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Plant pathology

Crown gall incidence in plant nurseries of Algeria, characteristics of *Agrobacterium tumefaciens* strains, and biological control of strains sensitive and resistant to agrocin 84

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Summary — Crown gall was observed in 99% of the plant nurseries surveyed in the major fruit tree production region of Algeria. Among 11 tree species surveyed, those with highest disease incidence were peach (5.39%), almond (3.43%), cherry (1.50%), apple (1.47%), and olive (1.30%). None of the quince and fig trees inspected had crown gall. Significant differences in the frequency of galled plants were correlated with the rootstock used by nurserymen for peach, cherry, apple and pear trees. Analysis of 60 galled samples, which yielded 154 *Agrobacterium tumefaciens* strains, revealed that biovar 2 and agrocin 84-sensitive strains were predominant among local agrobacteria. The ratio of agrocin-sensitive to agrocin-resistant was more than 3 times higher for biovar 2 (6:1) than for biovar 1 (1.67:1) strains; thus, suggesting that biovar 2 strains are most likely to harbor a nopaline/agrocinopine A-type Ti-plasmid. Biological control of crown gall was achieved in a field experiment in which peach 'Missour' seedlings were dipped first in a suspension of *A radiobacter* K84 and subsequently in a suspension of a local agrocin-sensitive strain. In the same experiment, K84 reduced the incidence of galling by 94% in seedlings inoculated with a local agrocin-resistant *A tumefaciens* strain. These data indicate that K84 could be used in Algeria despite the presence of agrocin- resistant strains.

crown gall / field susceptibility / biocontrol

Résumé — Fréquence du *crown gall* dans les pépinières fruitières d'Algérie, caractérisation des souches d'*Agrobacterium tumefaciens* et contrôle biologique par la souche antagoniste *Agrobacterium radiobacter* K84. La galle du collet ou *crown gall* sévit dans 99% des pépinières de production de plants fruitiers de l'Algérois. À l'exception des figuier et cognassier, toutes les espèces étudiées sont atteintes par la maladie. Les espèces les plus touchées sont le pêcher (5,39%), l'amandier (3,43%), le cerisier (1,50%), le pommier (1,47%) et l'olivier (1,30%) (Tableau I). Au sein d'une même espèce, la proportion de plants atteints de *crown gall* varie en fonction du porte-greffe utilisé par le pépiniériste. Les porte-greffes les plus sensibles sont le GF 677, le Missour et le GF 305 pour le pêcher, le St Lucie pour le cerisier, le MM 106 pour le pommier et le Kirsenchaller pour le poirier (Tableau II). La caractérisation phénotypique de 154 souches d'*A tumefaciens*, isolées de 60 échantillons, suggère que les souches du biovar 2 sensibles à l'agrocine 84 sont les souches les plus fréquemment rencontrées localement (Tableau III). Le rapport entre le nombre de souches sensibles à l'agrocine 84 et celui des souches résistantes est 3 fois plus grand chez le biovar 2 (6/1) que chez le biovar 1 (1.67/1), ce qui suggère une affinité du plasmide de type nopaline/agrocinopine A pour le fond chromosomique de type biovar 2 (Tableau V). En parcelle d'expérimentation, l'incidence du *crown gall* sur pêcher cv Missour est réduite de 94% lorsque ces plants sont initialement trempés dans une suspension d'*A radiobacter* K84 avant d'être inoculés avec une souche tumorigène sensible et/ou résistante à l'agrocine 84 (Tableau VI). La protection par K84 des plants inoculés avec une souche résistante à l'agrocine 84 suggère l'existence en plus de la production d'agrocine, d'autres mécanismes favorisant la performance de cet antagoniste. Ces résultats montrent que K84 pourrait être utilisé en Algérie et ce, en dépit de la présence de souches résistantes à l'agrocine 84.

