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Water activity at the fruit surface: a potential indicator of grape berry susceptibility to *Botrytis cinerea*

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Abstract: Water activity (Aw) is a physical property of a humid solid, which is of prime importance in food microbiology. This parameter is an assessment of available water, unbound to nutrient molecules, which allows damaging bacteria, yeasts and fungi to grow in a food product. As most moulds which do not grow below an Aw level of *ca*. 0.8, *Botrytis cinerea* was unable to grow on artificial media at an Aw of 0.93 and below. In 2008, in an experimental vineyard (*Vitis vinifera* L. cv. Merlot noir) near Bordeaux, *Botrytis* bunch rot development was assessed and the evolution of water activity was investigated at the surface of healthy grape berries during fruit development. Our results showed that Aw decreased steadily from the berry herbaceous stage to maturity and was correlated negatively and significantly (P = 0.01) with disease incidence. Furthermore, an experiment under controlled conditions showed the significant effect of relative humidity (RH) on the water activity level at the berry surface. When healthy berries at two different development stages ("herbaceous" and "beginning of colour change") remained at 80% RH for 6 days, the Aw values decreased significantly (P = 0.05). On the basis of the close relationship between water activity and Botrytis bunch rot development, the potential of Aw for use as a new disease indicator is discussed as well as the influence of climatic conditions on water activity measured at the grape berry surface.

Key words: grey mold, integrated control, ontogenic resistance, water availability

Introduction

Grey mould or *Botrytis* bunch rot, caused by the phytopathogenic fungus *Botrytis cinerea* Pers.:Fr., is one of the main aerial diseases in grapevine (*Vitis vinifera* L.) because it can reduce drastically both yield and wine quality. Epidemic progress on fruit is driven by various factors including notably i) the environmental conditions, particularly climate and microclimate (Fermaud *et al.*, 2001); ii) physiology and vegetative growth of the grapevine host plant (Valdés-Gómez *et al.*, 2008); iii) the biochemical and mechanical properties of the berry skin (Deytieux-Belleau *et al.*, 2009). The mechanical properties may be affected by the presence of micro-cracks or pores (Mlikota-Gabler *et al.*, 2003) as well as macroscopic injuries, such as those caused by insects allowing the fungus to penetrate into the host.

Among the berry skin features, water activity (Aw) at berry surface is a critical factor affecting both *in vitro* growth and metabolism of fungi, yeasts and bacteria (Rousseau and Donèche, 2001). For *B. cinerea*, spore germination and fungal growth are induced "*in vitro*" by a high free water level associated with a high Aw value (Lahladi *et al.*, 2007). More recently, the relationship between Aw and susceptibility of the grape berry to *B. cinerea* has been established using the cultivar Sauvignon blanc (Deytieux-Belleau *et al.*, 2009). In the present study, similar data have been further investigated to confirm the results on the black susceptible cultivar "Merlot noir".

The first objective of this work was to examine the temporal variations in Aw measured at the surface on healthy berries according to the fruit developmental stage. The second goal was to establish a relationship between the Aw evolution and the disease development in the vineyard. Lastly, the potential effect of ambient relative humidity (RH) on Aw at different developmental stages of the grape berry was also investigated.

Material and methods

Experimental vineyard, disease and maturity assessments

In 2008, grape berries (cv. Merlot noir) were collected from an INRA experimental vineyard near Bordeaux (France) from the end of July (herbaceous stage) to over-maturity at the beginning of October. The vineyard was planted in 1991 at the rate of ca. 5350 vines per ha with a traditional training system. The experimental vine plot was not treated with anti-*Botrytis* fungicides during the growing season. Disease development was assessed regularly by visual assessments of the percentage of the cluster surface showing typical rot symptoms with *B. cinerea* sporulation. At each assessment date, all the clusters from 7 blocks, each consisting of 5 adjacent vines, were sampled and rated visually for disease incidence, *i.e.* a total of approximately 280 clusters.

