEHL and HEINTZ 1987). PEARSON and GADOURY (1987) demonstrated that perithecia are the source of primary

infection in New York. But in other regions overwintering of mycelium in the dormant bud is considered as a primary inoculum (BULIT and LAFON 1978; VAN DER SPUY and MATTHEE 1977; SALL and WRYSINSKY 1982; BERNARD 1985) and till now the sexual reproduction cycle of powdery mildew is not well known.

This paper describes an *in vitro* inoculation procedure and assay system for *U. necator*, and a characterization of several isolates by their aggressiveness, their resistance to systemic fungicides and their heterothallic properties.

## Materials and methods

### 1. Dual culture of grapevine and powdery mildew *in vitro*

One-node cuttings of grapevine cultivars Cinsaut and Muscadelle were propagated by tissue culture method (GALZY 1969) and maintained by subculturing them every 3-4 months. Isolates of various geographical origin were obtained by collecting conidia from young colonies and inoculating them onto sterile Cinsaut plants *in vitro*. Conidia of each isolate were transferred onto sterile plants at 3-4 weeks intervals.

### 2. Bioassay system

Host plants: Sterile shoots of Cinsaut and Muscadelle were divided into one-node cuttings including the leaf. These systems of leaf-petiole-stem were planted in Petri dishes containing agar medium (1.5 % agar in distilled water).

Preparation of inoculum as spore suspension: Some colonies (3 weeks old) were washed in sterile distilled water containing Tween 20 in order to remove conidia from conidiophore into washing liquid which was centrifuged twice. The concentration of spores was adjusted to a level of $3 \times 10^4$ conidia/ml.

Inoculation: One drop (2 $\mu$l) of the spore suspension was inoculated onto each detached leaf with a micro-pipette. Sterile filter paper (5 mm x 5 mm) was put on the drop to absorb water, and was removed 4 d later. Petri dishes were sealed and incubated in a chamber (26-27 °C during 16 h illumination, 20-22 °C during 8 h dark).

### 3. Characterization of isolates of powdery mildew

a) Development of sporulation

Time from inoculation to sporulation and growth of colony diameter were used as criteria for the comparison between several isolates on Cinsaut and Muscadelle leaves *in vitro*. Observations were made at 24 h intervals. Number of conidia produced were estimated 3 weeks after inoculation for average size of colony. Conidia were collected in accordance with the method of preparation of inoculum described above. Estimation of conidia number was based on the numerical values after calculation with bacterial counter.

b) Resistance to triadimenol fungicide

A study was performed to determine resistance of isolates to the systemic fungicide triadimenol which was added to the agar medium at concentrations of 5 mg/l, 0.5 mg/l and 0.05 mg/l. Several isolates (Bordeaux, Greece and 2 from Portugal, the latter supplied by Plant Pathology Research Station of INRA-Bordeaux) were inoculated onto Cinsaut leaves. Observations were made on colony growth according to the method of DESAYMARD (1968).

Table 1: The number of days from inoculation to sporulation for 11 isolates of powdery mildew on two host cultivars Cinsaut and Muscadelle

| Isolate | Number of days | |
|---|---|---|
| | Cinsaut | Muscadelle |
| Greece M-1 | 5,33 ± 0,50(1) | 6,47 ± 0,41 |
| Greece M-2 | 5,22 ± 0,32 | 8,20 ± 1,57 |
| Azambuja | 5,47 ± 0,35 | 7,38 ± 0,89 |
| Montemor | 5,17 ± 0,19 | 6,81 ± 0,49 |
| Dijon | 6,61 ± 0,52 | 8,06 ± 0,31 |
| Montpellier | 5,88 ± 0,54 | 6,56 ± 0,58 |
| Italy | 5,22 ± 0,32 | 6,50 ± 0,74 |
| Switzerland | 4,71 ± 0,40 | 5,78 ± 0,51 |
| Bordeaux 86 M-2 | 5,95 ± 0,42 | 7,64 ± 1,13 |
| Bordeaux 86 M-3 | 6,15 ± 0,48 | 7,44 ± 0,80 |
| Bordeaux 87 | 7,29 ± 1,03 | 10,22 ± 1,95 |
| Total | 5,64 ± 0,15 | 7,28 ± 0,28 |

(1) Standard error of the mean

c) Aspects of perithecia formation

Determination of mating types: 5 clonal isolates were obtained by separation of single spores with a small fragment of sterile razor. These single spore clones were multiplied and maintained on Cinsaut leaves. Paired combinations were made by inoculating 2 of them on the same leaf of Cinsaut. Inoculation was carried out by dusting conidia onto the system of detached leaves described before. Inoculated leaves were incubated in a chamber for 4 weeks and the presence of perithecia was observed.

