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Toxicity of phytohemagglutinin (PHA) from *Phaseolus vulgaris* L. to the bird cherry-oat aphid (*Rhopalosiphum padi* L.)

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Abstract: Recent progress in plant transformation for insect resistance has increased the interest in the potential toxicity of lectins to insect pests. The aim of the present study was to examine the effects of phytohemagglutinin (PHA) on survival, development and fecundity of the bird cherry-oat aphid, when tested on artificial diet. The laboratory tests did not reveal any phagostimulating properties of lectin PHA to *R. padi*, although the presence of the PHA in the diet decreased fecundity of the adult aphids compared to aphids fed on control diets. Tested lectin had a negative effect on the insects' weight and induced their mortality. The data presented here suggest that PHA possesses a high insecticidal activity towards the bird cherry-oat aphid and might be considered as protein biopesticide against that aphid pest.

Keywords: feeding, survival, insecticidal, aphid, lectin

INTRODUCTION

Plants synthesize several substances to protect themselves against predators, including many secondary metabolites as well as a battery of defense proteins. Genetic engineering offers the possibility of incorporating foreign plants genes into genomes of some crop species, such as a gene encoding an entomotoxic protein [1]. As *Bacillus thuringiensis* are not effective against all types of insect pests, the search for new resistance factors needs to continue [2]. The work on proteins of alternative resistance factors is still limited to two major classes of plant proteins,

viz., protease inhibitors and lectins [3]. Plant lectins are a group of proteins with highly specific sugar binding activity that have been shown to have insecticidal effects on a variety of pest insects [4]. The examples in literature show clearly that the physiological effects of plant lectins, as well as their effects on insect behavior, can be very diverse, depending largely on the insect species and the lectin used [5]. Phytohemagglutinin (PHA), the lectin from *Phaseolus vulgaris*, is a tetrameric protein with a molecular weight of 120 kDa and sugar specificity for D-galactose/ N-acetyl-D-galactosamine residue. The toxic effects of PHA on different insects species is well documented [6, 7], but its toxicity to aphids has not been investigated. *Rhopalosiphum padi* /L./ (bird cherry-oat aphid) is a nearly worldwide aphid pest of grains. It is a part of a complex of cereal aphids that infests grains, and *R. padi* can often be a dominant cereal aphid species. Infestations of *R. padi* cause yield loss to small grains by reducing yield components such as numbers of spikelets and seeds. *R. padi* vectors barley yellow dwarf virus (BYDV), which can induce disease and further yield loss in grains [8].

The aim of the present study was to examine the effects of phytohemagglutinin on survival, development and fecundity of the bird cherry-oat aphid when tested on artificial diet.

MATERIAL AND METHODS

Laboratory experiments were carried out under control: at temperature of 24 °C and 18 °C (day-night), relative humidity of 65 ±5% and photoperiod of 16:8 hours. The experiment was conducted on wingless females of the bird cherry-oat aphid, which in control group were offered artificial diets of optimal composition, containing essential nutrients [9]. Moreover, the examined diets that were prepared contained additionally soluble lectin PHA at eight concentrations: 10, 50, 250, 500, 750, 1000, 1250, 1500 $\mu g \cdot cm^{-3}$. The prepared diets were introduced between layers of Parafilm M®, which were placed on plastic rings (h = 1.5 cm, Ø = 3.5 cm). Inside the rings prepared in this way, that 5 individuals of *R. padi* were placed, securing their basis with blotting cloth.

The phagostimulating test was aimed to determine the number of feeding aphids on controlled diets and those which contained specific concentrations of PHA for 1 and 24 hours since the onset of the testing. Moreover, for the following 8 days the number of larvae on the tested diets was recorded in order to determine the average daily fecundity of *R. padi*. Weight tests were also carried out to determine the body mass of the wingless females of bird cherry-oat aphid before the onset of the test and after 24 and 96 hours of feeding. Observations

on the impact of the analysed density of the lectin PHA on *R. padi* surival were carried out (8 days), registering the number of living wingless females of bird cherry-oat aphid.

