Effect of three antitranspirant films on *Botrytis cinerea* activities *in vitro*

B. Nasraoui[1], A. Barbier[2] and P. Lepoivre[2]

(1) Ecole Supérieure d' Agriculture du Kef, 7119 LE KEF, Tunisia.
(2) Faculté Universitaire des Sciences Agronomiques, 5030 Gembloux, Belgium.

Abstract

Nasraoui, B., A. Barbier and P. Lepoivre. 1996. Effect of three antitranspirant films on *Botrytis cinerea* activities *in vitro*. Arab J. Pl. Prot. 14(2): 98-101.

This work deals with the effect of the antitranspirant films Folicote, Nu Film P and Vapor Gard on *Botrytis cinerea* activities *in vitro*. Vapor Gard was fungitoxic to the conidial germination and the mycelial growth of the fungus when it was incorporated to the medium. However, as a film, it did not prevent the penetration of the fungus in the medium. In contrast, Folicote and Nu Film P have no fungitoxic effect. Furthermore, Nu Film P enhanced the fungal extracellular esterase activity induced by juniperic acid. This enzyme release was nullified by Folicote. Mode of action hypotheses related to cutinase released by fungi to penetrate plant cuticle was discussed.

Key words: *Botrytis cinerea*, Antitranspirant film, esterase, cutinase.

Introduction

The development of fungal phytopathogenic strains which are fungicide-tolerant and the rising costs of the fungicides have encouraged researchers to investigate alternative means to control fungal plant diseases. One of these means is the use of antitranspirant films which allow plants to save water and protect them against numerous fungal infections as summarized by Nasraoui (9). Some of these films act as physical barrier, others by chemical mechanism. Antitranspirant films like Safe Pack, Colfix, Vapor Gard, Wilt Pruf and Biofilm were found to be fungitoxic to germination and/or growth of some fungi (3, 4).

Furthermore, some compounds protect plants by acting as nonfungitoxic antipenetrant materials (1, 13). One of these cases is the inhibition of fungal cutinase by fatty acids acting without fungitoxic effect. Cutinase inhibition would prevent penetration and then infection of the pea by *Mycosphaerella pinodes* (10).

Since many antitranspirant films have lipidic nature, it is possible that some of these films act as antipenetrant by inhibiting cutinase used by phytopathogenic fungi to digest cuticle and penetrate the host plant (6, 7). This work aims to study the effect of some antitranspirant films on the extracellular esterase activity of *Botrytis cinerea* induced by juniperic acid. This esterase is assumed to be a cutinolytic enzyme (cutinase) as was the case of *Ascochyta pisi* (8). However, it is known that *B. cinerea* produce cutinase (12). Prior to that, it was appropriate to investigate if the antitranspirants used were fungitoxic to the conidial germination and the mycelial growth of this fungus.

Materials and methods

Fungus: The fungal species used was *B. cinerea* (strain isolated from strawberry in the Plant Pathology Laboratory, Gembloux Faculty of Agronomic Sciences, Belgium). This fungus was routinely grown on Oat Meal Agar medium at 26°C. Ten days-old cultures were used as inoculum.

Antitranspirants: Three antitranspirant films tested were:
1. Folicote (wax emulsion) from Attraco (Belgium),
2. Nu Film P (poly-1-*p*-menthene, 96%) from Miller (USA),
3. Vapor Gard (di-1-*p*-menthene, 96%), from Miller (USA).

Culture media: Conidial germination of *B. cinerea* was studied in distilled water containing 1, 3 or 5% (v/v) of each antitranspirant (4 replicates).

Fungal mycelial growth was measured in petri dishes (8 replicates) on Potato Dextrose Agar used as culture medium. The antitranspirants (1, 3 or 5%, v/v) were either mixed with the medium after autoclaving for 20 min at 120°C or floated on the surface of the medium in petri dishes and then removed in such a way only a film remained on the surface.

To induce esterase activity, *B. cinerea* was grown for 6 days in a modified Czapeck-Dox's liquid medium (2), pH 5, containing: 3g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl and 0.01 $FeSO_4$, $7H_2O$ in 1 litter of distilled water containing 0.05% (p/v) of juniperic acid (16-hydroxyhexadecanoic acid) from Aldrich-Chemie (8). To study this esterase activity, 0.5% (p/v) of ricinoleic acid ([R]-12-hydroxy-cis-9-octadecanoic acid) from Sigma, 0.5% (p/v) of glucose or antitranspirants Folicote or Nu Film P (0.5, 1, 2, 3 or 5%, v/v) was added to the medium. Spore suspensions of *B. cinerea* were inoculated to the liquid medium in erlenmeyer flasks, to reach a final volume of 20 ml containing 10^5 spores/ml. The flasks were incubated in a rotary shaker at 26°C. Each experiment was replicated three times.

