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## **Influence of phenolic acids isolated from blackcurrant and sour cherry leaves on grain aphid (*Sitobion avenae* F.)**

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**Abstract:** Effect of plant phenolics isolated from leaves of blackcurrant (*Ribes nigrum* L.) and sour cherry (*Prunus cerasus* L.) as potential biopesticides against the grain aphid was examined. The extracts from the sour cherry contained a twice higher level of salicylic acid than extract from leaves of the blackcurrant. Moreover, concentration of chlorogenic acid was higher in sour cherry and the content of tannic acid within both plant tissues was at the same level.

Both examined extracts had a negative effect on development of the grain aphid when the aphid fed on wheat plants sprayed with 1.0%, 2.5% and 5.0% solutions. The length of prereproductive period (time from birth to maturity) of the aphids was extended from 5.9 days on control plants (not sprayed) to 8.1 days, when the aphids were fed on the treated plants. Apterous females of *Sitobion avenae* fed on sprayed plants showed lower fecundity and lower values of intrinsic rate of natural increase.

**Keywords:** extracts, sour cherry, blackcurrant, phenolics, grain aphids

### INTRODUCTION

The secondary metabolites are widespread in plant kingdom and their concentration is usually highest in peripheral tissues. They take part in numerous environmental interactions and protect plants from invasion of herbivorous insects and pathogens. Such properties of plant secondary metabolites result in application of these compounds as biopesticides for insect pest control. Several classes of compounds, including alkaloids [1], flavonoids and related compounds [2] and diterpenes [3] are toxic or deterrent to insects. Aphids are one of the

main pests of cereal crops and the results of earlier studies indicated that they can reduce not only yields but also quality of the injured grains [4-6]. The rising costs of pesticides, their adverse effects on the environment and the increasing resistance of insects have caused undertaking the research on the identification and application of plant extracts as natural biopesticides [7, 8].

Several biochemical and physiological studies have demonstrated the involvement of natural plant phenolics in response of cereal plants to aphids. Leszczyński *et al.* [9, 10], and also Ciepiela and Chrzanowski [11] have found that high level of constitutive resistance of cereals to the grain aphid (*Sitobion avenae* F.) was related to the high content of gallic, ferulic and salicylic acids. Furthermore, the grain aphid feeding caused an increase of the salicylic acid level in injured resistant cultivars [12].

Sour cherry is quite a common shrub or tree (*Rosaceae*), whereas blackcurrant is a deciduous shrub found in Europe, Asia, and America that belongs to *Grossulariaceae*, *ribes* species [13, 14]. Rauha *et al.* [15] showed antimicrobial properties of extract from *R. nigrum* and in Polish folk medicine, blackcurrant and sour cherry plants were recommended as anti-inflammatory and antimicrobial factors.

The aim of this work was to examine influence of plant phenolic extracts from leaves of the blackcurrant and the sour cherry on development of the grain aphid.

## MATERIALS AND METHODS

### ***Plant material and phenols extraction***

The leaves of blackcurrant cv. "Bona" and sour cherry cv. "Łutówka" were collected in June 2004, and air dried in shadow at 37 °C and then ground in the electric grinder.

One g of the leaf powder (after purification with petroleum ether and chloroform) was extracted with 150 cm<sup>3</sup> of 80% methanol. The extraction was performed in Soxhlet apparatus for 6 h (at boiling point). The methanol extracts were evaporated under vacuum to 10 cm<sup>3</sup>, and 50 cm<sup>3</sup> of 5% NaHCO<sub>3</sub> was added, vigorously mixed and left for 3 h. The solution was filtered, acidified to pH 3 with 18% hydrochloric acid and fraction of free phenolic acids was obtained by extraction with diethyl ether. The residue after ether evaporation was dissolved in 50 cm<sup>3</sup> of methanol (a crude extract). HPLC method was used to determine composition of the phenolic acids [16]. The separation was performed in three independent repetitions and level of the phenolics was

expressed as mean value  $\pm$  standard error, in mg per one gram of dry matter ( $\text{mg g}^{-1}$  D.M.).

### ***Entomological experiments***

Three concentrations (1.0%, 2.5% and 5.0% v/v) of solutions from crude extract of the both plants were applied. All entomological experiments were conducted in climatic chamber ( $24 \pm 2$  °C for light cycle and  $16 \pm 2$  °C for dark cycle) under 16:8 h photoperiod in  $65 \pm 5\%$  relative humidity.

The effect of the phenolic extracts on the grain aphid development was evaluated, on 10 seedlings of winter triticale – cv. Marko sprayed with plant extracts (approx. 5 cm<sup>3</sup> per pool). After 1 hour plants were inoculated with wingless female (one female per one seedling) and isolated with Plexiglass tube.

