Environmental Conditions Affect *Botrytis cinerea* Infection of Mature Grape Berries More Than the Strain or Transposon Genotype

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Accepted for publication 2 March 2015.

ABSTRACT

Ciliberti, N., Fermaud, M., Roudet, J., and Rossi, V. 2015. Environmental conditions affect *Botrytis cinerea* infection of mature grape berries more than the strain or transposon genotype. Phytopathology 105:1090-1096.

Effects of environment, *Botrytis cinerea* strain, and their interaction on the infection of mature grape berries were investigated. The combined effect of temperature (T) of 15, 20, 25, and 30°C and relative humidity (RH) of 65, 80, 90, and 100% was studied by inoculating berries with mycelium plugs. Regardless of the T, no disease occurred at 65% RH, and both disease incidence and severity increased with increasing RH. The combined effect of T (5 to 30°C) and wetness duration (WD) of 3, 6, 12, 24, and 36 h was studied by inoculating berries with conidia. At WD of 36 h, disease incidence was approximately 75% of affected berries at 20 or 25°C, 50% at 15°C, and 30 to 20% at 30 and 10°C; no infection occurred at 5°C. Under favorable conditions (100% RH or 36 h of WD) and unfavorable conditions (65% RH or 3 h of WD), berry wounding did not significantly affect disease incidence; under moderately favorable conditions (80% RH or 6 to 12 h of WD), disease incidence was approximately 1.5 to 5 times higher in wounded than in intact berries. Our data collectively showed that (i) T and RH or WD were more important than strain for mature berry infection by either mycelium or conidia and (ii) the effect of the environment on the different strains was similar. Two equations were developed describing the combined effect of T and RH, or T and WD, on disease incidence following inoculation by mycelium (R^2 = 0.99) or conidia (R^2 = 0.96), respectively. These equations may be useful in the development of models used to predict and control Botrytis bunch rot during berry ripening.

Additional keywords: gray mold, *Vitis vinifera*.

Botrytis cinerea Pers., the anamorph of *Botryotinia fuckeliana* (de Bary) Whetzel, is a necrotrophic fungal pathogen responsible for gray mold, which affects a wide range of plants (28). In grape (*Vitis vinifera* L.), *Botrytis cinerea* can affect all the herbaceous organs but is particularly damaging on ripening berries, leading to severe losses and considerable reduction in wine quality (33,51). Susceptibility of grape clusters to Botrytis rot increases gradually from veraison to ripening (11,32), and there is a positive relationship between berry sugar content and incidence of Botrytis rot (26,40). Wounded mature berries are more susceptible than unwounded ones (28,32,40,43,44). Botrytis rot of ripening berries can originate from (i) latent infections caused by conidia during flowering or berry set, (ii) direct berry infection by conidia produced on various inoculum sources, and (iii) berry-to-berry infection caused by mycelium originating from previously infected berries within the cluster (16,25). Environmental conditions conducive to infection by *B. cinerea* conidia have been extensively studied in grape flowers (4,8) and ripening berries (4,5,28,32,42–45). In these reports, berry infection by conidia was favored by temperatures (T) between 15 and 25°C accompanied by 12 to 24 h of wetness duration (WD). At 5, 10, and 30°C, infection occurred but at lower levels. At any T, the infection incidence increased with increasing WD. At 12°C, infection by conidia occurred at 90 and 100% relative humidity (RH) but not at 80% RH. Aerial mycelium of *B. cinerea* developed quickly on infected berries at 21°C and 94% RH in the absence of wind but no mycelium developed at 69% RH under windy conditions (53).

The influence of the environment on conidial infection of berries is well documented. The effects of environment on berry-to-berry infection, however, have not been studied. Another important gap in our understanding concerns the phenotypic variability among *B. cinerea* strains. Except for Broome et al. (4), who used two isolates that were not genetically characterized, in all previous studies on berry infection, the high phenotypic variability among *B. cinerea* strains was not considered, including the recently published molecular and phenotypic information concerning the diversity in *B. cinerea* population.

Recent studies based on molecular and phenotypic markers (18,22,55) considered the pathogen to be a complex of two cryptic species: *B. pseudocinerea* and *B. cinerea* sensu stricto. *B. pseudocinerea* isolates have been rarely detected (17,27,29,54) or detected, mostly in spring, at low frequencies, representing only 1 to 15% of the whole *B. cinerea* sensu lato population (1,18,21,22,31,38,39,48). *B. cinerea* sensu stricto populations are represented by four transposon genotypes: (i) *transposa*, (ii) *flipper* only (containing only the Flipper transposable element), (iii) *boty* only (containing only the Boty transposable element), and (iv) *vacuma* (1,10,13,17,20,21,27,35,36,38,41). Results of recent studies showed that *B. cinerea* transposable elements are involved in the production of small RNAs that silence the expression of host defense genes (3,57).

