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NATURAL AND INDUCED RESISTANCE OF TABLE GRAPES TO POSTHARVEST DECAY

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Abstract

Table grapes were found to express different degrees of susceptibility to natural infection and inoculation by *Rhizopus stolonifer*. Natural resistance was related to cultivar and stage of berry development, but not to climatic conditions. Ultra-violet irradiation, induced the production of resveratrol and pterostilbene, which were fungitoxic to both *R. stolonifer* and *B. cinerea*. The incidence of decay which developed on *Rhizopus* -inoculated berries of different cvs. at various stages of maturity, was very significantly correlated to the level of resveratrol elicited by irradiation in the skin of the same berries.

Keywords

RHIZOPUS STOLONIFER, BOTRYTIS CINEREA, RESVERATROL, PTEROSTILBENE, UV-IRRADIATION.

1. INTRODUCTION

Grapes are cultivated extensively in the Mediterranean area and much of the crop for fresh consumption is exported to northern European countries, either by sea or by air freight. The predominant cause of postharvest deterioration is decay, which is not always effectively controlled by the use of SO_2 generators. However, without these generators the marketing of table grapes would be much more difficult and, though in many countries SO_2 has been banned from use for most of its applications in the food industry, it is still permitted for packaging of table grapes. The chief reason for this is probably the lack of an effective alternative. The great advantage that SO_2 has over other fungicides with regard to grapes, is that it is effective as a volatile and the fruit does not have to be wetted. Other volatiles which have so far been found to have fungicidal activity, such as acetaldehyde and chlorine, were both less effective and had additional disadvantages when compared with SO_2 (unpublished data).

The most common cause of postharvest decay of table grapes worldwide is *Botrytis cinerea* (Pearson and Goheen, 1988). However, in Israel we have found the cluster decay caused by *Rhizopus stolonifer* to be of greater economic importance and more predominant than *B. cinerea* (Barkai-Golan, 1981; Ben-Arie et al.1994).

Firstly, *R. stolonifer* is less sensitive to SO_2 than other rot causing fungi on grapes and the dose required for effective control may cause berry bleaching. Secondly, the fungus has a relatively short incubation period in the grape berry and a very rapid growth rate, so that under ideal conditions, a box of fruit can become a mass of fungal mycelium and spores within a two or three days (Shacham, 1989). Initial attempts to develop methods to control the decay caused by *Rhizopus* indicated that the susceptibility of the grape berry to decay may vary between cultivars, from one year to the next, and at different stages of development and maturity. The aim of this presentation is to describe some of the possible sources of resistance to decay, which are not necessarily specific for *R. stolonifer*.

2. MATERIALS AND METHODS

Grape clusters were sampled during 3 seasons fruit from 'Thompson Seedless' vineyards in two regions with different climatic conditions. At the lower altitude (300 m above sea level), both temperature and humidity are higher than at the higher altitude (800 m above sea level). In the former, fruit is harvested in mid-August and in the latter - in mid-September. Two clusters of fruit were sampled every 9-12 days from each of 5 uniform vines, that were chosen in each of the above two vineyards, from approximately veraison until about 1 month beyond the commercial harvest. Samples of the berries on 5 clusters were weighed and juiced for determination of soluble solid content (SCC), titratable acidity (TA, calculated as tartaric acid). Additional samples were extracted in ethanol for determination of soluble phenols and 10 intact berries were used for artificial inoculation, as described below (method #2). Relative susceptibility (the inverse of resistance) was assessed by one of the following two methods of artificial inoculation.

Method 1: Twenty- four apparently intact berries, detached randomly from clusters with their stem-ends attached, were dipped in a spore suspension of *Rhizopus stolonifer* containing 10^9 spores/ml. The berries were held in closed, humidified, multiwell plastic dishes at 28°C and examined after 72 hours for disease symptoms. Susceptibility was expressed as the percentage of decayed berries.

Method 2: Ten apparently intact berries, detached with their stem-ends from 5 clusters, were dipped in a spore suspension containing 10^6 spores/ml and held in a humidified atmosphere at 30°C for 48 hours. A decay index of 0-4 was used to rate each berry. (0 - no decay, 1 - an initial symptom, 2 - a longitudinal crack, 3 - mycelium, 4 - mycelium + sporangia).

The natural incidence of decay was assessed by storing the remaining cluster from each vine in a polyethylene bag at 20°C for 7 days, after which the number of decayed berries and the cause of decay were determined.

3. RESULTS AND DISCUSSION

3.1 Natural resistance

Resistance to decay was never found to be absolute, but differing degrees of susceptibility were observed. Three types of factors likely to affect the degree of susceptibility were examined.

3.1.1. Annual and regional susceptibility

The natural incidence of disease which developed on the fruit during 1 week's storage at 20°C following harvest, was much higher in one region than in the other, and varied considerably from year to year. However, there was very little difference in the DI following artificial inoculation between regions and years (Fig. 1). We therefore do not attribute the differences in natural infection to apparently variable levels of disease resistance, but possibly to climatic conditions or to the amount of inoculum in the vineyard. It is likely that both these factors are involved as at the higher altitude the conditions are less conducive to disease development, but there was also a tendency to increasing DI in both vineyards, indicative of a build-up of inoculum .