crown gall / *sensibilité au champ* / *lutte biologique*

INTRODUCTION

Crown gall is a ubiquitous neoplastic disease caused by tumorigenic strains of *Agrobacterium* (Moore and Cooksey, 1981). This organism is a soil inhabitant (Bouzar and Moore, 1987) that can attack 93 plant families, the majority of which are dicotyledon (De Cleene and De Ley, 1976). The pathogen enters the plant through wounds in the crown or roots. Because *A tumefaciens* (AT) requires a wounded plant for infection (Ream, 1989), most infected hosts occur in plant nursery stocks where root-pruning generates infection sites for the bacterium (Ross *et al,* 1970; Nesme *et al,* 1987). Although growth of cherry trees was not affected by the disease (Garrett, 1987), infection by tumorigenic strains of *Agrobacterium* may have significant detrimental effects on other hosts (Bouzar *et al,* 1983; Schroth *et al,* 1988; Nesme *et al,* 1990) generating major economic losses (Kennedy and Alcorn, 1980). Disease incidence can be reduced partially by cultural methods, but the best control of infection is the use of a pre-planting dip in *A radiobacter* strain 84 (*ie*, K84); this antagonist produces agrocin 84, an unusual bacteriocin that inhibits AT strains sensitive to agrocin 84 (Kerr, 1980). Biological control of crown gall with K84 has been successful in different regions of the world, including several Mediterranean countries (Bazzi and Mazzucchi, 1978; Farkas and Hass, 1985; Lopez *et al,* 1987). However, K84 has been ineffective on certain hosts (Moore, 1979; Grimm and Süle, 1981), against latent infections (Moore, 1976), and against biovar 3 and some biovar 1 and 2 strains of the pathogen that are resistant to agrocin 84 (Kerr and Panagopoulos, 1977).

The incidence of crown gall in Algeria has never been determined and no control measures other than culling have been practiced. This disease is of economic concern to local growers who cannot sell their galled nursery material. This paper reports the incidence of crown gall in the major fruit tree production region of Algeria, the characteristics of the strains isolated, and feasibility of K84 protection of wounds from AT infection.

MATERIALS AND METHODS

Plant materials and estimation of disease incidence

Fruit trees from 68 nurseries located in the Algiers' region were inspected for the presence of galls at the Coopérative Régionale Agricole de Production de Plants Fruitiers et de Service (CRAPPFS) of Chebli, Blida, the government-owned cooperative which is responsible for controlling local and imported nursery products. Estimation of crown gall incidence was determined on the following commercialized bare-root species: almond (*Prunus dulcis* D Webb), apple (*Malus sylvestris* Mill), apricot (*Pr armeniaca* L), fig (*Ficus carica* L), olive (*Olea europaea* L), peach (*Pr persica* Batsch), pear (*Pyrus communis* L), plum (*Pr domestica* L), pomegranate (*Punica granatum* L), quince (*Cydonia oblonga* Mill), and cherry (*Pr avium* L). Confidence intervals of the proportion of galled plants were calculated using the large sample normal approximation and a correction to account for the proportion of the total population sampled (Cochran, 1977).

Limited information is available concerning susceptibility of the different cultivars and hybrids used as rootstocks to AT infection. With the sample sizes of the different rootstocks being unequal, the statistical significance of the difference in disease incidence between 2 rootstocks was assessed by means of the *z* statistic (Fleiss, 1981).

Isolation and cultivation of crown gall bacteria

For isolation of AT strains, young tumors from 60 samples were washed in running tap water; 1 g of living tissue was minced in a few drops of sterile distilled water. After incubating the sample for 30 min at room temperature, the suspension was diluted and plated on mannitol-glutamate (MG) (Moore *et al,* 1988), medium 1A (selective for biovar 1 strains; Brisbane and Kerr, 1983), medium 2E (selective for biovar 2 strains; Brisbane and Kerr, 1983), and RS medium (selective for biovar 3 strains; Roy and Sasser, 1983). The seeded agar plates were incubated at 27 °C for one week. For each processed tumor, 7 colonies resembling *Agrobacterium* (Moore *et al,* 1988) were selected at random from each medium and purified by 2 successive streakings on King's medium B (King *et al,* 1954); colonies fluorescent under ultraviolet light were discarded. Bacterial cultures were stored at 4 °C in distilled water and maintained on slopes of potato dextrose agar (Difco Laboratories, Detroit, MI) supplemented with 0.05 % yeast extract, 0.05 % $CaCO_3$ and 0.02 % $MgSO_4$ $7H_2O$.

