Agronomie (1989) 9, 187-196

Influence of calcium nutrition on the susceptibility of *N. tabacum* to *Phytophthora parasitica*

S. Ferrario [1], N. Maia [2], L. Cardin [2] and P. Venard [2]

[1] *INRA, Station d'Agronomie et de Physiologie Végétale ;*

[2] *INRA, Station de Pathologie Végétale, 45, bd du Cap, B.P. 2078, F-06602 Antibes, France*

(received 2-3-1988, accepted 24-10-1988)

Summary — Calcium nutrition (0.1–15 meq · l^{-1} Ca; hydroponic culture under controlled conditions) had a significant influence on the susceptibility of *N. tabacum* to *Phytophthora parasitica*. In inoculated leaf experiments and in inoculated stem experiments; the tobacco plants grown in 2 meq · l^{-1} Ca (total calcium content = 2.5% of the dry matter) were the most resistant to *P. parasitica* var. *nicotianæ, i.e.*, in the compatible interaction. In the incompatible interaction, the tobacco plants grown above 0.5 meq · l^{-1} Ca (total calcium content > 1.5% of the dry matter) were the most resistant to *P. parasitica* in inoculated leaf experiments.

The pathogenesis-related protein (PR) accumulation was determined in the different interactions and compared with that obtained after induction by an abiotic inductor (a by-product of benzoic acid). Firstly, in the inoculated leaf experiments, the rapidity of PR appearance depended upon the inductor type or on its aggressivity and appeared to be correlated with the horizontal resistance in the two types of interactions. Then regardless of the inductor type and the tobacco cultivar (*Xanthi nc* or *Escambray*): the weaker the calcium nutrition level, the faster the PR appearance. These results suggested that there was no correlation between the rapidity of PR appearance and tobacco resistance level against the compatible and incompatible strains in interaction with calcium nutrition

Solanaceæ – hydroponic culture – plant – pathogen interaction – pathogenesis-related proteins – ELISA dosage

Résumé — Influence de la nutrition calcique sur la sensibilité du *N. tabacum* au *Phytophthora parasitica*. *La nutrition calcique (0,1 à 15 meq · l^{-1} Ca; culture hydroponique en conditions contrôlées) a un effet statistiquement significatif. Pour les tests sur disques de feuille comme pour les tests sur tiges de plants décapités, ce sont les tabacs de la dose 2 meq · l^{-1} de Ca (teneur en calcium total = 2,5% de la masse de matière sèche) qui semblent les plus résistants au* Phytophthora parasitica *var.* nicotianæ *(interaction compatible). Pour l'interaction incompatible et pour les tests sur disques de feuilles, ce sont les tabacs des doses supérieures à 0,5 meq · l^{-1} Ca (teneur en calcium total > 1,5% de la masse de matière sèche) qui semblent les plus résistants au* Phytophthora parasitica.

*La cinétique d'accumulation des protéines reliées à la pathogénèse (PRs) a été également observée pour les différents niveaux d'interaction et comparée à celle obtenue après induction par un inducteur abiotique (dérivé de l'acide benzoïque) en fonction de la nutrition calcique. D'une part, pour les tests feuilles, la rapidité d'apparition des PRs dépend du type d'inducteur ou de son agressivité et est corrélée au niveau de résistance horizontale pour les deux types d'interaction. D'autre part, quels que soient le type d'inducteur et la variété de tabac (*Xanthi nc *ou* Escambray*), l'apparition des PRs est d'autant plus rapide que le niveau de nutrition calcique est faible, ceci indique qu'il n'apparapas de corrélation nette entre rapidité de synthèse des PRs et niveau de résistance à la souche compatible ou incompatible en interaction avec la nutrition calcique.*

Solanacées – culture hydroponique – interaction plante – pathogène – "pathogenesis-related" protéines – dosage ELISA

Introduction

The influence of mineral nutrition on the resistance of plants to pathogenic agents has been studied since 1930. Indeed, the interaction between a host and a pathogenic agent depends not only upon their genomic characteristics but also on the numerous components of the plant and parasite surroundings. The combination of these environmental factors at any given time could later modify the extent of plant infection. Thus a diversity of influences might explain the different results that have been noted in the literature on this subject.

To show the influence of mineral nutrition, the variations in the other environmental factors should be limited. So we studied the influence of calcium nutrition on the susceptibility of *Nicotiana tabacum* to *Phytophthora* sp. all other conditions being maintained constant.

The first observations made by McCarter (1965) showed no significant effect of calcium nutrition on the susceptibility of tobacco to black shank (*Phytophthora parasitica* var. *nicotianæ*). According to Moore and Wills (1967) and Wills and Moore (1969), the percentage infection of tobacco plants grown in solution culture in the greenhouse and whose roots had been inoculated with *P. parasitica* var. *nicotianæ* is less for a calcium level between 1 and 2.5 meq · l^{-1} than for 10 and 12.5 meq · l^{-1}.

Other authors (Kincaid *et al.*, 1972) have linked the resistance of tobacco plants grown in the field to the ratio of Ca on soil cationic exchange capacity (CEC); a maximum of infection has been observed for a high level of Ca (67% of CEC). Finally, Muchovej *et al.* (1980) have observed a different effect according to the form of calcium salt: $CaSO_4$ and $CaCl_2$ had no effect, while $Ca(OH)_2$ and $CaCO_3$ enhanced death due to *Phythophthora capsici* of pepper plants grown in greenhouses soon after sowing.

The study of calcium is justified by the fact that *Phytophthora* sp. primarily invade the cell walls of host tissues. Indeed, the importance of calcium is well known in the maintenance of the plant cell wall structure and also in the activation or inhibition of some polygalacturonases of fungi (Edgington *et al.*, 1961; Bateman and Lumsden, 1965; Unbehaum & Moore, 1970).

