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Resveratrol Accumulation in Grapevine Infected with Grapevine Vein Necrosis Disease

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Abstract. Trans-resveratrol (4,3',5'-trishydroxystilbene) was identified in grapevine leaves with vein necrosis disease symptoms. The compound accumulates from 10 to 60 $\mu g.g^{-1}$ (fresh mass) in infected leaves.

It is supposed that the resistance of *Vitis* species to fungal infections is due to active defence mechanisms, which are triggered by the pathogen. Several antifungal substances which accumulate in grapevines as stress metabolites in response to infections by potential pathogens or to various forms of injury, have been associated with the resistance of vines to mildew and other diseases. Such responses include the production of a range of biosynthetically related di- and oligomers of a simple stilbene, trans-resveratrol (4,3',5'-trishydroxy stilbene), collectively termed viniferins and of trans-pterostilbene (3,5-bis-methoxy-4'-hydroxystilbene). In compatible interactions of *Plasmopara viticola* with susceptible cultivars of *Vitis vinifera,* the relative concentration of all these secondary metabolites is low in comparison to resistant species *V. riparia,* where ε-viniferin, α-viniferin and resveratrol are accumulated more rapidly and in greater amounts (Langcake and Pryce, 1976, Langcake and Pryce 1977a,b, Langcake *et al.* 1979, Langcake 1981). Pool *et al.* (1981) confirmed the findings of Langcake and his coworkers. They identified a higher resveratrol concentration induced by UV light in *Vitis cinerea* – a more resistant cultivar to *Botrytis cinerea,* than in Chelois × Ives – a less resistant cultivar. Stein and Hoos (1984) found also a negative correlation between the stilbene production and the susceptibility to *B. cinerea.* Thus the elicitation of phytoalexins or other fungitoxic substances may represent an effective method for screening disease resistance in the grape breeding programme.

The aim of our study was to isolate phytoalexins from the grapevine affected with grapevine vein necrosis disease, which is supposed to be a virus or by mycoplasma-like organisms induced disease (Bovey and Martelli 1986).

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MATERIAL AND METHODS

Fresh leaves of *Vitis berlandieri* × *rupestris* 110 Richter with symptoms of vein necrosis disease (Fig. 1) were used. Healthy leaves of the same cultivar were used as a control.

The method of phytoalexin isolation is a modification of techniques already described in literature (Langcake and Pryce 1976, 1977a, Pool *et al.* 1981, Stein and Hoos 1984, Šubíková and Kollerová 1988): infected leaves were homogenized for 2 minutes with 70 % methanol (1:10, w/v) in a Bühler HO 4 homogenizer at 20 000 rpm. The homogenate was filtered and clarified by low speed centrifugation. The supernatant was dried on a rotary evaporator at 40 °C and redissolved in water (5 ml) and ethyl acetate (10 ml). After mixing thoroughly, the phases were separated and the organic phase was evaporated, then redissolved in acetone (0,5 ml) and quantitatively applied to the activated silica gel plate Silufol^{R} (UV 254). The plate was developed three times in sequence using a chloroform:methanol mixture (25:1, v/v) as the developing solvent. Chlorophylls were removed from the plate after drying up. For extracting phytoalexins from plate methanol was used. After removing the silica gel the extract was evaporated and vacuum dried at 40 °C, resuspended in acetone and applied to the activated Silufol^{R} plate. The plate was then developed three times in sequence using a dichloromethane:methanol mixture (4:1, v/v) as the eluting solvent and dried.

After having been divided by thin layer chromatography phytoalexins were identified by their R_f values after spraying the plate with diazotized p-nitroaniline (DPNA) (Van Sumere *et al.* 1965) as well as from their ultraviolet absorbance spectra.

RESULTS AND DISCUSSION

Generally, the presence of leaf pigments, steroids and other interfering substances, makes the extraction of phytoalexins difficult. However the extraction of phytoalexins from grapevine leaves by methanol (70 %) and thin layer chromatography on Silufol^{R} plates appeared to be very effective.