The current study was conducted to investigate how infection of mature berries by mycelium or conidia is affected by environment, *B. cinerea* strain, and the interaction between environment and strain.

MATERIALS AND METHODS

***B. cinerea* strains and culture conditions.** Eight strains of *B. cinerea* sensu stricto, including four strains of the two main transposon genotypes, were used: 18.13T, 213T, 53T, and 344T for *transposa*; and 18.21V, 321V, 351V, and 155V for *vacuma*. All of these strains, which were characterized and used in previous studies as belonging to the *B. cinerea* species (8,37,39), were stored on potato dextrose agar (39 g liter^{-1}) (HiMedia Laboratories, Mumbai, India) at 5°C before being used in the experiments.

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http://dx.doi.org/10.1094/PHYTO-10-14-0264-R
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Different culture conditions were used to produce the inoculum for inoculation of berries. Strains were cultured in petri dishes (8.6 cm in diameter) containing malt agar medium (15 g of malt and 20 g of agar in 1 liter of distilled water) (Difco Laboratories, Detroit) at 20°C in darkness to obtain growing mycelium for berry inoculation by mycelium. To obtain conidiating cultures for berry inoculation by conidia, petri dishes containing V8 medium (70 g of double-concentrated tomato [75%] [Victoria, SAC S.p.A., Camagnola, Italy], 100 g of soup of legumes [Blédina, Danone, Paris, France], 3 g of phosphate dipotassium and 20 g of agar [Difco Laboratories], and 1 liter of distilled water) were used. Dishes were inoculated with plugs of mycelium (4 mm in diameter), incubated at 20°C, and exposed to a 12-h photoperiod using white and near-UV (UV-A at 370 nm) light (Black Light UV-A, L18 w/73; OSRAM, Munich) for 15 to 19 days. Conidia were suspended in water by adding 7 ml of sterile deionized water containing 0.05% Tween 20 (polyethylene glycol sorbitan monolaurate) (Sigma-Aldrich, St. Louis) to the cultures and gently rubbing the agar surface with a glass rod. The resulting suspensions were filtered through a double layer of sterile cheesecloth to remove remaining mycelium. The number of conidia in the suspension was determined and adjusted to 10^4 conidia ml^{-1} with a hemocytometer (Bünker, HBG, Giessen, Germany).

Berry collection and preparation. Three experiments (experiments 1, 2, and 3) were conducted with one of two inoculum types (either conidia or mycelium) and with different T, WD, and RH (Table 1). For experiments 1 and 2, ripening berries were collected in 2012 from an experimental vineyard ('Sauvignon blanc') at the INRA campus, Villenave d'Ornon. For experiment 3, ripening *V. vinifera* berries were collected in 2011 from a commercial vineyard ('Barbera') in Ziano Piacentino, North Italy. The two vineyards were managed as usual in the two areas but were not sprayed with specific fungicides against *B. cinerea*.

Mature berries that appeared to be healthy and unwounded were cut from the rachis with their pedicel attached, disinfested by immersion in calcium hypochlorite (50 g $liter^{-1}$; pH 7.2) for 10 min, rinsed three times with sterile water, and then air dried in a laminar flow hood. Sugar content, pH, titratable acidity, and mean berry mass were determined for a subsample of these berries to assess the mean level of berry maturity (Table 1). All berries used for the experiments were unwounded, unless noted otherwise.

Infection by mycelium (experiment 1). In experiment 1, berries were inoculated with mycelial plugs (see next paragraph) of each of the eight *B. cinerea* strains and were kept at four constant T regimes and four RH levels (Table 1). Additional berries were manually wounded along their equators using a sterilized syringe needle, inoculated, and kept at 20°C and at the four RH levels. As a control, both wounded and unwounded berries were treated with sterile water and incubated at 20°C and 100% RH.

As described by Martinez et al. (39), mycelial plugs (4 mm in diameter) were cut from the edge of 4-day-old colonies of *B. cinerea*, which were grown as previously described; these were placed individually on the equator of each berry, with the mycelium side touching the berry surface. Berries were then placed on a metallic grid in humidity chambers (plastic boxes, 19 by 13 by 4 cm, 15 berries per box), and the chambers were placed in incubators (EX- 111, Tabai Espec Corp., Osaka, Japan) in the dark at 15, 20, 25, or 30°C. To create different RH levels, 100 ml of different saline solutions or distilled water was placed in each chamber (Table 2). The real value of RH in the chambers was determined for the duration of the experiment with an electronic device (Tinytag Plus 2; Gemini Data Loggers, Chichester, UK). There was one humidity chamber for each combination of T and RH.

