European Journal of Plant Pathology **109:** 479–488, 2003.

Phenotypic differences between *vacuma* and *transposa* subpopulations of *Botrytis cinerea*

Fabian Martinez[1], Dominique Blancard[1], Pascal Lecomte[1], Caroline Levis[2], Bernadette Dubos[1] and Marc Fermaud[1,*]
[1]*Unité Mixte de Recherches en Santé Végétale INRA-ENITAB, Institut National de la Recherche Agronomique (INRA), BP 81, 33883 Villenave d'Ornon cedex, France;* [2]*Phytopathologie et Méthodologie de la Détection, INRA, Route de Saint-Cyr, 78026 Versailles cedex, France; *Author for correspondence (Phone: +33 5 57 122 622; Fax: +33 5 57 122 621; E-mail: fermaud@bordeaux.inra.fr)*

Accepted 28 January 2003

Key words: epidemiology, morphology, mycelial growth rate, transposable elements, virulence, *Vitis vinifera*

Abstract

One hundred and twenty-one single-spore strains of *Botrytis cinerea* isolated from Bordeaux vineyards were molecularly characterized as either *transposa* or *vacuma*, two subpopulations of *B. cinerea* distinguished by the presence of transposable elements. Forty-three *vacuma* and 68 *transposa* strains were distributed into two main classes (mycelial or sclerotial) by morphological phenotype according to the organ of origin. Strains isolated from overwintering sclerotia produced exclusively sclerotial colonies. The mycelial growth rate of 21 *transposa* and 13 *vacuma* strains was significantly influenced by agar-medium and temperature. The mycelial growth rate was significantly strain-dependent at favourable temperatures (15, 20 and 25 °C), but not at limiting ones (5 and 28 °C): *vacuma* strains showed the fastest growth rates. The strains of the two subpopulations were similar in virulence on both host species tested (*Vitis vinifera* and *Nicotiana clevelandii*). The grapevine leaves were significantly more susceptible to *B. cinerea* than those of tobacco. A significant negative correlation was established between virulence and mycelial growth rate. The epidemiological consequences concerning population structure of *B. cinerea* in vineyards are discussed.

Introduction

Botrytis cinerea Pers.: Fr., the anamorph of *Botryotinia fuckeliana* (de Bary) Whetzel, is a common and widespread ascomycete fungus which causes grey mould on numerous plants and can infect various tissues (Coley-Smith et al., 1980). An important biological feature is that the process of infection by *B. cinerea* is often associated with prior colonization of dead or dying plant debris as a nutrient-providing saprophytic base (Jarvis, 1977). In grapevine, the development of *B. cinerea* before harvest can result in serious fruit loss and in deterioration of wine quality (Dubos, 2000).

Many studies have reported that this fungus can exhibit great phenotypic diversity (Chardonnet et al., 2000; Di Lenna et al., 1981; Grindle, 1979; Kerssies et al., 1997; Lorbeer, 1980; Paul, 1929; Yourman et al., 2001). Because of the relative scarcity of genetic studies, somatic variability among isolates is often explained by the multinucleate and heterocaryotic nature of hyphae or conidia and the aneuploid state of nuclei (Büttner et al., 1994; Hansen and Smith, 1932). Part of this variation may also be due to the activity of transposable elements (Levis et al., 1997; McDonald, 1993; Smith and Corces, 1991). Giraud et al. (1997, 1999) reported that *B. cinerea* can be divided into at least two sibling sympatric subpopulations characterized by the presence of transposable elements. The strain *transposa* contains both transposable elements, Boty and Flipper, whereas the strain *vacuma* does not. Moreover, in vineyards in Champagne, the incidence of the *vacuma* subpopulation decreases regularly during

summer: from ca. 50% of the *B. cinerea* population in June to ca. 10% at harvest (Giraud et al., 1997).

The search for relationships between genetic patterns (e.g. using RAPD) and biological or ecological features has been generally unsuccessful (Alfonso et al., 2000; Kerssies et al., 1997; Vallejo et al., 1996). Nevertheless, a trend has been established concerning host specialization (Thompson and Latorre, 1999). In this study, the phenotypic differences within and between the two subpopulations considered were further characterized. Such differences are of interest because they can result directly from the presence of the transposable elements and because they can account for the drop in incidence of the *vacuma* subpopulation over the summer in the vineyard. Therefore, strains were collected in vineyards near Bordeaux, the molecular type (*vacuma* or *transposa*) was determined and some of their biological features were characterized. Morphology and virulence using two host species were studied as well as mycelial growth rate on artificial media to evaluate the saprophytic ability of strains to spread. These phenotypic characteristics are considered to be of prime importance to explain epidemiological differences between the two subpopulations.

Materials and methods

Fungal material and routine culture conditions

One hundred and twenty-one isolates of *B. cinerea*, originating from various locations near Bordeaux (Table 1), were collected in 1998 and 1999 from different diseased grapevine organs. After incubation of the plant material in moist chambers, the strains were obtained by single-spore isolation. All single-spore strains were stored at 4 °C on solid malt–agar medium ('MA': 15 g l^{-1} Cristomalt, Materne, France, 20 g l^{-1} agar). For each experiment, mycelial plugs (4 mm in diameter) were taken from cultures of *B. cinerea* on MA plates incubated for 4 days at 20 °C in the dark. The plugs were cut from the edge of the colonies and placed, individually, with mycelium underneath, at the centre of one Petri dish or of one leaf disc.