As eluting solvent systems dichloromethan:methanol (4:1, v/v) or toluene:ethyl acetate:methanol (25:8:1) were most suitable. R_f values of resveratrol in these systems were 0.31 or 0.33, respectively. It was found that resveratrol predominantly occured in grapevine leaves *Vitis berlandieri* × *rupestris* 110 Richter with symptoms of vein necrosis disease. This compound was identified on the basis of UV absorption spectrum with maxima at 293, 304 and 320 nm, and the minimum at 255 nm (Fig. 2) what is in agreement with the results of Langcake of Pryce (1976). Based on the known molar extinction coefficient $\varepsilon(306) = 26\,800$ (Langcake and Pryce 1977b) the concentration of resveratrol

in infected leaves was calculated. It ranged between 10–60 μg g^{-1} of fresh mass in infected leaves correlating with necrotization. Sometimes also resveratrol dimer ε-viniferin was identified. The compounds were not detectable in healthy leaves.

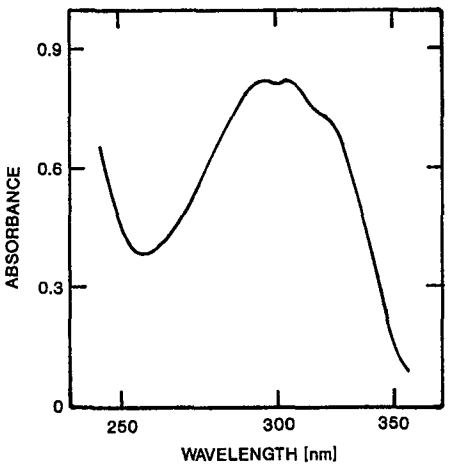

Fig. 2. UV absorption spectrum of resveratrol.

The production of phytoalexins is one of a number of detected biochemical changes important for virus localization in a hypersensitive reaction. Whether these substances are effective means of defence against certain pathogens, or whether they are of general importance is not quite clear at present. But in spite of that the elicitation of phytoalexins or other fungitoxic substances may represent an effective method for the selection of vines resistant to fungal pathogens (Pool *et al.* 1981).

In our preceeding experiments the accumulation of phytoalexins in bean leaves after infection with the tobacco necrosis virus and its isolated nucleic acid was estimated (Šubíková and Kollerová 1988). Their maximum concentration in leaves correlated with necrotization of lesions. On the basis of results obtained we supposed the accumulation of phytoalexins in grapevine leaves with vein necrosis disease symptoms and we were also successful to prove it.

These substances are related to the stress metabolites produced in response to infection, but their biological function *in situ* is so far not quite clear. Langcake and Pryce (1976) estimated resveratrol as an apparently normal constituent in the lignified stem tissue of grapevine Müller–Thurgau. It is supposed that these substances may have a different function in the normal development and act as a multicomponent defense response during the infection. Further precise measurements *in situ* are necessary for a better understanding of the role of these substances in plants.

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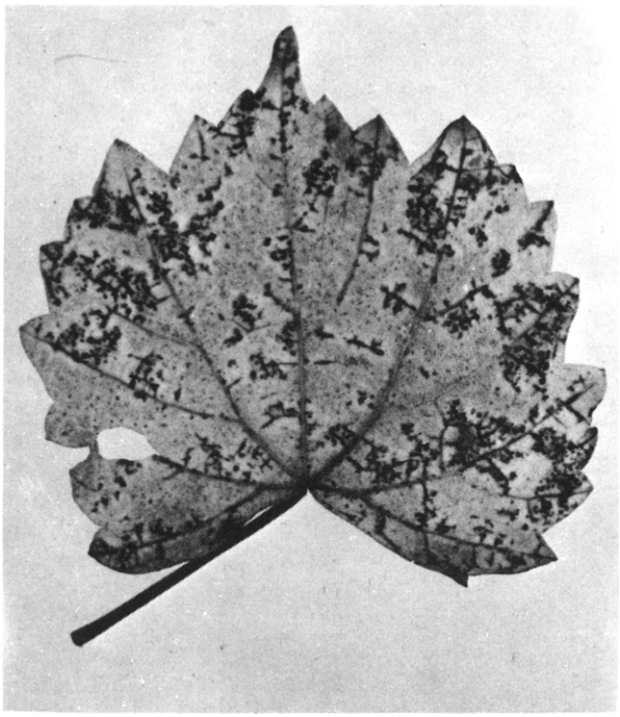

Fig. 1. Leaf of *Vitis berlandieri* × *rupestris* 110 Richter with symptoms of vein necrosis disease.