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Involvement of phenolic compounds in the resistance of grapevine callus to downy mildew (*Plasmopara viticola*)

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Abstract

Callus tissue of different grapevines (*Vitis* spp.) was inoculated with *Plasmopara viticola*. Short, highly-branched hyphae with necrosis, and long hyphae with heavy sporulation were observed on resistant and susceptible callus respectively. Thin-layer chromatography and spectrophotometric analysis showed that resistant callus contained greater quantities of gallocatechin derivatives than susceptible callus. Regression analysis between the field disease rating of each variety and its gallocatechin derivatives content indicated 92.2% correlation. Histochemical studies showed that, after infection with *P. viticola*, flavonoids appeared in the superficial cell walls of the callus, to a lesser degree on susceptible callus than on resistant callus. At a late stage of infection, the superficial cells of resistant callus were suberized, which did not occur in susceptible callus. This study showed that the preformed gallocatechin derivatives, the induced flavonoids and suberized superficial cells might play a role in the resistance of grapevine callus tissue to this fungus.

Abbreviations: Callus **I** = Callus of *V. riparia* var. Gloire de Montpellier; Callus **V** = Callus of *V. vinifera* var. Grenache; TLC = Thin Layer Chromatography; var. = variety; GAD = Gallic acid derivatives; GD = gallocatechin derivatives; RC= resistant callus; SC = susceptible callus.

Introduction

The downy mildew fungus *Plasmopara viticola* (Berk. et Curt.) (Berl et Toni), an obligate parasite of grapevine (*Vitis vinifera* L), causes an economically very important disease. It is difficult to maintain for experiments as it not only requires the culture of the host but also the provision of conditions favorable for infection and sporulation. The use of tissue culture to provide both material and ideal infection conditions in order to maintain this fungus was first employed by Morel [1944].

The method was used with modifications by Boubals [1959] to study mechanisms of grapevine resistance to downy mildew. He inoculated aseptic sporangia onto *in vitro* callus of a series of grape genotypes with different susceptibilities to this pathogen. Two types of aerial hyphae, long with sparse branches or short with serial small branches, formed on the SC and RC respectively. This phenomenon was attributed to a problem of nutrition [Boubals, 1959].

The aim of the present work was to try to explain this phenomenon with the aid of biochemical analysis and histochemical observation. The results give an overview of preformed and induced defense mechanisms found in callus of *Vitis* spp. with different susceptibility to this fungus, and the involvement of gallocatechin derivatives, flavonoids and suberin in resistance.

Material and methods

Material

Two American species (*Vitis. riparia* var. Gloire de Montpellier (**I**) and *V. rupestris* var. du Lot (**II**)), two complex hybrids between *V. vinifera* and American species (18.315 Seyve Villard (**III**), 12.375 Seyve Villard (**IV**)), and *V. vinifera* var. Grenache (**V**) were chosen for their range of resistance to downy mildew (from **I** to **V**, Boubals, 1959). Their field disease ratings are listed in Table 1.

Callus culture medium

Callus cultures of Vitis spp. were initiated and maintained on solid medium. The medium was composed of the base medium of Murashige and Skoog [1962] with macro- and micro-elements at pH 6.5, and with 20% (w/v) sucrose, 0.7% (w/v) agar, Galzy vitamins [1962], and α-naphthylacetic acid (NAA) (1 mg l^{-1}). The medium was aliquoted in polycarbonate tubes (250 $\times$ 25 mm), then sterilized by autoclaving at 110 °C for 30 min.

Initiation of grapevine callus

Branches of grapevines were taken from the grapevine collection at the Chaire de Viticulture, Ecole Nationale Supérieure Agronomique de Montpellier, France. Callus tissue was initiated from lengths of stem internode. Lengths (about 3 cm) of stem were dipped in 95% ethanol for 30 sec., surface sterilized in a saturated solution of calcium hypochlorite for 30 min and finally washed 3 times with sterile distilled water. The ends of the stems were then cut off aseptically. The lengths were placed upside down in the medium and incubated at 25 $\pm$ 1 °C for 15 days, then maintained at 24 $\pm$ 1 °C under constant illumination provided by a cool white fluorescent light (50 μE $m^{-2}s^{-1}$) on a 16 h photoperiod. After 35 days culture, the yellowish-green callus samples were inoculated with an aseptic suspension of *P. viticola*.