Molecular determination of the subpopulations

Mycelia and conidia were harvested by scraping a pure culture of *B. cinerea* on MA. Genomic DNA was extracted (Möller et al., 1992). Dot blot hybridizations for the detection of the transposable elements, Boty and Flipper, were performed (Giraud et al., 1997). Among the *vacuma* subpopulation, the strains of the 'group I' were detected using the original PCR–RFLP method (Fournier et al., 2002).

Morphological characterization

One hundred and eleven strains (Table 1) were selected in order to study both *vacuma* (V) and *transposa* (T) strains isolated from every grapevine organ (leaves: 19 V + 28 T, flowers: 11 V + 4 T, berries: 8 V + 22 T, canes: 4 V + 14 T). Each strain was cultured on MA plates (three replicates) at 20 °C in the dark. After 3 weeks, phenotypic observations were performed macroscopically on the basis of mycelial aspect, sporulation and sclerotial production. Eight different morphological types were defined (Table 2): four mycelial (M1, M2, M3 and M4) and four sclerotial (S1, S2, S3 and S4). The strains were distributed into these classes.

Mycelial growth rate assays

Thirty-four single-spore strains of *B. cinerea* (Table 1) collected in 1998 were studied under different temperature conditions on two different media: MA and PDA (39 g l^{-1} Potato–Dextrose–Agar, Difco Laboratories, USA). Three replicates per strain and per cultural condition were used. In the first set of assays, the mycelial growth rate was determined at one optimum temperature (20 °C) on both media, and at two suboptimum temperatures (15 and 25 °C) on MA medium. In a second set of assays, the following experimental design was replicated twice: 26 strains were compared on MA plates under three temperature conditions, two limiting for *B. cinerea* (5 and 28 °C) and, as a control, the optimum temperature (20 °C). Plates were incubated in the dark for 5 days at 20 and 25 °C, 8 days at 15 °C, and 15 days at 5 and 28 °C. Two perpendicular diameters per colony were measured daily and the mean colony radius was calculated.

Virulence tests

Thirty-two *B. cinerea* strains (Table 1) were tested using two host species: *Vitis vinifera* cv. Cabernet sauvignon and *Nicotiana clevelandii*. Three trials were

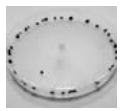
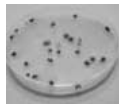
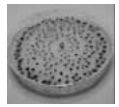
Table 1. Origin and molecular type of the 121 single-spore isolates of *B. cinerea* collected in Bordeaux vineyards (1998 and 1999)

Molecular type	Year	Strain no.[a]	Period	Organ	Cultivar; location
transposa	1998	**53**, **55**	Harvest	Berry	Sémillon; Sauternes
		110, 112–114	Veraison	Berry	Sauvignon; Pessac-Léognan
		132	Harvest	Berry	Sauvignon; Pessac-Léognan
		160, **163**	Pre-veraison	Berry	Sémillon; Pessac-Léognan
		179	Pre-veraison	Bunch	Sémillon; Pessac-Léognan
		193	Pre-veraison	Leaf	Sémillon; Pessac-Léognan
		213	Veraison	Leaf	Sémillon; Pessac-Léognan
		225	Veraison	Leaf	Sémillon; Pessac-Léognan
		234	Harvest	Berry	Sémillon; Pessac-Léognan
		250	Harvest	Leaf	Sémillon; Pessac-Léognan
		266	Bloom	Blossom	Sauvignon; Entre-Deux-Mers
		307, 314	Harvest	Leaf	Sauvignon; Entre-Deux-Mers
		326	Bloom	Blossom	Merlot; Médoc, St-Julien
		332	Bloom	Leaf	Merlot; Médoc, St-Julien
		343, **344**	Harvest	Berry	Merlot; Médoc, St-Julien
transposa	1999	468, 469, 473	Bloom	Blossom	Merlot; Médoc, St-Julien
		494, 495, 499, 501, 503, 507–514	Harvest	Berry	Sémillon; Pessac-Léognan
		590–592, 594, 595, 599, 604, 606, 607, 615–620	Harvest	Leaf	Merlot; Médoc, St-Julien
			Harvest	Leaf	Merlot; Médoc, St-Julien
		724–726, 729–731, 733, 734	Bloom	Leaf	Merlot; Médoc, Pauillac
		800, 801, 803–805, 807, 808, 819, 825, 827–829, 841, 842	Winter	Cane	Merlot; Médoc, St-Julien
vacuma	1998	80	Pre-veraison	Berry	Sauvignon; Pessac-Léognan
		106, 111	Veraison	Berry	Sauvignon; Pessac-Léognan
		115, 117	Veraison	Leaf	Sauvignon; Pessac-Léognan
		148, **155**	Harvest	Leaf	Sauvignon; Pessac-Léognan
		162, 171	Pre-veraison	Berry	Sémillon; Pessac-Léognan
		181	Pre-veraison	Bunch	Sémillon; Pessac-Léognan
		267, **268**	Bloom	Blossom	Sauvignon; Entre-Deux-Mers
		310	Harvest	Leaf	Sauvignon; Entre-Deux-Mers
		321, **322**, **324**	Bloom	Blossom	Merlot; Médoc, St-Julien
		329, 330	Pre-veraison	Leaf	Merlot; Médoc, Pauillac
		351, **357**	Harvest	Leaf	Merlot; Médoc, St-Julien
vacuma	1999	461	Bloom	Blossom	Sauvignon; Sauternes
		470, 471, 472, 474, 476, 477	Bloom	Blossom	Merlot; Médoc, St-Julien
		492, 523, 539	Harvest	Berry	Sémillon; Pessac-léognan
		593, 596–598, 600, 602, 603, 605, 610	Harvest	Leaf	Merlot; Médoc, St-Julien
		727, 728, 735	Pre-veraison	Leaf	Merlot; Médoc, Pauillac
		809, 824, 826, 838	Winter	Cane	Merlot; Médoc, St-Julien