3.1.2. Developmental susceptibiliy

The data from the third year, when the incidence of natural decay was highest, show a rise and fall in DI, with the peak occurring at the height of the commercial harvest season (Fig. 2). At the same time the DI on inoculated berries reached maximum values, but did not decline thereafter. Examination of some of the chemical components of the fruit, that change with ripening, may provide some explanation for the rise in susceptibility to decay, but do not afford any explanation for the decline in decay towards the end of the season. The increase in susceptibility which accompanies maturation and ripening may be due to changes in the chemical composition of the fruit, such as an increase in SCC and/or a decline in acidity. Phenolics, however, do not appear to play a part in changing susceptibility - in one vineyard they increased and in the other they decreased, both as the fruit ripened and during after-ripening. The decline in natural infection at the end of the season might not be an indication of increased resistance to infection (as was indicated by the sustained DI), but rather the result of environmental effects on the viability or the virulence of the fungus.

3.1.3. Varietal susceptibility

The response of 12 different cultivars to inoculation (method #1) when organoleptically ripe, indicated that varietal susceptibility (or resistance) to *R.stolonifer* does exist (Table 1). This is not surprising in that a similar situation has been shown to occur with a smaller number of cultivars with regard to decay caused by *B. cinerea* (Pezet and Pont, 1988). Though the possibility exists that resistance to decay is a result of anatomical structures, which form a barrier to invasion by the

pathogen (Jeandet and Bessis, 1989), this is not generally regarded as the only mechanism involved. Evidence has been presented that variable varietal susceptibility may be due, at least in part, to induced resistance, elicited by either biotic or abiotic agents (Pezet and Pont, 1992).

3.2 Induced Resistance

Induced resistance to a number of plant pathogens has been demonstrated in vines , predominantly in leaves (Langcake, 1981). This resistance has been related to a number of stilbene phytoalexins, namely - resveratrol, pterostilbene, α-viniferin and ε-viniferin. Their production has been shown to be elicited by infective fungi such as *Plasmopara viticola* and *B. cinerea,* and in addition by ultra-violet irradiation. Resveratrol and pterostilbene have also been elicited in grape berries and related to resistance to *B. cinerea* (Creasy and Coffee, 1988). We have isolated these two stilbenes from the skins of the above grape cultivars following either uv treatment or fungal inoculation. Berries at different stages of maturity, based on their SSC, were either exposed for 5 or 10 min to uv at 254 nm or inoculated as above with *R. stolonifer* or *B. cinerea* (method #2). The uv response was closer to the elicitation induced by *R. stolonifer* than by *B. cinerea* (Table 2), and resveratrol production was generally 10 fold that of pterostilbene, though the ratio between the two compounds was inconsistent. The incidence of decay, which developed on inoculated berries of different cvs. at various stages of development, correlated highly with the level of resveratrol detected in the skins of the uv-irradiated berries (Fig. 3). However, pterostilbene was the more potent fungicide in vitro (Table 3). In spite of this, and the fact that *R. stolonifer* was more sensitive than *B. cinerea* to both phytoalexins, the endogenous levels were far below the LD_{50} values for both fungi. This probably indicates that other sources of resistance to disease exist in the grape berry, in addition to its ability to produce phytoalexins, as has been suggested by Pezet and Pont (1992).

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Table 1: Cultivar susceptibility to artificial inoculation with *R. stolonifer*.

Cultivar	% Infected berries
Early Superior	91
Alphonse Lavalle	83
Spring Blush	79
Perlette	75
Thompson Seedless	62
Dabouky	51
Kishmish	48
Superior Seedless	34
Muskat of Hambourg	22
Dan Ben-Hanna	15

Table 2: Stilbene production in grape berries, measured 24 hours after elicitation (average values for 4 cvs. - ng/g f.w.).

Elicitation	Resveratrol	Pterostilbene
Botrytis cinerea	11.6	3.2
Rhizopus stolonifer	24.4	2.4
uv - 254 nm - 5 min	22.6	2.5
uv - 254 nm - 10 min	33.0	3.3
Control	3.3	1.3

Table 3: Biological effects of stilbenes on *R. stolonifer* and *B. cinerea* (% of control).

Stilbene concentration (ppm)	*Rhizopus stolonifer*		*Botrytis cinerea*	
	Spore germination	Mycelial growth	Spore germination	Mycelial growth
			Resveratrol	
62	101 a	110 a	91 b	86 abc
125	87 b	102 a	90 b	95 ab
250	59 c	66 cd	62 c	62 cd
500	14 f	27 e	27 de	30 e
			Pterostilbene	
62	28 de	58 d	30 d	79 bcd
125	13 f	63 cd	19 ef	48 de
250	0 g	0 f	0 f	0 f
500	0 g	0 f	0 f	0 f

a-f numbers in each column followed by different letters are significantly different at 95% probability.