Aseptic isolation of P. viticola

The pathogen was isolated from naturally-infected leaves of *V. vinifera* var. Grenache in a vineyard of the Chaire de Viticulture by the method previously described [Dai *et al.*, 1995a].

Infection of the callus and observation of aerial hyphae under the microscope

A suspension of sporangia (10^6 sporangia ml^{-1}) was prepared using sterile deionized water and spores from surface-sterilized contaminant-free leaves. Droplets (20 μl per droplet) of suspension were transferred with a slender Pasteur pipette to the callus. These were maintained at 23 $\pm$ 1 °C under illumination provided by a cool white fluorescent light (30 μE $m^{-2}s^{-1}$) on a 16 h photoperiod. Suspension and droplets were removed from the callus 24 h after inoculation. The healthy and inoculated calluses were sampled 3, 5, 8 and 12 days after inoculation. Sampled callus was lyophylized and analysed. All procedures were carried out at least in duplicate.

The mycelium and the sporangia were removed on a piece of cellotape 12 days after inoculation, mounted on a glass slide, stained with cotton blue (0.1% lactophenol solution) and examined under the light microscope.

Thin-layer chromatography (TLC) and spectrophotometric analysis of the healthy callus

After thirty-five days culture, callus was taken and lyophylized. Samples (25 mg) were weighed out and ground in a mortar with 5 ml of 80% methanol. The extracts were filtered through filter paper and the filtrates (0.5%) recovered, hereinafter referred to as 'crude extracts'.

The crude extracts (20 μl) were applied to cellulose TLC plates (5552, Merck). The phenolic compounds were separated by two – dimensional chromatography. The first solvent system (S1) was acetic acid – water (15:85, v/v). The second solvent system (S2) consisted of ethyl acetate – methanol – water – heptane (90:15:11:5, v/v). Two identical TLC sheets were developed. After drying, one was sprayed with Neu's reagent (2-aminoethyl diphenyl borinate (Fluka) 1% in absolute methanol) [Neu, 1956] and the other with vanillin-HCl (vanillin (Carlo Erba) 10% in a solution of HCl – ethanol (1:1, v/v) [Sarkar and Howarth, 1976] at room temperature.

Neu's reagent stains ortho-substituted phenols, for example: flavonoids [Neu, 1956], caffeic acid derivatives [Andary, 1975] and gallic acid derivatives (gallotannins) [Dai *et al.*, 1994], etc. Their colour reactions to Neu's reagent under UV light and maximum absorptions are shown in Table 1. With vanillin – HCl observation, at room temperature and under visible light, a red colour indicates the presence of condensed

Table 1. Characters of some phenols after treatment with Neu's reagent and observation under UV light

Phenols	Colour	Maximum absorption
gallic acid derivatives	deep blue	325 nm
gallocatechin derivatives	deep blue	325 nm
flavonoids	yellow to orange	410 nm
caffeic acid derivatives	whitish green	380 nm

tannins [Sarkar and Howarth, 1976]. The compounds that reacted with the two reagents were gallocatechin derivatives (epigallocatechins, gallocatechins or galloyl esters of catechins).

The gallocatechin derivatives (GD) were quantified as previously described [McMurrouch and McDowell, 1978] with 4-dimethylaminocinnamaldehyde (4-DMACH, Merck) reagent. Two and a half milliliter of reagent (100 mg 4-DMACH + 10 ml HCl 12 N + methanol qsp 100 ml) were mixed with 50 μl extracts. After 10 min of colour development, at room temperature, spectrophotometric evaluation was performed at 640 nm. The extract absorptions were compared to that of a standard methanolic solution of (+)-catechin (0.01 mg ml^{-1}) treated with the same reagent under the same conditions. Concentrations of GD in the callus were expressed as mg of (+)-catechin per g of dry callus.