[a] *B. cinerea* strain nos. are indicated in **bold** when assessed for both mycelial growth and virulence (in every trial), and, in *italic* for the few strains not characterized morphologically. Series of numbers are indicated by separating the first and last number by a hyphen. All isolates are available from authors upon request.

carried out using 6-week-old grapevine cuttings and 2-month-old tobacco plants grown in a greenhouse. Young leaves were detached and rinsed in sterile deionized water. Eight leaf discs, 2.5 cm in diameter, were inoculated per treatment (combination strain × host plant). A mycelial plug was placed centrally on the upper side of every leaf disc. The leaf discs were incubated at 22 °C for 3 days in moist chambers, i.e. plastic boxes (22 × 13 × 4 cm). Disease development on every leaf disc was assessed according to a visual semi-quantitative graded scale (0 = healthy, 1 = 10% rotten, 3 = 20%, 5 = 40%, 7 = 60%, 9 = 80%, 11 = 90% and 13 = totally rotten). A mean pathogenicity index was calculated.

Table 2. Phenotypic classification of *B. cinerea* strains on MA medium ($n = 111$)

Phenotype	Mycelial type 'M'				Sclerotial type 'S'			
	MI	MII	MIII	MIV	SI	SII	SIII	SIV
Mycelium	Short	Aerial	Mycelial masses	Thick and woolly	Rather short	Rather short	Rather short	Rather short
Sporulation[1]	−	+	±	−	±	±	±	−
Sclerotia[1]	0	−	−	−	In the edge of Petri dish	Often large, in circle	Often large, placed irregularly	Numerous, small and scattered
Number of strains[2]	8	4	6	14	31	10	28	23
Representative image								

[1]0: absence; −: absence or very rare; ±: more or less sporulating; +: sporulating profusely.
[2]13 strains were intermediate morphologically between two types and were then counted twice (in both classes).

Statistical analyses

The S+ software package was used for all statistical analyses (Statistical Sciences S-PLUS, 1993). The frequency distributions in two main morphological classes (mycelial or sclerotial) were compared between two organs, in pairs, using the χ^2 test with Yates' correction for continuity at $P = 0.05$. Prior to analysis, the numbers of mycelial ($M_1 + M_2 + M_3 + M_4$) and sclerotial ($S_1 + S_2 + S_3 + S_4$) strains were added up. Growth rate data were analysed by an ANOVA procedure using a factorial design with two main factors: strain and the experimental conditions (temperature × medium combinations). Two blocks were used for the analysis of the duplicated trials at 5, 20 and 28 °C. Virulence data, as mean disease grades, were also analysed by an ANOVA procedure on the basis of a factorial design with two main effects (the strain and the host species) and their interaction. The three replicated tests of virulence were analysed as three blocks. The sample correlation coefficient (Pearson r) was calculated using mean values for each of the 31 strains. The pathogenicity index was calculated as the overall mean from both hosts and the mean daily radial growth rate was calculated from the experiment at favourable temperatures (15, 20 and 25 °C).

Results

Molecular determination of the subpopulations

The presence or absence of two transposable elements (Boty and Flipper) was tested in every strain using dot blot hybridization (Figure 1). Strains from the *transposa* subpopulation contained both transposable elements, whereas the strains from the *vacuma* subpopulation did not. However, some *vacuma* strains showed a positive response to the 'Boty' probe only, as previously mentioned by Giraud (1998). The resulting molecular determination of each strain is presented in Table 1. Two *vacuma* strains (321 and 324) were of the 'group I' (Fournier et al., 2002; Leroux et al., 2002).

Morphological characterization

Colonies of *B. cinerea* on MA were classified visually into eight morphological types (Table 2). Two main morphological types, without marked differences in sporulation, were identified: 'mycelial' characterized by a quasi-absence of sclerotia and 'sclerotial', including colonies with many sclerotia. The strains were mostly of the sclerotial type under the present experimental conditions (Table 2).

The frequency distributions into the morphological classes are presented in Figure 2. The strains collected in winter from overwintering sclerotia on woody canes were of the sclerotial type only, irrespective of the subpopulation. Within the *transposa* strains, the distribution into the sclerotial and the mycelial classes was significantly different at $P = 0.05$ ($\chi^2 = 4.61$, df $= 1$) between woody canes and berries (100% and 63.6% of sclerotial strains, respectively). No significant difference was detected at $P = 0.05$ between leaves and berries ($\chi^2 = 0.31$, df $= 1$) or between leaves and woody canes ($\chi^2 = 2.59$, df $= 1$). As for the *vacuma* strains, the distribution into the two main classes was

