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A Proposal for an Improvement of the "Pourriture Noble" of Grapes†

OSWALD KAMOEN[1]* DENIS DUBOURDIEU[2], and BERNADETTE DUBOS[3]

[1]*Rijksstation voor Plantenziekten, C.L.O., B 9220 Merelbeke, Belgium*
Tel. 091-522083
[2]*Institute d'Oenologie, Univ. Bordeaux, II, F 33405 Talence, France*
[3]*Station de Pathologie végétale, I.N.R.A., F 33140 Pont-de-la-Maye, France*

Abstract

The "pourriture noble" is a *Botrytis* infection of ripe grapes. The fungus grows during the humidity of foggy nights. On sunny days the water, escaping from the injured cells, evaporates. The result is a lower water content and a higher sugar concentration.

The main problem for obtaining a good quality of "pourriture noble" are the years when the season is ending with rainy weather. Then the ripe grapes, not protected by fungicides, are infected. The water released by the cells is not evaporated during the rainy days and gives a grey rot and a bad wine quality.

A solution is proposed to obtain a good quality of "pourriture noble" even with a rainy maturation period. The principle is that the ripe grapes are protected during the humid period by a dicarboximide fungicide. As soon as a dry period is announced, a *Botrytis cinerea* strain resistant to dicarboximides is sprayed on the grapes, with a correct development of the "pourriture noble".

The requirements of strains suitable for use in the vineyards are examined.

Keywords: *Botrytis cinerea*, noble rot, pourriture noble, grape

Abbreviations: *Botrytis cinerea*: B.C.; "pourriture noble": P.N.

†Reviewed

* To whom correspondence should be addressed: Rijksstation voor Plantenziekten, Burg. v. Gansberghelaan, 96, B-9220 Merelbeke (Belgium)

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1. Introduction

Botrytis cinerea (B.c.) is a parasite of grapes causing normally grey rot but also a noble rot or "pourriture noble" (P.N.). P.N. is the B.c.-infection occurring only late in the season at full maturity during foggy nights and sunny days. This infection in appropriate circumstances is necessary to obtain the sweet wine with a special taste (vin liquoreux or Trokenbeerenauslese).

For the appropriate production of a P.N. at least the following prerequisites are necessary.

1. A good development of the grape during the season, without infections.
2. Infections only at full maturity when the grapes reach a sufficient content of sugar.
3. Small periods of humidity e.g. foggy nights in September or October. They are necessary for germination of the fungus.
4. Sunny days alternating with the humid nights are necessary for the evaporation of the water from the berries in order to obtain a relative increase of sugar concentration.

These prerequisites are an additional problem for the sweet wine production in comparison with the dry wines. Table 1 shows the years of failures for the P.N. in Sauterne from 1961 to 1985 compared with the quality of dry wine (Graves from 1961 to 1975). It appears from the table that success of the sweet wines is much more risky than for dry wine production. The years of failure (10/25) coincide often with higher rainfall in September or October (e.g. 1963, 1964, 1965, 1968, 1973 and 1974) or with long periods of cloudy humid maturation weather (e.g. 1978 and 1980). Then the grapes not protected by fungicides are easily infected. The water from the infected berries cannot evaporate during the rainy days and gives the undesired grey rot.

We now propose a solution to overcome the failures of the noble rot due to a rainy period during maturation of the grapes. The solution is based on the following principles.

1. If a rainy period coincides with maturity, the grapes must be sprayed with a dicarboximide fungicide.
2. A depression with rain generally ends after a few weeks.
3. A soon as a high pressure zone with sunny weather is forecast, a B.c. resistant to dicarboximides is sprayed at evening.
4. Then a correct development of the P.N. can be obtained.

Table 1. Years with bad quality for Sauterne (from 1961 to 1985) (compared with the quality of a dry wine in some corresponding years 1961–1975*)

Sauterne and Barsac		*White (Graves)*	
Year	Quality	Year	Quality
1963	bad	1963	bad
1964	a disaster	1964	excellent
1965	very moderate	1965	very moderate
1968	very moderate	1968	very moderate
1972	very moderate	1972	a good mean
1973	harvest destroyed by rain	1973	good
1974	unsuccessful	1974	mean quality
1977	bad, no infection, cold and dry		
1978	bad, rather grey rot		
1980	no concentration of sugars		

* Results from 1961 to 1975 from H. Johnson (1976)

The proposed procedure is a complete new method for noble rot production. It is evident that a number of difficulties must be solved and some wrong concepts must be rectified. In this paper we examine the following requirements of a resistant B.c. strain destinated for use in the vineyards.

1. The strain must have a sufficient resistance level (see 4.1).
2. A strain with a low competitive ability is wanted, to avoid survival in the vineyard for the next year (see 4.2).
3. We need a resistant strain with sufficient virulence for infection (see 4.3).
4. A good sporulation of the resistant strain is necessary for an industrial production of the inoculum (see 4.4).
5. An important point is also the stability of the strain (see 4.5).

