EFFECT OF INCUBATION TEMPERATURE AND RELATIVE HUMIDITY ON LESION DIAMETER OF *BOTRYTIS CINEREA* PERS. AND *PENICILLIUM EXPANSUM* LINK. ON APPLE FRUITS

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SUMMARY

Previous studies carried out in our laboratory demonstrated that the growth of *B. cinerea* and *P. expansum* was highly influenced by water activity and temperature in '*in vitro*' conditions. Regardless the temperature, a_w minimal for growth was ≤ 0.89 and equal to 0.89 for *P. expansum* and *B. cinerea* respectively. The effect of incubation temperature (5-25°C) and relative humidity (RH 75-98%) on the lesion diameter of two common fungi *B. cinerea* and *P. expansum* was studied and modelled in the controlled laboratory conditions in order to validate previous findings. The obtained results showed that only temperature had a significant effect on fungal growth on wounded apples. The relative humidity of air had no direct influence on growth of fungi. The part of variation explained by both studied factors is 52 and 55% respectively for *P. expansum* and *B. cinerea*. 'Lack of Fit' test was no significant for models, suggesting a greater difference between observed and predicted values. The difference between observed and predicted values was 14 and 29% respectively for *P. expansum* and *B. cinerea*. Theses results is in contradiction as compared to *in vitro* conditions and underlined that the humidity inside wounded apple sites is highly sufficient to start the growth of both postharvest pathogens of apples.

Key words: *P. expansum*, *B. cinerea*, Temperature, Relative humidity, Predictive model and apples.

INTRODUCTION

Penicillium expansum Link. and *Botrytis cinerea* Pers., two wound pathogens, are important postharvest diseases of apples and pears worldwide (Bondoux, 1992; Rosenberger, 1990), provoking economic losses in storage room (Jijakli *et al.*, 1999). Postharvest fungicide treatments are the main mean of controlling these losses. Imazalil and thibendazol remain the most important active substances applied for this purpose.

Water availability (water activity, a_w) and temperature are the major abiotic parameters determining the potential for germination and propagules growth of fungi on the fruit surface (Magan & Lacey, 1988; Plaza *et al.*, 2003). Previous *in vitro* studies highlighted the radial growth rate of both fungi was positively correlated with the water activity of medium and incubation temperature (Lahlali *et al.*, 2005; Lahlali, 2006). For both wound postharvest pathogens, Lahlali (1996) reported also that the optimal a_w for growth was ranged from 0.96 to 0.98 whatever the incubation temperature. The growth of *B. cinerea* stopped at a_w = 0.89 while *P. expansum* was able to grow below this

a_w value. Regarding the effect of temperature, the highest growth rates were observed *in vitro* conditions at 25°C. *In vivo*, Williams *et al.* (1995) reported that free water or high humidity (>93%) is required for germination and penetration of the host epidermis in the case of contact with *B. cinerea* conidia. Furthermore, moisture helps the pathogen to take up nutrients present on the host epidermis or pollen grain (Blakeman, 1980).
However the influence of relative humidity (which is directly related to a_w) and temperature on the growth (lesion diameter) of *P. expansum* and *B. cinerea* was never studied on apple fruit surface. In this context, the main objective of the present work was to determine the combined effect of relative humidity and temperature on the *in vivo* growth of both pathogens of apples fruits. For this purpose response surface methodology (RSM) was used because its implementation is fast and allows obtaining a high precision with lower cost of experiments due to a reduction of a number of combination between studied factors (Myers and Montgomery, 2002).

MATERIALS AND METHODS

Fungi

B. cinerea strain V and *P. expansum* strain vs2 were isolated, respectively from rotting strawberry and decayed apple (Plant Pathology Unit, FUSAGx, Belgium). For long-term storage, the strain was placed at -70°C in tubes containing 25% glycerol. In many experiments the initial conidial inoculum was taken from Petri-dish cultures on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) medium, preserved at 4°C for no more than 6 months.