Pathogenicity and biovar affiliation of A tumefaciens *strains*

Tumorigenicity of each isolate was determined by inoculating scalpel-wounded tissue of tomato (*Lycopersicon esculentum* cv Marmande) seedlings with 24 h-old bacterial growth. Strains that did not induce the development of a tumor on tomato plants were inoculated to sunflower (*Helianthus annuus* cv Nick 89-1) and *Kalanchoe daigremontiana*. Tumor formation was assessed visually 6 wk after inoculation.

Tumorigenic strains were examined for Gram reaction (Doetsch, 1981) and a battery of tests to determine biovar (Moore *et al,* 1988). These included: oxidation of lactose to 3-ketolactose, oxidase reaction, growth tolerance to 2% sodium chloride, pigmentation in ferric ammonium citrate medium, growth on Simmon's citrate medium and L-tyrosine, alkali production from malonic acid and tartaric acid, and acid production from *m*-erythritol and ethanol.

The following *Agrobacterium* strains were used for comparisons in the above-mentioned tests: B6 (tumorigenic, biovar 1; Baker R, Colorado State University), C58 (tumorigenic, biovar 1; Dickey R, Cornell University), K84 (nonpathogenic, biovar 2; Kerr A, University of Adelaide) and K47 (rhizogenic, biovar 2) (Kerr A); and 6/6 (tumorigenic, biovar 3; Sü le S, Hungarian Acad Sci).

Sensitivity of tumorigenic strains to agrocin 84

Because biological control of crown gall is highly correlated with agrocin sensitivity of the pathogen (Ellis *et al,* 1979), the strains were tested for agrocin sensitivity on MG agar plates following Stonier's method (1960) as modified by Cooksey and Moore (1980). Growth inhibition of the test strain was recorded after 3 days of incubation. The agrocin 84-sensitive strain C58 and the agrocin 84-resistant strain B6 were used as controls.

Field trials of crown gall biocontrol

One-year old peach Missour seedlings (5–7 mm in diameter) were imported from France by the CRAPPFS, stored for 2 months in a cold-chamber, and planted in a field located at the Institut Technique d'Arboriculture Fruitière et de Viticulture of Si Haroun, Blida. Although no tumors were visible at the time of planting, the roots were probably carrying AT strains since 18 out of 40 seedlings planted in autoclaved soil developed tumors within one growing season. Prior to planting, the seedlings were removed from storage, root-pruned, bundled into groups of 15, and dipped in either a bacterial suspension or water. In addition to the possible presence of tumorigenic agrobacteria in the soil and on the roots, some treatments consisted of inoculations with 2 tumorigenic biovar 2 strains (S and R, sensitive and resistant to agrocin 84, respectively) which were isolated from locally grown Missour seedlings. K84, S and R were grown on MG plates at 27 °C for 48 h, suspended in distilled water, and then stored overnight at 4 °C. At the time of inoculation, the bacterial suspensions were diluted with tap water up to the required volume; the final concentrations of antagonist and pathogen(s) were about 10^8 and 10^7 cfu/ml, respectively. The following treatments were used: 1), noninoculated, 2), inoculated with S, 3), inoculated with R, 4), inoculated with a 1:1 mixture of S and R, 5), inoculated with K84, 6), inoculated first with K84 and 10 min later with S, 7), inoculated first with K84 and 10 min later with R, 8), inoculated first with K84 and 10 min later with a 1:1 mixture of S and R. A randomized complete block design with six replications was used. There were 15 seedlings per replicate for each treatment in a block. Treated seedlings were planted 100 cm apart within rows (treatment) and rows were 100 cm apart. Plants were watered manually to avoid run off. Four months after planting, all plants were uprooted and examined for tumors. The data were subjected to analysis of variance (ANOVA) and Fisher's LSD using the SAS statistical program (Release 6.04; SAS Institute Inc, Cary, NC 27512, USA).

