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PATHOLOGIE VÉGÉTALE

Feasibility of rating fire blight susceptibility of pear cultivars (*Pyrus communis*) on *in vitro* microcuttings

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SUMMARY

Typical symptoms of fire blight (ooze production and necrosis) can easily be obtained from the inoculation of *in vitro* microcuttings of a host plant. The purpose of the study was to determine whether the susceptibility of pear cultivars to fire blight can be evaluated *in vitro*. In a work based on several *Pyrus* cultivars (susceptible and resistant) propagated *in vitro*, the effects of different external factors (method of inoculation, concentration of inoculum, growing conditions) on quantitative and qualitative expression of symptoms were analyzed. The variability of the results, already noticed in orchards, was not lessened on this plant material. Inoculations on *in vitro* microcuttings usually overestimated susceptibility. However, a decrease of the receptivity of susceptible cultivars was sometimes observed. Therefore tests on *in vitro* microcuttings do not seem to be suitable for accurate discrimination between cultivars based on their different levels of susceptibility. Nevertheless these tests may be useful for selecting out individuals with high levels of resistance from a population (*i.e.* after mutagenic treatment or induction of somaclonal variations).

Additional key words : *Temperature, light, conditions of inoculation.*

RÉSUMÉ

Est-il possible d'établir une échelle de sensibilité variétale au feu bactérien sur des microboutures de poirier (Pyrus communis).

Si les symptômes typiques du feu bactérien (production d'exsudat et nécrose) sont facilement obtenus sur microboutures *in vitro* de plantes-hôtes inoculées, est-il possible d'estimer la sensibilité d'un cultivar donné ? Travaillant sur différents cultivars de *Pyrus* (sensible et résistant) multipliés *in vitro*, l'effet de facteurs externes (méthode d'inoculation, concentration d'inoculum, conditions de culture) sur l'expression quantitative et qualitative de la maladie est analysé. La variabilité des résultats, déjà connue en verger, n'est pas réduite par l'utilisation de ce matériel végétal. Des inoculations sur microboutures donnent souvent une surestimation de la sensibilité mais on observe parfois une diminution de la réceptivité de cultivars sensibles. Ainsi, un test sur microboutures ne paraît pas approprié pour différencier précisément des sensibilités variétales. Cependant, il peut être utilisé pour sélectionner des individus très résistants à l'intérieur d'une population (par exemple après un traitement mutagène ou l'induction de variations somaclonales).

Mots clés additionnels : *Température, lumière, conditions d'inoculation.*

ABBREVIATION : CFu, colony.

I. INTRODUCTION

Fire blight, a disease of many Rosaceous plants (especially pear and apple), is caused by the bacterium *Erwinia amylovora*. When this disease is newly introduced into an area, at least two questions have to be answered rapidly. First, what is the susceptibility of locally grown cultivars ? A minimum of five years is needed to get this answer (LE LEZEC *et al.*, 1985). Second, will new resistant cultivars be available ? This second question will take as many as fifteen to twenty years to answer (LESPINASSE, PAULIN, 1984). In these two cases a test allowing for rapid screening would be of considerable interest.

According to VISEUR & TAPIA y FIGUEROA (1987), pear cultivar susceptibility can be rated fairly precisely on *in vitro* plant material. However, in our previous

work (DURON *et al.*, 1987) this was not so obvious. Thus, using *in vitro* propagated microcuttings of pear, the influence of several external factors on the expression of the disease has been studied in order to decide if a scale of varietal susceptibility could be properly established, based on such plant material.

II. MATERIAL AND METHODS

A. Plant material

The three pear cultivars : Passe Crassane, Doyenné du Comice (susceptible) and Old Home (resistant) were chosen because of their known behaviour in the field. The cultivars were propagated on a mineral solution of Lepoivre (QUOIRIN *et al.*, 1977) supplemented with the following : sucrose 30 g l^{-1} ; agar 6.5 g l^{-1} ; vitamins (mg l^{-1}) : inositol (100), thiamine (0.8), nicotinic acid (1) pyridoxine HCl (0.1), glycine (4) ; hormone (mg l^{-1}) : 6-benzyl aminopurine (0.5), indol-3-butyric acid (0.1).