Between tobacco and *Phytophthora* sp. several levels of interaction are known: 1) the compatible interaction between *N. tabacum* and *P. parasitica* var. *nicotianæ;* 2) the incompatible interaction between *N. tabacum* and *P. parasitica.*

In addition, within the compatible interaction, there are two types of tobacco resistance, vertical resistance due to R1, R2, R3 genes against the r1, r2, r3 races of *P. parasitica* var *nicotianæ*, and horizontal resistance or tolerance against the r0, r1, r2, r3 races of *P. parasitica* var. *nicotianæ.*

The study of the influence of calcium nutrition on these different interaction levels between *N. tabacum* and *Phytophthora* sp. could bring about a better understanding of the mechanisms which control these resistance levels.

Regarding this point, Bonnet *et al.* (1986) have suggested that the pathogenesis-related proteins (PRs) are good markers in the incompatible interaction for the resistance of tobaccos inoculated with *Phytophthora* sp. in the stem of topped plants. The PRs are also considered to be good markers of the general resistance of tobacco plants (Gianinazzi *et al.*, 1980; Matsuoka and Ohashi, 1986).

We studied the influence of calcium nutrition on the compatible and incompatible interactions and on the horizontal resistance of two tobacco cultivars. The kinetics of PR accumulation has been established in a few of these interactions and compared to that obtained during the appearance of the PRs induced by an abiotic inductor, a by-product of benzoic acid.

Materials and Methods

Plant material

Two tobacco cultivars of *Nicotiana tabacum* were used: *Xanthi nc* and *Escambray.* They differ in their general resistance level (*Escambray* > *Xanthi nc*).

Growing conditions

The experimental conditions were controlled and maintained constant (20°C, 16 h in the light (OSRAM HQI) 150–300 $\mu Em^{-2}S^{-1}$, 70% relative humidity). The tobacco plants were sown on sand, then 15–21 days later the young plants were transferred to nutrient solutions (hydroponic culture method).

The mineral composition of the nutrient solutions is explained in Table I. They were chosen to obtain different levels of total calcium in tobacco plants.

Experimental conditions

A complete randomized design was used involving 4–6 rows of 7–11 plants each.

In each experiment, and for all the treatments, only the concentration of calcium was modified in the nutrient solutions. The ionic balance was maintained by Cl^- or Na^+ additions (see Table I).

The different treatments carried out for each experiment are presented in Table II.

Inoculation methods

Two strains of compatible *P. parasitica* var. *nicotianæ* (nos. 183, 184 isolated from tobacco, no. 183 is more aggressive than no. 184) and one strain of incompatible *P. parasitica* (no. 44 isolated from *citrus*) were used. The mycelium was grown for 7 days on malt agar. Two types of inoculation were used: leaves, and stems of topped plants. Although *P. parasitica* is essentially a parasite that attacks plant roots, we have chosen these two methods because they allow a good and quick observation of the resistance of tobacco plants in compatible and incompatible interactions (Guyomard, 1985).

Inoculated leaf experiments

Three successive mature or nearly mature leaves were chosen on tobacco plants 50 days after sowing. Before being inoculated, leaf disks of 50 mm diameter were cut out of leaves and a small piece of mycelium was put in the center of the disk where a small 3-mm

Table I. Composition of the nutrient solutions in the different treatments.

	Ca (meq · l^{-1})						
	0.1	*0.5*	*1*	*2*	*4*	*10*	*15*
KNO_3	2	2	2	2	2	2	2
$Ca(NO_3)_2$	0.1	0.5	1	2	2	2	2
$MgSO_4, 7H_2O$	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NH_4NO_3	1	1	1	1	1	1	1
KH_2PO_4	1	1	1	1	1	1	1
$NaNO_3$	1.9	1.5	1	0	0	0	0
$CaCl_2, 2H_2O$	0	0	0	0	2	8	13

pH was adjusted to 6 with a solution of NaOH (N).
Oligoelements were added as a diluted commercial solution (0.1 ml · l^{-1})
Fe was added as a chelate (*Séquestrène*: 0.2 ml/l of a solution 33 g · l^{-1})

Table II. Summary of the different treatments in each experiment.

Experiment type	*Cultivars*	*Nutrient solutions (Ca meq · l^{-1})*	*Experimental conditons*	*Repetitions per treatment*	*Inoculation type:* P. parasitica *strain no.*	*Observation type*
Inoculated leaves	*Xanthi nc*	0.1; 0.5; 2 4; 10; 15	light and dark	6	184 (comp.) 44 (incomp.)	necrotic diameter
Inoculated leaves	*Xanthi nc Escambray*	0.5; 2; 10	light	11	184 (comp.) 44 (incomp.)	necrotic diameter
Inoculated leaves	*Xanthi nc*	0.1; 0.5; 1 2; 4; 10	light	9	184 (comp.) 44 (incomp.) benzoic acid	necrotic diameter and kinetics of PR accumulation
Inoculated leaves	*Xanthi nc Escambray*	0.5; 2; 10	light	11	184 (comp.) 44 (incomp.) benzoic acid	necrotic diameter and kinetics of PR accumulation
Inoculated stems	*Xanthi nc Escambray*	0.5; 2; 10	light	2	183 (comp.)	necrotic length and kinetics of PR accumulation

diameter hole had been made. Then, the leaf disks were floated on a benzimidazol solution (50 mg · l^{-1}) in Petri dishes and placed in a growing room at 20°C.

Experiment type1. In this experiment type, four leaf disks were cut out of each leaf for the three successive leaf levels. For each leaf, the two types of inoculation were practiced, two leaf disks were inoculated with the compatible strain (*P. parasitica* var. *nicotianæ* no. 184 weakly aggressive) and the two other leaf disks were inoculated with the incompatible strain. For each type of interaction, one leaf disk was placed in the light in the growing room, the other one in the dark. For four days, the daily observation of the infection consisted of measuring the necrotic diameter that appeared around the inoculation spot.

Experiment type 2. In this experiment type, five leaf disks were cut out of three successive levels of leaf. The treatments were as follows: 1) one disk inoculated with the compatible strain (*P. parasitica* var. *nicotianæ* no. 184); 2) one disk inoculated with the incompatible strain; 3) one disk non-inoculated; 4) one disk floated on a solution of 2-hydroxy-5-nitrobenzoic acid (5 x 10^{-4} M), which is a moderate abiotic inductor of PRs (Abad *et al.*, 1988b) in place of the benzimidazol solution.

