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SENSITIVITY OF THE FUNGUS *CYTOSPORA PERSOONII* TO THE FLAVONOIDS OF *PRUNUS CERASUS*

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Key Word Index—*Prunus cerasus*; Rosaceae; *Cytospora persoonii*; Sphaeropsidales; bioassay; fungal sensitivity; flavonoids.

Abstract—Bioassays compared mycelial growth of *Cytospora persoonii* on flavonoid glucosides and aglycones from resistant *Prunus cerasus*. Apart from the fact that flavonoid aglycones were more toxic than the corresponding glucosides, there was a difference in fungal reaction depending on the flavonoid type. The fungus was much more sensitive to changes in the concentration of the flavanone aglycones than to those of the corresponding glucosides. Apart from differences in the toxicity of the various flavonoids tested, the fungal sensitivity in the case of flavone and isoflavone aglycones was similar to the sensitivity of flavanone glucosides.

INTRODUCTION

Cytospora persoonii (Ehrenberg) v. Höhnel is the imperfect stage of the Ascomycete *Leucostoma persoonii* (Nits.) v. Höhnel. It causes perennial canker on the bark of peach, plum and sweet cherry [1]. Sour cherries (*Prunus cerasus* L.) are more resistant to this fungus [2]. Typical flavonoids of the bark of *P. cerasus* were extracted and isolated to study their effect on the fungal growth *in vitro*.

RESULTS AND DISCUSSION

The common method of testing fungal growth in Petri dishes is uneconomic because it consumes large quantities of test substances. Thus a new bioassay was developed using microtitreplates.

With the exception of dihydrowogonin 7-glucoside (**6**) and prunetin 5-glucoside (**12**), all tested flavonoids slowed down the mycelial growth at concentrations of 1 mM in the stock solution (Figs 1 and 2). Due to the diffusion of the stock solution through the agar, the effective concentrations must be less. Similar or higher concentrations were found *in situ* near the infection site [3]. There was a significant difference in the toxicity of aglycones and glucosides. Similar results were obtained by Bayer [4] in another test system with flavonoids from *P. avium* against *C. persoonii*. Together with the fact that flavonoid 5-glucosides are typical compounds of *P. cerasus* [5] and hydrolyse very easily [6, 7], the higher toxicity of the aglycones indicates a mechanism of resistance in *P. cerasus*. This hypothesis is confirmed by the observation that only small concentrations of flavonoid 5-glucosides are found in *P. avium* which is very susceptible to *C. persoonii* [3]. Additionally, in unwounded bark of *Prunus* spp., flavonoid aglycones occur only in traces. Therefore a mechanism for enhancing the toxicity of the constitutive flavonoid glucosides by hydrolysis is a very valuable defence reaction.

Naringenin (**2**) showed complete fungistatic effects in a stock solution at a concentration of 2.5 mM. Chrysin (**7**) stopped mycelial growth in most cases at 1.0 and 2.5 mM. Sakuranetin (**4**) had no effect at 0.1 mM but a significant one at higher concentrations (mostly fungistatic). The glucosides prunin (**3**) and sakuranin (**5**) exhibited moderate toxicity at all concentrations tested. Genistein (**10**) was less toxic, but in another trial it showed more toxicity than the corresponding 7-glucoside genistin. Tectochrysin (**8**) and prunetin (**11**) were more effective than their 5-glucosides **9** and **12**. For pinostrobin (**1**) no corresponding glucoside was tested. In contrast to the findings of Bayer [4], no general effect of the methoxyl group was found and **6** was not toxic at all.

Apart from the difference in activity between aglycones and glucosides, there was another observation with reference to the flavonoid type which is very interesting. The fungus is much more sensitive to changes in the concentration of the flavanone aglycones than to those of the corresponding glucosides (Fig. 1). Flavone and isoflavone aglycones (Fig. 2) showed similar curves as flavanone glucosides. Despite the large difference in the toxicity of **11** compared to **12**, there was no difference in the fungal sensitivity to the isoflavone aglycone and glucoside, because the regression curves showed similar slopes.