RESULTS

Crown gall incidence

Crown gall was present in 67 of the 68 nurseries surveyed; the single nursery with no detected galled stocks was located in Tipaza (northwest of Blida). Trees with highest disease incidence were peach (5.39%), followed by almond (3.43%), cherry (1.50%), apple (1.47%), and olive (1.30%) (table I). The proportion of diseased plants differed among the nurseries; in some peach and almond nurseries crown gall incidence reached 18 and 10 % respectively (data not shown). None of the quince and fig trees inspected had visible tumors.

Substantial differences in the frequency of galled nursery plants were observed among the different rootstocks used by nurserymen (table II). Missour, GF 677 and GF 305 rootstocks had higher levels of disease incidence than almond. None of the cherry trees grafted on Merisier sour cherry (*P cerasus* L) were galled; all diseased cherry trees recorded in this study were grafted on St Lucie (*P mahaleb* L). EM 26 and MM 111 apple rootstocks were not galled, whereas a relatively high proportion of MM 106 had crown gall tumors. The popular quince rootstock Cog BA 29 used for pear tree production was rarely galled; most galled pear trees were grafted on Kirsenchaller.

Isolation and characterization of A tumefaciens *strains*

From the tumor extracts plated on culture media, colonies were selected according to their *Agrobacterium*-like morphology. These colonies were

Table I. Crown gall incidence on nursery fruit trees in Algeria.

Species	*No of plants*			*% Diseased**
	Produced	*Sampled*	*Diseased*	
Peach	171 290	9 272	500	5.39 ± 0.44
Almond	83 010	1 400	48	3.43 ± 0.95
Cherry	6 590	4 730	71	1.50 ± 0.18
Apple	104 800	14 750	217	1.47 ± 0.18
Olive	7 500	2 000	26	1.30 ± 0.43
Apricot	148 920	11 000	49	0.45 ± 0.12
Pear	178 280	20 700	38	0.18 ± 0.05
Pomegranate	3 200	3 200	3	0.09 ± 0.00
Plum	73 190	18 310	5	0.03 ± 0.02

* 95% confidence interval.

opalescent, convex and circular (2–5 mm diameter) with an entire margin. Color of the colonies varied according to the medium; they were orange to brown on 1A, white on 2E, white with a burgundy red center on RS, and white on MG.

Table II. Variations in crown gall incidence among rootstocks.

Rootstock	*No of plants*		*Disease (%)*
	Sampled	*Diseased*	
Peach:			
GF 677 (almond x peach)	56	6	10.71
Missour	5 115	401	7.84
GF 305 (almond x peach)	186	12	6.45
Almond	3 915	81	2.07*
Cherry:			
St Lucie	1 797	71	3.95*
Merisier	2 993	0	0.00
Apple:			
MM 106	7 259	185	2.55*
Bitter-filder	5 585	32	0.57
EM 26	1 296	0	0.00
MM 111	610	0	0.00
Pear:			
Kirsenchaller	497	31	6.24
Cog BA 29	20 203	7	0.03*

* The proportion of galled rootstocks was significantly different ($P = 0.0001$) from other rootstock(s) used for the same species. All other rootstocks were not significantly different from each other.

One hundred and fifty-four Gram negative and nonfluorescent isolates induced overgrowths on test plants and were identified as *Agrobacterium*. All but 6 were pathogenic on tomato stems. Of the isolates nonpathogenic on tomato, 3 (one each from apricot, almond and peach) were tumorigenic on sunflower stems, and three (one each from apricot, plum and pomegranate) induced tumor formation on *Kalanchoe* leaves.