All these leaf disks were maintained in the light, since no PR protein accumulation can be observed in the dark (Abad *et al.*, 1986).

The kinetics of the necrotic area appearance were observed for one week. Simultaneously, the kinetics of the PRs appearance were observed. All leaf disks of two tobaccos were frozen daily until the extraction of the PRs and their quantification.

Inoculated stem experiments

The tobacco plants were topped at the tenth leaf from the bottom of the plant. A piece of mycelium of *P. parasitica* var. *nicotianæ* (no. 183) was placed on the topped stem and protected from desiccation by a cap made of aluminium. The invasion progress of the mycelium was observed for 10 days. Every day the necrotic length was measured outside of the stem. On the 10th day, the stem was cut lengthwise for the purpose of measuring the internal necrotic length.

Mineral quantification

The cations in the whole plants were quantified 50 days after sowing, using atomic absorption spectrophotometry (Ca, Mg) and atomic emission spectrophotometry (K).

Extraction and quantification of PRs

The PRs were extracted and quantified by an enzyme-linked immunosorbent assay (DAS-ELISA) as described by Abad *et al.* (1988a). Wells of microtiter plates (Falcon microtest III) were used, with alkaline phosphatase as the conjugated enzyme and *p*–nitrophenyl phosphate disodium (1mg · ml^{-1}) as the substrate in 10% diethanolamine buffer, pH 9.8. Values of absorbance at 405 nm were directly registered from a Titertek Twinreader. The PR contents of diluted plant extracts were computed from a standard curve with an Apple II program (Flow laboratory) (PR standard used was PRs (b1) purified by HPLC as in Abad *et al.* (1988a). Experimental conditions used for this ELISA were those described by Cardin *et al.* (1983).

In the leaf experiments, the PRs were quantified daily in the leaf disks until 5 days after the inoculation for the kinetics of their appearance in experiment type 2.

In the stem experiments, 9 days after the inoculation, the PRs were quantified in the 2 leaves and in the stem (just below the part of the stem invaled by the mycelium) and in the roots of these plants.

Statistical methods

Analysis of variance (ANOVA; Dagnélie, 1970) was used for the statistical interpretation of the results.

Results

Influence of calcium nutrition on development and on the mineral content

Increasing calcium contents and decreasing magnesium contents were obtained in the tobacco plants grown in nutrient solutions between 0.1 and 4 meq · l^{-1} of Ca, above which we observed a saturation level (Table III).

Influence of calcium nutrition on the susceptibility of N. tabacum *to* Phytophthora parasitica

Inoculated leaf experiments

Experiment type 1: N. tabacum *cv* Xanthi nc. In this experiment, we studied the influence of calcium nutrition in the light and in the dark on the susceptibility of three leaf levels of *N. tabacum* cv *Xanthi nc* to compatible (184) and incompatible strains of *P. parasitica.*

In general, the results obtained on the third day after the inoculation gave the most significant results. The influence of the experimental conditions was important. 1) Effect of the light: the susceptibility of tobacco plants to *P. parasitica* was dependent upon the light conditions (ANOVA: $p < 0.001$, 72 h after inoculation). *P. parasitica* seemed to be less aggressive in the light than in the dark: the incompatible strain and the compatible one were slowed or blocked in the light as compared with the dark (Fig. 1). 2) Effect of the leaf level: the oldest leaves were statistically the most susceptible (ANOVA: $p <$

Table III. Influence of calcium nutrition on the fresh matter (FM) and on the total calcium, potassium and magnesium contents (% dry matter) in *Nicotiana tabacum* cv *Xanthi nc* and cv. *Escambray.*

Xanthi nc					Escambray	
Ca (meq/l)	*FM (g)*	*Ca*	*K (% dry matter)*	*Mg*	*FM (g)*	*Ca (% dry matter)*
0.1	64.3	0.92	8.19	0.65	–	–
0.5	73.91	1.53	8.60	0.59	120.2	1.26
2	88.78	2.46	8.47	0.32	131.5	2.46
4	71.78	3	8.45	0.28	–	–
10	82.73	3.2	8.63	0.22	146.7	3.58
15	51.68	3.26	8.99	0.21	–	–

0.001, 72 h after inoculation), (non-illustrated data). 3) Effect of calcium nutrition: Regardless of the light conditions and the leaf levels, calcium nutrition modified the susceptibility of *N tabacum* cv *Xanthi nc* to *P. parasitica* with regard to the necrotic area.

Compatible interaction. The tobacco plants cultivated on the nutrient solution of 2 meq · l^{-1} of Ca were significantly more resistant to the fungus (ANOVA: $p < 0.001$, 72 h after inoculation). This result was also presented as a function of the total Ca content of tobacco plants in Fig. 1 and 2.5% of the dry matter seemed to be optimal for resistance.

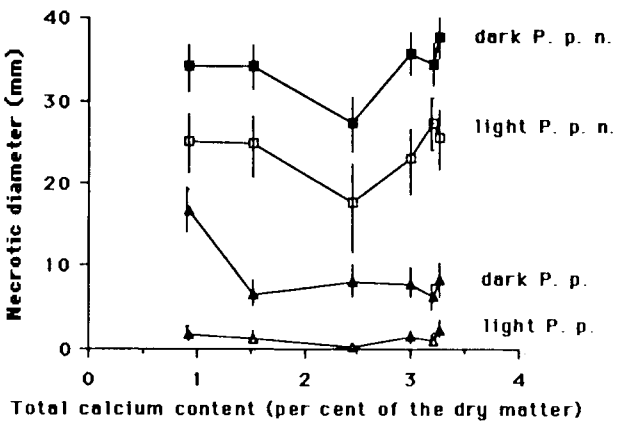

Fig. 1. Influence of calcium nutrition on the susceptibility of leaf disks of *N. tabacum* cv. *Xanthi nc* to *P. parasitica* var. *nicotianæ* (184) (P.p.n.) and to *P. parasitica* (P.p.), 72 h after inoculation. ■ : leaf disks placed in the dark inoculated with *P. parasitica* var. *nicotianæ* ; ❑ : leaf disks placed in the light inoculated with *P. parasitica* var. *nicotianæ*; ▲ : leaf disks placed in the dark inoculated with *P. parasitica* ; Δ : leaf disks placed in the light inoculated with *P. parasitica*. 1 standard deviation at 5% level.

