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Studies on the generative reproduction of grapevine powdery mildew (*Uncinula necator* BERK.)

by

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Etude de la reproduction sexuée de l'oïdium de la vigne (*Uncinula necator* BERK.)

Résumé : L'étude de la morphologie des organes reproducteurs d'*Uncinula necator* et des facteurs qui influencent leur développement s'est traduite par les résultats suivants.

Une corrélation significative a pu être établie entre les différents niveaux de résistance des plantes hôtes et la quantité des périthèces sur les feuilles.

Le taux de périthèces sur les plantes de serre était d'environ 80 % plus faible que sur des cépages de plein champ de sensibilité voisine.

Après conservation des périthèces à basse température une irradiation de 2 h aux rayons ultra-violets n'a pas entrainé leur ouverture; la lumière ou l'obscurité n'ont pas davantage eu d'influence. Bien au contraire une irradiation prolongée (de 5 h) a eu pour conséquence une diminution du pourcentage de périthèces ouverts.

Le facteur essentiel du processus d'ouverture s'est avéré être l'eau. Une atmosphère avec 100 % d'humidité relative n'est pas suffisante.

Les organes reproducteurs s'ouvrent aisément à +22 °C, mais ils ne s'ouvrent pas à une température égale ou inférieure à +5 °C.

Les périthèces contiennent jusqu'à 6 asques dont chacun possède en moyenne 6 ascospores. Après une conservation à basse température pendant 6 semaines, environ 50 % des ascospores ont conservé leur entière vitalité.

Key words: oidium, sexuality, growth, irradiation, temperature, water, resistance, variety of vine, morphology.

Introduction

Since its first appearance in Europe, more than a century ago, powdery mildew (*Uncinula necator*) has been one of the most severe grapevine diseases.

Usually no fungus damages occur if highly effective fungicides are applied at the appropriate time. Nevertheless the pathogen is able to develop, especially after the final spraying and before harvest when the fungicides have lost their efficiency. High temperature and low precipitation during that time favour growth. The conditions for the development of *Oidium tuckeri* have been studied (LORENZ 1976; NIEDER 1978, 1983; BRENDEL 1986) in some detail but not much is known about the sexual cycle of *Uncinula necator.* Morphology and development of the fruit bodies (cleistothecia) are described by BOUBALS (1961), GALET (1977), BULIT and LAFON (1983). Their appearance seems to be limited to particular years: for instance in October 1958 in Montpellier (BOUBALS 1961); in autumn 1971 in Bordelais (ROUSSEL and BOUARD 1971).

Although cleistothecia are of minor importance for perennation of the fungus (SALL and WRYSINSKY 1982), their development involves recombination of genetic material leading to — possibly fungicide resistant — new strains.

A number of reports on the abundant formation of fruit bodies in different wine growing regions during the last years (MUR 1985; WICKS and MAGAREY 1985; MAGAREY

and WICKS 1986; BERNARD and MUR 1986) as well as the increased production of these organs in our area in 1985 led us to investigate cleistothecia development and the conditions required for their opening in the following season. Another aspect, however, was to study morphology and development of the cleistothecium containing the asci and ascospores and to compare the behaviour of *Vitis vinifera* varieties and some hybrids with different susceptibility to powdery mildew in various environments.

Material and methods

Seriously infected leaves with cleistothecia of 7 greenhouse and 12 field-grown cultivars showing different resistance levels, were collected in October 1985. 15 leaves/variety originating from the basal part of the canes were chosen at random.

To determine the number of cleistothecia, discs (17 mm) were cut out of the leaves and all visible developmental stages were counted under the stereomicroscope.

At the same time, the infection rate of powdery mildew was determined on 10 vines of each cultivar by estimating patches with visible sporulations on 20 leaves/plant. The plants were classified according to their degree of resistance (0 = highly resistant, 5 = medium resistant, 9 = very susceptible).

All cultivars had been subjected to the same cultural practices without fungicide treatment.