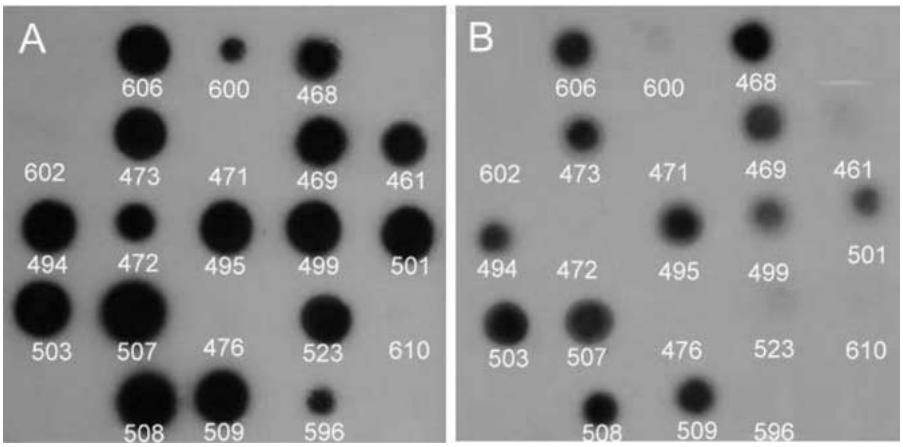


Figure 1. Example of an autoradiograph showing dot blot hybridizations using the 'Boty' probe (A) and the 'Flipper' probe (B). The molecular determination of *B. cinerea* strains (numbered as in Table 1) as *transposa* resulted from the presence of both transposable elements (Boty and Flipper).

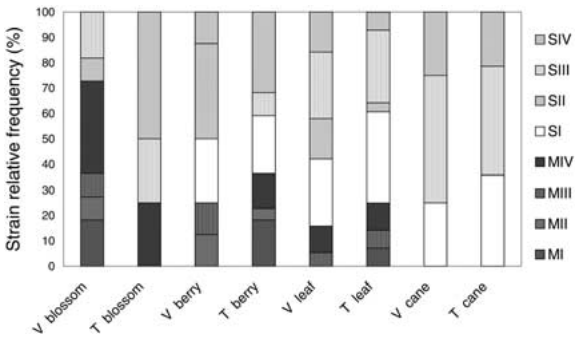


Figure 2. Frequency distributions of 111 *B. cinerea* strains in eight morphological classes on malt–agar medium at 20 °C (MI – MIV, mycelial types and SI – SIV, sclerotial types, see Table 2) according to the subpopulation, *vacuma* (V) or *transposa* (T), in combination with the grapevine organ from which the strain originated.

Table 3. Variance analysis of the daily mycelial growth rate of *B. cinerea*

Source of variation	df	Mean square	*F* value	*P*
ANOVA 1[1]				
Temperature × medium combinations	3	1.05	395.8	<0.0001
B. cinerea strains	33	0.03	10.0	<0.0001
ANOVA 2[2]				
Temperature	2	10.1	4652.7	<0.0001
B. cinerea strains	25	0.01	3.2	0.0001
Interaction	50	0.01	2.4	0.0003

[1]ANOVA 1, testing two main effects of strains and cultural conditions, i.e. four conditions resulting from three favourable temperatures (15, 20, 25 °C) combined with two agar-media (MA, PDA).
[2]ANOVA 2, testing two main effects of strains and limiting temperatures (5, 20, 28 °C).

significantly different at $P = 0.01$ ($\chi^2 = 7.42$, df $= 1$) between blossoms and leaves (72.7% and 15.8% of mycelial strains, respectively). No significant difference was detected at $P = 0.05$ between blossoms and berries ($\chi^2 = 2.53$, df $= 1$) or between leaves and berries ($\chi^2 = 0.0004$, df $= 1$).

Mycelial growth rate

At favourable temperatures (15 °C, optimum 20 and 25 °C), the strains showed significant differences in mycelial growth rate (Table 3). The ranking showed gradual differences between all strains (Figure 3). The *vacuma* strains, including the two 'group I' strains (nos. 321 and 324), were characterized by the greatest daily growth rates with one exception (no. 310). These were mostly greater than 1 cm d^{-1} when considering the average of the favourable temperatures tested. The growth rate was also influenced significantly by the combination of temperature and agar-medium tested (Table 3). The means were significantly differentiated and ordered as follows: 1.10 cm d^{-1} (a) on PDA at 20 °C, 1.05 cm d^{-1} (b) on MA at 20 °C, 0.99 cm d^{-1} (c) on MA at 25 °C and 0.71 cm d^{-1} (d) on MA at 15 °C.

At limiting temperatures (5 and 28 °C), both the temperature and the *B. cinerea* strain significantly affected mycelial growth rate (Table 3). The growth rate was markedly reduced at 5 and 28 °C compared with the optimum of 20 °C. The means varied significantly from one temperature to the next: 0.98 cm d^{-1} (a) at 20 °C; 0.33 cm d^{-1} (b) at 5 °C and 0.11 cm d^{-1} (c) at 28 °C. The differences between strains were significant only at 20 °C, but not at both limiting temperatures, as indicated by the significant interaction. The resulting strain ranking at 20 °C confirmed that from the experiment at 15, 20 and 25 °C (Figure 3). Similarly, the greatest growth rates, exceeding 1.02 cm d^{-1}, characterized in all the *vacuma* strains tested (nos 322, 162, 321, 181, 357, 155, 324, 267, 268) with one exception, no. 310.