Incompatible interaction. Above a total calcium content of 1.5% of the dry matter (0.5 meq · l^{-1} of Ca) the tobacco plants were more resistant to the fungus (ANOVA: $p < 0.001$, 72 h after inoculation (Fig. 1).

Experiment type 1: Comparison N. tabacum *cv* Xanthi nc *and* Escambray. Since *N. tabacum* cv *Escambray is* generally more resistant to *P. parasitica* than *Xanthi nc,* a comparison between the influence of calcium nutrition on these two cultivar susceptibilities to compatible and incompatible strains was made in the light.

Compatible interaction. An increased resistance of tobacco plants whose total calcium content was 2.5% of the dry matter (2 meq · $^{-1}$ Ca) was observed for the two cultivars as in the above experiment (Fig. 2). Furthermore, the *Escambray* cultivar has a greater general resistance level that slowed the progress of the mycelium in the leaf tissue.

Incompatible interaction. The progress of the mycelium was blocked by the light, so no effect of the calcium nutrition could be observed for the two cultivars.

Inoculated stem experiments

In this inoculation type of experiment, a more aggressive compatible strain (*P. parasitica* var. *nicotianæ* no. 183) than in the inoculated leaf experiment was required otherwise the stem would not be invaded by the fungus.

For the *Xanthi nc* cultivar, the tobacco plants grown on the 2 meq · l^{-1} of Ca level (total calcium = 2.5% of the dry matter) were the most resistant to the fungus. For the *Escambray* cultivar, it seemed that tobacco plants grown on the 2 and 10 meq · l^{-1} Ca of (total calcium ≤ 2.5% of the dry matter) were the most resistant. The most significant results were observed 9 days after the inoculation (Fig. 3).

Influence of calcium nutrition on the PR levels

Inoculated leaf experiments

Experiment type 2: N. tabacum *cv* Xanthi nc. The kinetics of the PR appearance in the leaf disks were dependent upon the leaf level, the inductor type and the calcium nutrition level.

Firstly, regardless of the calcium nutrition level or the inductor type, the highest accumulation of PRs was always observed for the youngest leaf level (non-illustrated data).

Secondly, induction with a by-product of benzoic acid led to a more rapid appearance of PRs than inoculation with the incompatible strain,

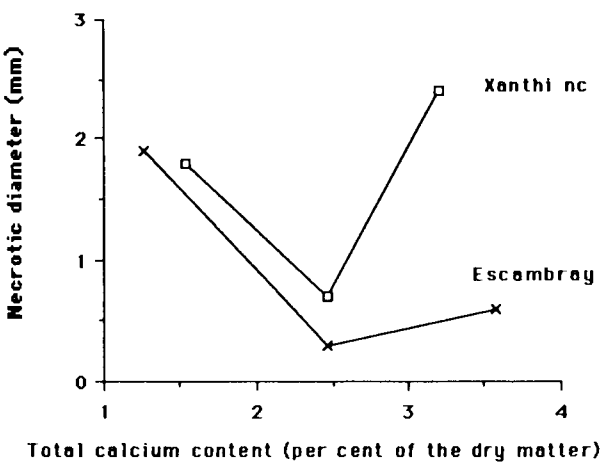

Fig. 2. Influence of calcium nutrition on the susceptibility of leaf disks of *N. tabacum* to *P. parasitica* var. *nicotianæ* (184), 96 h after inoculation. ❑ : *N. tabacum* cv. *Xanthi nc* ; X : *N. tabacum* cv. *Escambray*.

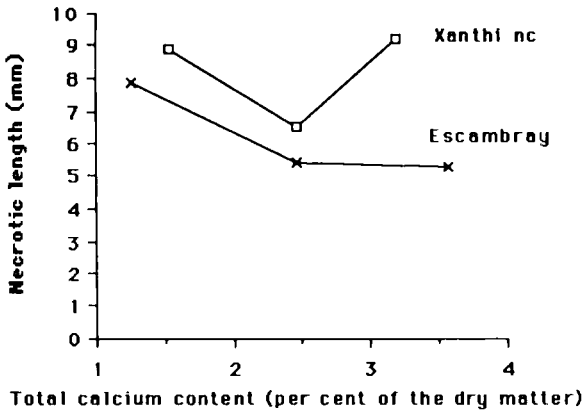

Fig. 3. Influence of calcium nutrition on the susceptibility of stems of *N. tabacum* to *P. parasitica* var. *nicotianæ* (183), 9 days after inoculation. ❑ : *N. tabacum* cv. *Xanthi nc* ; X : *N. tabacum* cv. *Escambray*.

which in turn was quicker than inoculation with the compatible strain (184) (Fig. 4a and b). Two days after inoculation, the differences between the three inductions were already marked and the PR levels in leaf disks inoculated with the incompatible strain or induced by the by-product of benzoic acid were already higher than the levels of non-inoculated leaf disks. Levels of PRs in leaf disks inoculated with the compatible strain 184 became different from the non-inoculated ones only three days after the inoculation. Levels of PRs in non-inoculated leaf disks were rather low (about 500 ng · g^{-1} of the fresh matter).