With Neu's reagent, the total quantity of (GAD + GD) was determined using the following method. The crude extracts (0.5%) were diluted 6-fold, then Neu's reagent solution added (100 μl for 3 ml of diluted extract). Spectrophotometric evaluation was performed at 325 nm. The extract absorptions were compared to that of a standard methanolic solution of gallic acid (0.14 mg ml^{-1}) treated with the same reagent under the same conditions. Concentrations of (GD + GAD) in the callus were expressed as mg of gallic acid per g of dry callus.

Histochemical studies

Sections (30 μm thickness) of test callus were cut with a freezing – stage microtome. After staining with diverse reagents, the sections were mounted in the staining reagents or in glycerine – water (15:85, v/v) and examined using a light microscope (Nikon Optiphot) with two filter sets: a UV filter set with 365 nm excitation and a 400 nm barrier filter, and a blue filter set with 420 nm excitation and a 515 to 560 nm barrier filter. The flavonoid compounds were detected using Neu's reagent [Neu, 1956]. Sections were immersed in 1% 2-amino-ethyldiphenylborinate (Fluka) in absolute methanol for 2–5 min, mounted in glycerine-water, and observed under epifluorescence. The results were confirmed with Wilson's reagent [Hariri *et al.*, 1991].

Vanillin-HCl reagent [Sarkar and Howarth, 1976] was employed to test for catechins and condensed tannins. Sections were immersed 5 min in 10% w/v vanillin in absolute ethanol-concentrated HCl (1:1 v/v), then mounted in this reagent and observed under the light microscope. All procedures were carried out at room temperature.

Suberin was examined using Sudan IV as previously described [Jensen, 1962]. Sections were treated with Sudan IV (Sigma) saturated in 70% ethanol for 15 min, then rinsed rapidly 3 times with 50% ethanol and observed under the light microscope.

Results

Callus symptoms and sporulation after inoculation

Callus symptoms after inoculation by *P. viticola* are listed in Table 3. Aerial hyphae appeared on callus of all varieties 6 days after inoculation. Twelve days after inoculation, short, highly-branched aerial hyphae and necrosis were observed on the callus of *V. riparia* var. Gloire de Montpellier (**I**) and *V. rupestris* var. du Lot (**II**). Long aerial hyphae and heavy sporulation covered almost the whole surface of the callus of *V. vinifera* var. Grenache (**V**). Sporulation, less intense of than that on callus **V**, and short, highly-branched hyphae (sometimes long hyphae) were observed on 18.315 SV (**III**) and on 12.375 SV (**IV**). These results agree with those of Boubals [1959].

Analysis of gallic acid derivatives (GAD) and gallocatechin derivatives (GD) in healthy callus

TLC analysis showed that the extracts of the 5 calluses contained GAD and GD. Their distribution in the 5 calluses, Rf values and color reaction are shown in Table 2. This table shows that the concentration of GD2 decreased as the susceptibility of callus to *P. viticola* increased.

The results of quantitative spectrophotometric determination of GD and (GAD + GD) in the crude extracts are listed in Table 3. This table shows the various varieties, their mean field disease ratings, symp-

Table 2. Rf values and colour reactions of gallic acid derivatives and gallocatechin derivatives in grape callus extracts subjected to TLC

Compounds[a]	Callus[b]	Rf values S1	Rf values S2[c]	Neu's reagent	Vanillin-HCl
GAD1	I–V	0.63	0.17	deep blue	–
GAD2	V	0.63	0.38	deep blue	–
GD1	I–V	0.35	0.45	deep blue	red
GD2	I–V	0.21[d]	0	deep blue	red