Infection by conidia (experiments 2 and 3). Berries were inoculated with conidial suspensions of each of the eight strains in experiment 2 and with strains 18.13T and 18.21V in experiment 3. The berries were then incubated with different combinations of T and WD (Table 1). In experiment 2, additional berries were manually wounded as described earlier, inoculated, and kept at 20°C for 36 h of WD.

In experiment 2, berries were placed in humidity chambers as described for experiment 1. One humidity chamber containing 100 ml of sterile water on the bottom was used for each experimental condition (i.e., T × WD). Berries were inoculated by adding 10 µl of a conidial suspension (prepared as described above) to the equator. Chambers were then kept in incubators in the dark with all combinations of four T regimes and five WD periods (Table 1). At the end of each WD period, 40 µl of calcium hypochlorite (1%, pH 7.2) was placed on the inoculated area on the berry surface to stop conidial germination. After 1 min, the berries were rinsed with 1 ml of sterile water to remove the residues of calcium hypochlorite. They were then placed in the 20°C incubator in the dark for approximately 3 weeks to enable the expression of berry rot symptoms. As a control, additional berries were treated with sterile water instead of a conidial suspension and were kept in humidity chambers at 20°C for 36 h of WD.

In experiment 3, pedicels were detached from the berries and berries were then disinfested as above. The disinfested berries were placed in petri dishes (14 cm in diameter) on a metallic grid so that they did not touch the two filter papers, which were saturated with sterile water, on the bottom of the dish. A 5-µl droplet of the conidial suspension of strain 18.13T or 18.21V, prepared as described above, was placed on the pedicel scar. The petri dishes were then sealed with Parafilm to maintain a saturated atmosphere and were kept in incubators (FTD2500-Lux; ISCO, Lincoln, NE) at different T (Table 2) in the dark. After 3, 6, 12, 24, and 48 h of WD, a 5-µl droplet of calcium hypochlorite (5%, pH 7.2) was placed on the inoculation site to remove any viable inoculum from the berry surface. Petri dishes were resealed and kept at 20°C in the dark for 2 weeks to stimulate Botrytis rot. For each of the two strains, there were three dishes (10 berries per dish) for each combination of T and WD. As a control, additional berries were treated with sterile water

TABLE 2. Relative humidity (± 2%) obtained at different temperatures using distilled water or salts to prepare saturated salt solutions (12,58)

Salt or distilled water	Relative humidity (%) at the indicated temperature			
	15°C	20°C	25°C	30°C
NH_4NO_3	70	…	…	…
$NaNO_2$	…	66	64	63
$(NH_4)_2SO_4$	81	81	80	80
$Na_2C_4H_4O_6$	94	92	92	92
Distilled water	100	100	100	100

TABLE 1. Grape cultivars, *Botrytis cinerea* strains, inoculum sources, and combinations of environmental conditions used in the three experiments

Experiment[a]	Cultivar	Strains	Inoculum source	Environmental conditions[b]
1	Sauvignon blanc	All	Mycelium	T: 15, 20, 25, 30°C and RH: 65, 80, 90, 100%
2	Sauvignon blanc	All	Conidia	T: 15, 20, 25, 30°C and WD: 3, 6, 12, 24, 36 h
3	Barbera	18.13T, 18.21V	Conidia	T: 5, 10, 15, 20, 25, 30°C and WD: 3, 6, 12, 24, 48 h

[a] Experiments 1 and 2 were performed once; experiment 3 was performed twice. The level of berry maturity was sugar at 225 g $liter^{-1}$, pH 3.31, 4.64 titratable acidity (H_2SO_4 g $liter^{-1}$), and 2.46 g of berry mass in experiment 1; sugar at 227 g $liter^{-1}$, pH 3.25, 4.48 titratable acidity, and 2.33 g of berry mass in experiment 2; and sugar at 239 g $liter^{-1}$, pH 2.95, 10.56 titratable acidity, and 2.49 g of berry mass in experiment 3.

[b] T = temperature, RH = relative humidity, and WD = wetness duration. All combinations of T and RH and of T and WD were tested systematically.

instead of a conidial suspension and were kept in humidity chambers at 20°C for 48 h of WD.

Assessment of disease incidence and severity. Berries were assessed as healthy or rotten, and disease incidence was calculated as the percentage of the total number of berries that were rotten. The percentage of berry surface area with rot was assessed visually (only in experiments 1 and 2); that the rot was caused by *B. cinerea* was confirmed by the observation of typical *B. cinerea* conidiophores when each berry was examined with a stereomicroscope. In experiment 1, the berries were assessed at 6, 14, and 22 days postinoculation (dpi). The berries were assessed at 7, 14, and 20 dpi in experiment 2 and at 14 dpi in experiment 3.

Data analysis. Because no rot developed in berries inoculated with sterile water without mycelium or conidia in any of the experiments (data not shown), these data were not used in statistical analysis.