At the end of incubation period, distilled water (control) or acetone (used as enzyme extractor) were added (20%, v/v) to the culture. The mixtures were incubated for 2 hr and filtered to discard the mycelium. Esterase activity was then measured (8, 10).

Esterase activity measurement: Esterase activity was evaluated by using *p*-nitrophenyl acetate (PNPA) as substrate and *p*-nitrophenol (PNP) as end product (11). The enzymatic reaction was carried out in 7 ml of 30 mM phosphate buffer (pH 7) mixed with 1 ml enzymatic preparation (the fungal culture filtrate) and 2 ml of 0.2 mM PNPA (obtained from a solution of 0.1 M PNPA in methanol). Incubation was carried

out at 37°C for 30 min. PNP was quantified by measuring absorbance at 400 nm.

Results

Conidial germination: Preliminary trials on *B. cinerea* conidial germination showed that such germination was maximal (more than 95%) in the distilled water alone or added with 1, 3 or 5% of Folicote or Nu Film P. No fungal germination was obtained by the addition of Vapor Gard even at 1% concentration.

Mycelial growth: Figures 1-a and 1-b showed that when antitranspirants Folicote or Nu Film P (at 1, 3 or 5%) were incorporated in the culture medium, no modification in the *B. cinerea* mycelial growth was obtained. In contrast, incorporation of Vapor Gard (at the same concentration) led to a severe reduction in fungal growth: nearly 15 mm, as compared to 90 mm of the control (Fig. 1-c).

When the antitranspirants were floated on the medium and then removed, no effect was observed in the cases of Folicote and Nu Film P (Fig. 2-a and 2-b). With Wapor Gard, the growth of *B. cinerea* was slightly reduced between the third and the seventh day (Fig. 2-c).

Esterase activity: Supplementing the mineral solution with 0.05% of juniperic acid, as carbon source, resulted in the release of a soluble extracellular esterase activity by *B. cinerea* mycelium after incubation for 6 days (Fig. 3-a). Post-treatment of such culture with acetone at the end of the incubation period, enhanced the release of esterase activity. This activity was highly reduced upon addition of 0.5% of ricinoleic acid or glucose to the medium, whether post-treated or not with acetone. No significant esterase activity was released when the fungus was grown in a mineral culture medium, even after acetone post-treatment.

When the 0.05% juniperic acid culture medium was supplemented with the antitranspirant Nu Film P (up to 5%), the released esterase activity increased (up to 2% Nu Film P) and then decreased (Fig. 3-b). The same situation was obtained with slightly higher esterase activity when culture was post-treated by acetone.

When 0.5 or 1% of Folicote was added to the 0.05% juniperic acid culture medium, the esterase activity was slightly increased (Fig. 3-c). This activity was little enhanced with acetone post-treatment. No significant esterase activity was obtained with supplementing of 2 to 5% Folicote, even when the culture was post-treated with acetone.

Discussion

Results obtained showed that Folicote and Nu Film P (up to 5%) did not have any toxic effect on *B. cinerea* (Fig. 1 and 2). In contrast, Vapor Gard was highly toxic to the conidial germination and the mycelial growth of this fungus (Fig. 1-c). However, it did not constitute a barrier when it was used as a film (Fig. 2-c). Thus, antitranspirant films do not act as physical barrier like in the case of *Colletotrichum gloeosprioides* with the GZM film noticed by Han (5). In the rest of the experiments only Folicote and Nu Film P were used because they did not seem to be fungitoxic.