Finally, after induction by a by-product of benzoic acid, PR accumulation was faster and greater the weaker the calcium nutrition level was. After inoculation with the compatible and the incompatible fungus strains, calcium nutrition influence was more complex: 1) 0.1 meq · l^{-1} of Ca showed a greater PR accumulation 2 days after inoculation with the compatible and incompatible strains, but without increasing resistance

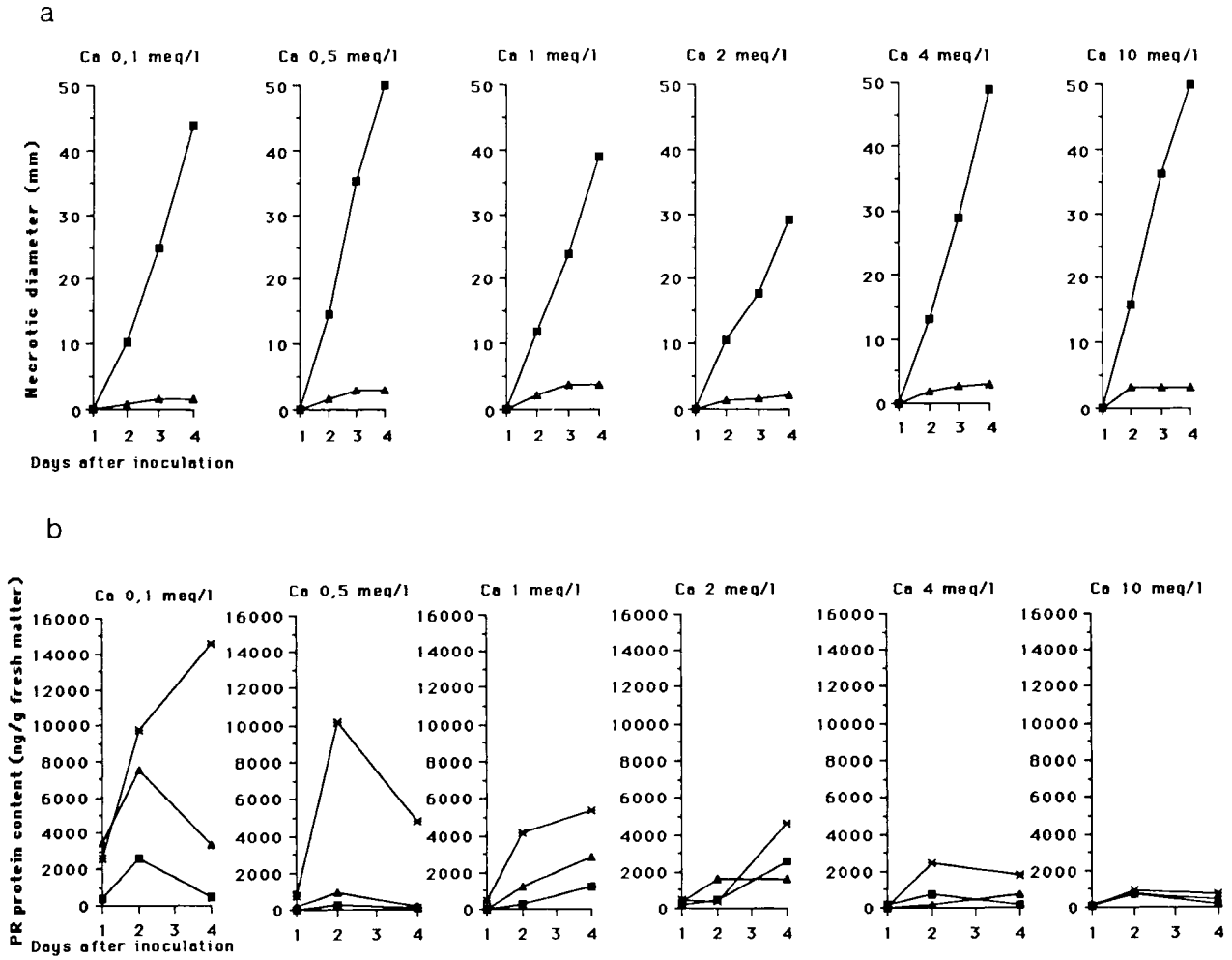

Fig. 4. Kinetics of the appearance of the necrotic diameters (**a**) and the PRs (**b**) in leaf disks of *N. tabacum* cv. *Xanthi nc* related to calcium nutrition and the inductor type. Each point represents the mean of duplicate assays. **a.** ▲ : leaf disks inoculated with *P. parasitica* ; ■ : leaf disks inoculated with *P. parasitica* var. *nicotianæ* (184). **b.** ✻ : induction of PRs by a by-product of benzoic acid; ▲ : induction of PRs after inoculation with *P. parasitica* ; ■ : induction of PRs after inoculation with *P. parasitica* var. *nicotianæ* (184).

(Fig. 4a and b); 2) on 1 and 2 meq · l^{-1} of Ca, a later PR accumulation correlated with a greater resistance which appeared on the fourth day after inoculation with the compatible strain. A similar PR accumulation was observed on the fourth day in the incompatible interaction, but without an apparent correlation with resistance (Fig. 4a and b). 3). Furthermore, on 2 meq · l^{-1} of Ca the PR tobacco level on the fourth day after inoculation was greater in the compatible interaction than in the incompatible one, which was not accompanied by necrotic lesions under the lighted experimental conditions.

Experiment type 2: Comparison of N. tabacum *cv* Xanthi nc *and* Escambray. The different kinetics of PR appearance as a function of inductor type were observed for the two cultivars. However, the PR levels were 5 to 6 time higher for *Escambray* than for *Xanthi nc* (Fig. 5), but PR levels in non-inoculated plants were already higher for *Escambray* than for *Xanthi nc* (Maia, unpublished data).

In general, regardless of the type of inductor, the faster and the greater the PR appearance was, the weaker the calcium nutrition level was (Fig. 5). The influence of calcium nutrition on the PR synthesis level observed for the two cultivars (*Xanthi nc* and *Escambray*) was similar to that observed in the first experiment, but the differences between PR levels induced by calcium nutrition were not so marked in correlation with a greater resistance (lower necrotic diameters).