Generalized linear models. Generalized linear models were fitted to investigate the effect of (i) T, WD, and their interaction (as fixed effects) on disease incidence in experiment 1; and (ii) T, RH, and their interaction (as fixed effects) on disease incidence in experiment 2. In experiment 2, only the data relative to 10, 20, and 25°C, and 80, 90, and 100% RH were considered because no disease developed in any inoculated berry at 30°C or 65% RH; considering all data did not permit model convergence. Fungal strains were considered as random effect in all analyses (52). An identity link function was used for the berry rot incidence in experiment 1 because it was considered as a quantitative trait (47), and a link logit function was used in experiment 2 because model fitting with original values gave convergence problems (data not shown); for both models, a conditional binomial distribution to the random effect was assumed. All models were fitted using the SAS package (ver. 9.3; SAS Institute, Cary NC).

Regression analysis. Data on disease incidence assessed at the end of each experiment were regressed against T and RH (in experiment 1) and against T and WD (in experiment 2). Disease incidence data of any strain were rescaled (expressed as proportion of the maximum on a 0-to-1 scale) by dividing each value by the incidence of that particular strain with the optimal combination of T and RH (the combination resulting in the maximum incidence) in experiment 1 or with the optimal combination of T and WD in experiment 2. Rescaled values were then independent of the capability of the strain to cause the disease; this enabled us to compare the combined effect of T and RH, or T and WD, for the different strains.

Different nonlinear regression models were used and compared based on the Akaike's Information Criterion (AIC). The model providing the smallest AIC value was then considered the most likely to be correct (6). The following models were considered for experiments 1 and 2, respectively: $y = [a \times Teq^b \times (1 - Teq)]^c/[1 + \exp(d - e \times RH/100]$ (equation 1) and $y = [a \times Teq^b \times (1 - Teq)]^c \times \exp[-d \times \exp(-e \times WD)]$ (equation 2), where y is the rescaled infection incidence; Teq is the equivalent of T calculated as (T – Tmin)/(Tmax – Tmin), where T is the temperature regime (in °C) and Tmin and Tmax are minimal and maximal T for infection, respectively, which were considered as equation parameters (59); RH is relative humidity; WD is wetness duration (in hours); a to c are the equation parameters accounting for the effect of T; and d and e are the equation parameters accounting for the effect of RH or WD. Equation 1 includes a logistic equation and equation 2 a Gompertz equation in the denominator (which define the disease increase at increasing RH or WD, respectively), which have an asymptotic value that depends on T according to an Analytis equation in the numerator (with parameters a, b, and c, which define the top, symmetry, and size of the curve, respectively) (2,7).

Parameters of the selected equations were estimated for pooled data of the eight strains. Deviations of the data for individual strains from the predicted data for pooled strains were then calculated as the absolute difference between the observed value and the predicted ones. Finally, the mean absolute error (MAE) and its standard error were calculated.

Regression analysis was performed using the SPSS statistical package (SPSS ver. 19.0; IBM SPSS Statistics, IBM Corp., New York).

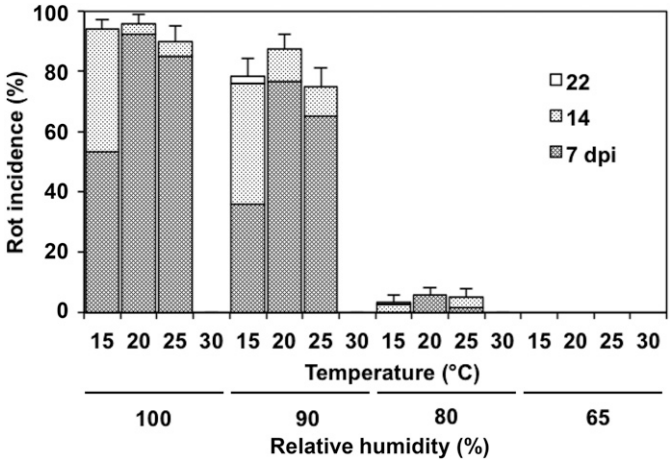

Fig. 1. Incidence of *Botrytis cinerea* rot at 7, 14, and 22 days postinoculation (dpi) in mature berries that were inoculated with mycelium and then subjected to four temperatures and four relative humidities. Whiskers are the standard error for eight strains.

RESULTS

Disease in berries inoculated with mycelium (experiment 1). Regardless of fungal strain and T, no disease occurred at 65% RH (Fig. 1). Regardless of fungal strain and RH level, no disease occurred at 30°C (Fig. 1). Disease incidence was affected by RH ($P < 0.001$) but no significant difference existed between incubation at 10, 15, and 20°C ($P = 0.57$); interaction T × RH also was not significant ($P = 0.98$). The covariance parameter estimate for the random effect of fungal strains was = 0.459, meaning that even though strains were influenced by T and RH in a similar way, they showed different capability of causing berry rot.