Of the 154 tumorigenic strains evaluated by biochemical tests, 84 were biovar 2 and 56 were biovar 1. The remaining 14 strains had intermediate reaction profiles and could not be classified. All plant species with the exception of pear yielded tumorigenic strains (table III). Biovar 1 strains were recovered from all hosts with the exception

Table III. Number of *A tumefaciens* strains recovered from different nursery fruit trees.

Host	*Biovar 1 (56)*[a]	*Biovar 2 (84)*	*Untyped (14)*
Peach (29)[b]	13	77	6
Almond (7)	19	–	–
Cherry (6)	10	4	3
Apricot (3)	7	–	–
Olive (3)	3	–	3
Plum (3)	2	2	–
Apple (3)	2	–	1
Pomegranate (3)	–	1	1
Pear (3)	–	–	–

[a] No of strains. [b] No of tumors processed.

of pomegranate (table III). Almond and apricot tumors yielded only biovar 1 strains. Biovar 2 strains originated mainly from peach galls. The culture media used for isolation were different in their recovery efficiency. More than 80% of AT strains recovered from medium 2E were identified as biovar 2 (table IV). Medium 1A allowed isolation of less than 30 % of biovar 1 strains; 63% of the strains isolated on this medium were biovar 2. Half of biovar 1 strains were recovered from the RS biovar 3 medium; this medium favored the development of a large number of *Agrobacterium*-like colonies which later proved to be fluorescent pseudomonads (data not shown). The non-selective MG medium allowed the isolation of only 10 tumorigenic strains.

In vitro tests revealed that 73 % of AT strains were sensitive to agrocin 84 (table V). Sensitivity to agrocin 84 occurred in 86 % and 63 % of the biovar 2 and biovar 1 strains, respectively (table V).

Control of crown gall in the field

Treatment with strain K84 significantly reduced the percentage of galled peach seedlings (table VI). Index of crown gall control was > 87% in every treatment where K84 was applied. The protective action of K84 was as effective against the agrocin-sensitive strain (S) as it was against the agrocin-resistant tumorigenic strain (R); K84 reduced the level of infection by strain R from 53.2% to only 2.7%. The mixture of tumorigenic strains was as effectively controlled by K84 as were individual tumorigenic strains.

Table IV. Number of tumorigenic *Agrobacterium* strains recovered from different isolation media.

Medium	Biovar 1	Biovar 2	Untyped
1A	16	37	6
2E	6	43	3
RS	28	3	2
MG	6	1	3

Table V. Number of *A tumefaciens* strains sensitive to agrocin 84.

Agrocin 84	*Biovar 1*	*Biovar 2*	*Untyped*
Sensitive	35	72	6
Resistant	21	12	8

DISCUSSION

Crown gall is an economically important disease in the largest fruit tree growing region of Algeria. Nearly all nurseries surveyed were affected by this disease. Losses estimated in this study did not take into account one third of the production which was sold outside the government controlled market and thus was not submitted to control by the phytosanitary commission. In addition, some growers pruned galls from their stocks prior to submitting them to phytosanitary control. Besides affecting estimates of crown gall incidence, such practices may negatively affect survival of the young trees following planting and propagate the disease by contaminating newly planted orchards.

Species of fruit trees most susceptible to crown gall in descending order were peach, almond, cherry, apple and olive. Substantial differences were associated with certain peach, cherry, apple and pear rootstocks. Peach trees rootstocks Missour, GF 305 and GF 677 were more susceptible to crown gall than almond; susceptibility of GF 305 and GF 677 has previously been reported in France (Lopez, 1978) and Spain (Lopez *et al,* 1987). Ste Lucie cherry and MM 106 apple had higher galling frequencies than other rootstock(s) used for the same species. Rootstock Cog BA 29 used for pear was rarely galled. These observations warrant further investigation into the differential resistance of rootstocks to crown gall; however, disease resistance studies should focus on a single species as was the case for grapevine (Ferreira and van Zyl, 1986; Szegedi *et al,* 1989) and poplar (Nesme *et al,* 1990).