Water activity (Aw) and maturity monitoring

For maturity assessments, three samples of 100 cut fresh berries were randomly selected at each date and processed independently according to Deytieux-Belleau *et al.* (2009). As for Aw measurements, every week, a random sample of 10 berries, all being apparently healthy, was used to measure Aw according to Deytieux-Belleau *et al.* 2009.

Table 1. Experimental conditions to test the effect of relative humidity on water activity

Experiment	Berry stage	Sampling date and first Aw assessment	DAA (days after anthesis)	Berry no.	Incubation period (day) at 80% or 100% R.H.
1	Herbaceous	07/18/08	46	8	6
2	Beginning of veraison	08/05/08	64	8	6
3	Over-maturity	10/17/08	137	8	3

In order to test whether Aw and relative humidity (RH) may interact at the berry surface, 8 fresh and healthy grape berries (pedicel attached) were randomly sampled at three development stages (Table 1). Beforehand, the pedicel was surrounded with paraffin to avoid water exchanges and the Aw-meter was calibrated using a solution of K_2SO_4 (Aw = 0.973 at 25°C). To measure Aw at the fruit surface, each berry was placed in the chamber of a GBX Aw-meter (model “FA-st/1”) for *ca.* 1 hour at room temperature (between 25 and 29°C). The berries were then placed under controlled conditions for 3 to 6 days, using climatic chambers (EX-111; TABAI ESPEC Corp., Japan), at a constant temperature of 20°C in the darkness. At each berry stage, half of the berries (n = 4) were maintained at 100% RH whereas the other 4 berries were placed at 80% RH. After the RH-treatment, Aw at the berry surface was measured again as described above. The experimental conditions are summarized in Table 1. Data were analysed by ANOVA, followed by the test of Newman-Keuls for mean comparison, and based on two main factors: the phenological stage and the RH treatment.

Results and discussion

Climatic conditions and grey mould development

In 2008, the climatic conditions were conducive to *Botrytis* bunch rot as shown by frequent precipitations (Figure 1). Under our experimental conditions on cv. Merlot noir and without any anti-Botrytis fungicide, the epidemic progress curve was characterized by an incidence of *Botrytis* bunch rot that increased regularly from mid-veraison onwards (Figure 2). At the end of September, i.e. just after maturity, the disease incidence reached ca. 100% of rotted clusters.