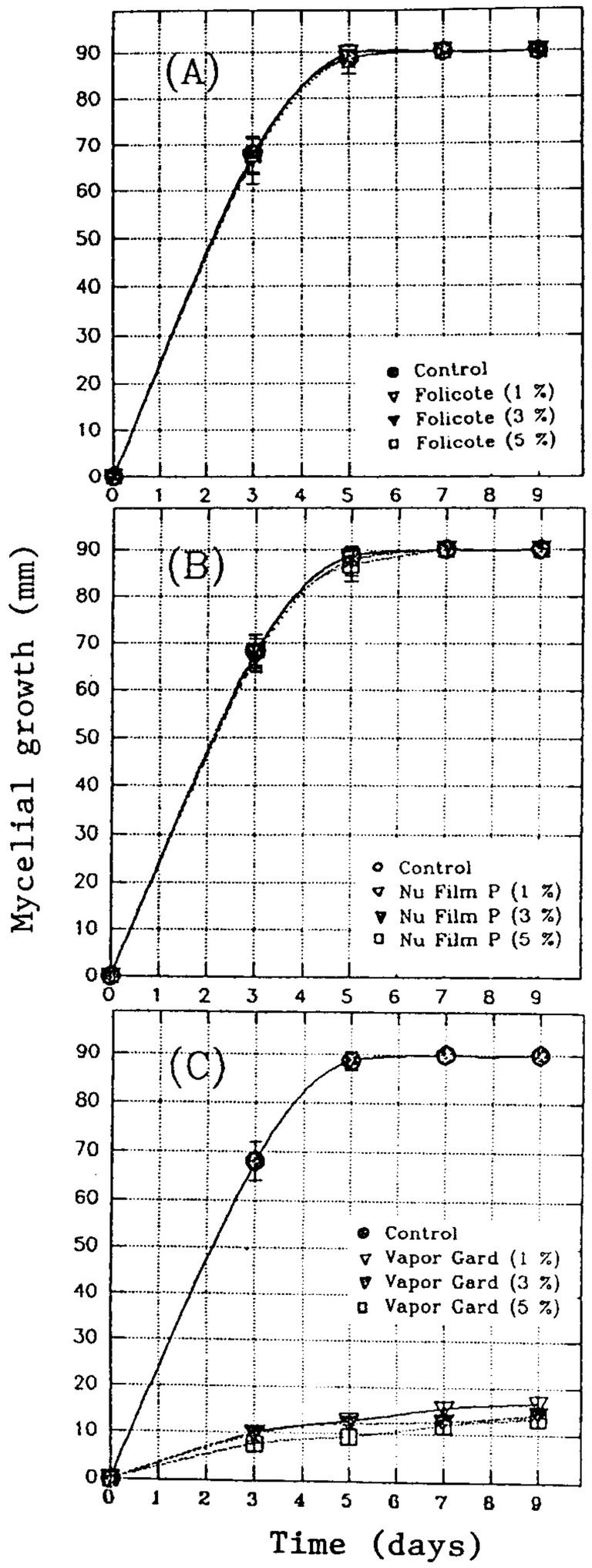


Figure 1. Effect of antitranspirants Folicote (A), Nu Film P (B) and Vapor Gard (C) incorporated to PDA culture medium, on the *in vitro* mycelial growth of *Botrytis cinerea*. (I: SD).

Presented results in Figure 3-a confirmed that mineral solution supplemented with juniperic acid induced an extracellular esterase in *B. cinerea*. Supplemention of ricinoleic acid or glucose to this medium inhibited the release of the esterase activity as reported earlier for *A. pisi* and *M. pinodes* (8, 10).

The post-treatment with acetone showed that, with juniperic acid, there was a part of esterase activity which was induced but not released. This was not the case with ricinoleic acid or glucose addition because the inhibition of the esterase would concern both induction and release of the enzyme. All of these results compared with those of *A. pisi* and *M. pinodes* (8, 10) led us to assume that this *B. cinerea* esterase would be the cutinolytic enzyme: cutinase, reported by Salinas *et al.* (12).

