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Short Paper

# Evaluation of resistance in seven apple cultivars to rosy apple aphid, *Dysaphis plantaginea* (Hemiptera: Aphididae) under greenhouse and field conditions

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**Abstract**: Rosy apple aphid, *Dysaphis plantaginea* (Passerini), with worldwide distribution is an important pest of apple orchards. Host plant resistance is an essential component of integrated management of this insect pest. We investigated the resistance of seven apple cultivars, *Malus domestica* (Borkhausen), namely Granny Smith, Starkrimson, Golden Smoothee, Red Delicious, Golden Delicious, Boshghabi, and Shaki to rosy apple aphid under field and greenhouse conditions. The damage level by rosy apple aphid was significantly different among the tested cultivars precisely three weeks after infestation in both field and greenhouse conditions. The least leaf curling was observed on Shaki, while the most deformation was detected on Golden Delicious. There was positive correlation between damage in greenhouse and field studies. In addition, the lowest and highest numbers of aphids were observed on Shaki and Golden Delicious, respectively. Consequently, our results demonstrated that among the cultivars tested the Shaki cultivar is moderately resistant to rosy apple aphid and has the potential to be used in the integrated management of this aphid.

**Keywords:** Rosy apple aphid, Damage level, *Malus domestica*, Aphid abundance, Host plant resistance

## Introduction

Apple, *Malus domestica* (Borkhausen), is one of the most prevalent and widely grown fruits in different parts of the world. In Iran, apple is mostly grown in cool climate regions such as Ardabil province. In apple orchards rosy apple aphid, *Dysaphis plantaginea* (Passerini), threatens apple trees and its damage on apple is more considerable than any other aphid (Miñarro *et al.*, 2005).

Rosy apple aphid is a holocyclic and heteroecious species, with apple as primary host and *Plantago* spp. as secondary host plants (Blackman and Eastop, 2006). The aphids overwinter as eggs on the primary host and their infestation starts early in the spring by settling on the underside of young apple leaves. The aphids cause severe damage to apple trees by curling leaves which lead to leaf abscission and distortion of growing shoots. Continuing the feeding activities in the leaves that are near the developing fruit results in production of deformed apples of reduced quality. Furthermore, secretion of honeydew on fruit via large population of aphids would be synchronized with sooty mold fungi, influencing final apple product and making them

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unmarketable. In the late spring and early summer, by migration of the winged morphs to the secondary host plants, damage on apple would be lessened (Forrest and Dixon, 1975; De Berardinis *et al.,* 1994; Blommers *et al.,* 2004).

The economic threshold of rosy apple aphid is one fundatrix in 100 buds (Blommers, 1994) and the aphid may cross the economic threshold due to the high reproductive capacity and short life cycles where chemical applications would be required. The widespread use of the chemical applications has led to increasing resistance to insecticides in aphid populations (Wyss and Daniel, 2004). Also, application of the insecticides has negative effects on natural enemies and decreases their effective control of the aphid population (Theiling and Croft, 1988). On the other hand, costs for chemicals and ecological risks have to be taken into account. Besides, Cross *et al.* (2007) reported that rosy apple aphid's control is difficult in organic orchards. Especially, the severe leaf-curlings, protect the aphids from direct exposure to insecticides (Kindler and Springer, 1991, Cross *et al.,* 2007). So, it seems necessary to provide an alternative strategy for control of this aphid that does not rely on insecticides. Host plant resistance, the essential part of the integrated pest management program, can be appropriate choice for this purpose. This strategy not only reduces pest population in one growing season but also, it probably could affect the next growing season (Dorn *et al.,* 1999). Use of insect-resistant cultivars offers a very effective way to aphids' control by affecting pest population density, pest's damage, efficiency of natural enemies and reducing pesticide applications in agroecosystems, providing the best long term solution (Smith, 1989).

Different apple cultivars with various characteristics play an important role in host plant resistance programs. Some researchers have examined their potential effects on the aphid populations. Angeli and Simoni (2006) investigated the acceptance of the apple cultivars by rosy apple aphid and reported Golden Orange and Querina Florina as resistant cultivars and Red Delicious, Renetta Canada, Golden Lasa, and Golden Delicious as susceptible hosts. In another study, Arnaoudov and Kutinkova (2006) assessed the susceptibility of some apple cultivars to the rosy apple aphid. They introduced Golden Delicious, Jonagold and Melrose as very susceptible cultivars and Vista Bella, Mollie's Delicious and Aivanija as partially resistant cultivars to infestation by *D. plantaginea.* Rat-Morris (1993) reported that the resistance of the Florina cultivar to *D. plantaginea* can be as a result of antibiosis and tolerance of this cultivar to the aphid infestation. So, the susceptibility or resistance of different cultivars to rosy apple aphid may vary widely. Different morphological characteristics of the cultivars such as trichomes and hardness or thickness of leaf tissues can play an important role for the aphid preference. Furthermore, chemical characteristics, like nutritional composition of plant tissues known as primary metabolites, and the plant's secondary metabolites, responsible for defense against herbivores, may affect aphid population build up on the different apple cultivars (Angeli and Simoni, 2006).