Effect of cultivars on perithecia productivity: 2 isolates of opposite mating types (from Greece and Bordeaux) were inoculated on Cinsaut and Muscadelle leaves with a mixed spore suspension (2 $\mu$l). Detached leaves were planted in Petri dishes and were incubated in chamber for 4 weeks. Percentage of colonies which induced perithecia formation and number of perithecia with yellow to brown color were observed periodically.

## Results and discussion

### 1. Development of sporulation

Times from inoculation to sporulation varied according to isolates and to host plant cultivars. Times on Cinsaut leaves tended to be shorter than that on Muscadelle for all isolates. Swiss isolate sporulated in shorter time on both cultivars, isolate of Bordeaux 87 took the longest time on these cultivars. Other isolates sporulated in 5.2-6.6 d on Cinsaut leaves, whereas in 6.5-8.2 d on Muscadelle leaves (Table 1).

Evolutions of colony diameter were shown in Fig. 1. Except the isolate of Bordeaux 87 all of them increased in colony diameter more rapidly on Cinsaut leaves than on Muscadelle leaves. Generally the differences in diameter between two host plants were more evident on the 7th d than on the 11th or 14th d after inoculation. The Montemor isolate grew most rapidly among the tested

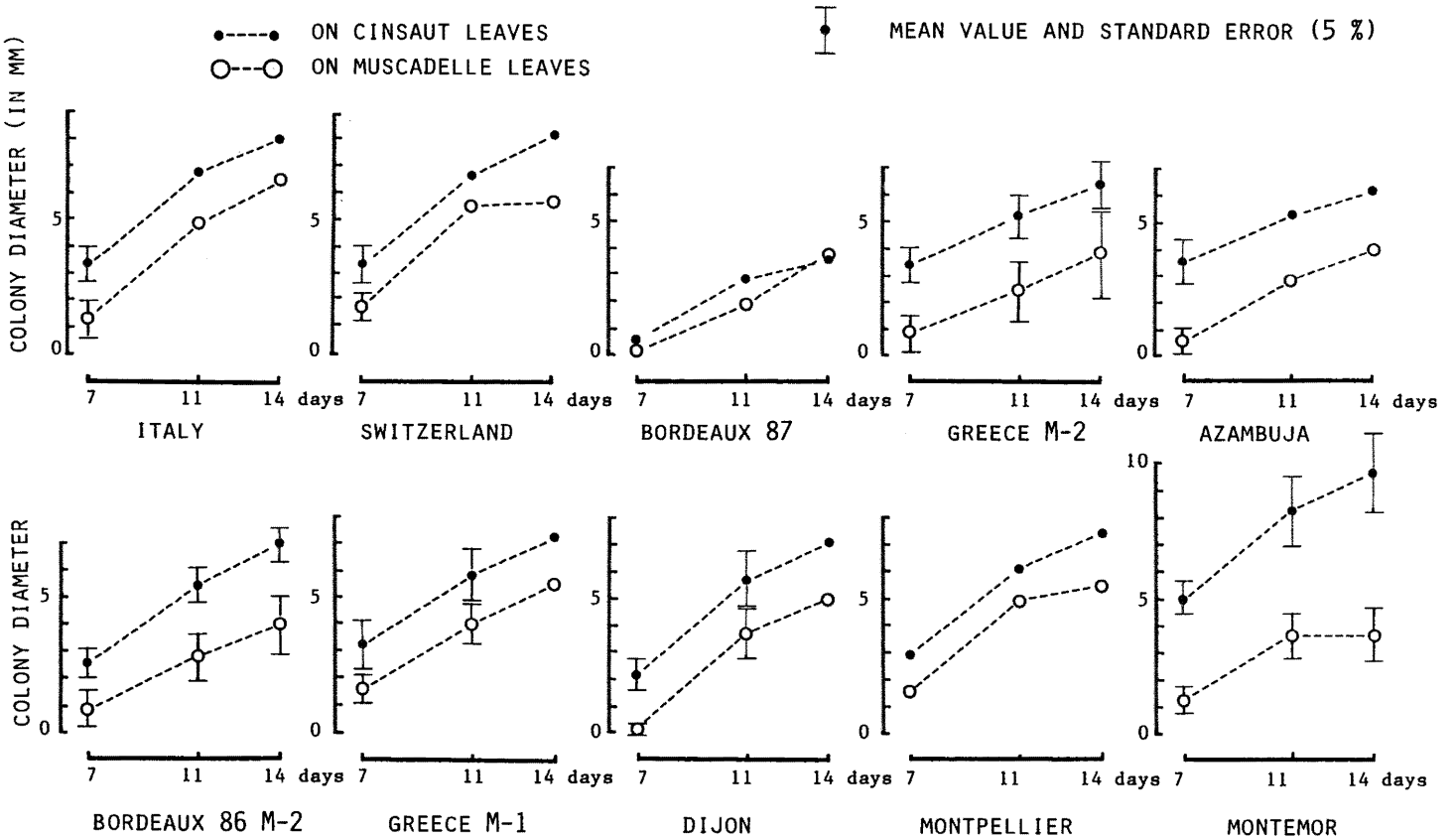


Fig. 1: Evolution of colony diameter for 10 isolates on two host cultivars.

Table 2: Estimation of conidia formation on the 21th d after inoculation on Cinsaut and Muscadelle leaves

| Isolate | Number of conidia ($x10^4$/leaf) | |
|---|---|---|
| | Cinsaut | Muscadelle |
| Greece M-1 | 13.19 ± 5.30(1) | 2.02 ± 1.63 |
| Montemor | 23.28 ± 5.58 | 1.95 ± 1.88 |
| Dijon | 12.87 ± 7.78 | 2.55 ± 2.29 |
| Montpellier | 11.72 ± 3.17 | 2.35 ± 1.50 |
| Italy | 16.24 ± 4.20 | 4.69 ± 4.19 |
| Switzerland | 19.17 ± 4.58 | 2.12 ± 2.27 |
| Bordeaux 86 M-2 | 13.14 ± 3.75 | 2.84 ± 3.11 |
| Bordeaux 87 | 1.60 ± 1.55 | 1.49 ± 2.01 |

(1) Standard error of the mean