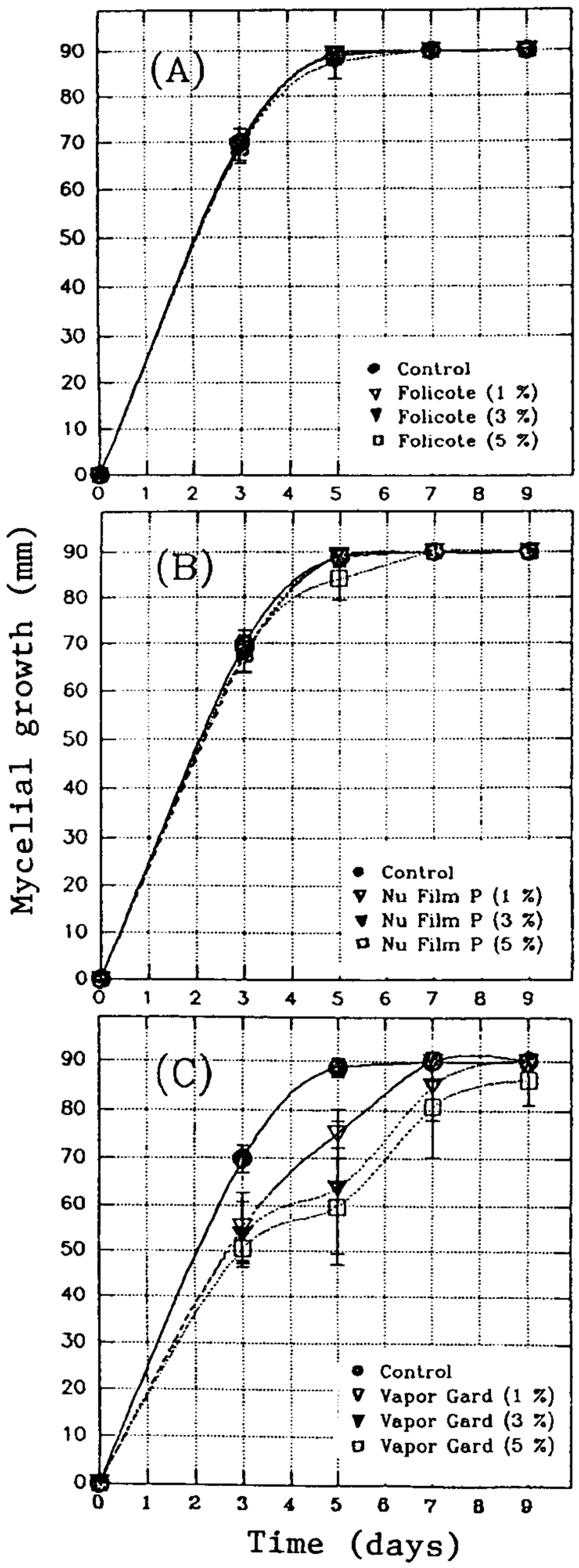
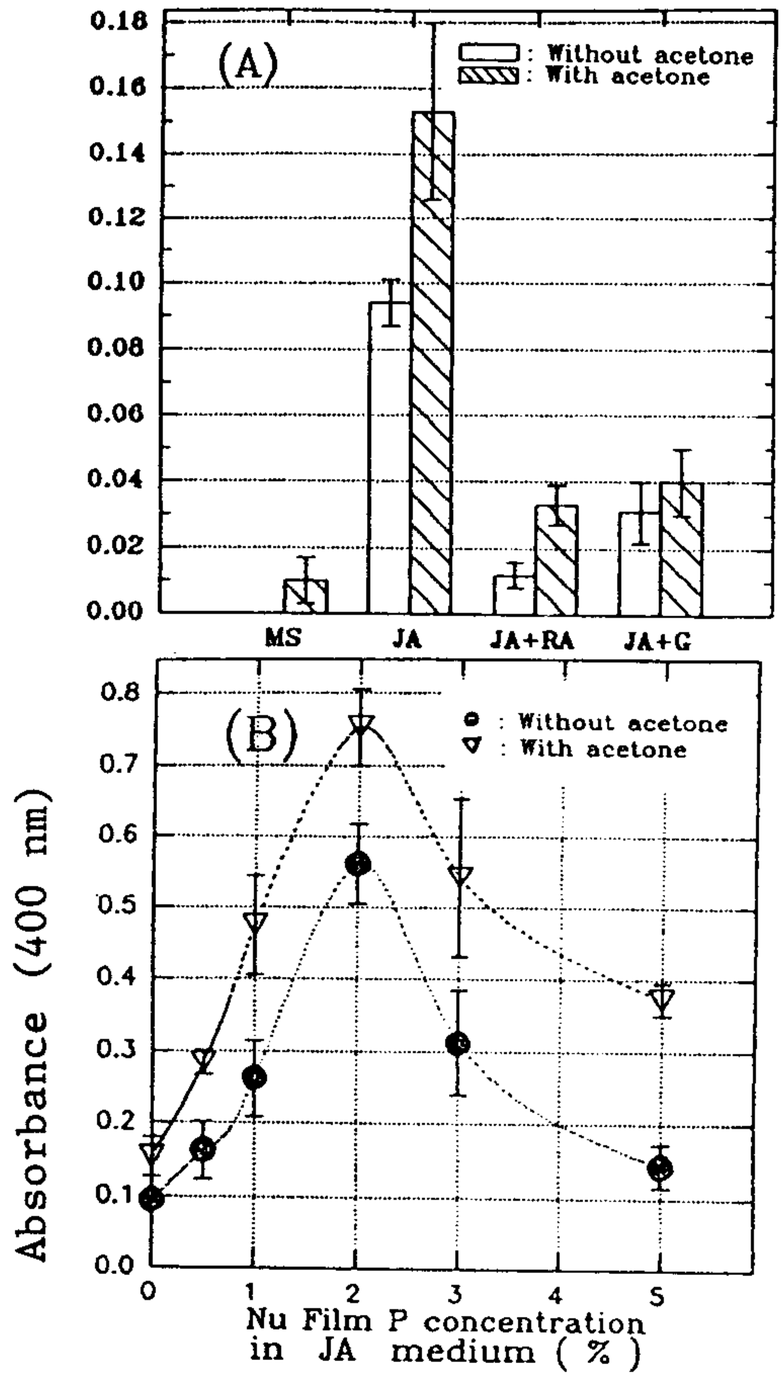