1. Experiments with cleistothecia

Cleistothecia were stored in cold-chambers (−5 and −20 °C) for further investigations. After a 6-week storage, they were placed either on glass slides or in petri dishes filled with water or left on the leaves and then irradiated with UV-light for 0, 2 or 5 h. After irradiation, those cleistothecia that had been left on the leaf surface were transferred to fresh leaf discs or into water. Except of the ones in water, the cleistothecia were then incubated in 100 % RH, without liquid water at +22 °C. The percentage of spontaneously opened fruit bodies was evaluated after 0, 1, 7, 14 d by counting 200 cleistothecia of each sample. Values were statistically separated by χ^2-tests as described by LINDER and BERCHTHOLD (1979).

In order to test the influence of temperature and light intensity during incubation, samples treated as described above were stored at +22 and +5 °C either under normal daylight (16 h light, 8 h dark) or in the dark. Monitoring followed the same procedure as in our previous experiment.

In this test, also cleistothecia hibernated in the greenhouse were included.

2. Microscopical studies

For microscopic examinations, completely opened cleistothecia were used. If moisture was not high enough, the fruit bodies closed again and no further studies were possible, thus they had to be kept constantly in liquid water.

To examine the vitality of the ascospores, a method described by STEIN and BACHMANN (1982) was used. 10 µl fluorescein diacetate (0.5 % in acetone) and 20 µl Evans blue (1 % w/v in H_2O) were added to 500 µl distilled water. 10 µl of the solution were placed on the opened cleistothecia. After 5 min the sample could be observed with a Zeiss Standard microscope equipped with an incandescent UV-light device.

The observations under the scanning electronmicroscope required a special preparation as described by BLAICH *et al.* (1984).

Results and discussion

1. Opening conditions of cleistothecia

a) Humidity: The opening of cleistothecia depended on the conditions of incubation. Unlike fruit bodies in water, dry-stored specimens did not open at all. Storage temperature in the cold chambers had no influence on this behaviour. Cleistothecia incubated in water opened vigorously, even 2 weeks after the start of the experiment. Transferring the fruit bodies into water after irradiation did not improve the opening rate significantly. Contrary to the vegetative reproduction of powdery mildew — conidia requiring a high air humidity but no liquid water (DELP 1954; STEIN *et al.* 1985) — generative organs need liquid water for further development. Already YOSSIFOVITCH (1923) perceived the important role of liquid water for cleistothecia burst and germination of the ascospores. Cleistothecia need moisture mainly

Table 1

Influence of storage temperature and UV irradiation on the opening of cleistothecia, as per cent, after a 6-week storage

Influence de la température de conversation et de l'irradiation aux ultra-violets sur le pourcentage d'ouverture des périthèces, après une conservation de 6 semaines

Incubation (d)	−5 °C — UV (h)			−20 °C — UV (h)		
	0	2	5	0	2	5
0	1.0	1.7	1.5	1.0	1.5	1.5
7	10.5	13.0	10.0	8.0	11.5	7.5
14	20.0	25.5	16.0	13.0	13.5	6.0
Final $\bar{x}$		20.5			10.8	

Table 2

Influence of UV irradation, incubation temperature and light conditions on the opening of greenhouse-cleistothecia, as per cent, after a 6-month storage on leaves

Influence de l'irradiation aux ultra-violets, de la température d'incubation et de la lumière sur le pourcentage d'ouverture des périthèces, après une conservation de 6 mois sur le feuillage

Incubation (d)	0 h UV				2 h UV	
	+5 °C		+22 °C		+5 °C	+22 °C
	dl	d	dl	d	dl	dl
0	1.0	2.0	1.0	2.5	3.0	2.5
7	1.5	0	4.0	5.0	3.5	1.5
21	1.0	0.5	12.0	12.5	2.5	8.5
Final $\bar{x}$	1.2		12.2		2.5	8.5

dl: daylight (16 h light, 8 h dark).
d: dark (24 h darkness).

for swelling, the opening of their envelope being a mechanical process due to a high inside pressure rather than a biochemical reaction.