isolates on Cinsaut leaves, whereas on Muscadelle leaves it was the Italian one. The Bordeaux 87 isolate grew very slowly on both cultivars.

The number of conidia produced for each colony was estimated on the 21st d after inoculation (Table 2). A great variability was observed according to isolates and cultivars. All isolates except Bordeaux 87 produced more conidia on Cinsaut than on Muscadelle. Some of them such as Montemor, Switzerland and Italy were very productive on Cinsaut leaves but the Bordeaux 87 isolate was less productive.

Boubals (1961) rated Cinsaut and Muscadelle as very susceptible, whereas in this study it seems that the degree of susceptibility could be affected by isolates. With Bordeaux 87 for example, these two cultivars showed the same rapidity of colony growth but the Montemor isolate grew more rapidly on Cinsaut leaves than on Muscadelle. These results show great variability in sporulation time and in colony growth in response to isolate. Doster and Schnathorst (1985) suggested that the time needed for sporulation allows susceptibility discrimination between host plants. Therefore, it could be an expression of the aggressiveness of an isolate. In our test, negative correlations ($r = -0.87^{**}$ for Cinsaut, $r = -0.86^{***}$ for Cinsaut and Muscadelle combined) were observed between time and colony diameter on the 7th d. The coefficient of correlation between time and the number of conidia estimated on the 21st d was not very clear for Muscadelle, but significant for Cinsaut ($r = -0.86^{***}$) and for Cinsaut and Muscadelle combined ($r = -0.69^{**}$).

Aggressiveness of isolates could be considered as a degree of rapidity of colony growth and at the same time as abundance of conidia production which would be an inoculum for the secondary infection. These results might suggest that the sporulation time can express the aggressiveness of isolates.

At any rate, great variability of aggressiveness was found in response to isolates on two very susceptible cultivars. This variability in aggressiveness should be taken into consideration in further studies.