When females produced their first offspring, one larva was left, whereas other insects were removed. The larvae were observed daily until their sexual maturity. The duration of prereproductive period (PRP) and daily fecundity (DF) were calculated. These data were also used to calculate value of the intrinsic rate of natural increase ( $r_m$ ) according to Wyatt and White [17].

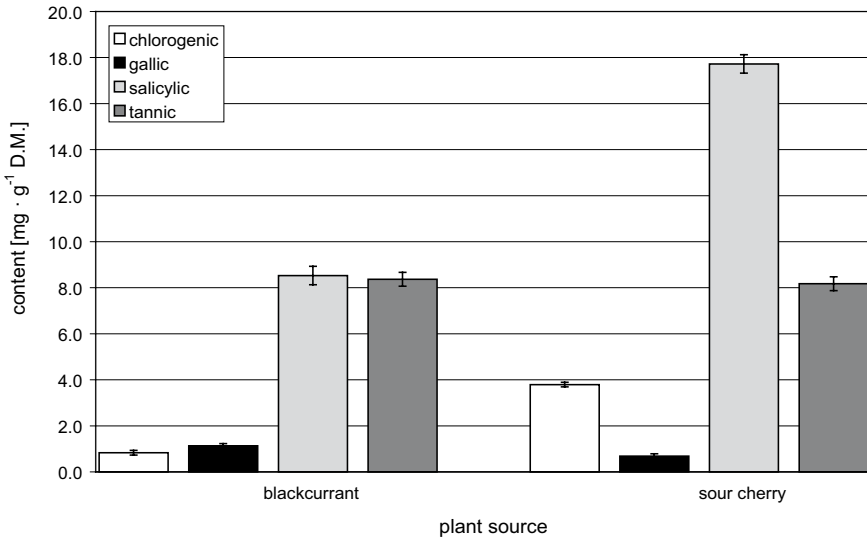
### ***Statistical analysis***

Significant differences between the average values of the antibiosis parameters were calculated using analysis of variance (ANOVA), and Tukey's post-hoc test at  $P \leq 0.05$ .

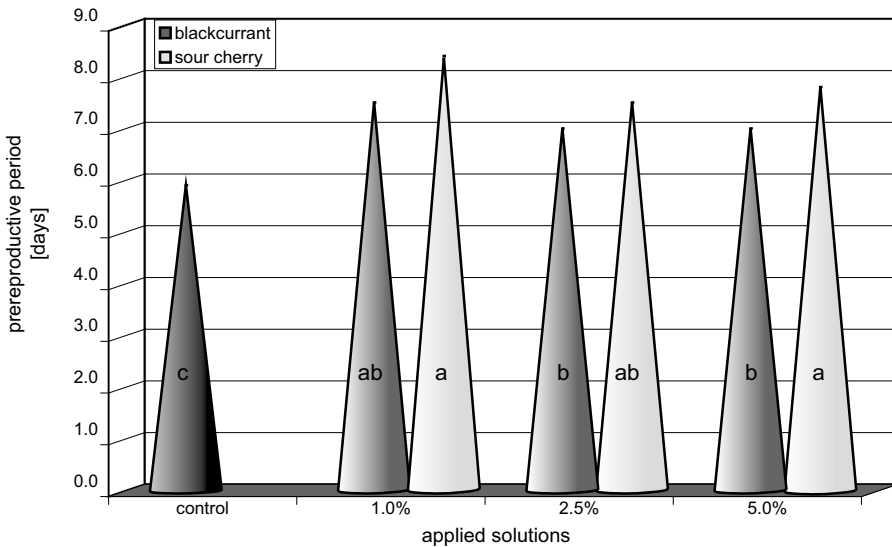
## RESULTS AND DISCUSSION

HPLC analyses showed that studied extracts from sour cherry and blackcurrant contained eight phenolic acids: salicylic, chlorogenic, tannic, gallic, caffeic, ferulic, *p*-coumaric and *trans*-cinnamic. Predominant compound in sour cherry was salicylic acid, while in blackcurrant salicylic and tannic acids (Figure 1). Gallic and chlorogenic acids occurred within leaves of the studied plants in lower concentrations, and other identified phenolic acids in trace amounts.