All the tests were carried out in 10 replicates for each concentration of lectin and controlled diet and their significance were subjected to variant analysis (ANOVA). Differences in the reaction of the studied concentrations of lectin PHA on fecundity of *R. padi* were determined by Duncan's test.

RESULTS AND DISCUSSION

In this study it was the first time that the biological reaction of an aphid to the determined concentrations of PHA was tested. Phagostimulating test did not show any clear interdependence between the concentration of lectin in the tested diets and the number of wingless females of bird cherry-oat aphid both after the first and the 24th hour of feeding (Figure 1). Similar results were obtained by Sprawka and Leszczyński [10], who showed that PHA which they tested did not have the properties of feeding stimulator and did not exert any deterrent effect on behavior of *S. avenae*. Rahbe and Febvay [11] showed that none of the proteins tested (twenty five proteins) had deterrent effect to pea aphid (*Acyrthosiphon pisum* H.). It is consistent with the thesis formed by Yamashita e*t al*. [12], that proteins are commonly devoid of phagostimulatory properties, except for some very specific compounds such as polypeptidic sweeteners or taste modifiers.

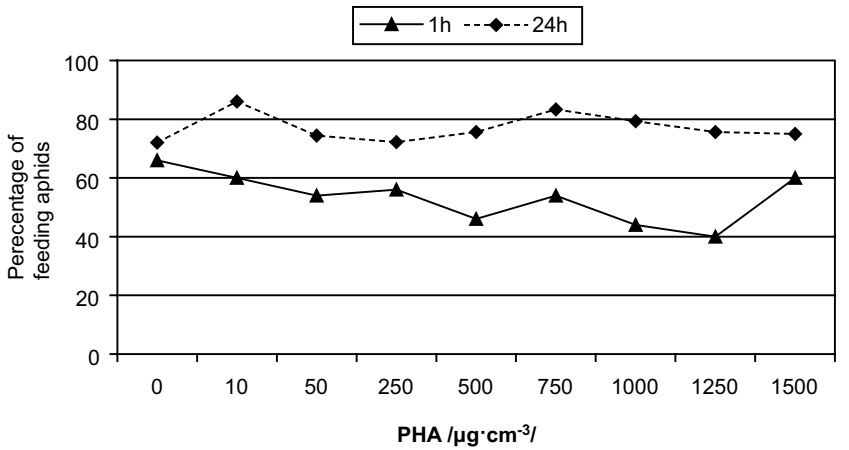

Figure 1. Effect of PHA on the feeding of wingless females of bird cherry-oat aphid.

Table 1. Daily fecundity of wingless females of bird cherry-oat aphid feeding on diet conained different concentrations of PHA

PHA /µg·cm^{-3}/	Average daily fecundity (number of larvae/female/day)
0 (control)	1.42 a
10	1.38 b
50	1.33 b
250	1.27 c
500	1.22 c
750	1.09 d
1000	1.04 d
1250	0.92 e
1500	0.85 f

Values in the column followed by various letters are significantly different at P≤0.01 (Duncan's test).

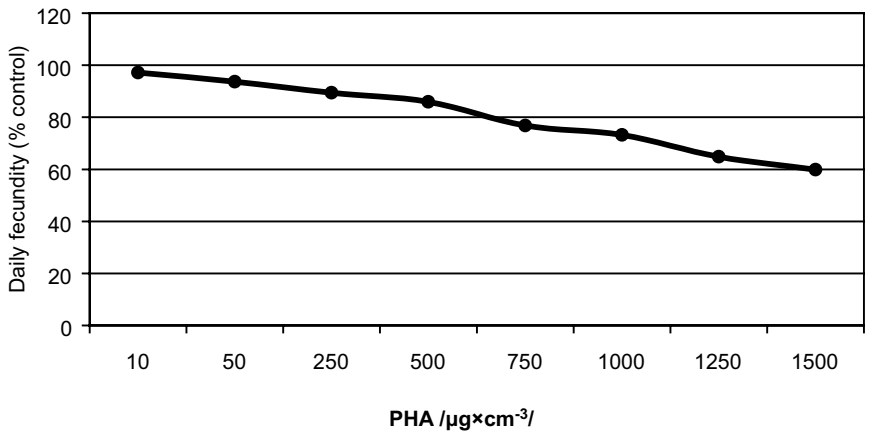

Figure 2. Effect of PHA on daily fecundity of wingless females of *R. padi*.