Disease incidence was approximately 30 to 15 times higher at 100 and 90% RH than at 80% RH. Disease developed faster at 100% RH than at 90% RH (i.e., incidence was greater at 7 and at 14 dpi for the higher RH value) (Fig. 1). At 100% RH at 7 dpi, disease incidence was 93 and 85% at 20 and 25°C, respectively, and 53% at 15°C; by 14 and 22 dpi, however, incidence at 100% RH was >90% at the three T (Fig. 1).

Disease incidence and severity as affected by three T regimes and three RH levels at 22 dpi were closely related (Fig. 2). An exponential equation in the form $y = 0.944 \times e^{4.9876x}$ (where x is disease severity and y is disease incidence) provided a good fit of these data, with $R^2 = 0.84$.

Equation 1 provided a good fit of the combined effect of T and RH on rescaled disease incidence at 22 dpi for pooled data from the eight strains (Fig. 3) and for the single-strain data. The equation had the following parameter estimates: Tmin = 0, Tmax = 30, $a = 7.75 \pm 0.47$, $b = 2.14 \pm 0.11$, $c = 0.47 \pm 0.10$, $d = 35.36 \pm 2.52$, and $e = 40.26 \pm 2.82$, with $R^2 = 0.99$. The MAE for the single-strain data were = -0.04 ± 0.01, and most of the observed-predicted residuals were within ± 0.1. Deviation was highest for strain 213T (MAE = 0.07 ± 0.03) and lowest for the *vacuma* strain 18.21V (MAE = 0.002 ± 0.03).

Disease in berries inoculated with conidia (experiments 2 and 3). In experiment 2, disease incidence was affected by T ($P = 0.003$) and WD ($P < 0.001$) but not by their interaction ($P = 0.07$). The covariance parameter estimate for the random effect of fungal strains approached 0, meaning that the variability due to the strain effect was likely to be irrelevant.

Regardless of fungal strain, disease incidence at 20 dpi and at 20°C was highest with 36 h of WD (Fig. 4A). For the same WD period (i.e., 36 h), disease incidence was 80 and 76% at 20 and 25°C,

respectively; 57% at 15°C; and 35% at 30°C (Fig. 4B). Similar results were obtained in experiment 3 (Fig. 5); at 14 dpi, no infection occurred at 5°C, and infection incidence was similar at 10 and 30°C (Fig. 5B).

Equation 2 provided a good fit of the combined effect of T and RH on rescaled disease incidence at 20 dpi for pooled data from the eight strains (Fig. 6) and for the single-strain data. The equation had the following parameter estimates: Tmin = 5°C, Tmax = 35°C, a = 6.416 ± 1.22, b = 1.292 ± 0.073, c = 1.42 ± 0.21, d = 2.30 ± 0.19, and e = 0.05 ± 0.02, with R^2 = 0.96. The equation provided a good fit for the pooled data for eight strains. The average MAE for single strains was 0.0003 ± 0.014, with most of the observed-predicted within ± 0.3. The deviation was highest for strain 18.13T (MAE = 0.17 ± 0.05) and lowest for strain 344T (MAE = 0.01 ± 0.04).

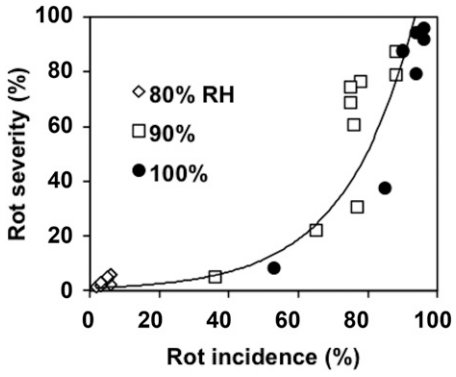

Fig. 2. Relationship between incidence and severity of *Botrytis cinerea* rot at 6, 14, and 22 days postinoculation in mature berries that were inoculated with mycelium of eight strains and then subjected to three temperatures (15, 20, and 25°C) and three relative humidities (RH) (80, 90, and 100% RH). Separate symbols for temperature and days postinoculation are not presented in this figure. The curve fits the data based on the following equation: $y = 0.944 \times e^{4.9876x}$, $R^2 = 0.84$.