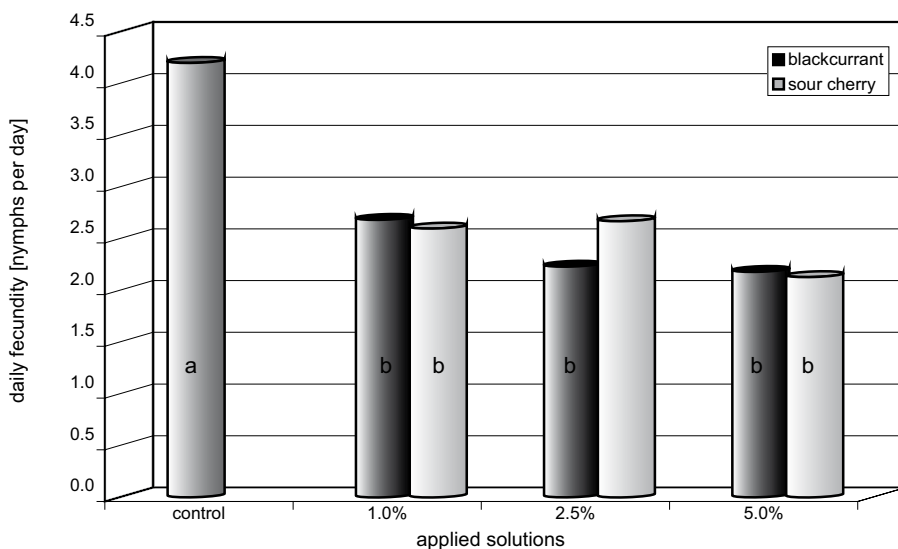
Entomological experiments showed that both studied extracts at all tested concentrations negatively influenced the grain aphid development and fecundity. The aphid prereproductive period was prolonged about 1-2 days and slightly stronger by sour cherry phenolic acids (Figure 2). Both studied extracts reduced daily fecundity of the grain aphid on average about 40% (Figure 3). The plant phenolic acids also reduced about 25-30% values of the intrinsic rate of natural increase (Figure 4).



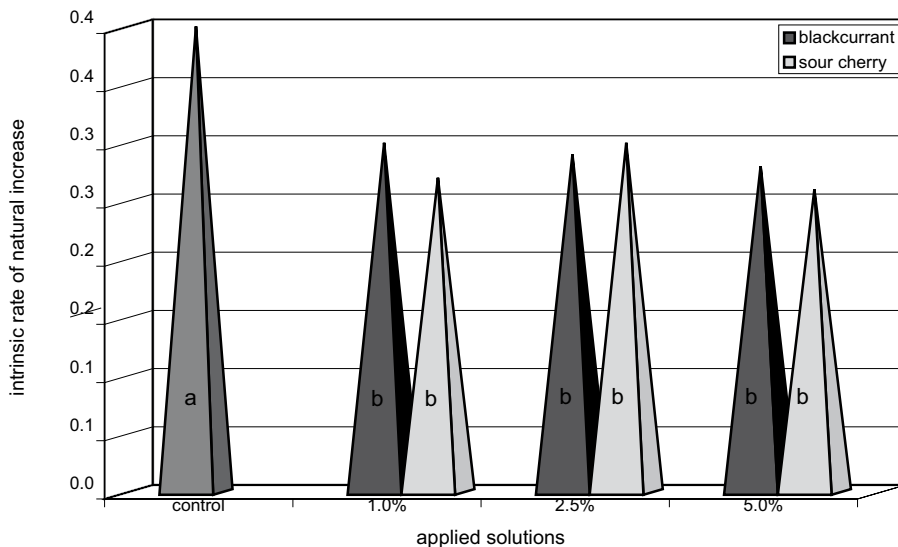
**Figure 1.** The content of the identified phenolic acids ( $\text{mg g}^{-1}$  D.M.) in leaf tissues of the blackcurrant and sour cherry.



**Figure 2.** Effect of plant extracts on the duration of prereproductive period of the grain aphid. Different letters on the cones indicate statistically significant differences at  $p \leq 0.05$  (Tukey's test).



**Figure 3.** Effect of the phenolic acids from plant extracts on the daily fecundity of the grain aphid. different letters on the cylinders indicate statistically significant differences at  $p \leq 0.05$  (Tukey's test).



**Figure 4.** Intrinsic rate of natural increase of the grain aphid in response to control and extracts from leaves of blackcurrant and sour cherry. Different letters on the pyramids indicating statistically significant differences at  $p \leq 0.05$  (Tukey's test).

Todd *et al.* [18] have shown that phenolic compounds and flavonoids, especially dihydroxyphenols with hydroxyl groups in position *ortho* (e.g. chlorogenic acid), exerted a negative effect on cereal aphid *Schizaphis graminum*. It was also suggested that isochlorogenic acid should be considered as a resistance factor of lettuce to the aphid *Pemphigus bursarius* [19]. Leszczyński *et al.* [9] found that ferulic, caffeic and chlorogenic acids at concentration 62.5 mg dm<sup>-3</sup> inhibited development of the bird cherry-oat aphid, *Rhopalosiphum padi*. In addition, it was also suggested that higher concentrations of *p*-coumaric, gallic, and *trans*-cinnamic acids reduced feeding of this aphid species.

The presented results let draw a conclusion that phenolic acids present in the leaves of the sour cherry and blackcurrant act as natural biopesticides against the grain aphid. However, further study are needed to establish the most effective doses of these natural substances, and to examine antibiotic properties of the identified individual phenolic acids.

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