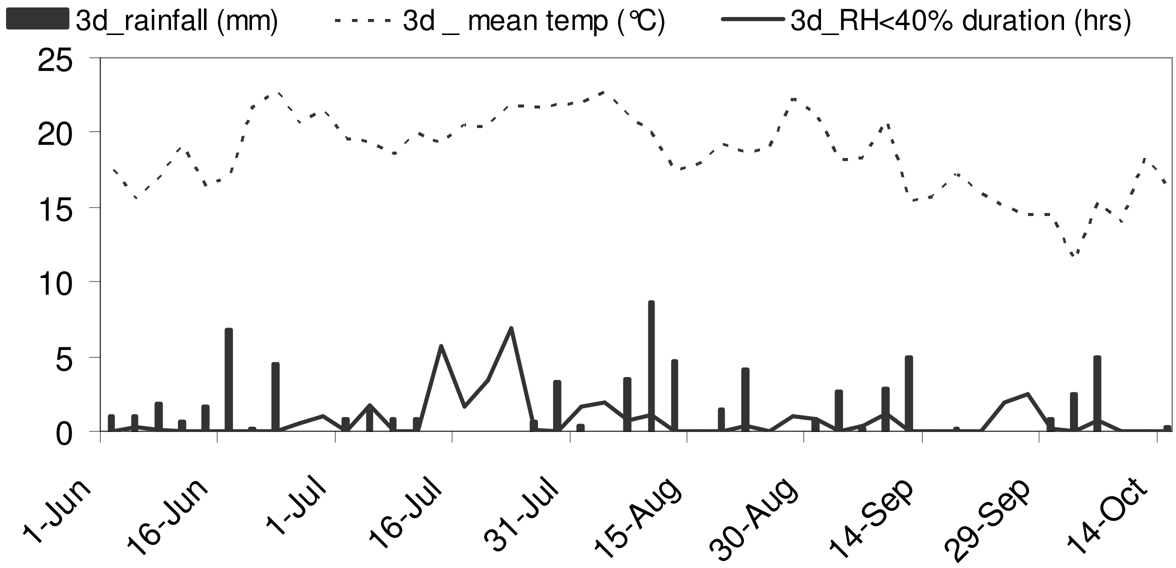

Figure 1. Climatic conditions in 2008 corresponding to the experimental vineyard (Villenave d'Ornon, France). Means are calculated from three consecutive days for air temperature (°C), rainfall intensity (mm) and duration of relative humidity RH<40% (no. hrs per day)

Grape berry maturity evolution

As shown in Figure 3, grape berry maturity increased regularly during the season. At maturity, the maturity index reached ca. 50 which was calculated as the following ratio: sugar content (g/l) divided by total acidity (g H_2SO_4/l).

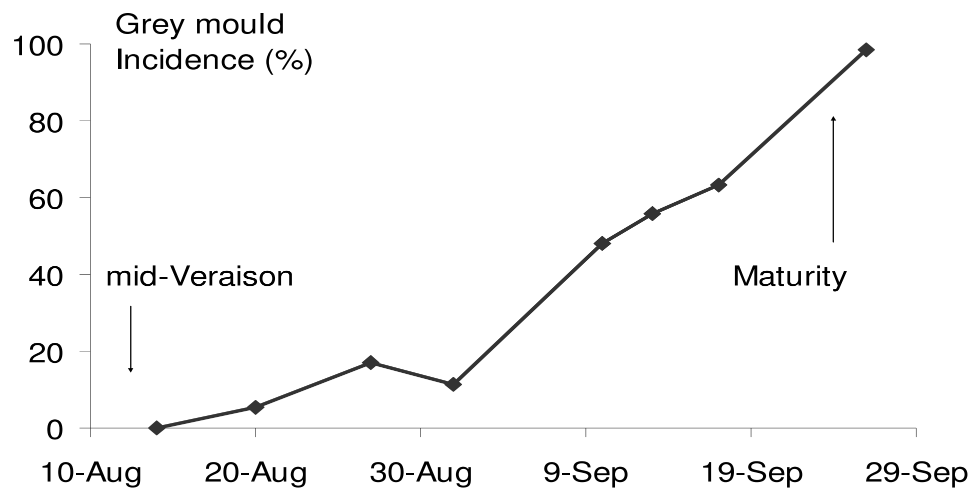

Figure 2. Epidemic progress curve of *Botrytis* bunch rot in 2008 (cv. Merlot noir) on the experimental vineyard (Villenave d'Ornon, France). Incidence is the percentage of diseased clusters assessed from mid-veraison "midV" to the maturity "M" stage.