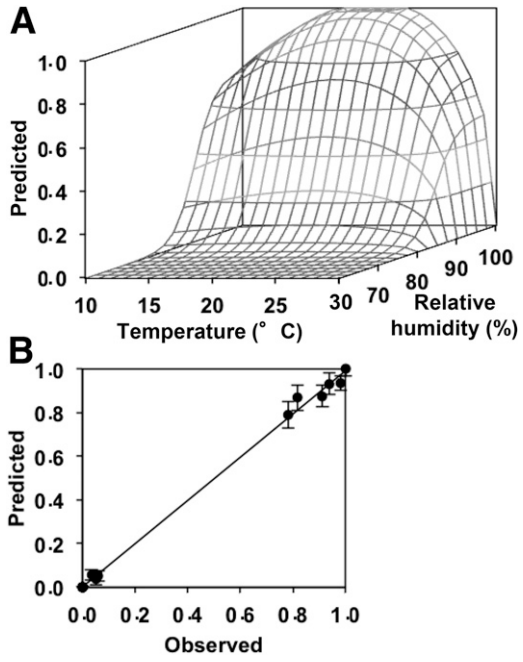

Fig. 3. A, Predicted incidence of *Botrytis cinerea* rot at 22 days postinoculation in mature berries inoculated with mycelium as affected by temperature (T) and relative humidity (RH) and **B,** plotted against observed values in experiment 1. Predictions in A are based on equation 1. Equation parameters were estimated for the pooled data of eight strains; disease incidence data of any strain were rescaled by dividing each value by the infection incidence of that particular strain with the optimal combination of T and RH (the combination resulting in the maximum incidence). In B, whiskers are the standard error for the inoculations with eight strains.

Effect of wounding. In berries inoculated with mycelium (Table 1, experiment 1), disease incidence at 80% RH was approximately five times higher for wounded than for intact berries. This difference was much lower at 90% RH and was absent at 100% RH (Fig. 7). No fungal development was observed at 65% RH even in wounded fruit. Similarly, in berries inoculated with conidia, disease incidence was higher for wounded than for unwounded berries with 6 and 12 h of WD but not with 3 and 36 h of WD (Fig. 8).

Infection by mycelium versus conidia. A plot of disease incidence at 22 dpi following mycelium inoculations in experiment 1 versus disease incidence at 20 dpi following conidial inoculations in experiment 2 (Fig. 9) showed that strain ability to cause infection was similar for inoculations made with mycelium and conidia, with Spearman's rank correlation coefficient ρ = 0.81 (P = 0.015).

DISCUSSION

In the present study, we determined how *B. cinerea* infection of mature berries inoculated with either mycelium or conidia is affected by environment (T, RH, and WD), *B. cinerea* strain, and the interaction between the environment and strain.

The combined effect of T and RH was studied by inoculating mature berries with mycelium plugs. The influence of the environment on mycelial infection has been neglected (15) and, to our knowledge, similar studies have not been published. Thomas et al. (53) and Nelson (45) also evaluated the effect of T and RH on berry infection but these authors inoculated mature berries with conidia. Therefore, our study refers to the berry-to-berry infection pathway whereas those of the authors mentioned above refer to the "pathway V" described by Elmer and Michailides (16). Pathway V corresponds

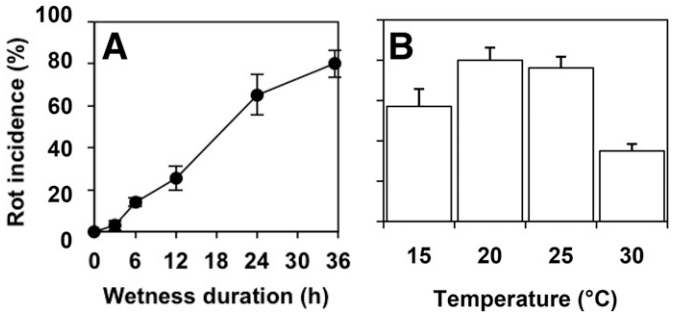

Fig. 4. Incidence of *Botrytis cinerea* rot at 20 days postinoculation in mature berries inoculated with conidia and kept at **A,** 20°C for six wetness duration (WD) periods and **B,** four temperatures for 36 h of WD. Whiskers are the standard error for the inoculations carried out with eight strains.

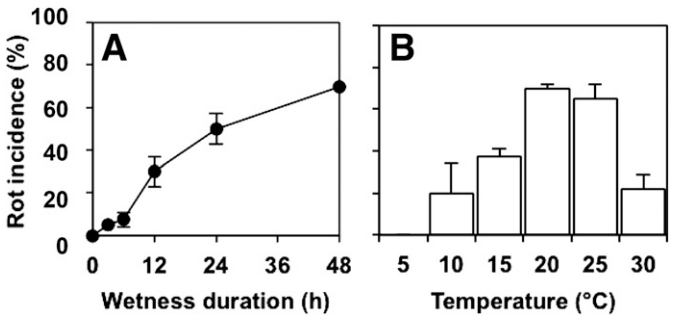

Fig. 5. Incidence of *Botrytis cinerea* rot at 14 days postinoculation in mature berries inoculated with conidia and kept at **A,** 20°C for six wetness duration (WD) periods and **B,** six temperatures for 48 h of WD. Whiskers are the standard error for the inoculations carried out with two strains.

to direct conidial infection of ripening fruit. Infection by mycelium was strongly influenced by RH: no disease occurred at 65% RH and both disease incidence and severity increased with RH regardless of T. This study then shows that berry-to-berry infection is favored by high humidity, as was previously demonstrated for infection by conidia (45). We also found a relationship between incidence and severity in mycelium-infected berries; such a relationship has been previously reported for other diseases (50) but not for disease caused by *B. cinerea*.

