Biochemical Systematics and Ecology 55 (2014) 137–144

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Effect of saponins and apigenin mixtures on feeding behavior of the pea aphid, *Acyrthosiphon pisum* Harris

Sylwia Goławska*, Iwona Sprawka, Iwona Łukasik

Department of Biochemistry and Molecular Biology, Siedlce University of Natural Sciences and Humanities, Prusa 12, 08-110 Siedlce, Poland

ARTICLE INFO

Article history:
Received 18 September 2013
Accepted 15 March 2014
Available online

Keywords:
Insect feeding
EPG
Pea aphid
Agarose-sucrose gels
Saponins
Flavonoids

ABSTRACT

Electrical penetration graphs (DC EPG) were used to monitor the feeding behavior of the pea aphid *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) exposed to mixtures of saponins: 3GlcA, 28AraRhaXyl medicagenic acid glycoside (MAG) and zanhic acid tridesmoside (ZAD) with flavonoid apigenin, on agarose-sucrose gels. In general, mixtures of saponins with apigenin incorporated into agarose-sucrose gels resulted in a reduction in both the number of aphid probes and delayed their duration. Aphid feeding on gels impregnated with mixtures of the tested chemicals also showed a reduction in salivation into the gels and elongation of passive ingestion from the gels (EPG patterns g-E1 and g-E2, respectively). There were significant differences among the feeding behaviors of the pea aphids on gels containing mixtures of either MAG or ZAD with apigenin. These differences were also depended on the concentration and proportions of these compounds.

1. Introduction

Food consumption and growth rates of herbivores are depressed when their diets contain saponins or flavonoids (Goławska and Łukasik, 2012; Goławska et al., 2012a, 2013). Flavonoids and saponins are among the more common classes of compounds used as defensive agents against insects (Oleszek et al., 1992) and can be expected to influence herbivore feeding behavior (Kubo, 2006; Goławska et al., 2008, 2010).

Saponins occur in ca 100 plant families including *Medicago sativa* L., known as alfalfa, which is an important livestock feed crop (Small, 1996). Saponins can be classified into two major groups based on their aglycone structure as either steroidal saponins or triterpenoid saponins and a third group are classified as steroidal alkaloids (Bruneton, 1995).

Alfalfa differ in the content of saponins, such as medicagenic acid, zanhic acid, and soyasapogenol glycosides (Oleszek et al., 1992; Pecetti et al., 2006). Saponin concentrations between 0.2 and 2% have been reported in different varieties of alfalfa (Pedersen and Wang, 1971; Majak et al., 1980). The studies indicated that herbivorous are clearly affected by saponin concentrations of 0.01%, 0.1%; 0.2% and 0.5% in alfalfa (Nozzolillo et al., 1997; Adel et al., 2000).

Saponins are reported to be important in varietal resistance of alfalfa to the pea aphid (*Acyrthosiphon pisum* Harris) and are useful in breeding programs to develop resistant varieties of alfalfa (Goławska, 2007; Goławska and Łukasik, 2009). Agrell et al. (2003) reported that saponins are involved in the herbivore-induced defense of alfalfa. Three alfalfa zanhic acid tridesmoside saponins; 3GlcA, 28AraRhaXyl medicagenic acid glycoside, and 3GlcA, 28AraRha medicagenic acid glycoside,

* Corresponding author.
E-mail address: sylwia@uph.edu.pl (S. Goławska).