Fruits preparation

'Golden delicious' fruits were disinfected by soaking during two minutes in sodium hypochlorite solution (10 %) then rinsed twice in sterile distilled water. After drying for one hour, apples were wounded in two sites at their equatorial zone with a depth of 4 mm and 1-2 mm in diameter. Each wound was inoculated with a suspension of 10 µl of *P. expansum* or *B. cinerea* applied at 10^6 spores/ml. Small growth chambers (desiccators) with a maximal capacity of 1 liter of water have been used in these experiments. The different approximate values of equilibrium relative humidity (98±1, 86.5±1 and 75±1%) inside desiccators were controlled using the saturated salt solutions with respect to studied temperatures: K_2SO_4 (98%), KCL (86.5%) and NaCl (75%) (Xu *et al.* 2001). Desiccators with different relative humidity were incubated for 48 h at experimental temperature (5, 15 or 25°C) before introduced the inoculated wounded apples fruit with both pathogens *P. expansum* and *B. cinerea*. The relative humidity in each desiccator was controlled by means a thermo-hygrometer.
Three trials were carried out over time. Each treatment contained 3 replicates per trial, one replicate consisting in a desiccator of four apples. After 30 days of incubation, the diameter lesion was measured.

Experimental design

Response Surface Methodology (RSM) with a 3^k full factorial design was applied with Design-Expert® version 6., (Stat-Ease, Inc., Minneapolis, USA) statistical software. Temperature (5, 15 and 25°C) and relative humidity (75, 86.5 and 98%) were investigated. The design contains 9 experiments. A 2nd order polynomial equation was then fitted to the data by a multiple regression procedures. For both factor systems the model equation is,

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1} \beta_{ij} X_i X_j$$

where Y is the response (lesion diameter), β_o is a constant coefficient, X_i are coded variables ranging from -1 to +1, β_i represent linear coefficients, β_{ij} are the second-order interaction coefficients, and β_{ii} are the quadratic coefficients. All values of model coefficients were calculated by multiple regression analysis. Interpretation of the data was based on the signs (positive or negative effect on the response) and statistical significance of coefficients ($P<0.05$). Interactions between two factors could appear as an antagonistic effect (negative coefficient) or a synergistic effect (positive coefficient).

Statistical models validation

The internal statistical validation of response surface model carried out according to following statistical criteria: coefficient R-square values (R^2), Lack of Fit-value as compared to F-Table, Root mean square error (RMSE) and bias and accuracy factors were calculated as followed:

$$\text{RMSE} = \sqrt{\frac{\text{RSS}}{\text{df}}} = \sqrt{\frac{\sum(\mu_{\text{observed}} - \mu_{\text{predicted}})^2}{\text{df}}}$$

$$\text{Bias factor} = 10^{[\sum \log(\mu_{\text{observed}}/\mu_{\text{predicted}})/n]}$$

$$\text{Accuracy factor} = 10^{[\sum |\log(\mu_{\text{observed}}/\mu_{\text{predicted}})|/n]}$$

where RSS is the residual sum of squares, df is the number of degrees of freedom, μ_{observed} is the experimental values and $\mu_{\text{predicted}}$ is the predicted values given by models.

RESULTS AND DISCUSSION

The study of the combined effect of temperature and relative humidity on the lesion diameter of *P. expansum* and *B. cinerea* was performed on wounded apples (two wounds by apple). After wounds inoculation with an aliquot of 10

µl per wound at a concentration of 10^6 spores/ml of *P. expansum* or *B. cinerea*, apple fruits were placed in desiccators (4 apples per desiccator) incubated at various relative humidities and temperatures during a period of 30 days. The apples infection (lesion diameter) with *P. expansum* and *B. cinerea* increased with the increase of incubation temperature at a maximum of 25°C. The variance analysis showed that the effect of the relative humidity on the growth of both postharvest pathogens of apples fruits appeared to be significant at incubation temperature of 25°C for *P. expansum* and at 15 and 25°C for *B. cinerea* (Figure 1).