The inoculations were performed on the youngest, unfolded leaf of unrooted microcuttings. Tips of actively growing stems (about 1.5 cm long) were subcultured on LEPOIVRE medium one week before inoculation.

B. Inoculum

All the inoculations were made with an aggressive strain of *E. amylovora :* strain CFBP 1430 (Collection française de Bactéries Phytopathogènes - I.N.R.A. - Angers), cultivated on KING's medium B (KING *et al.*, 1954). Bacterial suspensions were prepared with cultures in the exponential stage of growth.

C. Method of inoculation

One leaf per microcutting was punctured with teeth-nosed dissecting forceps previously dipped into a bacterial suspension. This method was more efficient as the number of punctures increased (up to 3) (DURON *et al.*, 1987).

D. Growing conditions

Before inoculation, the conditions of the growth room were 16 h of light (3 000 lux, 26.9 µmol $m^{-2} s^{-1}$, 400-700 nm) at 24 °C, 8 h of darkness at 20 °C.

After inoculation, the incubation temperatures applied (17, 24, 26, 30 °C) were constant day and night. The experiments were done under dark conditions except for a particular experiment that studied the effect of light, in which case the photoperiod was 18 h light, 6 h darkness. Light was supplied by fluorescent tubes and incandescent lamps. Different qualities of light were obtained with filters according to LE DEUNFF (1971) : a sheet of red Sceneroid n° 227 to obtain red light : three sheets of Sceneroid (one green n° 216, one blue n° 212, one red n° 227) to obtain far red light.

Microcuttings were placed under the filters in order to receive an illuminance around 15 000 lux. That means in photon flux density, for each quality of light : white illumination, 275.2 µmol $m^{-2} s^{-1}$ (400-700 nm) ; red, 166.3 µmol $m^{-2} s^{-1}$ (580-700 nm) ; far red, 79.3 µmol $m^{-2} s^{-1}$ (700-850 nm).

E. Symptom assessment

Microcuttings were considered diseased if necrosis extended at least to the petiole of the inoculated leaf, which is assumed to be the beginning of a progressive infection. Readings were made every 2 or 3 days up to a maximum of 14 days after inoculation.

The number of plants per treatment was : 3 replicates of 6 plants per treatment for the experiment studying the effect of the concentration of inoculum ; 3 replicates of 4 plants per treatment and per cultivar for the experiment studying the effect of temperature ; 3 replicates of 16 plants per treatment for the experiment studying the effect of the quality of light.

III. RESULTS

A. Concentration of inoculum

Susceptibility varied with the concentration of inoculum up to a threshold level after which a plateau was reached (fig. 1). The turning point appeared to be around 5×10^7 cfu/ml. Thirteen days after inoculation there were 89 to 100 % diseased microcuttings with 5×10^7 cfu/ml or more and only about 39 % of the microcuttings showed symptoms with 1×10^7 and 5×10^6 cfu/ml.

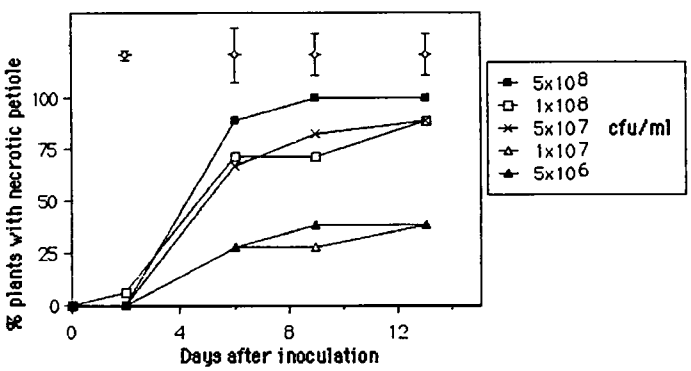

Figure 1

Effects of inoculum concentration on in vitro susceptibility to fire blight of the pear cultivar Doyenné du Comice.