Virulence

All the strains were pathogenic on both host species: *V. vinifera* and *N. clevelandii* (Figure 4). The grapevine leaves were significantly more susceptible to the pathogen than those of tobacco (F value $= 137.4$; df $= 1$; $P < 0.0001$). The mean pathogenicity indexes were 9.1 and 5.4, respectively. There were also significant differences in virulence between the *B. cinerea* strains (F value $= 3.4$; df $= 31$; $P < 0.0001$). In grapevine, the most pathogenic strains colonized more than 90% of the leaf disc surface (index ≥ 11), whereas the weaker ones colonized less than 50% of the leaf disc surface (index ≤ 6). The relative strain ranking was consistent on both hosts (Figure 4) as indicated by a non-significant interaction between both host and strain effects (F value $= 0.8$; df $= 31$; $P = 0.73$). However, the test did not discriminate between most of the strains because they were sorted into overlapping groups of homogeneous means. The *vacuma* strains did not differ in pathogenicity from the *transposa* strains.

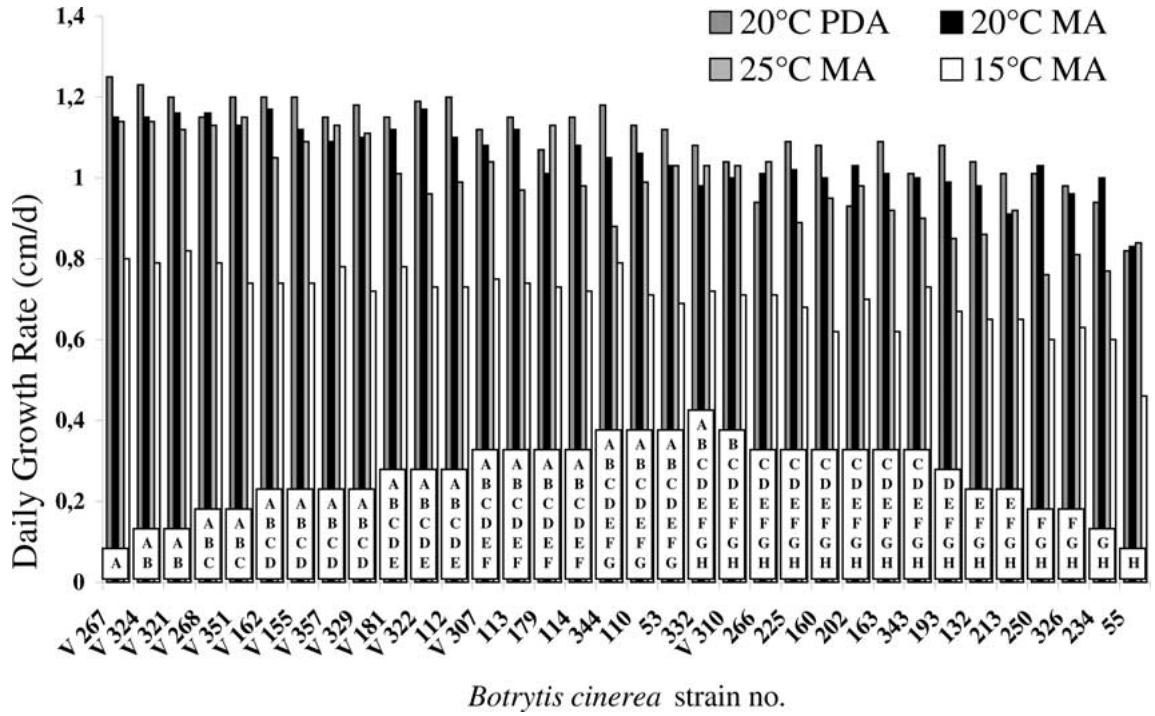


Figure 3. *B. cinerea* mycelial growth (cm d^{-1}) on two agar-media and at three favourable temperatures (15 °C, optimum 20, 25 °C). The significant effect of the strain ($n = 34$) were reflected in the greatest growth rates characterizing the *vacuma* strains ('V' in front of the strain number). Overall means for every strain (bars) with the same letter are not significantly different according to Newman and Keuls' test after ANOVA ($P = 0.05$).

Relationship between mycelial growth rate and virulence

There was a negative correlation between the mean pathogenicity index and the daily radial growth rate. The correlation was significant at $P = 0.01$, when both subpopulations were analyzed together (df = 29, $r = 0.49$). The negative correlation was also significant at $P = 0.05$ when analyzing the *transposa* strains only (df = 20, $r = 0.47$) with the equation: '$y = -0.0241\,x + 1.126$'. A similar trend was noticeable for the *vacuma* strains analyzed separately, but the correlation was not significant (df = 7, $r = 0.61$, just below the r threshold value of 0.67 at $P = 0.05$).