Figure 2. Effect of antitranspirants Folicote (A), Nu Film P (B) and Vapor Gard (C) flowed on PDA culture medium and then taken off, on the *in vitro* mycelial growth of *Botrytis cinerea*. (I: SD).



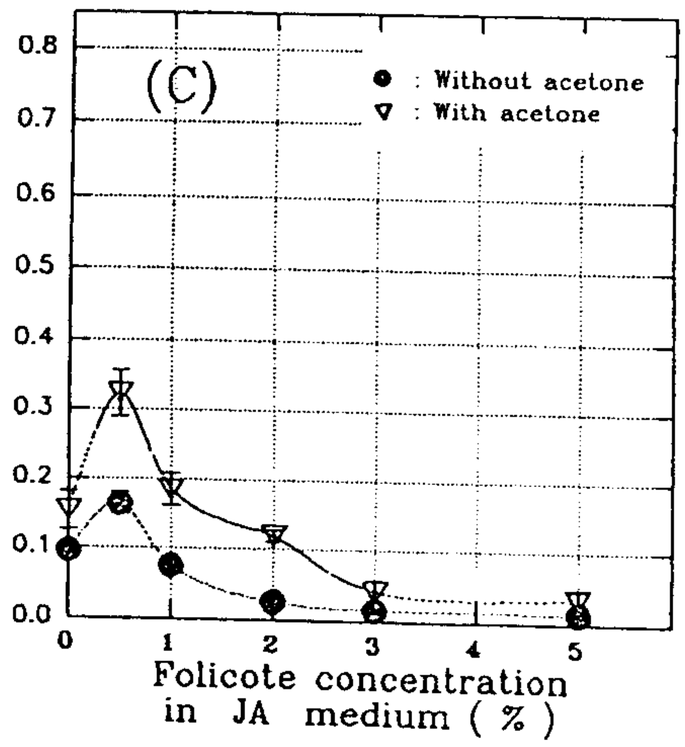


Figure 3. Esterase activity of *Botrytis cinerea* induced by juniperic acid medium containing ricinoleic acid or glucose (A) or the antitranspirants Folicote (B) or Nu Film P (C). (I: SD). (MS= Mineral solution; JA= Juniperic acid; RA= Ricinoleic acid; G= Glucose).

This hypothesis allowed us to study the eventual effect of Folicote and Nu Film P on the induction and the release of *B. cinerea* esterase (cutinase). Figure 3-b showed that adding Nu Film P enhanced the induction of esterase activity from which a part was released only upon acetone post-treatment. Thus, Nu Film P seems to protect plants by a mean other than cutinase inhibition.

In the Folicote case, the esterase activity was slightly enhanced and then decreased until it stopped completely. The supplementing of Folicote (2 to 5%) to the juniperic acid medium could inhibit induction of the fungal cutinase.

Our overall results confirm that the mode of action of antitranspirant films are different from one case to another. It can act by fungitoxicity (Vapor gard) or by some other mechanisms (Nu Film P and Folicote). With Folicote, mode of action seems to be associated with possible inhibition of *B. cinerea* cutinase. Such inhibition would prevent the fungus to penetrate the host across the cuticle when the tissue is unwounded. Elad *et al.* (3) have noticed that the use of Folicote reduced the infection by *B. cinerea* of bean, tomato, peper and cucumber by 40 to 60%. Thus, Folicote seems to

act as antipenetrant like other compounds reported by Sisler (13) and Ali *et al.* (1). This hypothesis will be more studied in a future *in planta* bioassay.