b) U V i r r a d i a t i o n : Table 1 shows that a 2 h irradiation had no effect on opening as compared to the control. Moreover, a 5 h irradiation inhibited opening. This negative effect of long-time irradiation is valid for cleistothecia stored at both −5 °C and −20°C. According to MUR (1985), however, UV-irradiation is a prerequisite for the opening of 2-year-old cleistothecia stored at −27 °C (discussed below).

c) S t o r a g e t e m p e r a t u r e had a significant influence on cleistothecia opening, as shown in Table 1: the total average of opened fruit bodies reached 20.5 % after a storage at −5 °C, but only 10.8 % at −20 °C. After a 6-month cold storage the cleistothecia had lost their vitality: both after the −5 °C and after the −20 °C storage, the rate of opened fruit bodies was never significantly different from 0. Neither light intensity nor different incubation temperatures influenced the opening rate.

In contrast, cleistothecia hibernated on dry leaves in the greenhouse developed more frequently after a 3-week incubation, although their average opening rate was lower than it had been 6 months earlier.

As mentioned in the preceding test, there were no significant differences between irradiated samples (2 h) and the control (Table 2).

In our experiments, fruit bodies lost their vitality after a cold storage of only half a year at −5 °C and even after 6 weeks at −20 °C. MUR (1985) obtained survival after a

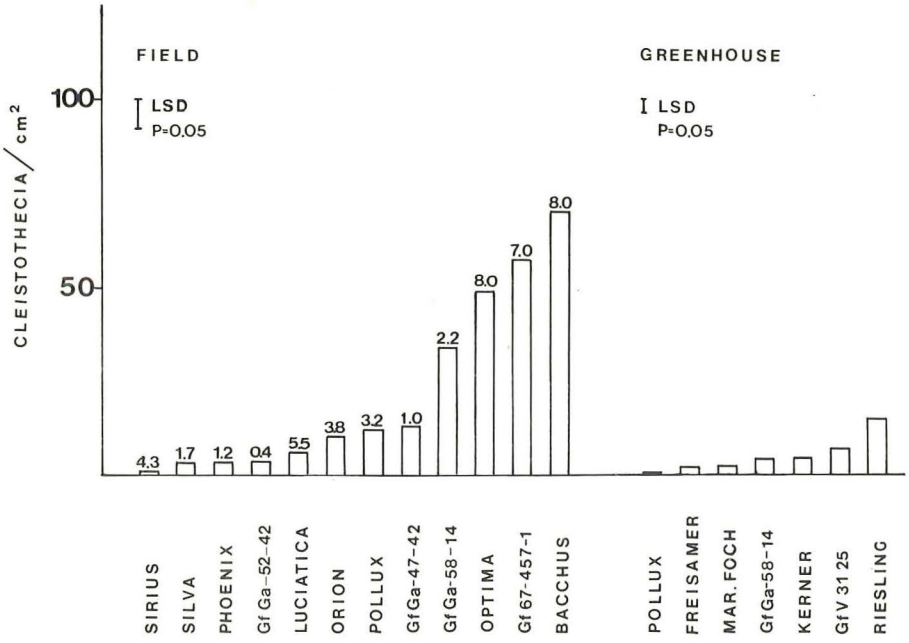

Fig. 1: Cleistothecia density on leaves of several cultivars under different local conditions. Figures above columns indicate mean degree of susceptibility.

Densité des périthèces présents sur les feuilles en fonction du cépage et de l'environnement. Les chiffres au-dessus des colonnes représentent une note moyenne de sensibilité.

2-year storage at an even lower temperature (−27 °C). This difference could be due to the early sampling time in October 1985 when the cleistothecia possibly had not yet reached their full maturity, which could also explain the contradictory results of UV-treatment as mentioned above. Greenhouse-hibernated fruit bodies, however, kept their vitality, the greenhouse temperature not having been lower than −5 °C.