Discussion

In the range of favourable temperatures for *B. cinerea* (15–25 °C), most of the *vacuma* strains showed the fastest mycelial growth rates on both agar-media. The assumption can be made that the growth rate on a highly nutritive medium constituted an indirect assessment of the saprophytic colonization potential of the strain. This could indicate a better ability of *vacuma* strains to spread saprophytically on nutritive moribund plant substrates. This interpretation is substantiated, particularly at flowering in the fields, by two major pieces of evidence. First, for various plant species, isolates from flowers, petals and stamens resulted in the prevalence of *vacuma* strains (59%) in June (Giraud et al., 1999). Similarly, in different vineyards near Bordeaux (unpublished data) and in Champagne (Giraud et al., 1997), *vacuma* strains were isolated most often just after flowering and exceeded 50% of the *B. cinerea* population. Second, it has been demonstrated that the pathogen develops principally saprophytically on senescing grapevine floral parts, especially, debris of petal calyptras and necrotic stigmas or styles (Bulit and Lafon, 1977; McClellan and Hewitt, 1973). More generally, in many host species, the floral tissues are naturally highly susceptible to *B. cinerea*, especially when senescing: ornamentals (gerbera, rose), grapevine (Nair and Allen, 1993) or kiwifruit (Fermaud et al., 1994).

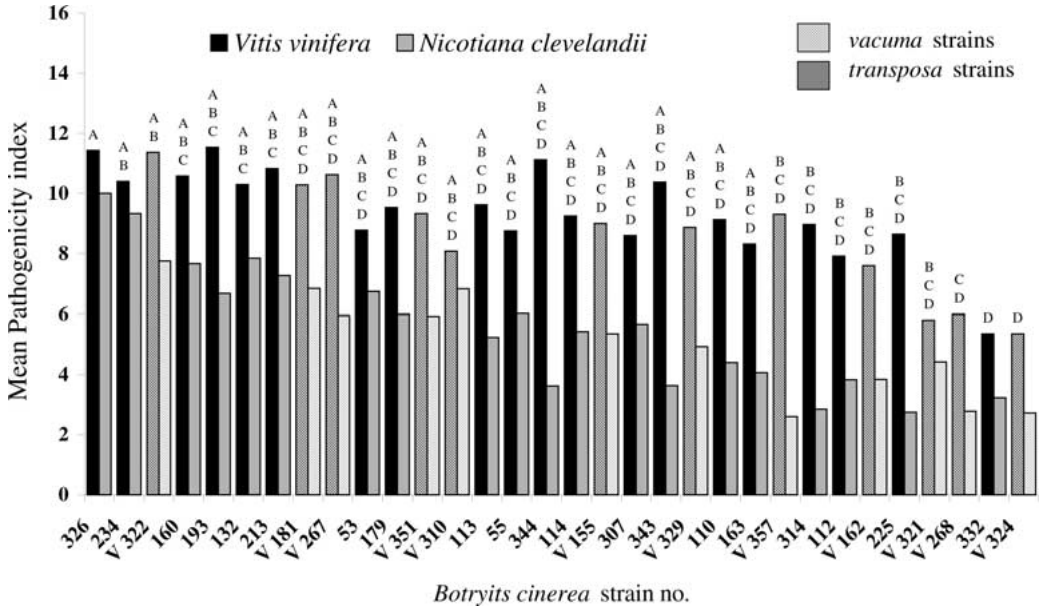


Figure 4. *B. cinerea* virulence (visual graded scale: 0 = healthy, 13 = maximal leaf colonization) according to significant strain ($n = 32$) effect and significant host species effect (*V. vinifera* and *N. clevelandii*). The strains of both subpopulations, *transposa* (shaded) and *vacuma* (hatched), were not differentiated. Means (bars), calculated from both hosts, with the same letters are not significantly different according to Newman and Keuls' test after ANOVA at $P = 0.05$ (same group of homogeneous means).

The activity of transposable elements may have influenced those genomic regions that are involved in vegetative growth. Integration of transposable elements in fungal chromosomes has been shown to have influenced chromosomal features, DNA sequence and gene expression (Daboussi, 1997). Presence and/or activity of transposable elements may negatively influence fungal fitness (McDonald, 1993; Rose and Doolittle, 1983); this may be relevant for natural *transposa* strains. A further evaluation of the fitness of subpopulations with and without transposable elements occurring in vineyards throughout the growing season on various organs will help to clarify this point (Martinez, 2002).

The mycelial growth rate of isolates belonging to both subpopulations, *vacuma* and *transposa*, was similarly affected by temperature. The greatest mycelial growth rate was observed at 20 °C, then, in a decreasing order, at 25, 15, 5 °C and the smallest mycelial growth rate was noted at 28 °C. This confirmed the temperature effect on infection of grape berries by *B. cinerea*, found *in vitro* at 15, 20 and 25 °C (Nair and Allen, 1993) and on the development of aerial mycelium on grape berries (Thomas et al., 1988). Thus, the strains of the two subpopulations showed a similar thermic optimum. Nevertheless, a small difference in thermic optimum could occur within a narrower interval of variation than that tested (less than 5 °C). At both limiting temperatures, 5 and 28 °C, the mycelial growth rate was not different between strains. This seems to imply a lack of difference in the adaptation to higher temperatures between *vacuma* and *transposa* strains. Therefore, these results cannot account for the prevailing occurrence of *transposa* strains in vineyards during summer, i.e. after bloom (Giraud et al., 1997).

Every isolate from grapevine, caused lesions on both the grapevine leaves and the less susceptible tobacco leaves. This indicated that *B. cinerea* shows no strict host plant specificity (MacFarlane, 1968). Differences in virulence between strains were consistent on both hosts. These differences resulted, at least partially, from a differential ability to colonize intercellularly and macerate leaf tissues. Considering the absence of a difference between the two subpopulations, the experimental conditions may have favoured strains which develop better saprophytically (100% relative humidity, supplying nutrients within the mycelial plugs) and not favoured triggering a plant defence response. The mycelial colonization of tissues lacking of defence response could be representative of the prominent

symptom development on ripening grape berries which progressively lose their potential for stilbene synthesis towards fruit maturity (Keller et al., 2000).