[a] GAD, gallic acid derivatives; GD, gallocatechin derivatives.
[b] **I**, *Vitis. riparia* var. Gloire de Montpellier; **II**, *V. rupestris* var. du Lot; **III**, 18.315 Seyve Villard, **IV**, 12.375 Seyve Villard, **V**, *V. vinifera* var. Grenache.
[c] S1, acetic acid – water (15:85, v/v). S2, ethyl acetate – methanol – water – heptane (90:15:11:5).
[d] The 5 callus extracts varied in the spot size of GD2. Semi-quantitation was carried out using visual comparison. The concentrations of GD2 were in the order of: **I** (+++), **II** (+++), **III** (++), **IV** (+) and **V**(±).

toms on the callus, mean GD content (mg (+)-catechin g^{-1} dry weight), and mean (GD + GAD) content (mg gallic acid g^{-1} dry weight).

Regression analysis between the field disease rating of each variety and its GD content gave a 92.2% correlation, and with (GD + GAD) content gave a 95.0% correlation.

Table 3 showed that there was a correlation between the sporulation capacity, the growth of mycelium and the GD content of the callus. The more GD they contained, the weaker the sporulation capacity and the more highly-branched the growth of mycelium.

Histochemistry

Calluses of a resistant variety (*V. riparia* var. Gloire de Montpellier, **I**) and a susceptible variety (*V. vinifera* var. Grenache, **V**) were chosen for histochemical studies.

Healthy callus showed a red colour after staining with vanillin-HCl, which indicates the presence of condensed tannins. This color was stronger on callus **I** than on callus **V** (Fig. 1A, 1B), indicating that condensed tannins were more concentrated in resistant callus (RC) than in susceptible callus (SC). With inoculated callus, no change was observed.

Healthy callus tissue emitted a deep blue fluorescence with Neu's reagent under UV light, which indicates the presence of GAD and/or GD. This fluorescence was made evident only with Neu's reagent [Dai *et al.*, 1994], and it was more intense in callus **I** than in callus **V**.

Histochemical study also showed that compounds in the callus reacted with both Neu's reagent and vanillin-HCl. These results confirmed those of TLC analysis. The callus contained GD and GAD, and the RC was richer than SC in GD.

Three days after inoculation, a yellowish-white fluorescence was observed under UV light in the superficial cell walls of callus (Fig. 1C, 1D) and an orange-yellow fluorescence was observed under blue light after treatment with Neu's reagent, indicating the presence of flavonoids [Neu, 1956]. The fluorescence was more intense in callus **I** than in callus **V**, indicating higher levels of these molecules in RC than in SC. These fluorescences intensified with time. With Wilson's reagent, the superficial cell walls also emitted a yellow fluorescence under UV light, which confirmed the results obtained with Neu's reagent.

The superficial cells of callus **I**, but not callus **V**, showed an orange-red color under visible light after staining with Sudan IV, 12 days after inoculation, which indicates the presence of suberin in RC.

Discussion

The results show that there is a great difference in reactions among the calluses of *V. vinifera* var. Grenache (**V**), the two American species (*V. riparia* var. Gloire de Montpellier (**I**) and *V. rupestris* var. du Lot (**II**)), and the two hybrids (18.315 SV (**III**) and 12,375 SV (**IV**)), following infection with the downy mildew pathogen *P. viticola*. The major symptomatic differences between these varieties were in the growth and ramification of aerial hyphae, in necrosis, and in sporulation capacity. The susceptible callus (**V**) bore long hyphae with conspicuous sporulation, whilst the two American species (**I** and **II**) bore short, highly-branched hyphae and showed necrosis without sporulation. The two hybrids (**III** and **IV**) produced both types of hyphae and with a lesser degree of sporulation.