Inoculated stem experiments

Nine days after the inoculation with the compatible strain (183), the PR level was influenced by the calcium nutrition level except in roots where it

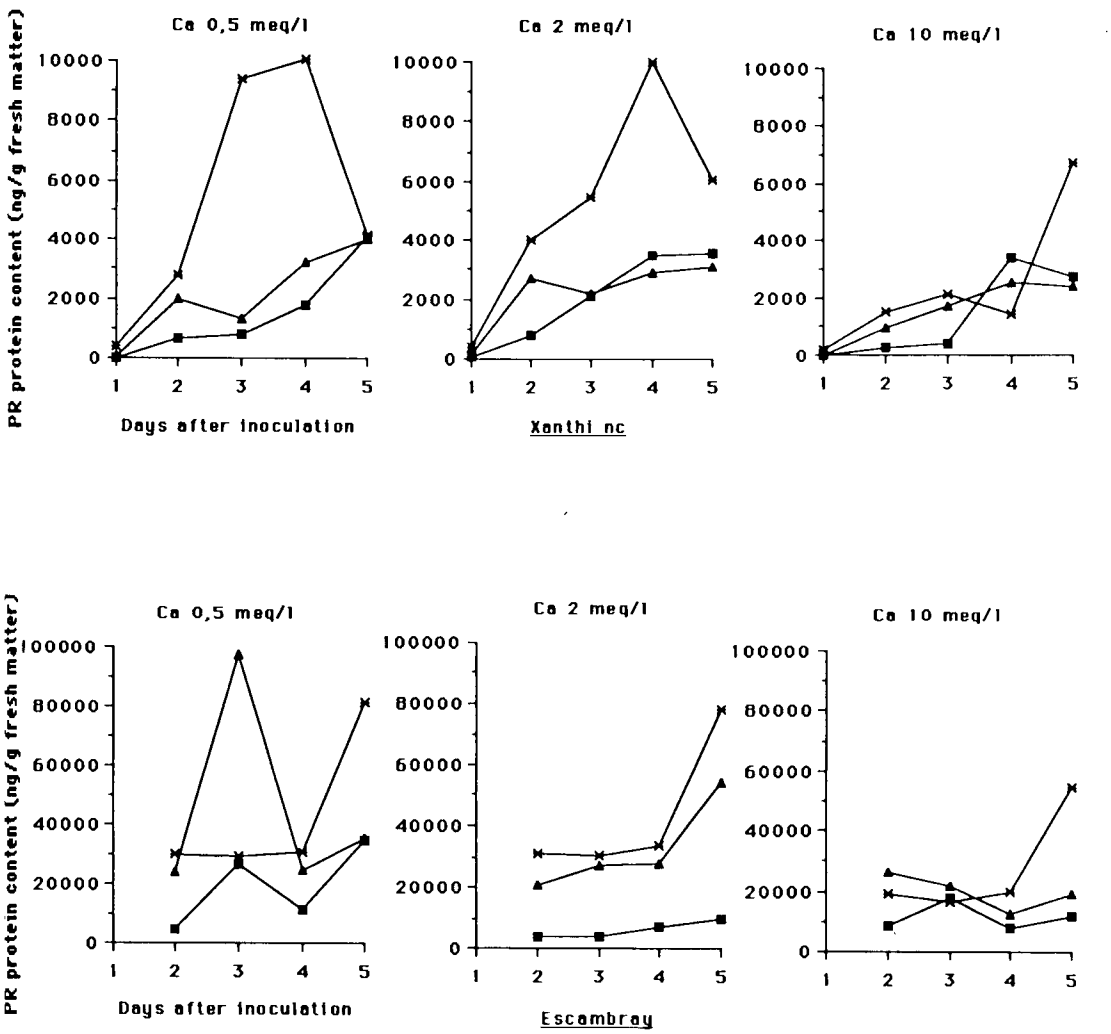

Fig. 5. Kinetics of the appearance of the PRs in leaf disks on *N. tabacum* cv. *Xanthi nc* and cv. *Escambray* related to calcium nutrition and the inductor type. Each point represents the mean of duplicate assays. x : induction of PRs by a by-product of benzoic acid; ▲ : induction of PRs after inoculation with *P. parasitica* ; ■ : induction of PRs after inoculation with *P. parasitica* var. *nicotianæ* (184).

was low. For *Xanthi nc* and *Escambray* cultivars in the two leaves just below the part of the stem invaded by the mycelium, a greater PR level was correlated with a lower calcium nutrition level. However, for *Xanthi nc* cultivar in the stem just below the part invaded by the mycelium, a greater PR level was correlated with a greater calcium nutrition level, and, for *Escambray* cultivar, a greater PR level was observed for 2 and 10 meq · l^{-1} of Ca (total calcium content ≤ 2.5% of the dry matter) (Fig. 6). The greater PR levels for *Xanthi nc* cultivar could be explained by a late sampling, since the kinetics of PR accumulation were quicker and higher for *Escambray* than for *Xanthi nc* (Maia, unpublished data).

Discussion and Conclusion

In this study we observed the influence of calcium nutrition on the compatible and incompatible interactions *N. tabacum–Phytophthora parasitica*. Our results are new in regard to the literature concerning two points.

First, the determination of total calcium content in whole plants was different from that described by other authors. Indeed, our method of growing plants allowed us to obtain total calcium contents from 0.92 to 3.26% of the dry matter, while Wills and Moore (1969) obtained tobacco plants in which Ca varied from 0.37 to 1.42% and from 0.19 to 1.38% for Moore and Wills (1967). Our nutritive solutions were not different from those used by these authors, in regard to the calcium concentration. However, the Mg and K cations were less concentrated in our mineral solutions. Therefore, the antagonism phenomenon between these cations was weaker in our experiments and allowed us to obtain greater total calcium content in whole tobacco plants. Despite the fact that we did not use the same inoculation methods, the shift between these total calcium contents in whole plants enabled us to complete the results already obtained by these other authors.

Second, inoculated leaf and stem experiments were utilised for which the literature had not yet reported significant results.

Furthermore, the influence of calcium nutrition on the susceptibility of *N. tabacum* to *P. parasitica* and on the kinetics of PR accumulation seemed reproducible.

The results we obtained for the two types of inoculation experiments and for the two cultivars were in agreement: 2 meq · l^{-1} of Ca induced a greater tobacco resistance level against the compatible strains. Expressed as a function of the total calcium content of the whole plant, tobacco plants with Ca = 2.5% of the dry matter seemed more resistant to *P. parasitica* var. *nicotianæ*. We preferred this latter expression of the results because calcium content in plants could arise from different calcium nutrition levels if the other mineral nutrients varied.

For the incompatible interaction, it was only the very low Ca level (< 0.5 meq · l^{-1}, *i.e.*, total calcium content < 1.5% of the dry matter) which seemed to induce a greater susceptibility of *N. tabacum* cv *Xanthi nc* to *P. parasitica*.