2. Materials and Methods

2.1 Origin of isolates

Three strains sensitive to vinclozolin were homocaryotic selections obtained from Lauber in 1971. They are indicated as B.c.L.5G6, B.c.L.10G6 and B.c.L.69G6. Two resistant isolates are from strawberries and selected in the laboratory, on orange serum agar (Difco nr. 0521-01-9) with vinclozolin (50 ppm) and 20% glucose. Two homocaryotic strains were selected from these cultures (Lauber, 1971) indicated as B.c.R.a and B.c.R.c. Three

resistant isolates were obtained from H. Maraite and indicated as B.c.RR.1, B.c.RR.2, B.c.RR.3.

2.2 Estimation of the resistance of the strain

The first method to estimate the resistance of the strain was by measuring the radial growth of the fungus on different concentrations of vinclozolin on Czapek-Dox-agar in petridishes (9 cm). The ED 50 and ED 95 were calculated using a computer program with probit and log transformation of respective percent growth inhibition and vinclozolin concentration.

A second method was the estimation of the frequency of resistance in spore populations. The method of Maraite et al. (1980) was used with slight modifications, viz. a Czapek-Dox agar (Merck nr. 5460) was used with a vinclozolin addition up to 100 ppm. The spore suspensions were harvested after 14 days from orange serum agar in petridishes.

2.3 Estimation of the saprophytic development

Saprophytic development was measured by mycelium growth, by sporulation and by sclerotium formation.

For mycelial growth, agar plugs (4 mm diam.) with growing hyphae were transferred to fresh Czapek-Dox-agar. The diameter of the colonies was measured. The mycelial growth is expressed as the growth rate of the hyphae per day.

Sporulation was evaluated by washing cultures on orange serum agar after 14 days with sterile water containing 0.01% tween 80. The number of conidia was counted by means of a Burker slide.

Sclerotia were estimated after one month on the petridishes with Czapek-Dox-agar used for ED 50 determinations.

2.4 Test for competition in mixed populations

Both sensitive and resistant strains were inoculated together in stripes on orange serum agar. This was called the generation zero. After ten days, the cultures were gently dusted over a fresh medium. This was repeated during several generations. After each generation the frequency of resistance in the spore populations was tested (see 2.2).

2.5 Estimation of the virulence of dicarboximides resistant strains

Pathogenic capacities of the strains were tested on cucumber slices and on strawberries.

Cucumber slices about 5 mm thick were inoculated with mycelial plugs of 4 mm diameter and incubated on moist filterpaper in petridishes.

For the test on strawberries freshly harvested ripe fruits were inoculated at their basis with an agar-mycelium plug of 4 mm diameter and incubated in humid chambers. The distance of browning was measured after three days.

3. Cellular Basis of "Pourriture Noble" Development

Before examining the requirements of a B.c strain suitable for use in the vineyards, a description of the P.N. infection process at the cellular and physiological levels may be useful.

During the growth of the grape, microfissures appear in the skin of the berries especially around the stomata, through which the germtubes can penetrate (Pucheu-Planté and Mercier, 1963). If there are periods of sufficient humidity, e.g. foggy nights, germination and penetration for the hyphae becomes more and more successful with progressing maturation because the resistance of the grapes disappears. This is probably due to a lower inhibition of the polygalacturonase by a decrease of the proanthocyanidins and maybe also due to a lower phytoalexin synthesis (Stein, 1984).

After penetration, the hyphae are growing in the intercellular spaces and mostly in the external part of the berry. The pectin cement of the cell walls is destroyed by the pectin degrading enzymes (Pucheu-Planté and Mercier, 1983). The plasma membrane disrupts due to the lack of support from these destroyed cell walls (Basham and Bateman, 1975; Kamoen et al., 1978). The cell content not further contained within the cell membrane escapes in the intercellular spaces from where the cell water evaporates at dry weather.

4. Results

4.1 Estimation of the resistance of the strains

For our laboratory experiments we chose eight strains. Their ED 50 and ED 95 values for vinclozolin are given in Table 2.

4.2 Survival of the resistant fungus in the vineyards

The solution for the problem of survival is that strains resistant to dicarboximides mostly have a low competitive ability. This aspect can be examined *in vitro* by successive mixed cultures of resistant and sensitive strains on media without fungicides (Maraite et al., 1980). Some resistant strains survived during ten generations. In the other mixed cultures the resistant strain disappeared after three to nine generations ($> 0.001\%$ resistant spores). We found similar results, however further work remains necessary.