Effet de la concentration d'inoculum sur la sensibilité in vitro au feu bactérien de la variété Doyenné du Comice.

Vertical bars are standard errors of means.

Les segments verticaux représentent les intervalles de confiance des moyennes.

B. Growing conditions

1. *Temperature*

The length of time between inoculation and the first observation of symptoms on susceptible cultivars

decreased as the temperature increased. But whatever the temperature, the final susceptibility rating was the same. Figure 2 shows the results of Passe Crassane. Those of Doyenné du Comice were similar.

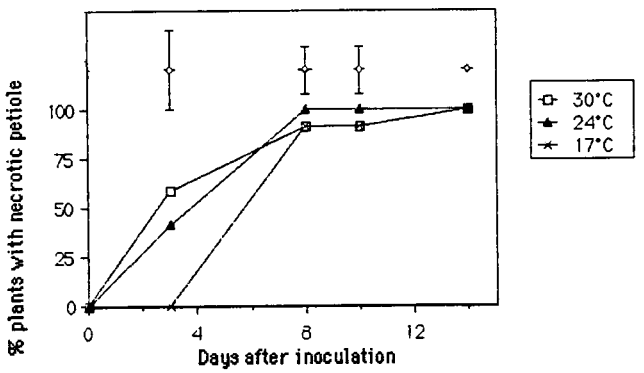

Figure 2

Effect of temperature on in vitro *susceptibility to fire blight of the pear cultivar Passe Crassane.*

Effet de la température sur la sensibilité in vitro *au feu bactérien de la variété Passe Crassane.*

Vertical bars are standard errors of means.

Les segments verticaux représentent les intervalles de confiance des moyennes.

For the cultivar Old Home, an increase of temperature tended to decrease the resistance of microcuttings although our results were not statistically significant (fig. 3).

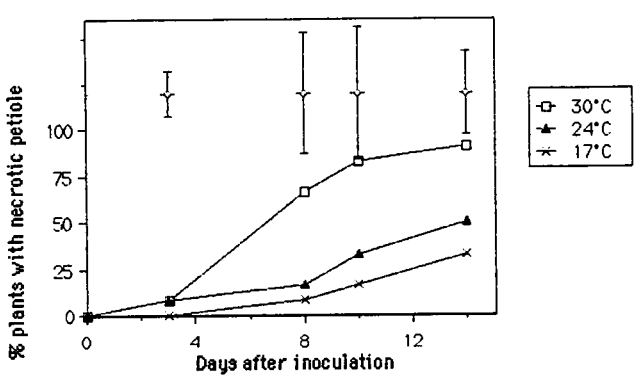

Figure 3

Effect of temperature on in vitro *susceptibility to fire blight of the pear cultivar Old Home.*

Effet de la température sur la sensibilité in vitro *au feu bactérien du cultivar Old Home.*

Vertical bars are standard errors of means.

Les segments verticaux représentent les intervalles de confiances des moyennes.

2. *Conditions of illumination*

Susceptible microcuttings (Passe Crassane) placed in the dark or under far red illumination were significantly more susceptible than those placed under normal conditions of illumination. Microcuttings illuminated with red light showed an intermediate susceptibility (fig. 4).