It was demonstrated by TLC, spectrophotometric assay and histochemical studies that gallocatechin derivatives (GD) were heavily concentrated in resistant callus. Although spectrophotometric assays have shown that the content of (GD + GAD) was also correlated with the resistance of the callus, TLC analysis demonstrated that the concentration of GD2 plays the most important part in the quantity of (GD + GAD). In healthy callus, there was a correlation between the GD

Table 3. Field disease rating, symptoms on callus, and mean GD and (GD + GAD) content of each grapevine callus analysed in this study

Variety[a]	Field rating[b]	Symptoms on callus		Mean GD content (mg (+)-catechin g^{-1} DW)[d]	Mean (GAD + GD) content (mg gallic acid g^{-1} DW)[d]	Significance[e]
		Sporulation[c]	Aerial hyphae			
I	1	0	short highly-branched	5.52 ± 0.13	85.68 ± 4.54	A
II	2	0	short, highly-branched	6.68 ± 0.26	91.98 ± 4.54	A
III	3	+	short, and long	3.37 ± 0.15	65.94 ± 4.05	B
IV	4	+	short, and long	2.21 ± 0.08	43.00 ± 4.05	B
V	5	+++	long	0.26 ± 0.04	23.52 ± 1.45	C

[a] **I** = *V. riparia* var. Gloire de Montpellier; **II** = *V. rupestris* var. du Lot; **III** = 18.315 Seyve-Villard; **IV** = 12.375 Seyve-Villard; **V** = *V. vinifera* var. Grenache.
[b] Field disease rating according to Boubals (1959), 1 = high resistance; 5 = high susceptibility.
[c] 0 = no sporulation; + = 25–50% leaf surface bearing sporulation; +++ = 75–100% leaf surface bearing sporulation.
[d] GD = gallocatechin derivatives. GAD = gallic acid derivatives. Each result is the mean of 3 replicates; ± = standard error; DW = dry weight. The correlation between the field disease rating and the concentration of GD was 92.2%, and with content of (GD + GAD) was 95.0%.
[e] Values followed by the same letters are not significantly different (T test, $P = 0.01$).

content and the degree of resistance in the field (Table 3). Resistance to *P. viticola* is therefore strongly linked to the molecules containing a gallic acid nucleus in the callus. The GD content was also correlated with the growth and ramification of the mycelium on the callus (Table 3). Gallocatechins are heavily concentrated in the root endodermis and hypodermis of cotton seedlings of resistant variety [Brammall and Higgins, 1988]. It is suggested that the absence of gallocatechin in the root cap of cotton may be causally related to the susceptibility of this tissue to fungal penetration and colonization [Brammall and Higgins, 1988]. Gallic acid extracted from the flowers of *Rosa chinensis* Jacq. exhibited strong antifungal properties against as many as 17 fungi [Tripathi and Dixit, 1977]. This implies that GD play a role in the resistance mechanism of callus to *P. viticola* and are preformed inhibitory compounds.

Flavonoid compounds were detected by a specific fluorescence with Neu's reagent in superficial cell walls of callus 3 days after inoculation, and to a lesser degree in susceptible than in resistant callus. Flavonoids represent one component of the resistance phenomenon, as has been shown for other pathogens such as viruses [Cho and Goodman, 1979], bacteria [Sequeira and Dezoeten, 1977] and fungi [Friend, 1981] or parasitic plants such as *Viscum album* [Hariri *et al.*, 1991]. These flavonoids were fixed to the cell walls and were impossible to extract, in their native forms, with organic solvents, even in either acid or alkaline medium. This may indicate the advantages of histochemistry, with which more detailed and more localized reactions can be observed. With traditional methods, only extractible compounds can be determined.

The superficial cells of RC were suberized at a late stage of infection, a feature generally thought to aid cell wall resistance to fungal penetration and extension [Beckman and Talboy, 1981; Aist, 1983; Ride, 1983]. In a previous work, we have demonstrated the presence of lignin in cell walls of grapevine leaves that appeared as a defense mechanism at a late stage of infection with *P. viticola* [Dai *et al.*, 1995b]. The deposition of flavonoids and suberin substances in these cells may either be directly detrimental to fungal metabolism or cause a loss of the normal elasticity of the hyphae [Brammall and Higgins, 1988].

It is possible that the superficial cell structure was also contributing to resistance. These cells, in SC, were round, more loosely packed and larger than those of RC (Fig. 1).