The biochemical characterization of AT strains isolated from a variety of hosts and nurseries showed that the majority of the strains were biovar 2; similar results were reported in Italy (Zoina *et al,* 1987) and neighboring Morocco (Benjama and Daoud, 1989). Although the present study was not designed to statistically analyze the distribution of the different biovars among the different hosts, it is remarkable that 85 % of the strains isolated from peach galls were biovar 2 and that the strains isolated from

Table VI. Effectiveness of strain K84 in reducing crown gall incidence on peach seedlings grown in the field [a].

Treatment [b]	*No of plants*		*Galled seedlings (%)*	*Index* [c] *of crown gall control*
	Examined	*Diseased*		
Untreated	85	30	35.3	–
S	72	45	62.5	–
R	79	42	53.2	–
S + R	75	37	49.3	–
K84	70	3	4.3	87.82
K84 + S	85	3	3.5	94.40
K84 + R	73	2	2.7	94.92
K84 + S + R	71	1	1.4	97.16

[a] Data were combined after statistical analysis revealed no difference among blocks. No multiple comparisons between factors were necessary; only the presence of K84 was significant at $P = 0.0001$. [b] Seedlings treated with K84 were drained for 10 min before being inoculated with the agrocin 84-sensitive tumorigenic strain S, the agrocin 84-resistant tumorigenic strain R, or the combination of both.

[c] $\text{Index} = 100 - \frac{\text{\% disease in treatment x 100}}{\text{\% disease in corresponding control}}$

almond and apricot tumors were biovar 1. The different isolation media varied in their recovery efficiency and their selectivity. Medium 2E was efficient in selecting for biovar 2. Medium 1A was less efficient in recovering biovar 1 strains than RS medium. The latter favored also the development of a large number of fluorescent pseudomonads whose colonies could not be visually discerned from those of *Agrobacterium*. MG medium was the least useful of the four media for isolation of agrobacteria. In studies designed to determine the relative abundance of the different biovars it is necessary to employ different media which may complement each other in isolating different groups of agrobacteria; some agrobacteria have been shown to be fastidious, growing only on media supplemented with several salts and trace elements (Canfield and Moore, 1989).

A large proportion of AT strains present in the plant nurseries surveyed were sensitive to agrocin 84. Thus, it appears that use of K84 would prevent most AT infections in these nurseries. The biovar affiliation of agrocin-sensitive strains indicate that biovar 2 strains from Algeria are more likely to be sensitive to agrocin 84. The ratio of agrocin-sensitive to agrocin-resistant was more than three times higher for biovar 2 (6:1) than for biovar 1 (1.67:1) strains. Because agrocin-sensitivity is a Ti-plasmid borne trait (Hayman and Farrand, 1988), these data suggest that biovar 2 strains are most likely to harbor a nopaline/agrocinopine A-type Ti- plasmid. This affinity of a Ti-plasmid for a particular chromosomal background is supported by a population study of indigenous agrobacteria isolated from a single field (Bouzar *et al,* 1991) which suggests that the type of Ti-plasmid maintained in a cell is dependant on the chromosomal background.

It is clear from the results of field applications that strain K84 successfully prevented the majority of tumorigenic agrobacteria from infecting wounded peach 'Missour' rootstock. The proportion of galled seedlings was extremely low when one considers that the plants were artificially inoculated and also challenged by resident pathogenic agrobacteria on the plant itself (*cf, Materials and methods* section) and in the soil; the latter was found to be heavily infested (Bouzar et al, 1991). Surprisingly, K84 protection was as effective against the agrocin-resistant strain as it was against the agrocin-sensitive strain. Similar protection against agrocin- resistant strains has also been reported in neighboring Spain on peach and plum trees (Lopez *et al,* 1989). These data suggest that other factors, in addition to agrocin 84, contribute to the effectiveness of K84 (Cooksey and Moore, 1982). The efficacy of K84 for biological control of crown gall has been demonstrated again, and in this instance despite the presence of agrobacteria resistant to agrocin 84. The present data indicate that K84 may prove useful in controlling crown gall of stone fruit trees

under Algerian conditions. Because of the rare but possible breakdown in K84 biocontrol which is caused by the transfer of the agrocin plasmid pAgK84) from K84 to pathogenic agrobacteria, a transfer-deficient deletion mutant of pAgK84 has recently been constructed (Jones *et al,* 1988). This genetically-engineered strain which controlled crown gall as effectively as its parental strain K84 (Jones and Kerr, 1989) will be tested under Algerian conditions as a substitute for K84 when proper authorization is granted.