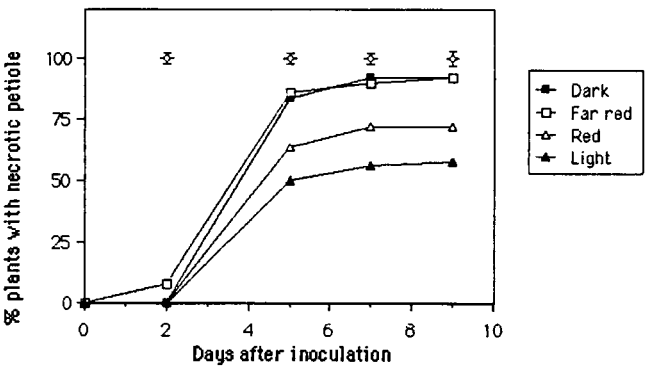

Figure 4

Effect of conditions of illumination on in vitro *susceptibility to fire blight of the pear cultivar Passe Crassane.*

Effet des conditions d'éclairement sur la sensibilité in vitro *au feu bactérien de la variété Passe Crassane.*

Vertical bars are standard errors of means.

Les segments verticaux représentent les intervalles de confiance des moyennes.

IV. DISCUSSION

Artificial inoculations cannot be performed on *in vitro* microcuttings as they are in orchards. The inoculum in an orchard is forced into the shoot below the apex with a syringe. *In vitro*, the microcuttings are first wounded and then the inoculum is deposited on the wound. There can be a risk that the plantlet is too heavily wounded to remain receptive to inoculation. The method of inoculation with teeth-nosed dissecting forceps has been found to be satisfactory, provided that the concentration of inoculum is high enough (5×10^7 to 5×10^8 cfu/ml).

Environmental factors have great influence on the development of fire blight. This influence, well-known in the orchard, also exists on *in vitro* microcuttings, and is particularly easy to study on such material.

As far as temperatures are concerned, our results with susceptible varieties can be discussed in the light of those of BILLING (1974). She studied *in vitro* the effect of temperature on the growth of *E. amylovora* and found that 18 °C was a turning point. Below 18 °C, growth increased rapidly with increasing temperature. Above 18 °C, the increase of growth with temperature was less. Actually in our inoculations with susceptible cultivars, an increase of temperature (from 17 °C to 30 °C) did have the expected effect on the growth rates of *E. amylovora*, leading to earlier symptoms. Nevertheless, the overall rating of the cultivars remained the same, because the final amount of disease was constant whatever the temperature. In contrast, for the resistant cultivar, an increase of temperature induced a higher frequency of diseased microcuttings. One explanation could be that high temperature (30 °C) acts on the plant physiology, and this lessens resistance of such cultivars.

In regard to the illumination conditions, we have previously shown that dark conditions increased the development of necrosis when compared with photoperiodic conditions (DURON *et al.*, 1987). In the present study, darkness and far red light increased susceptibility of our microcuttings, as compared with red or white

illumination. It is to be emphasized that the far red and dark conditions promote the photoconversion of Pfr (phytochrome in the far red absorbing form) to Pr (phytochrome in the red absorbing form). The higher susceptibility of the microcuttings could then possibly be associated with a low Pfr content : this hypothesis remains to be tested.

The studies on the effects of the environmental factors described here would have been performed with great difficulty in the orchard or even in the greenhouse. This points out the interest in the use of *in vitro* propagated plant material. But although this method permits the control of most if not all external factors, the results show a surprising variability and are not systematically better than those obtained in the orchard. For example, inoculations of Doyenné du Comice with the two concentrations of inoculum : 1×10^7 and 5×10^7 (fig. 1) gave two significantly different frequencies of diseased microcuttings after 13 days : 39 and 89 respectively. Such a difference in the rating of the same variety is not usually observed in the orchard where the experimental conditions (including concentration of inoculum) are not so strictly controlled.

On account of this variability, we think that it is difficult to determine a real scale of varietal susceptibility based on *in vitro* propagated microcuttings. Nevertheless, it is possible to select highly resistant individuals within a population. The response of such resistant microcuttings to artificial inoculation is of low variability whatever the conditions (except under extreme temperature conditions : 30 °C). An *in vitro* test therefore can be used as a preliminary and rapid screening after any treatment of plants aimed at the induction of variability among a homogeneous population, such as mutagenesis, somaclonal variation and transformation.

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