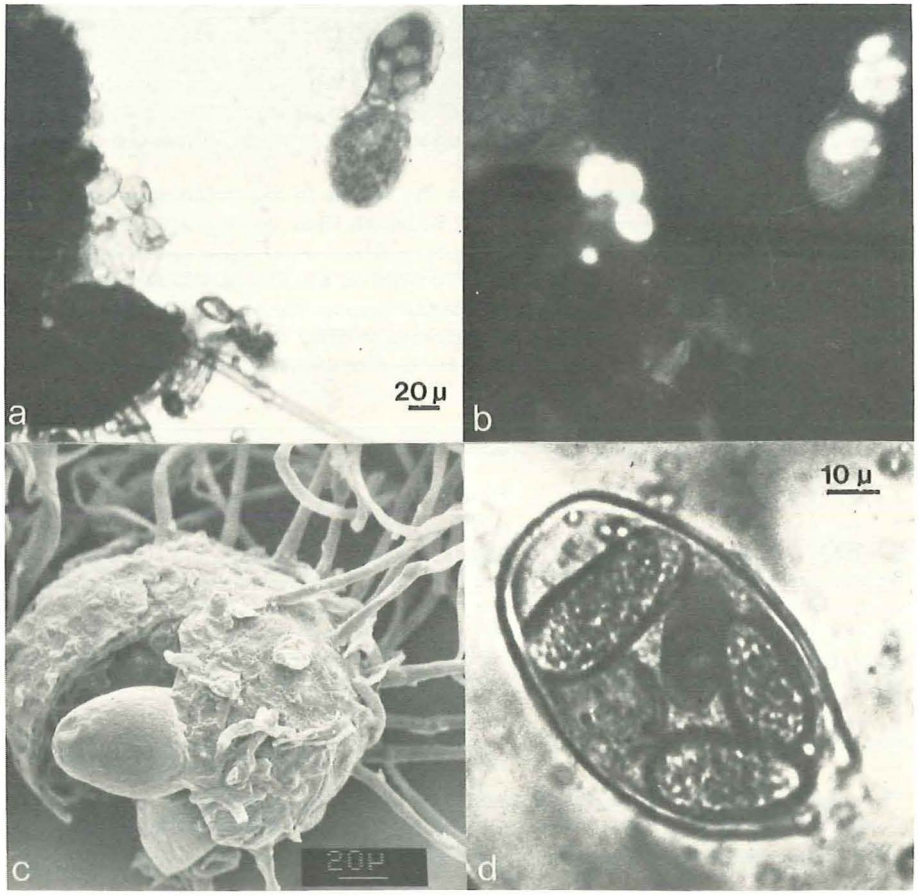

Fig. 2: a and b) Vitality test with fluorescein diacetate. Cleistothecia of *Uncinula necator* have been squeezed to liberate the asci. Three of them are visible containing 5—7 ascospores: a) under normal light; b) stained with fluorescein diacetate to differentiate viable ascospores which show a yellow fluorescence under the UV-microscope. — c) Scanning electron micrograph of an opened cleistothecium showing parts of an ascus. — d) Ascus of *Uncinula necator* with 5 ascospores after staining with Evans blue specific for dead cells. This is the case for the two ascospores in the middle.

a et b) Test de vitalité au diacétate de fluorescéine. Les périthèces d'*Uncinula necator* ont été écrasés pour libérer les asques. On en aperçoit trois d'entre eux, contenant 5 à 7 ascospores, a) sous éclairage normal, b) sous éclairage ultra-violet, les ascospores vitales présentent une fluorescence jaune. — c) Microscopie électronique à balayage. Périthèce ouvert laissant apparaître partiellement un asque. — d) Asque d'*Uncinula necator* contenant 5 ascospores, après coloration au bleu d'Evans, mettant en évidence les cellules détruites uniquement. C'est le cas ici des deux ascospores au centre.

d) Incubation temperature: In contrast to cleistothecia incubated at +5 °C, those kept at +22 °C opened significantly better (Table 2). Actually, temperatures are not only necessary for the opening process but also for the mycelium which needs at least +6 °C for its development (DELP 1954).