The highest fecundity (next to individuals on control diets) was found in wingless *R. padi* females which fed on diets containing the lowest concentration of PHA (Table 1). The increase of concentration of lectin in a diet lowered the daily fecundity of the insect in relation to the insects feeding on the control diet. The highest reduction of fecundity, about 40%, was recorded for the wingless females of bird cherry-oat aphid which fed on diets containing the highest tested concentration of phytohemagglutinin (Figure 2). The weight tests that were carried out showed that the increase in concentration of PHA in a diet,

as well as lengthening of feeding time, induced the lowering of the body mass of wingless females (Figure 3). With respect to the analysed concentration of PHA about 40% decrease of body weight of the studied *apterae* was registered (Figure 4). The laboratory tests that were carried out showed that the increase in lectin concentration induced the decrease of *R. padi* survival after 24 hours of the test in operation. Such tendency was also observed after 96 hours of insect feeding (Figure 5).

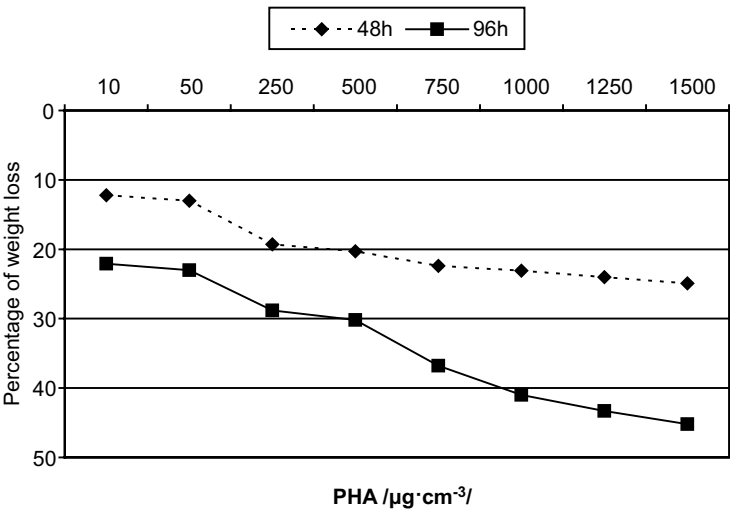

Figure 3. Changes in weight of wingless females of bird cherry-oat aphid induced by tested concentrations of PHA.

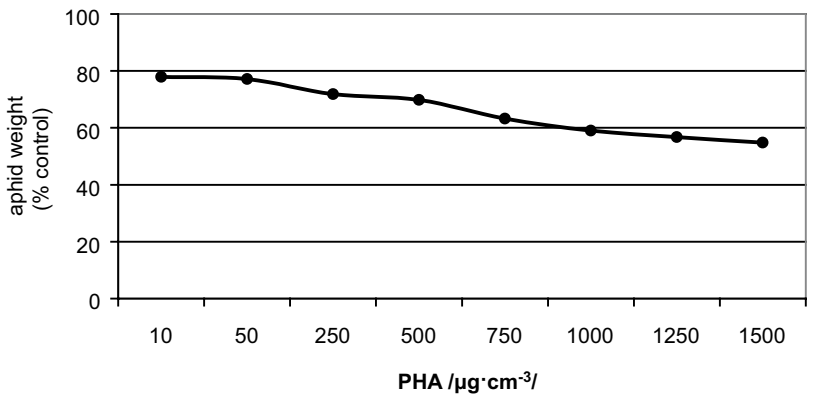

Figure 4. The influence of PHA on weight of wingless females of bird cherry-oat aphid.