http://dx.doi.org/10.1016/j.bse.2014.03.010
0305-1978/

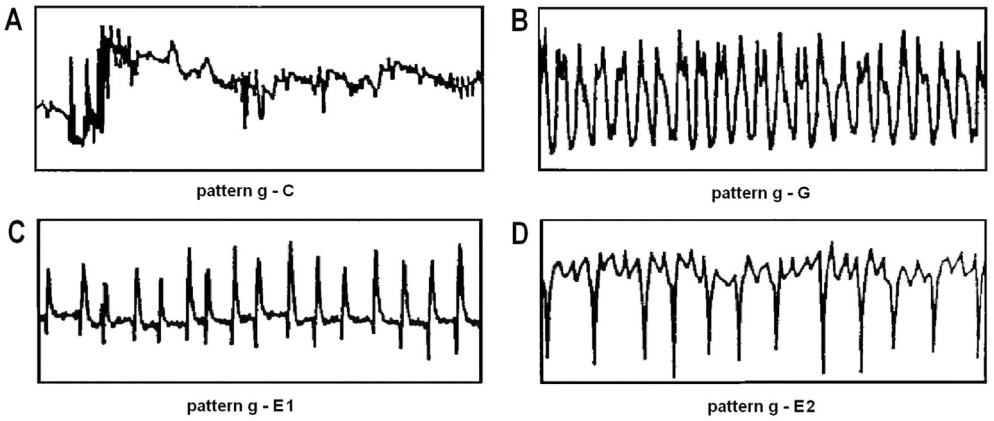

Fig. 1. The major EPG waveforms of aphids feeding on agarose-sucrose gel. The plots indicate stylet activity over time. The following EPG patterns were distinguished: A. Stylet activity in the gel; B. Salivation into the gel; C. passive ingestion of the gel; D. active ingestion of the gel.

showed inhibition effects on feeding behavior of the pea aphid (Goławska, 2007). Another saponin 3GlcA28AraRhaXyl medicagenate, was found to induced mortality in the rice weevil *Sitophilus oryzae* (Da Silva et al., 2012).

Flavonoids can also influence plant–insect interactions (Oleszek et al., 1992). Recent work on alfalfa flavonoids reported the presence of apigenin glycosides of which 7-*O*-[2-*O*-feruloyl-*β*-D-glucuronopyranosyl(1 → 2)-*O*-*β*-D-glucuronopyranosyl]-4′-*O*-*β*-D-glucuronopyranosideapigenin was the dominant apigenin glycoside present in alfalfa plants (Goławska et al., 2010). The total apigenin glycosides concentration in aerial parts of alfalfa's ranged from 3.6 to 4.0 mg/g dry matter. However, studies have shown that apigenin and its glycosides affect aphids' behavior (Goławska et al., 2010) and performance (Rice-Evans, 2000) with a negative correlation between the concentration of total apigenin glycosides in the alfalfa plants and pea aphid abundance and phloem sap ingestion (Guerin et al., 1983; Agrell et al., 2003; Goławska et al., 2010).

Although there has been research on the effects of saponins and flavonoids on insects, there is no data on how saponins and flavonoids together affect insect behavior, especially feeding behavior. Synergistic interactions among saponins were showed (Adel et al., 2000). Saponins may also act synergistically with other groups of allelochemicals (Sutherland et al., 1982; Berenbaum, 1985). Therefore, in this paper, the effects of isolated alfalfa saponins and apigenin on pea aphid feeding behavior are examined in detail. Two major alfalfa saponins – 3GlcA, 28AraRhaXyl medicagenic acid glycoside (MAG), and zanhic acid tridesmoside (ZAD) and the flavonoid – apigenin were used in *in vitro* experiments. These compounds have been reported in alfalfa (Stochmal and Oleszek, 2007; Goławska et al., 2012b). In the present study electrical penetration graph method (EPG) was used to monitor the feeding behavior of pea aphids exposed to different combinations of saponins with apigenin, incorporated in an agarose-sucrose gel. Understanding the activity of these compounds should (1) help researchers in using these compounds to construct transgenic plants that resist insects; (2) clarify the appropriateness of using the candidate genes for a given agronomical purpose (Sauvion et al., 2004).

2. Materials and methods

2.1. Aphid culture

The *A. pisum* green clone used in this study was descended from single parthenogenetic females collected on alfalfa in Poland. Aphids were reared on broad beans (*Vicia faba* L. var. Start (Fabaceae)) in plastic pots in environmental chamber at 21 ± 1 °C, L16:D8 photoperiod, and 70% RH at the Siedlce University of Natural Sciences and Humanities, Poland. Adult apterous females were used for the agarose-sucrose gel experiments. Aphid cohort production was as described earlier (Rahbe and Febvay, 1993).

2.2. Chemicals and gels

Individual saponins (MAG and ZAD) were obtained from the Institute of Soil Science and Plant Cultivation, Puławy, Poland, where they were isolated from alfalfa as described by Oleszek et al. (1990). Apigenin were purchased from Sigma–Aldrich (EC No. 208-292-3). The effect of the tested compounds on pea aphid feeding behavior was investigated *in vitro*, using agarose-sucrose gels. Gels were prepared by incorporating 1.25% agarose (Sigma A-0169) into a 30% sucrose solution and then adding tested saponins and apigenin to obtain concentrations of 0 (control), 10 and 100 μg cm^{-3}. After the mixtures were stirred, they were heated in a water bath (75 °C for 30 min) and then poured into plastic rings (10 mm high; 15 mm diameter) covered with a stretched Parafilm M® membrane. Transparent gels formed after 1–2 min and were offered to aphids for probing.