The mechanisms of infection and/or resistance in these two types of interaction are different: the progress of the compatible mycelium in plant tissue is not blocked, while the incompatible one is

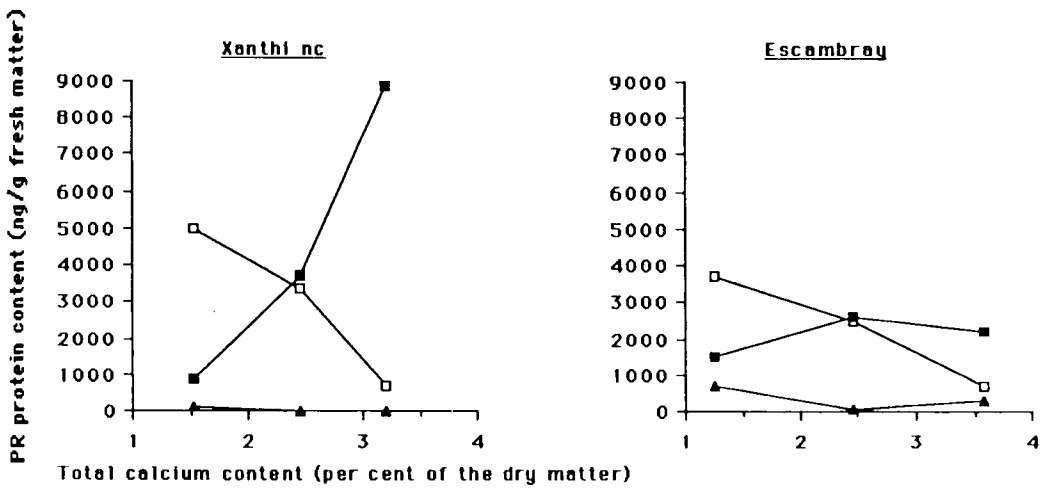

Fig. 6. Influence of calcium nutrition on the accumulation of the PRs in different tissues of *N. tabacum* cv. *Xanthi nc* and cv. *Escambray* after the inoculation of stems with *P. parasitica* var. *nicotianæ* (183), 9 days after inoculation. ❑ : leaves; ■ : stems; ▲ : roots.

stopped in the light and slowed in the dark. Therefore, it is interesting to observe that the influence of calcium nutrition seemed different in the two types of interaction and thus we hypothesize that calcium is involved in different means of resistance according to the interaction type.

The interpretation of the role of calcium in these interactions between *N. tabacum* and *P. parasitica* is not easy. A better knowledge of the physiology in the interactions between host and pathogen is indispensable in interpretting the influence of a mineral element in the defense reaction of infected plant tissues.

On the one hand, we may assume that the nutrititional requirements of an obligate parasite, such as *P. parasitica*, are more or less supplied by plants in which the total calcium content varies. As a matter of fact, the growth of *P. parasitica* needs some nutrients, such as thiamine, sugars, amino acids (or NO_3^-), SO_4^{2-}, $PO_4H_2^-$, K^+, Mg^{2+} and Ca^{2+} in culture media (Hohl, 1983).

Some authors have already reported correlations between the susceptibility of plants and their nutrient contents useful for *Phytophthora* sp. Alten and Orth (1940) have observed that a lower amino acid content in potatoes induced a lower susceptibility to *P. infestans*. Grainger (1956, 1968) has reported a correlation between sugar content and susceptibility to *P. infestans* in potato plants.

In general, variations in total calcium content are accompanied by variations in other mineral contents, such as magnesium or potassium. In our experiments, magnesium and calcium contents varied inversely. Therefore, it is possible that we observed an additional effect of these two mineral variations. For the compatible interaction, a Ca content of 2.5% of the dry matter (0.3% for Mg) could be a critical mineral balance. In addition we observed the consequences of the variations of Ca or Mg contents of tobacco plants on the *P. parasitica* development.

Moreover, calcium variations may consequently induce variations in the concentrations of amino acids and sugars in the apoplast of plant tissue due to a calcium role in the permeability of biomembranes.

According to Waterfield *et al.* (1982), black shank disease development was significantly lower in the roots of susceptible tobacco plants grown at low Ca than those of the same cultivar grown at high Ca. Furthermore, they showed that the reduced disease development of plants grown at low Ca appeared to be associated with changes in membrane permeability, which may be correlated with changes in the various sterol fractions.

In our experiments, low calcium content increased the susceptibility of tobacco plants to the incompatible strain. This could be explained by a higher availability of sugars or amino acids for mycelium growth. In future experiments, it would be interesting to test these hypotheses.

On the other hand, an influence of calcium on resistance mechanisms could be added to a trophic effect on *P. parasitica*.

This was one of the reasons why we studied the influence of calcium nutrition on the kinetics of PR accumulation because Bonnet *et al.* (1986) have observed that PR level was dependent upon the interaction type.

In the inoculated leaf experiments, the PR accumulation was greater in the incompatible interaction, which is in agreement with the observation of Bonnet *et al.* (1986), except for 2 meq · l^{-1} of Ca on the fourth day after inoculation, which could be explained by a later PR appearance in the compatible interaction than in the incompatible one. The kinetics of this protein appearance was qualitatively similar for the two cultivars *Xanthi nc* and *Escambray*. Also, the influence of calcium nutrition on the kinetics of the PR accumulation in the host–pathogen interactions and after induction by an abiotic inducer was significant. In general, regardless of the type of inductor, the faster and the greater the PR appearance was, the weaker the calcium nutrition level was except for 2 meq · l^{-1} of Ca on the fourth day after inoculation with the compatible strain of *P. parasitica*.

In the inoculated stem experiments, the variation in the PR levels in leaves just above the part of the stem invaded by the mycelium was opposite to those in the stem, in regard to calcium nutrition.

It would be interesting to follow the PR kinetics in these two different plant parts, so as to have a better knowledge of the relationship between them and the hypothetical PR migration between leaves and stems that could be influenced by the total calcium content of the plant.

Indeed, there was no correlation between the rapidity of the PR synthesis and the resistance level against the compatible or incompatible strains in interaction with calcium nutrition. These results are in agreement with the observation of other authors that could dissociate appearance and accumulation of PRs and resistance phenomena in tobacco (Fraser, 1982; Abad *et al.*, 1986).