Acknowledgments

We thank the "C.G.R.I.-Communauté Française de Belgique", Brussels, Belgium, for support to B. Nasraoui.

الملخص

نصراوي، بوزيد، آن باربيه وفيليب لوبوافر 1996. تأثير ثلاثة أغشية مانعة للنتح (التعرق) في أنشطة الفطر *Botrytis cinerea* تحت ظروف المختبر. مجلة وقاية النبات العربية. 14(2): 98-101.

يعالج هذا البحث تأثير ثلاثة أغشية مانعة للتعرق في أنشطة الفطر *Botrytis cinerea* مسبب العفن الرمادي تحت ظروف المختبر. وأظهرت النتائج أن خلط Vapor Gard مع المستنبت الغذائي يحدث سمية للفطر ويؤثرفي إنبات أبواغه الكونيدية وغزله الفطري. على أنه كغشاء لم يمنع الفطر من الإختراق والوصول إلى المستنبت. وعلى نقيض ذلك، لم يظهر الـ Folicote و Nu Film P أية آثار سامة؛ بل إن Nu film P زاد من نشاط الفطر في إفراز إنزيم الإستيراز المستحث بوساطة حمض الشربين Juniperic acid علماً أن الـ Folicote يمنع تحرير هذا الإنزيم. ويناقش الباحثون الفرضيات المختلفة بطريقة العمل المرتبطة بتحرير الفطور لإنزيم الكيوتيناز والذي يسمح لها باختراق طبقة القشيرة/ الكيوتيكل.

كلمات مفتاحية: *Botrytis cinerea*، الأغشية المضادة للنتح، إستيراز، كيوتيناز.

References

1. **Ali, M.K., B. Nasraoui, P. Lepoivre and J. Semal.** 1992. Chémoprotection indirecte contre les champignons phytopathogènes: Concept et applications.-Cahiers Agric. 1: 47-54.
2. **Dickman, M.B. and S.S. Patil.** 1986. A rapid and sensitive plate assay for the detection of cutinase produced by plant pathogenic fungi. Phytopathol. 76: 473-475.
3. **Elad, Y., N. Ayish, O. Ziv and J. Katan.** 1990. Control of grey mould (*Botrytis cinerea*) with film-forming polymers. Pl. Pathol. 39:249-254.
4. **Elad, Y., O. Ziv, N. Ayish and J. Katan.** 1989. The effect of film-forming polymers on powdery mildew of cucumber. Phytoparasitica 17:279-288.
5. **Han, J.S.** 1990. Use of antitranspirant epidermal coatings for plant protection in China. Pl. Dis. 74:263-266.
6. **Kolattukudy, P.E.** 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. Annu. Rev. Phytopathol., 23:223-250.
7. **Koller, W.** 1991. The plant cuticle, a barrier to be overcome by fungal plant pathogens. *In*: The fungal spore and disease initiation in plants and animals. Carry, T.C. and C.H. Harvey (eds). Plenum Press, New York, USA, 219-246.
8. **Nasraoui, B., P. Lepoivre and J. Semal.** 1992. Effects of commercial fatty acids on cutinase release by *Ascochyta pisi*. J. Phytopathol. 136:238-246.
9. **Nasraoui, B.** 1993. Role des films antitranspirants dans la phytoprotection contre les maladies fongiques. Anna. Inst. Nat. Rech. Agro. Tunisie, 66:125-135.
10. **Nasraoui, B., P. Lepoivre, G. Lognay and J. Semal.** 1994. Effect of extract of cutin hydrolysate on the *in vitro* release of esterase activity and on the infection of pea leaflets by *Mycosphaerella pinodes*. Med. Fac. Landbouww. Univ. Gent, 59:835-846.
11. **Parkkinen, E., O. Oura and D.V. Richmond.** 1978. The esterase of baker's yeast. I. Activity and localization in the yeast cell. J. Inst. Brewing, 84:5-8.
12. **Salinas, J., F. Warnaar and K. Verhoeff.** 1986. Production of cutin hydrolyzing enzymes by *Botrytis cinerea in vitro*. J. Phytopathol. 116-299-307.
13. **Sisler, H.D.** 1986. Control of fungal diseases by compounds acting as antipenetrants. Crop Protec. 5:306-313.