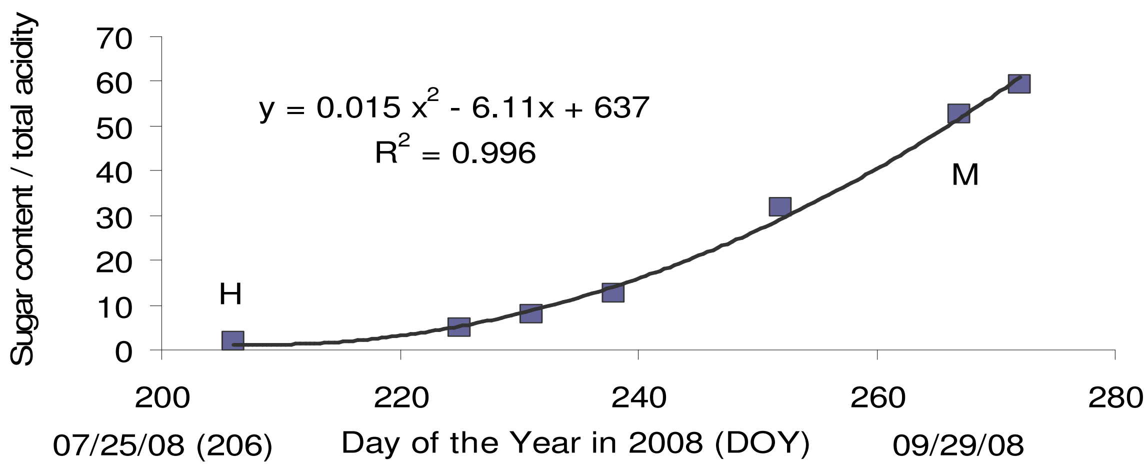

Figure 3. Evolution of grape berry maturity (cv. Merlot noir) shown using the maturity index "Sugar / Total acidity". Sugar content is expressed in g/L and total acidity in g H_2SO_4/L. Grape berry stages: "H": herbaceous immature berries, "M": maturity.

Evolution of water activity at the berry surface

Water activity at the berry skin surface decreased progressively during grape berry development from ca. 0.96 to a minimum of ca. 0.89 on 22 September (Figure 4). As a general trend, Aw decreased linearly up to maturity (112 DAA). However, in over-mature grape berries, Aw increased at the end of the season (from 22 September onwards).

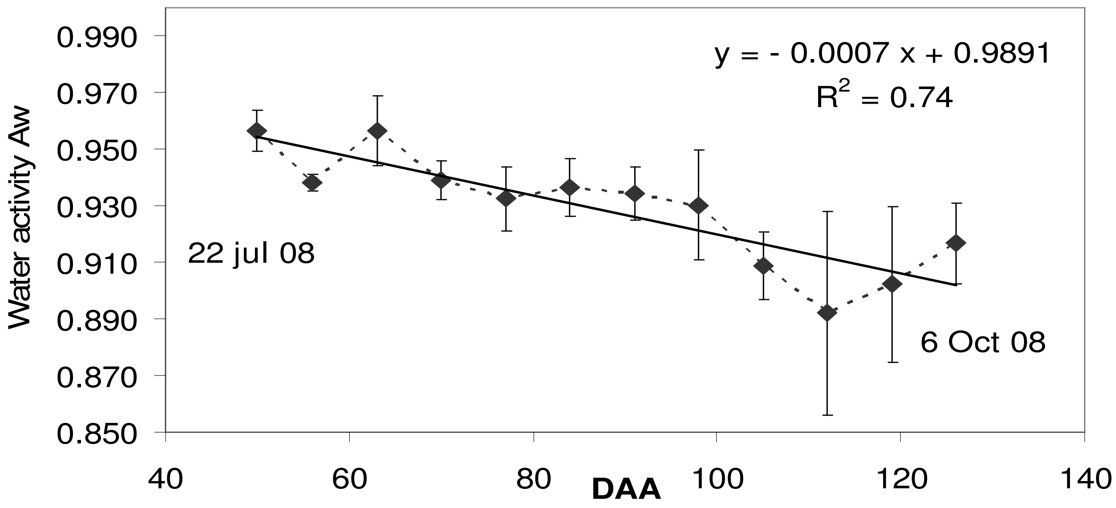

Figure 4. Variations in water activity at the berry surface in 2008 on cv. Merlot noir. (± *standard deviation: error bars*).

Relationship between disease incidence and water activity

Our results showed that Aw was correlated negatively with disease incidence. The linear relationship was highly significant ($P < 0.01$, Pearson's R = 0.96; ddf = 6) (Figure 5).