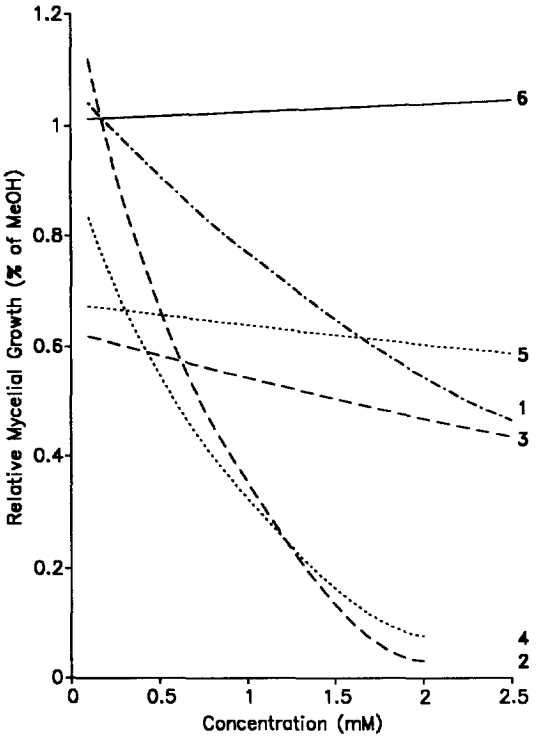

Fig. 1. Regression of the relative mycelial growth on flavanones. Compounds: **1** pinostrobin; **2** naringenin; **3** prunin; **4** sakuranetin; **5** sakuranin; **6** dihydrowogonin 7-glucoside. Scale on y-axis: 1 = 100%.

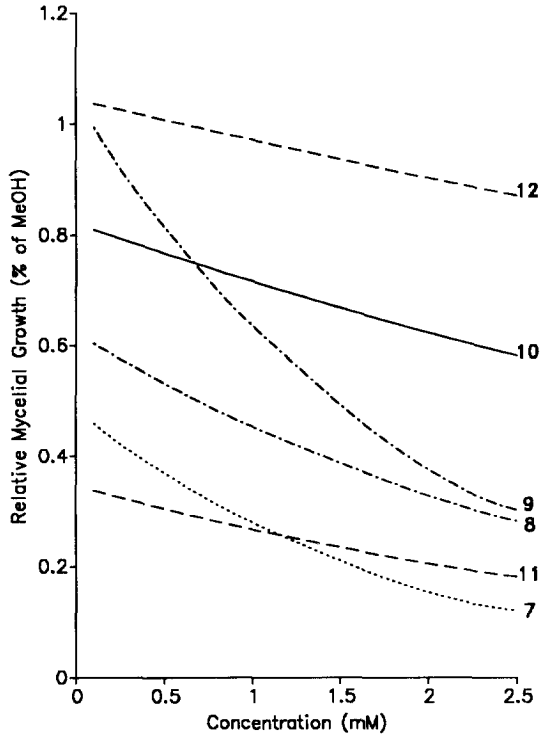

Fig. 2. Regression of the relative mycelial growth on flavones and isoflavones. Compounds: **7** chrysin; **8** tectochrysin; **9** tectochrysin 5-glucoside; **10** genistein; **11** prunetin; **12** prunetin 5-glucoside. Scale y-axis: 1 = 100%.

Table 1. Oxidation pattern and flavonoid type of the tested substances

Oxidation pattern			Flavonoid type		
OMe	OH	O-Glc	Flavanone	Flavone	Isoflavone
	5, 7			**7**	
7	5		**1**	**8**	
7		5		**9**	
	5, 7, 4′		**2**		**10**
	5, 4′	7	**3**		
7	5, 4′		**4**		**11**
7	4′	5	**5**		**12**
8	5	7	**6**		

Tectochrysin 5-glucoside (**9**) was the only flavone glucoside to be tested. Interestingly, the regression curves show that fungal sensitivity to this glucoside is more comparable with that of the flavanone aglycones (**1**, **2** and **4**) than with all glucosides tested (**3**, **5**, **6** and **12**).

Bioassays comparing different flavonoid types should be carried out at more than one concentration. Because of the different sensitivities, contrasting results will be obtained depending on the concentration tested. The reason for the different sensitivities of the fungus is not clear. Perhaps the flavanone aglycones are detoxified by the fungus at lower concentrations only and exhibit a strong toxicity at higher concentrations.

EXPERIMENTAL

Source of the flavonoids. Compounds **4**, **5**, **6**, **8**, **9** and **12** were isolated from bark of *P. cerasus* and purified [5, 7]. Although compounds **1**, **2**, **3**, **7**, **10** and **11** are known to occur in bark of *P. cerasus*, they were obtained from a commercial source (Carl Roth GmbH & Co., Karlsruhe, Germany).

Medium. Malt extract (0.1%) (Biomalz, Kirner Vitabornwerk Andres GmbH, Kirn, Germany), 0.5% agar, 0.03% streptomycinsulphate.

Method. Each hole in a microtitreplate (96 holes) was filled with 50 μl medium. After solidification, 8 μl of a methanolic solution of each flavonoid at a concentration of 0.1, 0.5, 1 and 2.5 mM was added. For inoculation, mycelium discs (r = 0.5 mm) were transferred to each hole.

Evaluation. The area covered with mycelium was estimated under the binocular microscope twice per trial in steps of 10% (in trial 1 on days 3 and 6 after inoculation; in trial 2 on days 5 and 8 after inoculation).

Regression. Sixty-four values per flavonoid (2 trials × 2 dates × 4 concentrations × 4 parallels).

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