A negative correlation was established between mycelial growth rate and the virulence index. A similar negative correlation has been reported for *Leptosphaeria maculans*, causing the blackleg disease in crucifers (Koch et al., 1989; McGee and Petrie, 1978; Rouxel et al., 1994). This relationship would support the assumption that the *vacuma* strains, due to their faster mycelial growth rate, may be less aggressive than the *transposa* strains. In order to determine potential differences more discriminatively, especially between the two subpopulations, further studies should be carried out under more stringent conditions (sub-optimum temperature or/and light inoculations using conidia).

In the present morphological study, sclerotial colonies predominated, as reported previously (Goto et al., 1980). Interestingly, the strains isolated from cane bark in winter were of the sclerotial type exclusively *in vitro*. This can be related to the fact that the winter isolates were obtained only from sclerotia, the conspicuous overwintering state, and not from mycelium or conidia as for the other stages. The strains from the two subpopulations were not different: every morphological type was found in both subpopulations. Nevertheless, a difference was evident within the *vacuma* strains, between strains from blossoms (72.7% of mycelial strains) and leaves (15.8% of mycelial strains). This could suggest again that the state of *vacuma* strains at bloom in the vineyards is mostly mycelial, and results primarily from a hyphal colonization of the floral organs.

In conclusion, the *vacuma* subpopulation was characterized by a faster mycelial growth than the *transposa* subpopulation. In the context of fungal ecology (Pugh, 1980), it can be hypothesized that the *vacuma* strains have a life-strategy of the 'ruderal' type, more markedly so than the *transposa* strains. This life-strategy, also known as an 'r-strategy', has been applied to fungi exhibiting fast growth and high sporulation. Profuse sporulation is generally considered to be a typical feature of *B. cinerea* in the field (Jarvis, 1977). However, we did not observe any difference in sporulation intensity between the two subpopulations. This could be due to the experimental conditions which may be not representative of field conditions, in that darkness lowers sporulation. Therefore, a possible difference in sporulation ability between the two subpopulations should be further investigated. The life-strategies are related to two features of the environment: 'stress', which essentially leads to a shortage of food, and 'disturbances' which prevent fungal development (e.g. fungicides) or promote it (e.g. intermittent additions of nutrients, such as leaves falling in the autumn) (Grime, 1979; Pugh and Boddy, 1988). Thus, in the vineyard at the end of flowering, the fall of senescent petal calyptras in profusion would constitute a disturbance. Other disturbances, such as cultural practices (leaf removal, pruning, trimming) could also promote the *vacuma* subpopulation by providing dead or decaying organic matter. However, a more marked ruderal lifestyle and saprophytism fitness may not be the only components accounting for the difference in the prevailing subpopulation in the vineyard. Population migrations from one plot or region to another may also contribute to population differences.

Acknowledgements

We are indebted to D. Fortini, A. Chaigne, G. Monique, and X. Capdevielle for their technical participation to this work. Special thanks are also due to C. Gadenne and K. Mayo Candresse for helpful contribution during manuscript writing and to Prof. G. Holz, Dr. J. Köhl and Dr. P.A.G. Elmer for valuable comments on the manuscript. This study was supported by different research grants: from the 'Institut National de la Recherche Agronomique' (INRA; AIP Ecopath), from the 'Région Aquitaine' (contract number: 990307004) and from the 'Conseil Interprofessionnel du Vin de Bordeaux' (CIVB).

References

Alfonso C, Raposo R and Melgareja P (2000) Genetic diversity in *Botrytis cinerea* populations on vegetable crops in greenhouses in south-eastern Spain. Plant Pathology 49: 234–251

Bulit J and Lafon R (1977) Observations sur la contamination des raisons par le *Botrytis cinerea* Pers. (pp 61–69). In: Travaux dédiés à G Viennot-Bourgin. Société Française de Phytopathologie, Paris, 416 pp

Büttner P, Koch F, Voigt K, Quidde T, Risch S, Blaich R, Bruckner B and Tudzynski P (1994) Variations in ploidy among isolates of *Botrytis cinerea*: Implications for genetic and molecular analyses. Current Genetics 25: 445–450

Chardonnet CO, Sams CE, Trigiano RN and Conway WS (2000) Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. Phytopathology 90: 769–774

Coley-Smith JR, Verhoeff K and Jarvis WR (1980) The Biology of *Botrytis*. Academic Press, London

Daboussi MJ (1997) Fungal transposable elements and genome evolution. Genetica 100: 253–260

Di Lenna P, Marciano P and Magro P (1981) Comparative investigation on morphological and physiological features of three isolates of *Botrytis cinerea*. Phytopathologische Zeitschrift 100: 203–211

Dubos B (2000) Grey mould. In: Féret (ed) Parasitic Fungal Diseases in the Vine. Bordeaux, France

Fermaud M, Gaunt RE and Elmer PAG (1994) The influence of *Thrips obscuratus* on infection and contamination of kiwifruit by *Botrytis cinerea*. Plant Pathology 43: 953–960

Fournier E, Levis C, Fortini D, Leroux P, Giraud T and Brygoo Y (2002) Characterization of *Bc-hch*, the *Botrytis cinerea* homolog of the *Neurospora crassa* het-c vegetative incompatibility locus, and its use as a population marker. Mycologia (in press)