## 2. Resistance to the triadimenol fungicide

2 isolates (Azambuja, Montemor) from Portugal could grow in the presence of higher concentration of triadimenol. Azambuja isolate sporulated at 0.5 mg/l of triadimenol as well as control. Montemor isolate also sporulated at this concentration, but colony growths were significantly lower than that of the control. Bordeaux and Greece isolates developed mycelium but did not sporulate. Sporulation of these isolates at 0.05 mg/l was less active than the control, with

the Greece isolate being very sensitive to triadimenol. At 5 mg/l of triadimenol, no sporulation occurred in any isolate (Fig. 2).

STEVA *et al.* (1988) reported that some Portuguese isolates seemed to be resistant to triadimenol. Our results confirm the tendency of these isolates to be resistant to this inhibitor of the sterol biosynthesis. Azambuja isolate is the most resistant, while isolates of Bordeaux and Greece were very sensitive to triadimenol. These properties concerning resistance to a fungicide could be introduced by selection pressure of fungicide treatment in the vineyard. LEE and WICKS (1982)

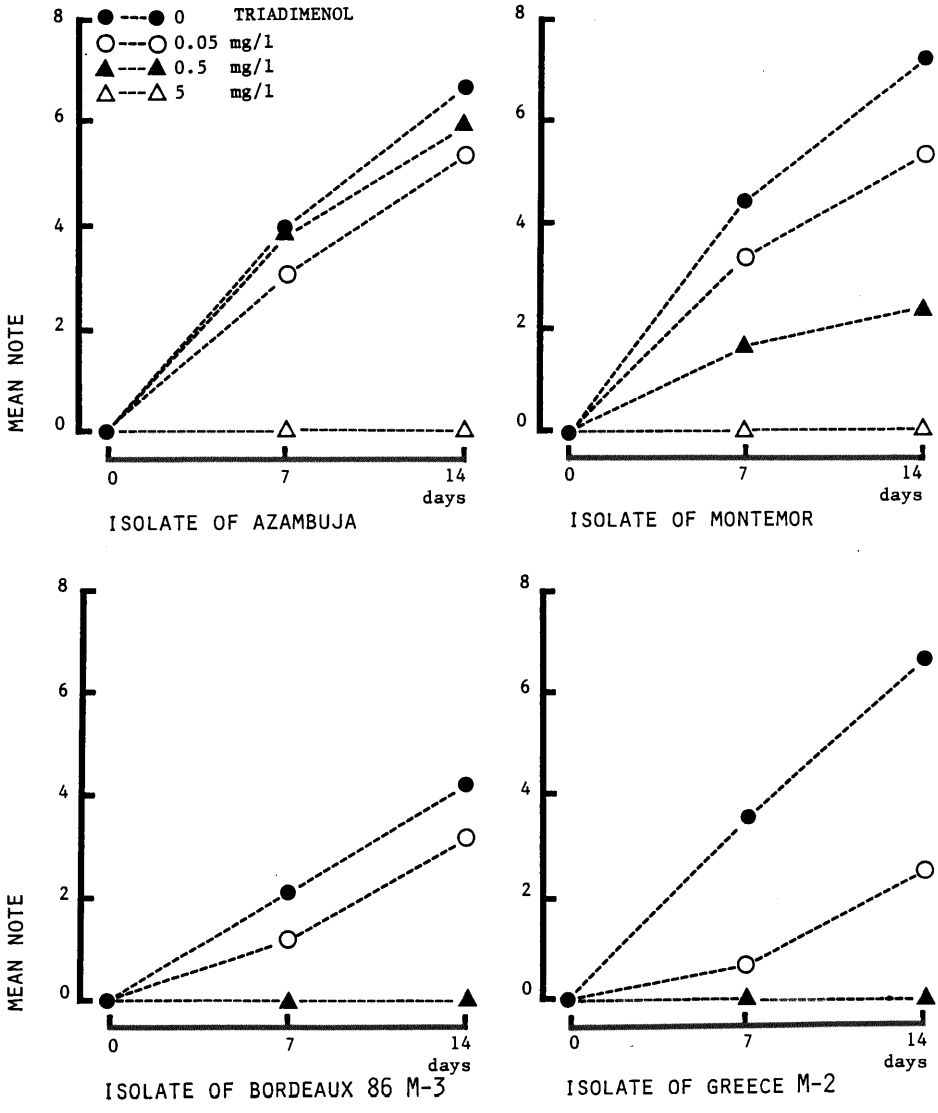


Fig. 2: Evolution of mean note of colony development for 4 isolates at several concentrations of triadimenol: Each note (note 0 to 10) represents the percentage of leaf surface infected by sporulation. Note 0: 0 %, 1: 0-2.5 %, 2: 2.5-5 %, 3: 5-15 %, 4: 15-30 %, 5: 30-50 %, 6: 50-70 %, 7: 70-85 %, 8: 85-95 %, 9: 95-97.5 %, 10: 97.5-100 %.

Table 3: Perithecia formation by paired combination of isolates

| ISOLATE | BORDEAUX M-2 | SWITZERLAND | GREECE M-1 | GREECE M-2 |
|---|---|---|---|---|
| BORDEAUX 86 M-1 | --- | --- | + | + |
| BORDEAUX 86 M-2 | | --- | + | + |
| SWITZERLAND | | | + | + |
| GREECE M-1 | | | | --- |

+ : PERITHECIA FORMED

--- : NON-PERITHECIA FORMATION

developed an experimental system with dual culture that can be used for fungicide studies on grapevine downy mildew. Our assay system is very convenient for the evaluation of systemic fungicides against grapevine powdery mildew.