Dual culture of obligate parasites with tissue-cultured callus has been used as a simplified experimental system for the investigation of structure and physiology of host-parasite interaction and has provided a source of contaminant-free spores or mycelium for physiological experiments [Ingram, 1977]. In this study, the dual culture of obligate parasite with tissue-cultured callus was used to study the interaction between grapevine callus and *P. viticola*.

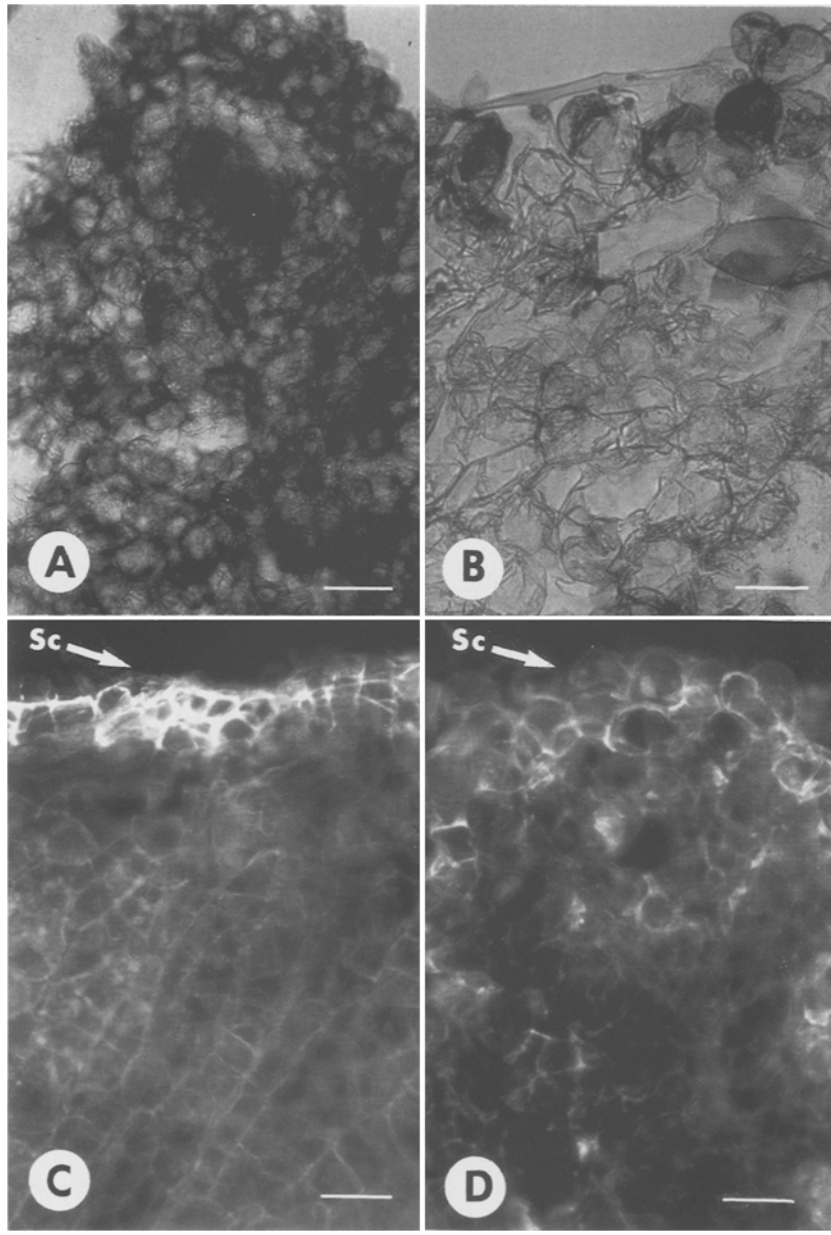

Fig. 1. Callus of *V. riparia* var. Gloire de Montpellier (resistant) and *V. vinifera* var. Grenache (susceptible), treated with Vanillin-HCl, observed with visible light (A, B). A, callus of var. Gloire de Montpellier; B, callus of var. Grenache. The red color indicates the presence of condensed tannins in the callus. These two species treated with Neu's reagent, 3 days after inoculation, examined with UV light (C, D). C, callus of var. Gloire de Montpellier; D, callus of var. Grenache. The yellowish-white fluorescence indicates the presence of flavonoids on the superficial cell walls (Sc). Bar = 100 μm.