We studied the combined effect of T and WD by inoculating mature berries with conidia of eight *B. cinerea* strains. Our data confirmed most previously reported results, which were based on single strains (4,34,42–44), although we detected no infection at 5°C whereas other authors reported a low infection incidence at 5°C (34,42,43).

Our comparison of disease incidence in wounded and intact berries showed that, under highly favorable conditions (e.g., 100% RH or 36 h of WD) and highly unfavorable conditions (e.g., 65% RH or 3 h of WD), there was no significant effect of wounding. Under intermediate humidity or wetness conditions (e.g., 80% RH or 6 to 12 h of WD), however, disease incidence was higher in wounded than in unwounded berries. We suggest that, with intermediate humidity or wetness, wounds provide the fungus with fluid and thereby reduce the stress resulting from lower RH or shorter WD. In addition, the availability of nutrients in the wound area may increase the fungal growth rate (14,46). Because carbohydrates, including fructose and glucose, stimulate conidial germination (9), conidia may germinate more rapidly on wounded than on unwounded berries and, therefore, may require a reduced WD. However, no effect of wounding was observed with only 3 h of wetness; the stimulation provided by the wound is evidently incapable of compensating for such a short WD. Similarly, the failure of wounding to enhance infection by mycelium at low humidity (65% RH) may be due to the rapid dehydration of mycelium. Low-RH conditions also decrease water activity at the

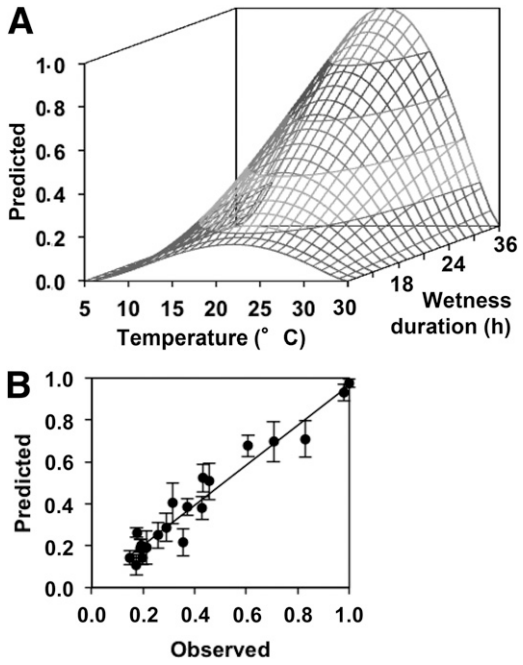

Fig. 6. A, Predicted incidence of *Botrytis cinerea* rot in mature berries inoculated with conidia as affected by temperature (T) and wetness duration (WD) and **B,** plotted against observed values in experiments 2 and 3. Predictions in A are based on equation 2. Equation parameters were estimated for the pooled data of eight strains; disease incidence data of any strain were rescaled by dividing each value by the infection incidence of that particular strain with the optimal combination of T and WD (the combination resulting in the maximum incidence). In B, whiskers are the standard error for the inoculations with eight strains.

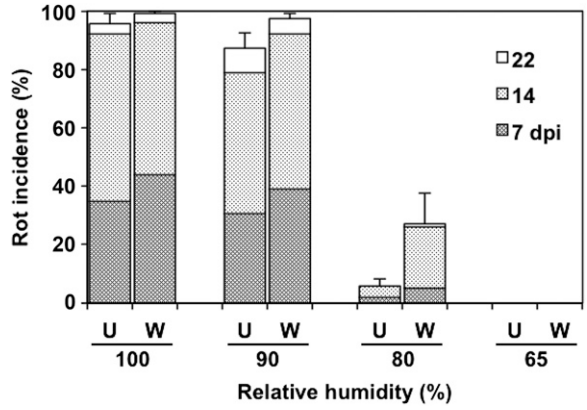

Fig. 7. Incidence of *Botrytis cinerea* rot at 7, 14, and 22 days postinoculation (dpi) in wounded (W) and unwounded (U) mature berries that were inoculated with mycelium and kept at 20°C with four levels of relative humidity (RH). Whiskers are the standard error for the inoculations carried out with eight strains.