## 3. Aspects of perithecia formation

### a) Mating types

None of the single-spore isolates ever formed perithecia by themselves. But paired combinations of some of them induced perithecia formation (Table 3). Initiation was observed on the 14th d after inoculation. The isolates Bordeaux M-1, Bordeaux M-2 and Swiss did not produce perithecia when combined with each other. Likewise, the combination between 2 Greece isolates did not induce perithecia. Meanwhile, combinations between these two groups induced perithecia formation.

Perithecia appear late in the season in the vineyard, but their frequency varies in response to year and region (BERNARD and MUR 1986; MAGAREY and WICKS 1986; DIEHL and HEINTZ 1987). HIURA (1962) reported heterothallism for *Erysiphe graminis* and SMITH (1970) first observed this character on *U. necator*. Our results confirm this phenomenon, and 5 isolates could be divided into 2 groups. The first one includes 2 Bordeaux isolates and the Swiss isolate, the second group includes 2 Greece isolates. The combination of isolates belonging to opposite groups induced perithecia formation. HIURA (1962) reported that this phenomenon is controlled by one pair of genes in *Erysiphe*, and it is also likely to be true in *U. necator*.

The combination of two opposite mating types is a necessary condition to induce perithecia formation. But this does not account for the great variations in perithecia number. We suggest that several factors such as weather which affect the metabolism of the host and the fungus may play an important role in the frequency of occurrence of appropriate mating types on a given host, thus resulting in abundance of perithecia.

### b) Effect of cultivars on perithecia

First perithecia formation was observed on the 11th d after inoculation on Cinsaut leaves and on the 14th d on Muscadelle leaves. The percentage of colonies which induced perithecia formation increased to 100% for Cinsaut leaves and 67% for Muscadelle leaves on the 21st d (Fig. 3). Perithecia productivity (Fig. 4) was significantly different between Cinsaut and Muscadelle after the 14th d of inoculation.

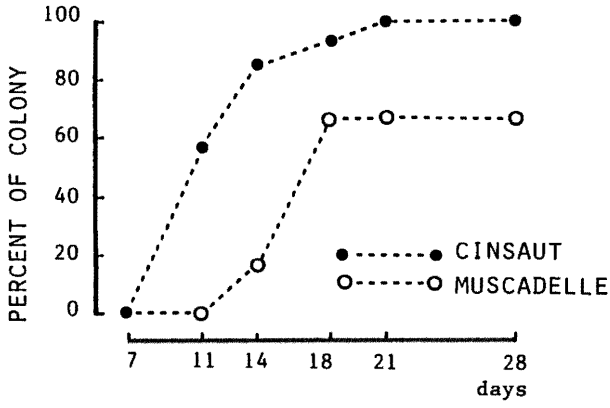


Fig. 3: Evolution of percentage of colony which induced perithecia formation on two host cultivars.