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References

Aist JR (1983) Structure responses as resistance mechanisms. In: Bailey JA, Deverall BJ (eds) The dynamics of host defense (pp 33–70). Academic Press, Australia

Andary C (1975) Contribution à l'étude botanique, chimique et pharmacodynamique d'*Orobanche rapum-genistæ* Thuill (Orobanchacées). Ph.D Thesis, Université de Montpellier I, France

Beckman CH and Talboy PW (1981) Anatomy of resistance. In: Wace ME, Bell AA, Beckman CH (eds) Fungal wilt disease of plants (pp 487–521). Academic Press, New York

Boubals D (1959) Contribution à l'étude des causes de la résistance des vitacées au mildiou de la vigne (*Plasmopara viticola* (B. et C.) Berl. et T.) et de leur mode de transmission héréditaire. Ann Amel Plantes 1: 5–233

Brammall RA and Higgins VJ (1988) A histological comparison of fungal colonization in tomato seedlings susceptible or resistant to *Fusarium* crown and root rot disease. Can J Bot 66: 915–925

Cho EK and Goodman RM (1979) Strains of soybean mosaic virus: classifcation based on virulence in resistant soybean cultivars. Phytopathology 69: 467–470

Dai GH, Andary C, Mondolot-Cosson L and Boubals D (1995a) Histochemical responses of leaves of *in vitro* plantlets of *Vitis* spp. to infection with *Plasmopara viticola*. Phytopathology 85: 149–154

Dai GH, Andary C, Mondolot-Cosson L and Boubals D (1994) Polyphenols and resistance of grapevines (*Vitis* spp) to downy mildew (*Plasmopara viticola*). Acta Horticulturae 381: 763–766

Dai GH, Andary C, Mondolot-Cosson L and Boubals D (1995b) Histochemical studies on the interaction between the grapevines (*Vitis* spp.) and downy mildew (*Plasmopara viticola*). Physiol Mol Plant Pathol 46: 177–188

Friend J (1981) Plant phenolics, lignification and plant disease. In: Reinhold L, Harborne JB, Swain T (eds) Progress in Phytochemistry, Vol 7 (pp 197–261). Pergamon Press, Oxford

Galzy R (1962) Confirmation de la nature virale du court-noué de la vigne et essais de thermothérapie sur des cultures *in vitro*. C R Acad Sci 253: 706–708

Hariri B, Sallé G and Andary C (1991) Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.). Protoplasma 162: 20–26

Ingram DS (1977) Application in plant pathology. In: Street HE (ed.) Plant Tissue and Cell Culture (pp 463–500). University of California Press, Berkeley

Jensen WA (1962) Botanical Histochemistry. Freeman, San Francisco, California, pp 264

McMurrouch I and McDowell J (1978) Chromathographic separation and automated analysis of flavanols. Analyst Biochem 91: 92–100

Morel G (1944) Le développement du mildiou sur des tissus de vigne cultivés *in vitro*. C R Acad Sci 218: 50–52

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473–497

Neu R (1956) A new reagent for differentiating and determining flavones on paper chromatograms. Naturwissenschaften 43: 82

Ride JP (1983) Cell walls and other structural barriers in defense. In: Callow JA, Wiley J (eds) Biochemical plant pathology (pp 215–236). Ltd, New York

Sarkar SK and Howarth RE (1976) Specificity of the vanillin test for flavanols. J Agric Food Chem 24: 317–320

Sequeira L, Gaard G and Dezoeten GA (1977) Interaction of bacteria and host cell walls: its relation to mechanisms of induced resistance. Physiol Plant Pathol 10: 43–50

Tripathi SC and Dixit SN (1977) Fungitoxic properties of *Rosa chinensis* Jacq Experientia 33(2): 207–209