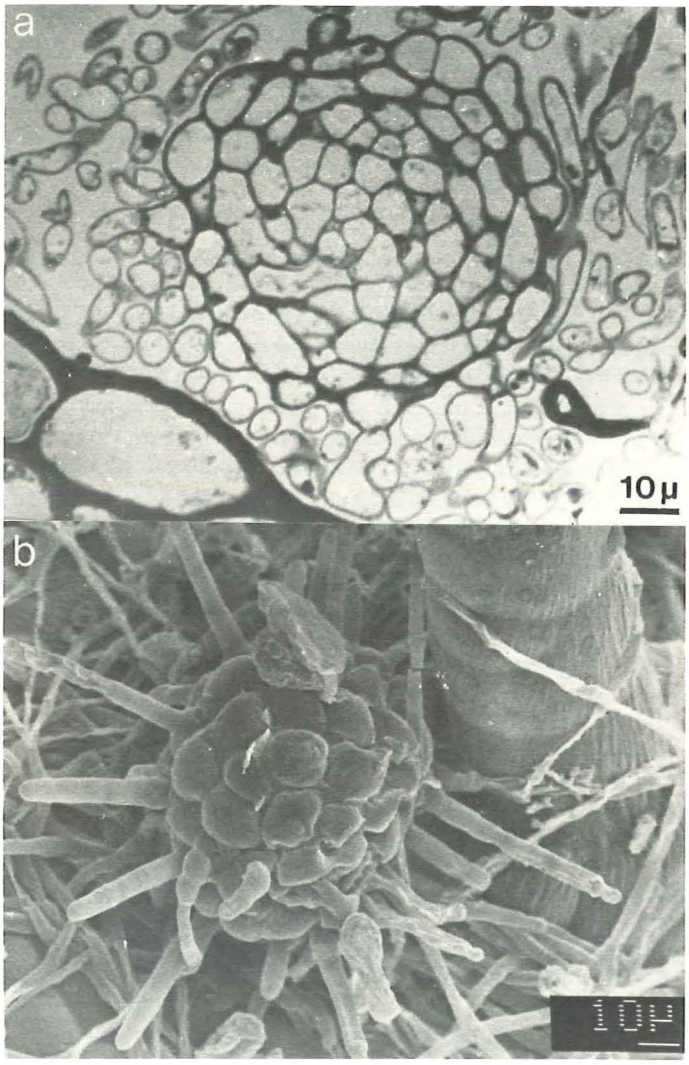

Fig. 3: a) Section (1 µm) through a young cleistothecium on leaf epidermis. The developmental stage corresponds to (b), no asci are being formed yet. — b) Scanning electron micrograph of a young cleistothecium near a leaf hair. Appendices start developing.

a) Coupe transversale (1 µm) d'un périthèce immature sur un épiderme foliaire. Son stade de développement correspond à (b), les asques ne sont pas encore formés. — b) Microscopie électronique à balayage. Jeune périthèce près d'un poil de la face inférieure d'une feuille. Le développement des appendices n'est pas encore achevé.

e) Incubation light: Different light conditions had no influence (Table 2). Opening occurred in the dark as well as in the light (1000 lux, photoperiod 16 h/8 h).

2. Influence of the genotype

The degree of powdery mildew infections and the number of cleistothecia within the varieties (Fig. 1) showed a significant correlation ($r = +0.75^{**}$) in the field. Less cleistothecia were formed on the leaves of resistant varieties, e. g. Phoenix or Silva, than on susceptible cultivars like Bacchus or Optima. BECKER and SCHRODT (1962) report similar observations. Such relations could not be found in the greenhouse on severely infected varieties with similar level of susceptibility as the ones tested in the field. One possible explanation for this fact is the periodical irrigation of the greenhouse cultivars, so that dry conditions like outdoors could not arise.