In our opinion, the resistance mechanism which is actually efficient is not yet known. However, other resistance mechanisms should be studied in interaction with calcium nutrition,

such as phytoalexin or callose syntheses, since the activity of β1–3 glucan synthase is dependent *in vitro* upon Ca^{2+} in soybean cells (Kauss *et al.*, 1983).

For another type of plant–pathogen interaction, Bayles and Aist (1987) observed that resistance to *Erysiphe graminis* f.sp. *hordei* conditioned by the *ml–o* gene in barley was inhibited with treatments that were expected to lower the concentration of cytoplasmic, ionized calcium in the host cells. These results led these authors to hypothesize that calcium is required for the activation of the resistance mechanism pershaps (the activity of β1–3 glucan synthase) and that the *ml–o* mutation affects calcium regulation in the cell, resulting in an elevated cytosolic calcium ion level in the resistant isoline.

In the *N. tabacum–P. parasitica* interaction we studied, it would also be interesting to observe the effect of provoked variations in the cytosolic calcium ion level on the metabolism in tobacco plants and on the resistance mechanisms against *P. parasitica* infection.

References

Abad P., Ponchet M., Ferrario S., Poupet A. & Cardin L. (1986) Influence de la lumière sur l'hypersensibilité de *Nicotiana tabacum* var. *Xanthi* n.c. au virus de la mosaïque du tabac, absence de corrélation entre l'induction de protéines b et l'expression de la résistance à la multiplication virale. *C.R. Acad. Sci. Paris* 302-33, 603-608

Abad P., Cardin L., Poupet A. & Ponchet M. (1988a) Comparison between PRb1 quantification obtained by ELISA and HPLC technology. *J. Phytopathol.* (in press)

Abad P., Marais A., Cardin L., Poupet A. & Ponchet M. (1988b) The effect of benzoic and derivatives on *N. tabacum* growth in relation to PR-b accumulation. *Antiviral Res.* (in press)

Alten F. & Orth H. (1940) Untersuchungen über den Aminosäurengehalt und die Anfälligkeit der Kartoffel gegen die Kraut- und Knollenfäule (*P. infestans* de By.) *Phytopathol. Z.* 13, 243-271

Bateman D.F. & Lumsden R.D. (1965) Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhizoctonia solani*. *Phytopathology* 55, 734-738

Bayles C.J. & Aist J.R. (1987) Apparent calcium mediation of resistance of an ml-o barley mutant to powdery mildew. *Physiol. Mol. Plant. Pathol.* 30, 337-345

Bonnet P., Poupet A., Abad P., Venard P. & Cardin L. (1986) Induction de nécroses foliaires, de protéines b et de résistance dans les interactions tabac–*Phytophthora*. *Agronomie* 6, 829-837

Cardin L., Salle D., Poupet A. & Ponchet M. (1983) Dosage immunoenzymatique du VMT dans différents tissus de tabacs cultivés *in vitro*. *Agronomie* 3, 983-988

Dagnélie P. (1970) *Théorie et Méthodes Statistiques, Applications Agronomiques. Vol II. Des Méthodes de l'Inférence statistique.* Duculot, Gembloux, pp. 45

Edgington L.V., Corden M.E. & Dimond A.E. (1961) The role of pectic substances in chemically induced resistance to fusarium wilt of tomato. *Phytopathology* 51, 179-182

Fraser R.S.S. (1982) Are "pathogenesis-related" proteins involved in acquired systemic resistance of tobacco plants to tobacco mosaic virus? *J. Gen. Virol* 58, 305-313

Gianinazzi S., Abad P., Cornu A. & Scalla R. (1980) First report of host b-protein appearance in response to a fungal infection in tobacco. *Physiol. Plant Pathol.* 16, 337-342

Grainger J. (1956) Host nutrition and attak by fungal parasites. *Phytopathology* 46, 445-456

Grainger J. (1968) Cp/Rs and the disease potential of plants. *Hortic. Res.* 8, 1-40

Guyomard G. (1985) Apport des cultures *in vitro* à l'étude de l'interaction Nicotiana - Phytophthora. Pathogenie, deviations métaboliques associées, relation avec la morphogénèse. DEA, Univ. Aix Marseille III

Hohl H.R. (1983) Nutrition of *Phytophthora* In: *Phytophthora - Its Biology. Taxonomy, Ecology, and Pathology.* (Erwin D.C. Bartnicki - Garcia S. & Tsao P.H., eds.), Am. Phytopathol. Soc., St.Paul, MN, pp. 41-54

Kauss H., Kohle H. & Jeblick N. (1983) Proteolytic activation and stimulation by Ca^{++} of glucan synthase from soybean cells. *FEBS Lett.* 158, 84-88

Kincaid R.R., Martin F.G. & Rhoads F.M. (1972) Regression of tobacco black-shank index on soil calcium. *Phytopathology* 62, 302

Matsuoka M. & Ohashi Y. (1986) Induction of pathogenesis-related proteins in tobacco leaves. *Plant Physiol.* 80, 505-510

McCarter S.M. (1965) Effect of certain soil environmental factors and host nutrition on black shank disease development in tobacco. *Diss. Abstr.* 26, 6955

Moore L.D. & Wills W.H. (1967) Calcium nutrition in relation to the black shank disease of tobacco. *Plant. Dis. Rep.* 51, 641-644

Muchovej J.J., Maffia L.A. & Muchovej R.M.C., (1980) Effect of exchangeable soil aluminium and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper. *Phytopathology* 70, 1212-1214

Undehaum L.M. & Moore L.D. (1970) Pectic enzymes associated with black root rot of tobacco. *Phytopathology* 60, 304-308

Waterfield W.F., Moore L.D. & Orcutt D.M. (1982) The effect of calcium nutrition on the sterol content of tobacco roots and their susceptibility to *Phytophthora parasitica* var. *nicotianæ*. *J. Plant. Nutr.* 5, 1387-1400

Wills W.H. & Moore L.D. (1969) Calcium nutrition and black shank of tobacco. *Phytopathology* 59, 346-351