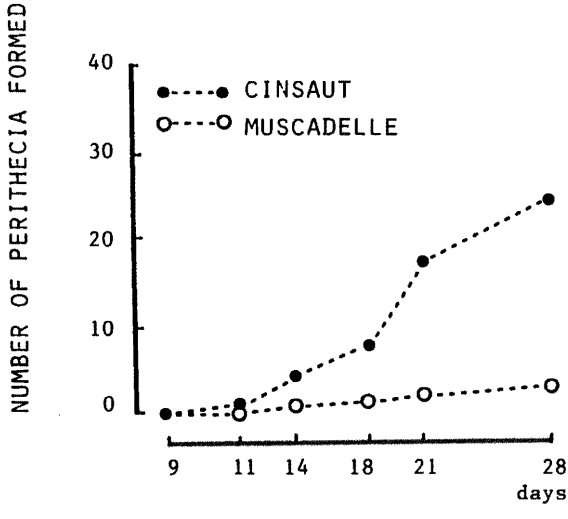


Fig. 4: Effect of host cultivars on the productivity of perithecia: number of perithecia initiated on Cinsaut and Muscadelle leaves by a paired combination of isolates (Greece and Bordeaux isolate).

Cinsaut leaves facilitated more perithecia formation than Muscadelle leaves. Diehl and Heintz (1987) reported a significant correlation between powdery mildew infections and number of perithecia under field conditions. Cinsaut and Muscadelle are rated as very susceptible (Boubals 1961), in this study, however, it seemss that the degree of susceptibility of these two cultivars is variable according to isolate: with isolates from Greece and Bordeaux, Cinsaut is significantly more susceptible than Muscadelle. It is too early for conclusions as to the relationship between susceptibility and production of perithecia, but it seems reasonable that productivity correlates with susceptibility.

## Conclusion

With the *in vitro* method using detached leaves with petiole and stem, we investigated the pathogenicity of *U. necator*. This system was applied to characterization of isolates.

Aggressiveness of several isolates was compared on Cinsaut and Muscadelle leaves, and great variability on time from inoculation to sporulation, colony development and conidia production was found in response to isolate. Sporulation time correlates fairly well with colony development and conidia production. These parameters correlating with each other, sporulation time could indicate the aggressiveness of isolate. The resistance of isolates to systemic fungicide was also studied; those originating from Portugal were found to be more resistant to triadimenol than other ones. It seems that such resistance was introduced by natural selection in the vineyard under the pressure of fungicide treatment.

Perithecia formation was demonstrated in our assay system. Single spore isolates were heterothallic and they were divided into two mating type groups. Perithecia production was more abundant on Cinsaut leaves than on Muscadelle leaves, and initiation of perithecia formation was earlier on Cinsaut leaves than on Muscadelle leaves. The productivity of perithecia seems to correlate with the susceptibility of host.

The variable reactions of the host to its parasite as well as the genetic variability of *U. necator* should be carefully considered in every breeding programme for resistance.

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# Phenol and silica incrusts in epidermal cells of *Vitis* spp. as a general defence mechanism

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A b s t r a c t : The host-parasite interactions between the grapevine *Vitis vinifera*, powdery mildew *Uncinula necator (Oidium tuckeri)* and grey mold *Botrytis cinerea* were studied by light microscopy-histochemistry and electron microscopy.

Chemical defence mechanism involves incrusting of the walls of the infected cell and of neighbouring cells with phenolic substances associated with a cell wall bound peroxidase activity. This indicates the formation of lignin-like components. In addition, silica deposits were observed in whole cell walls or parts of them. Pure, mechanically resistant silica skeletons remained after a treatment with conc. $H_2SO_4 + H_2O_2$ at 400 °C and washing with conc. HCl. They consisted of groups of 1-20 cells of the upper epidermis with adhering parts of the corresponding palisade cells or of the lower epidermis (including stomatal cells) with adhering spongy parenchyma. Not only cell walls but also wrinkles of the upper epidermis, defence papillae and fungal haustoria were silicified. Silica accumulations were greater in resistant than susceptible cultivars.

These reactions are induced not only by parasitic fungi but also by mechanical damage of the leaf. Our studies corroborate observations in other host-parasite systems and indicate the existence of an unspecific, fast-reacting mechanism serving as an early defence line which allows the activation of slower, more specific defence reactions.