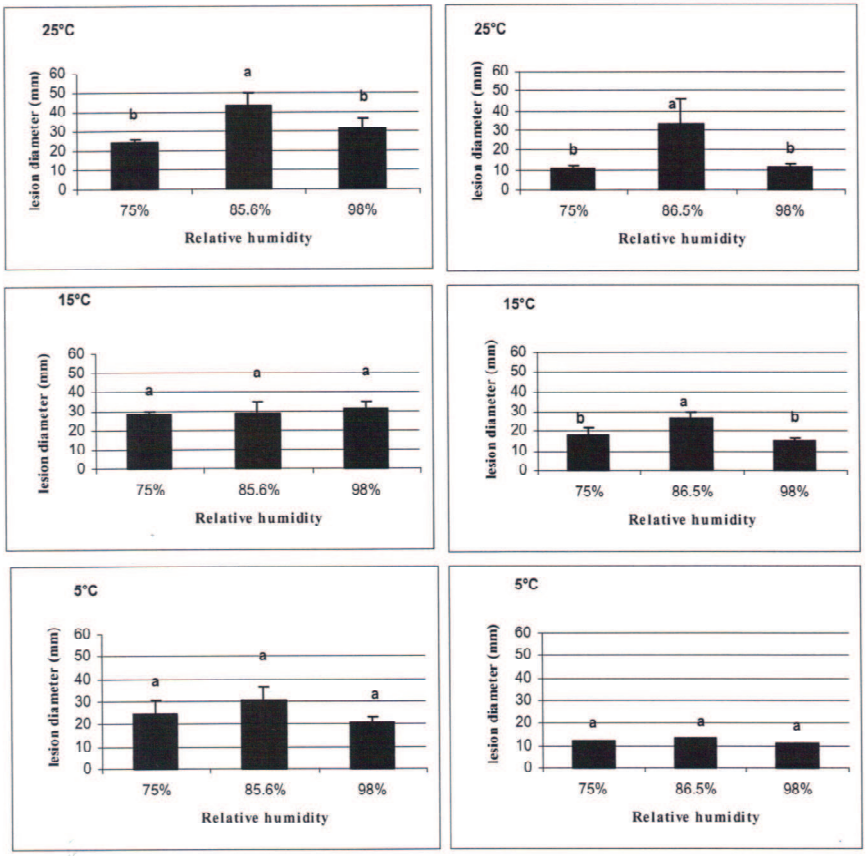

Figure 1. Lesion diameters (mm) of *P. expansum* (left) and *B. cinerea* (right) obtained at various treatments of relative humidity (75, 86.5 and 98 %) for each incubation temperature 25, 15 and 5°C. Treatments having the same letters are not significantly different according to Duncan's multiple range test at ($P<0.05$).

The modeling of the combined effect of temperature and relative humidity on lesion diameters development showed that the temperature has a significant effect on the growth of both fungi (Table 1). In opposite, there was no signifi-

cant effect of relative humidity, quadratic coefficient of temperature and interaction between temperature and relative humidity. This can indicate that the humidity inside fruits is sufficient to support the growth of both fungi. The part of variation explained by both factors was 52 and 55% respectively for *P. expansum* and *B. cinerea* (Table 2).

Table 1. Coefficients significance of full factorial design used for predicting the combined effect of relative humidity and incubation temperature on lesions diameters of *P. expansum* and *B. cinerea*.

Parameters		*P. expansum*		*B. cinerea*	
		Coefficients	P	Coefficients	P
Response mean	β_0	34.66	0.0001*	27.23	0.0031*
T	β_1	3.96	0.0082*	3.28	0.0405*
HR	β_2	1.06	0.4443^{ns}	-0.51	0.7394^{ns}
T^2	β_{11}	-0.30	0.9010^{ns}	-4.27	0.1161^{ns}
HR^2	β_{22}	-7.79	0.0033*	-11.08	0.0004*
T×HR	β_{12}	2.71	0.1175^{ns}	0.56	0.7626^{ns}

P= Probability * Significant

Table 2. ANOVA of quadratic response surface models for lesions diameters of *P. expansum* and *B. cinerea* on apple fruits.

	P. expansum	*B. cinerea*
R^2	0.5204	0.5506
RMSE	5.76	6.38
Adjusted R^2	0.4062	0.4436
Predicted R^2	0.2283	0.2874
Accuracy adequation	6.398	6.548
F-value of model	4.56	5.15
Lack of fit F-value	4.00	6.01
F-Table	3.16	3.16
Bias indices	1.063	1.067
Accuracy indices	1.14	1.29

The 'Lack of Fit' test of both models appeared to be significant, indicating that experimental points were represented in the limits of quadratic model. These results underlined the necessity to include more terms in the model in order to increase the part of variation explained by both factors (cubic model). Due to the low quality of fitted models, the interpretation must be expressed in term of general tendencies. The bias factor was 1.1 respectively for *P. expansum* and *B. cinerea*. While the accuracy factor revealed a difference between the observed and predicted values of 14 and 29% respectively for *P. expansum* and *B. cinerea*.