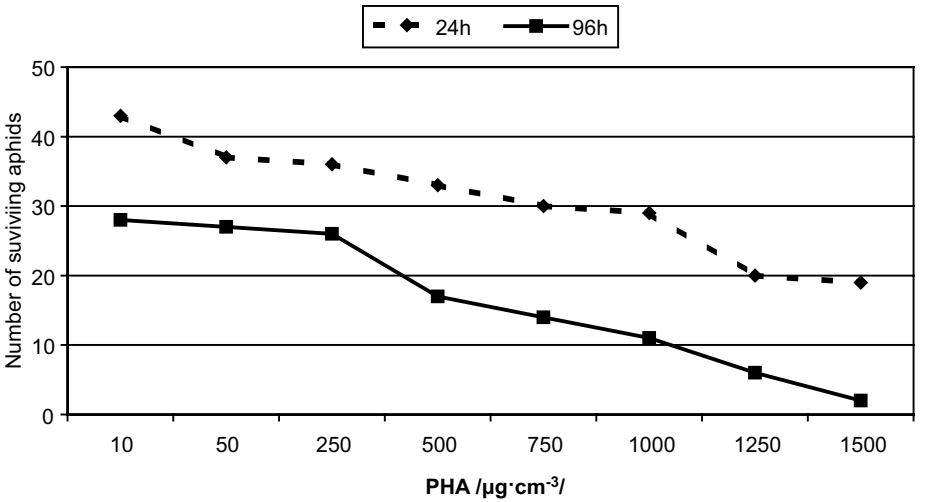

Figure 5. The influence of PHA on survival of wingless females of bird cherry-oat aphid.

Similar results were reported for grain aphid (*Sitobion avenae* F.). Sprawka and Leszczyński [10] showed that the presence of the PHA in the diet decreased fecundity of the wingless females of grain aphid up to 30% compared with aphids fed on control diets. Moreover, aphids exposed to PHA showed reduction in weight by about 20%. PHA also caused high levels of mortality of the grain adult aphids. Rahbe *et al*. [13] showed that PHA lectin influences negatively the survival and mass increase in *A. pisum*. Moreover, Habibi *et al.* [14], who studied the impact of 15 plant lectins on the survival of *Empoasca fabae* /H./ (*Homoptera*) females found that the phytohemagglutinin is one of the most toxic in relation to this insect species. It has been pointed out that the concentration of 200 $\mu g \cdot cm^{-3}$ significantly increased the mortality of this pest. On the second day of the experiment total mortality of *E. fabae* females was observed, after the females were feeding on a diet containing the highest concentration of PHA (1000 $\mu g \cdot cm^{-3}$, 1500 $\mu g \cdot cm^{-3}$). The same tendency was observed in the case of a bird cherry-oat aphid, its mortality was correlated with the concentration of lectin in the diet and with the time of feeding. The toxic effect of lectin PHA in relation to *Callosobruchus maculatus* /F./ was confirmed also by Sadeghi *et al.* [15]. It was shown that lectin significantly reduced female fecundity and was one of the most toxic of all. Similar results were obtained by Machuka *et al*. [16], who observed a negative impact of lectin PHA on the mortality of larvae *Maruca vitrata* /F./.

There is also little data on the impact of other lectins on cereal aphids bionomy. Stroger *et al.* [17] showed that transgenic wheat plants from lines expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA) decrease the fecundity, but not the survival, of grain aphid. The same tendency was observed in the case of another cereal aphids, a rose-grass aphid (*Metopolophium dirhodum* Walk.). Wheat plants expressing GNA did not show any significant detrimental effect on aphid survival, although they were able to reduce the fecundity of *M. dirhodum* by 25% when compared with insect fed on control plants [18]. This differential effect may be due to differences in sugar specificities: GNA binding to mannose residues, whereas PHA is specific D-galactose/ N-acetyl-D-galactosamine residues. From earlier study reports [19, 20], the toxicity of plant lectins has a strong connection with their specificity towards carbohydrate, which directs interactions with given receptors placed in the insects' digestive tracts. Moreover, it has been postulated that binding of lectin to the epithelial membrane of the insect gut and sugar moiety of any of the glycosylated digestive enzymes is the predetermining factor for insecticidal activity [21].

The data presented here suggest that PHA possesses a high insecticidal activity towards the bird cherry-oat aphid and might be considered as a protein biopesticide against that aphid pest, but further studies on mechanisms of the toxicity of PHA to cereal aphids are necessary.

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