3. Vitality and germination of ascospores

Fruit bodies contained up to 6 asci with 4—8 ascospores. An odd number was not uncommon (Fig. 2 d), the average number being 6. According to the vitality test, about 50 % of them were still alive (Fig. 2 a and b). After a 6-month cold storage (on leaves at $-5\,^{\circ}C$), ascospores were used to inoculate surface-sterilized leaf discs. In some cases a new infection with visible sporulation was obtained.

4. Microscopical studies

Semi-thin sections (0.5 µm) through old leaves bearing cleistothecia allowed the observation of different developmental stages. Fig. 3 a shows a section through a young fruit body before formation of asci. The SEM micrograph (Fig. 3 b) of a similar stage reveals short appendices. The fulcra of mature ones are much longer exhibiting the characteristic crosiers at their tips. Under dry conditions ripe fruit bodies show wrinkles and depressions, in water they are turgescent and often burst releasing their asci (Fig. 2 c).

Conclusion

The formation of sexual organs of *Uncinula necator* seems to be due to particular weather conditions (BECKER and SCHRODT 1961, 1962; BOUBALS 1961; LORENZ 1976; BERNARD and MUR 1986). In years with a high frequency of cleistothecia, the precipitation during their time of development in September was rather low as compared to the average precipitation of the last 30 years (Table 3). It should be pointed out, though, that dry conditions are not the only reason for the formation of fruit bodies, whose appearance only in particular years is certainly of a much more complex nature. Among others, the physiological condition of the hostplant at the end of the vegetative period, as well as the high day temperatures during that time and the age of the fungus, seem to be involved. On the one hand high temperatures favour the development of the fungus, on the other hand dry conditions are stress factors to the hostplant, leading perhaps to a restriction of fungal growth due to a lack of nutrients (ARNAUD and ARNAUD 1931; REISS 1981) — conditions which in fungi usually induce the formation of fruit bodies.

As a consequence of these observations a reconsideration of the present treatment strategies might be worthwhile. Indeed precipitations, which inhibit germination of conidia overwintered in the buds, might favour the appearance of infection focuses caused by ascospore germination.

Table 3

Average temperatures and precipitations in September · Years with abundant cleistothecia formation at Geilweilerhof

Températures et précipitations moyennes en septembre · Années avec une formation abondante de périthèces à Geilweilerhof

	Temperature (°C)	Precipitation (mm)
1959	16.5	4.9
1961	17.2	28.3
1975	16.0	51.0
1985	15.0	24.9
∅ 1948—1977	14.6	59.0

In autumn 1986, again some cultivars have been oberved which presented cleistothecia in the field as well as in the greenhouse. Thus the sexual cycle of powdery mildew — possibly involving the appearance of fungicide resistant and more virulent strains — is perhaps more frequent in temperate regions than hitherto supposed.

Actually a great number of fungi are heterothallic, forming no fruit bodies or only small numbers of them if the appropriate mating type is missing.

The comparatively low frequency of fruit bodies (80/cm²) in our case might well indicate genetic differences like the lack of compatible mating types or the presence of incomplete homothallism in European strains. This assumption is corroborated by some reports on differences of virulence between North American and European strains. Such genetic differences are very likely because it must be assumed that only a limited number of genes had been introduced to Europe in the last century.

Summary

Morphology and factors influencing the development of *Uncinula necator* fruit bodies were studied.

The resistance levels of hostplants and the amount of cleistothecia on their leaves were significantly correlated under field conditions.

The number of cleistothecia was about 80 % lower on greenhouse plants than on field-grown vines with similar susceptibility.

An UV irradiation (2 h) did not induce the opening of the cleistothecia after cold storage, neither did light or darkness. A longer UV treatment (5 h), however, decreased the percentage of open cleistothecia.

Liquid water was essential for the opening process. An atmosphere with a 100 % relative humidity was not sufficient.

The fruit bodies opened readily at +22 °C, but not at +5 °C and below.

Cleistothecia contained up to 6 asci with an average content of 6 ascospores. About 50 % of the ascospores had still their full vitality after 6-weeks cold storage.

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