Applying a same quadratic polynomial equation for each factor, Xu *et al.*, (2001) reported similar results on wound apple pathogen *Monilia fructigena* where the temperature has a more important effect on the colonization than that of relative humidity. Furthermore, 75% of the total variation was due to the temperature whereas 8 to 10% were explained only by the relative humidity. Besides, the effect of relative humidity appeared to be no significant.

The contour curves (Figure2) showed that the optimal growth of *P. expansum* and *B cinerea* was observed at incubation temperature included between 20 and 25°C and at relative humidity ranging between 80 to 92%. One part of

these results showed that the storage room was the ideals environment for decays development because their relative humidity was always included between 85 and 90% (Brown & Miller, 1999). The maximum growth was observed at 25°C for both pathogens.

Pasanen *et al.* (1991) studied at the laboratory conditions the influence of environmental factors temperature (4-30°C) and air humidity (11-96%) on the growth of both fungi *Aspergeillus fumigatus* and *Penicillium* sp. and reported that a short period of favorable conditions was sufficient to start fungal growth and that the temperature was not a limiting factor for fungal growth on building materials, because fungi are capable of growing at even below 10°C. The authors also underlined that the relative humidity of air had no direct influence on the growth of fungi. Fungi may grow at very low levels of air humidity if water is available on the surface. Consequently, repeated or persistent moisture condensation or water leakage is sufficient for fungal germination and growth on building materials. These results support our hypothesis according to which the humidity inside fruits apples is rather sufficient for starting fungal germination and growth.

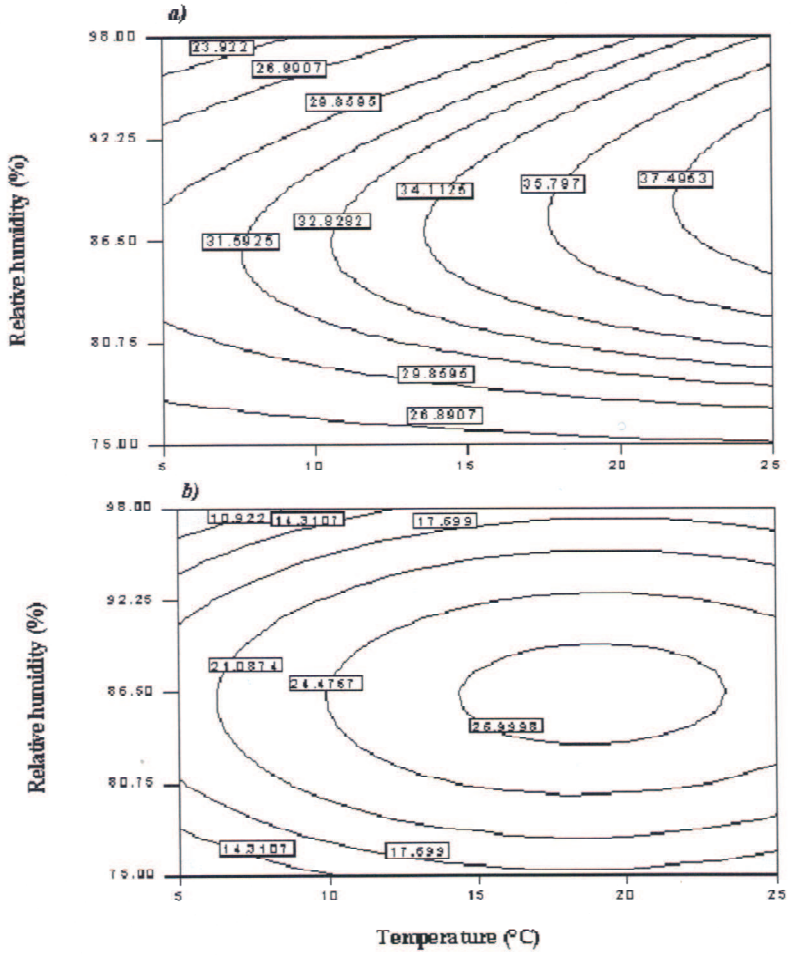

Figure 2. Contour curves showing the predicted effect of incubation temperature and relative humidity on lesions diameters of *P. expansum* (a) et de *B. cinerea* (b) on apple fruits.

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