Giraud T (1998) Etude de la diversité génétique, du mode de reproduction et de la structure des populations du champignon phytopathogène *Botrytis cinerea*. Thèse Université Paris VI

Giraud T, Fortini D, Levis C, Leroux P and Brygoo Y (1997) RFLP markers show genetic recombination in *Botrytinia fuckeliana* (*Botrytis cinerea*) and transposable elements reveal two sympatric species. Molecular Biology and Evolution 14: 1177–1185

Giraud T, Fortini D, Levis C, Lamarque C, Leroux P, LoBuglio K and Brygoo Y (1999) Two sibling species of the *Botrytis cinerea* complex, *transposa* and *vacuma*, are found in sympatry on numerous host plants. Phytopathology 89: 967–973

Goto S, Terabayashi T and Yokotsuka I (1980) Identification, cultural properties and pathogenicity of grey mould of grape, *Botrytis cinerea*. Nippon Nôgeikagaku Kaishi 54: 117–121

Grime JP (1979) Plant Strategies and Vegetation Processes. John Wiley, Chichester, New York, Brisbane and Toronto

Grindle M (1979) Phenotypic differences between natural and induced variants of *Botrytis cinerea*. Journal of General Microbiology 111: 109–120

Hansen HN and Smith RE (1932) The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopathology 22: 953–964

Jarvis WR (1977). *Botryotinia* and *Botrytis* species – taxonomy, physiology and pathogenicity. Monograph no. 15, Ottawa, Research Branch, Canada Department of Agriculture

Keller M, Steel CC and Creasy GL (2000) Stilbene accumulation in grapevine tissues: Developmental and environmental effects. Acta Horticulturae 514: 275–286

Kerssies A and Bosker-van Zessen AI (1997) Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. Plant Disease 81: 781–786

Koch E, Badawy HMA and Hoppe HH (1989) Differences between aggressive and non-aggressive single spore lines of *Leptosphaeria maculans* in cultural characteristics and phytotoxin production. Journal of Phytopathology 124: 52–62

Leroux P, Fournier E, Brygoo Y and Panon ML (2002) Biodiversité et variabilité chez *Botrytis cinerea*, l'agent de la Pourriture grise. Phytoma-La défense des végétaux 554: 38–42

Levis C, Fortini D and Brygoo Y (1997) *Flipper*, a mobile Fot1-like transposable element in *Botrytis cinerea*. Molecular and General Genetics 254: 674–680

Lorbeer JW (1980) Variation in *Botrytis* and *Botryotinia*. In: Coley-Smith JR, Verhoeff K and Jarvis WR (eds) The Biology of *Botrytis* (pp 19–39) Academic Press, New York

Martinez F (2002) Etude de la structure génétique et des aptitudes biologiques des populations de *Botrytis cinerea* Pers. dans le vignoble bordelais. Thèse Université de Bordeaux II

McClellan WD and Hewitt WB (1973) Early *Botrytis* rot of grapes: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology 63: 1151–1157

McDonald JF (1993) Evolution and consequences of transposable elements. Current Opinion in Genetics and Development 3: 855–864

McFarlane HH (1968) Plant host–pathogen index to volumes 1–40 (1922–1961). Review of Applied Mycology. Commonwealth Mycological Institute, Kew

McGee DC and Petrie GA (1978) Variability of *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology 68: 625–630

Möller EM, Bahnweg G, Sandermann H and Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research 20: 6115–6116

Nair NG and Allen RN (1993) Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. Mycological Research 97: 1012–1014

Paul WRC (1929) A comparative morphological and physiological study of a number of strains of *Botrytis cinerea* Pers. with special reference to their virulence. Transactions of the British Mycological Society 14: 118–135

Pugh GJF (1980) Strategies in fungal ecology. Transactions of the British Mycological Society 75: 1–14

Pugh GJF and Boddy L (1988) A view of disturbance and life strategies in fungi. Proceedings of the Royal Society of Edinburgh 94B: 3–11

Rose MR and Doolittle WF (1983) Molecular biological mechanisms of speciation. Science 220: 157–162

Rouxel T, Galla C and Balesdent MH (1994) Du polymorphisme au complexe d'espèces: Combien d'agents pathogènes sont impliqués dans la nécrose du collet du Colza? Agronomie 14: 413–432

Smith PA and Corces VG (1991) Drosophila transposable elements: Mechanisms of mutagenesis and interactions with the host genome. Advances in Genetics 29: 229–299

Statistical Sciences, S-PLUS (1993) Guide to Statistical and Mathematical Analysis S-PLUS version 3.2. Stat Sci, a division of Mathsoft, Inc., Seattle

Thomas CS, Marois JJ and English JT (1988) The effects of wind speed, temperature, and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. Phytopathology 78: 260–265

Thompson JR and Latorre BA (1999) Characterization of *Botrytis cinerea* from table grapes in Chile using RAPD–PCR. Plant Disease 83: 1090–1094

Vallejo I, Santos M, Cantoral JM, Collado IG and Rebordinos L (1996) Chromosomal polymorphism in *Botrytis cinerea* strains. Hereditas 124: 31–38

Yourman LF, Jeffers SN and Dean RA (2001) Phenotype instability in *Botrytis cinerea* in the absence of benzimidazole and dicarboximide fungicides. Phytopathology 91: 307–315