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Structure of epidermis wall, cuticle and cuticular microcracks in nectarine fruit

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Summary — The fine structure of the epidermis of the nectarine fruit ("Tasty-fruit" cultivar) has been investigated. The work was mainly focussed on cuticular microcracks which occur on the ripe fruit. The outer wall of the epidermal cell is thick and is composed of different layers which exhibit various textures. A layer that could be interpreted as a cutinized, dense network of polysaccharidic fibrils, clearly appears at the junction between the cuticle and the wall. Cracks result in an abrupt interruption of the cuticle, but the layer at the junction of the cuticle and the wall and in some instances the waxes remain at the surface of the cell wall. The wall itself shows the same aspect in the cracks and under the normal cuticle. The susceptibility of cracks to the penetration by phytopathogenic fungi is discussed.

nectarine / cuticle / cell-wall / epidermis

Résumé — Structure de microfissures cuticulaires dans la paroi épidermique de nectarine. *La structure fine de l'épiderme de nectarine (cultivar "Tasty-fruit") a été étudiée en microscopie optique et en microscopie électronique à transmission. Le travail a été principalement orienté sur des microfissures cuticulaires qui surviennent sur les fruits mûrs. En microscopie optique (fig 1–4) les microfissures sont nettement visibles et paraissent limitées à la couche cuticulaire, sans prolongement dans la paroi épidermique. En microscopie électronique, la paroi externe des cellules épidermiques apparaît épaisse et constituée de plusieurs couches (fig 5). À la jonction entre la paroi et la cuticule, une couche formée d'un réseau de microfibrilles polysaccharidiques enrobées dans une matrice opaque d'origine cuticulaire est clairement visible (fig 6). Les fissures se traduisent par une interruption brutale de la cuticule (fig 10–11), mais la paroi présente le même aspect dans les fissures et sous la cuticule normale. La couche constituant la jonction entre la paroi et la cuticule, et dans certains cas des cires, subsistent à la surface de la paroi cellulaire (fig 12). Dans les fissures s'étendant sur plusieurs cellules épidermiques, des crevasses profondes ont été observées à la jonction entre 2 cellules adjacentes (fig 14) bien que plus généralement l'interface entre cellules semble renforcée par une structure cuticulaire (fig 15). La texture de la paroi épidermique (c'est-à-dire la disposition des fibrilles de cellulose) a été étudiée sur des échantillons traités par la méthylamine afin d'extraire la matrice amorphe de la paroi. La paroi externe des cellules épidermiques est constituée de couches de textures variées (fig 17–24). La texture des parois situées sous la cuticule normale ne paraît pas différente de celle des parois situées sous les fissures cuticulaires. La sensibilité des fissures à la pénétration par des champignons phytopathogènes est discutée.*

nectarine / cuticule / paroi cellulaire / épiderme

INTRODUCTION

The fungal penetration of the epidermis of plants generally involves the degradation of cuticle by cutinolytic enzymes (Kolattukudy and Koller, 1983). Spontaneous microcracks have been noticed in the cuticle of many fruits – apple (Mourichon and Bompeix, 1979), grape (Bessis, 1972), nectarine (Fogle and Faust, 1975 and 1976) – and could play an important role in the penetration of fungal pathogens.

On mature nectarine fruits, scanning electron microscope investigations have shown that, in some instances, the fungal pathogens *Monilia laxa* (Aderh and Ruhl) Honey and *Rhizopus stolonifer* (Ehren B ex Fr) Lind obviously pene-