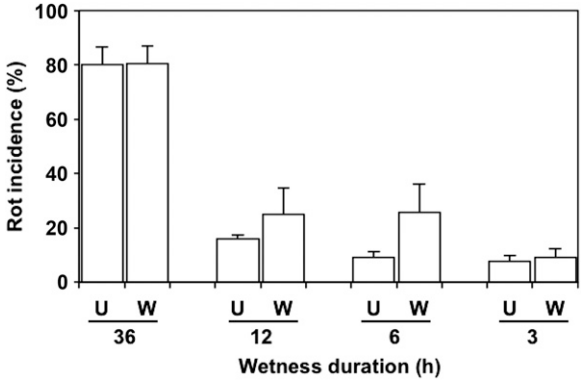

Fig. 8. Incidence of *Botrytis cinerea* rot at 20 days postinoculation (dpi) in wounded (W) and unwounded (U) mature berries inoculated with conidia and kept at 20°C with wetness durations of 3, 6, 12, and 36 h. Whiskers are the standard error for the inoculations carried out with eight strains.

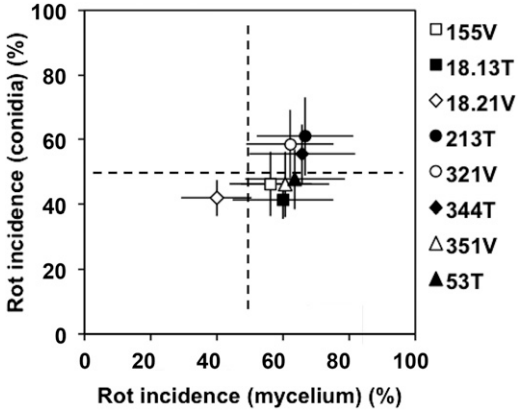

Fig. 9. Plot of incidence of *Botrytis cinerea* rot at 22 days postinoculation (dpi) with mycelium of eight strains (in experiment 1) versus disease incidence at 20 dpi with conidia of the same eight strains (in experiment 2). Points are averages, and whiskers are the standard error for the inoculations carried out with eight strains at 15, 20, and 25°C with 80, 90, and 100% relative humidity in experiment 1 and with the same temperatures but with 12, 24, and 36 h of wetness duration in experiment 2.

berry surface (19), which may reduce fungal growth. *B. cinerea transposa* strains require a water activity level >0.96 (11).

Although a recent study used representative strains of the *vacuma* and *transposa* genotypes to investigate *B. cinerea* infection of young bunches and inflorescences (8), earlier studies dealing with the effect of environmental conditions on infection of mature berries (4,34,42–45) failed to consider the genetic diversity in the pathogen population (18,21–24,37–39,49,56). In the current study, strain and the interactions between strain and the environment accounted for only a negligible part of the total experimental variability. Thus, our results suggest that (i) T and RH or WD were more important than strain in causing mature berry infection by either mycelium or conidia and (ii) the effect of the environment on the different strains was similar.

In conclusion, given that the effect of T, RH, or WD was similar among the eight *B. cinerea* strains in the current study, we developed two equations that describe the combined effect of T and RH, or T and WD, on rescaled infection incidence following inoculation by mycelium or conidia, respectively. Rescaled infection incidence is calculated by dividing each observed value of a particular strain by the infection incidence of the strain at the optimal combination of T and RH, or T and WD. This rescaled infection incidence is independent of the capability of each strain to cause disease. These equations may be useful for the development of new models or for the refining of existing models (4,5,15,30,42) that predict Botrytis bunch rot and that guide Botrytis bunch rot management during berry ripening. The equation for conidial infection could be used to predict infection of clusters through the infection pathway V according to Elmer and Michailides (16). The equation for mycelium infection could be used to predict berry-to-berry infection, which is a crucial component of the overall severity of Botrytis bunch rot at harvest. In our experiments on mycelium infection, rescaled disease incidence was either <0.1 or >0.8 and this could affect model fitting and its application (Fig. 3). An experimental design including additional T and RH combinations (e.g., 17.5°C and 85% RH, and 12.5°C and 95% RH) and, maybe, a higher sample size (i.e., more berries per treatment) could provide further information about the combined effect of T and RH on disease incidence following inoculation by mycelium.

ACKNOWLEDGMENTS

N. Ciliberti conducted this work within the Doctoral School on the Agro-Food System (Agrisystem) of the Università Cattolica del Sacro Cuore (Italy). We thank the technical staff, particularly J. Jolivet, at UMR SAVE-Bordeaux, where most of the experiments were carried out. In Bordeaux, this study has been carried out in the framework of the Cluster of Excellence COTE.

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