Table 2. Some properties of the used strains

Strain	ED 50 ppm	ED 95 ppm	Growth mm/day	Conidia $10^5/cm^2$	*Sclerotia**	Growth mm/d (± S.D.) on cucumber	on strawb.
B.c.L.5G6	0.2	0.8	6.9	−*	0	4.3±0.3	4.5±3.3
B.c.L.10G6	0.7	4.3	3.6	−*	0	2.4±0.4	2.2±0.4
B.c.L.69G6	1.9	13	5.6	60±6	−*	3.5±0.2	5.0±2.0
B.c.R.a	6.9	91	4.7	80±17	+	1.8±1.3	7.3±0.5
B.c.R.c	6.9	83	4.2	77±20	++	1.6±0.7	5.4±1.3
B.c.RR.1	>50		4.1	0.8±0.2	++	2.9±2.7	4.7±1.8
B.c.RR.2	16	300	4.3	$\leq 10^3$	0	2.0±0.3	2.9±0.4
B.c.RR.3	>50		2.3	$\leq 10^3$	+++	2.0±0.2	1.6±0.3

* −no results, 0:no sclerotia, +:sclerotia present, +++ many sclerotia.

Also sclerotia may be a factor of survival. Consequently strains with high sclerotia formation must be avoided. Table 2 shows that there are great differences between the strains.

4.3 Virulence of dicarboximides resistant strains

Table 2 gives a survey of the mycelial growth speed per day on cucumber slices and on strawberries. There is no parallelism between the results on cucumber and on strawberries. Two resistant strains and one of our sensitive strains (B.c.L.10 G6) showed a lower growth on strawberries.

The reason for the lower virulence is not easy to explain. A test on cucumber slices may be a wrong basis for testing virulence for soft fruits, e.g. grapes. One reason for lower virulence on soft fruits may be the sensitivity of the highly resistant strains for osmoting pressures (Leroux and Gredt, 1982). Nevertheless some high resistant colonies are isolated straight from strawberries (Maraite et al., 1980) but more rarely from grapes (Leroux et al., 1982). All our strains except B.c. R.R.3 grew well on grapejuice with > 100 g/l sugar.

Other negative factors in virulence may be the lower germination or a slower growth rate as is shown in Table 2.

4.4 Production of inoculum and commercialization

Many resistant strains but not all have a much lower sporulation capacity than the sensitive ones (Table 2) and this may create problems for the industrial production of infection propagules. Therefore a strain with good sporulation must be selected. For spore production in the laboratory, mostly surface cultures are used; those can also be used on industrial scale (Van Damme, pers. comm.).

4.5 Stability of the resistant strain

Several resistant strains in a subculture without fungicide are giving sectors with a variable percent of sensitive conidia or with more abundant mycelium or sclerotia. *Botrytis cinerea* is a fungus with heterocaryosis. As long as the strain intended for spraying in the vineyards is heterocaryotic, we are not sure that all the requirements to fulfill remain stable. Five of the strains that we used in our experiments are homocaryotic (B.c.L. and B.c.R.), and no sectors were observed in any one of our cultures.

5. Discussion

For the introduction of a new method difficulties may be expected. This will be true for this proposal for the P.N. improvement. Up till now we examined only the problems related to the suitability of the fungal strains. We made as much as possible concerning the analytical approach of the problems that may be expected. Apparently, all such problems can be solved on the basis of the laboratory experiments and literature information. Nevertheless, field experiments with a more holistic approach will be necesary to persuade the growers, the advisors and the industry. However, not all problems of our proposal can be tested in the field.

A field trial for survival of the introduced resistant strain is difficult to apply due to the actual presence of resistant strains in the vineyards: e.g. in Sauterne-Barsac 14% in 1980 (Leroux et al., 1982). The use of dicarboximides during the growth season in the vineyard and even in the near surrounding will compromise the good function of our system and must be avoided. If we want to use a resistant strain in the vineyards it must be tested previously for competitive ability in the laboratory.

Also the test of the stability of the chosen strain is no subject for field experiments, no more than the experiments for inoculum production and commercialization.

In the vineyard the strains selected in the laboratory must be tested to see if they are suitable for infection, for growth of the grape and for sugar tolerance. An important question is at what moment of the pathogenic process the fungus comes in direct contact with the sugar fluid escaped from the cell. Moreover a limitation of the fungal growth at the end of the pathogenic colonization of the berry may even be beneficial for the P.N.

Another reason of lack for virulence may be a lower rate in enzyme production as found by Lorenz and Pommer (1984). Pectic enzymes, e.g. are essential for noble rot development (see 3). Consequently the strains must be tested in this respect.

When we switch over from laboratory to field experiments we need a good knowledge of the weather conditions required for a successful infection and an optimal development of the P.N. In this field, similar research is desirable, as that performed for the grey rot (Strizyk, 1983). We also need a sufficient precise weather-forecast, predicting the foggy nights and the sunny days over at least one week. Finally, a correct interpretation of possible failures in the early field experiments will be necessary to realize success rapidly.

In our laboratory we have already selected a few homocaryotic strains possessing most of the mentioned requirements. On the other hand, a good knowledge of the pathogenis of *Botrytis cinerea* is indispensable. Nevertheless, some further research is necessary in this specific field. We call now for industries interested to start preliminary field trials, industrial inoculum production and commercialization.

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