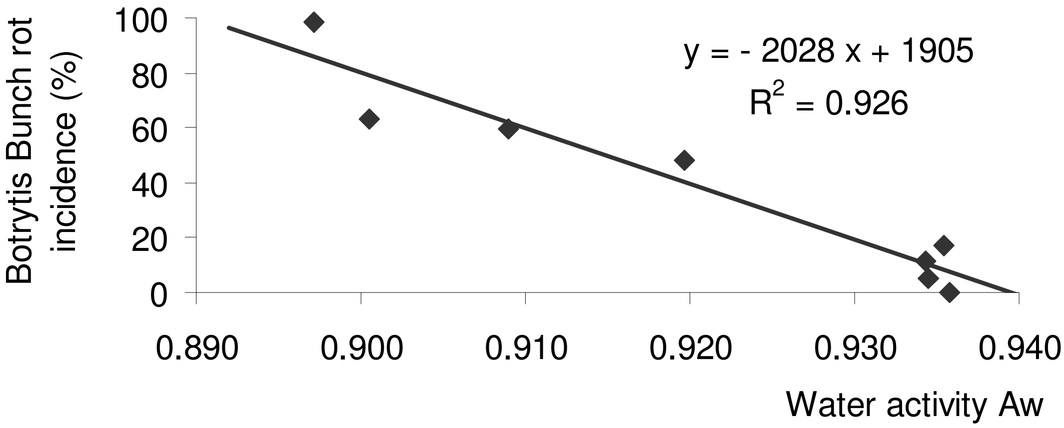

Figure 5. Significant correlation and linear relationship between Aw and incidence of botrytis bunch rot

Effect of relative humidity on water activity at the berry surface

At the surface of grape berries, cv. Merlot noir, the effect of RH on Aw, is showed in Table 2. The treatment consisting of a period of 3 to 6 days at 100% RH did not affect significantly Aw at the berry surface ($P = 0.57$). On the other hand, the treatment of the berries at 80 % RH resulted in a significant ($P < 0.001$) decrease in Aw at both "herbaceous" and "beginning of colour change" berry stages. However, at the "over-maturity" stage, no effect was detected as shown by the second ANOVA. This resulted from a significant interaction ($P = 0.0002$) between the two main factors tested.

Table 2. Effect of relative humidity on water activity, assessed at the surface of healthy grape berries (cv. Merlot noir), according to the berry developmental stage.

Berry stage	*ANOVA 1*		*ANOVA 2*	
	Control [a]	HR 100% [b]	Control [a]	HR 80% [b]
Herbaceous	0.907 A	0.912 A	0.908 a	0.863 c
Beginning of colour change	0.903 A	0.885 A	0.888 b	0.839 d
Over-maturity	0.857 B	0.858 B	0.805 e	0.804 e

[a] Aw assessed at the beginning of the experiment
[b] Aw assessed at the end of the treatment period by HR

Conclusion

Water activity Aw at the berry surface is considered as a possible major determinant governing grey mould development in the vineyard. These results on the black cultivar Merlot noir in 2008 strengthen those obtained in 2006 on the susceptible white cultivar Sauvignon (Deytieux-Belleau *et al.*, 2009). Therefore, besides fruit maturity, Aw might be used to estimate the evolution of the intrinsic berry susceptibility to infection by *B. cinerea*. The temporal pattern was a decrease in water activity until berry maturity which may be related to an increasing amount of solutes or exudates (exosmosis) at the berry surface. Aw decreased presumably because, at the fruit surface, solutes bind with free water reducing, then, available water. In this context, we have established a clear linear regression between water activity and disease incidence in the vineyard. These findings indicated that the measurement of water activity could constitute a good indicator to characterize grape susceptibility from bunch closure to fruit maturity. However, because several environmental factors may also affect the development of *B. cinerea*, extrapolation of these results to various other natural situations; i.e. other grapevine growing regions, cultivars and/or years, should be made with caution.

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