The present study was planned to compare the level of resistance or susceptibility of seven apple cultivars against *D. plantaginea* in Iran under field and greenhouse conditions. The findings of this study could be used to design a comprehensive scheme for IPM program of this pest.

## Materials and Methods

In order to assess the intrinsic susceptibility of different apple cultivars and natural infestation of them, greenhouse and field studies were planned, respectively. This study was conducted in Ardabil province from May to June 2011.

### Plant materials

One-year old seedlings of five common apple cultivars in Iran including Granny Smith, Starkrimson, Golden Smoothee, Red Delicious, Golden Delicious, and two local cultivars Boshghabi and Shaki were selected and



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obtained from Horticulture Research Station of Meshkinshahr, Ardabil, Iran. These cultivars were chosen based on their greater acreage in Meshkinshahr. Before planting, to have a perfect bond with the surrounding soil and protect them from desiccation, the plants' roots were immersed in a mud bath (mixture of clay, rotten manure, and fungicide in water). During the experiments, investigations were done on the principal stem and every growing secondary shoots were pruned.

**Aphid colony**

The rearing of rosy apple aphid was started from apterous females collected from apple orchards in Meshkinshahr, early in the spring 2011. Aphid colony was maintained on a local cultivar of apple in greenhouse conditions for about one month. At the beginning of the experiments, four apterous adults were randomly selected from the stock colony and transferred with a brush on the youngest leaf of each plant in greenhouse and field. In the first week of the experiments to assure of staying of the mentioned numbers of aphids on different cultivars, replacings were done if necessary.

**Greenhouse studies**

This experiment was conducted in four replicates in a completely randomized design. In the greenhouse, each replicate of the cultivars were planted in plastic pots (35cm diameter × 40cm height). The pots filled with a mixture of soil, sand and rotten manure in the ratio of 2: 1: 1 and maintained at 24 ± 4 °C, 65 ± 5% RH and the natural photoperiod. The seedlings were irrigated three times a week. The plants were checked and cleaned daily to prevent colonization of any other arthropods.

**Field studies**

Studies were carried out in the Agricultural Research Station of the University of Mohaghegh Ardabili, Ardabil, Iran (elevation: 1,332 m; longitude: 48°18'E; latitude: 38°15'N). Five replicates of each cultivar were planted in holes (35cm diameter × 40 cm deep) which were dug with a post hole digger and filled with the same mixture of soil that were used in greenhouse pots. Completely randomized design was used in the experiment. Row and plant spacings were maintained at 5 m and 4 m, respectively. The field was managed according to the local practice with weekly flood irrigation, and no pesticides were applied.

**Damage assessment**

Three weeks after infestation, observations on the plants were made (Miñarro and Dapena, 2001, 2004, 2007, 2008). According to Rat-Morris (1993), leaf damage was graded on the following six-point scale: 0 = no damage, 1 = leaf slightly curled at the edge, 2 = leaf slightly curled longitudinally, 3 = typical rosy apple aphid leaf rolling, 4 = 2 to 5 typically rolled leaves; and 5 = more than 5 typically rolled leaves. Besides, aphid abundance on the cultivars was categorized by a four - point scale (Miñarro and Dapena, 2007): 0 = no aphids, 1 = 1 to 5 aphids per leaf, 2 = 6 to 25 aphids per leaf; and 3 = more than 25 aphids per leaf.

**Statistical analysis**

Prior to analysis, data on aphid abundance and plant damage were transformed by Kolmogorov – Smirnov test to standardize the variance, and then analyzed using one–way ANOVA in SPSS 16.0. Comparisons among means were carried out using the Student - Newman - Keuls (SNK) test at $\alpha = 0.05$. Spearman correlation coefficients were calculated between (1) damage in greenhouse and field, (2) aphid abundance and damage in the field.

**Results**

**Greenhouse studies**

The leaf deformation caused by rosy apple aphid differed significantly among apple cultivars tested in the greenhouse ($F = 3.048$; df = 6, 21; $P < 0.05$). The apterous aphids which fed on Golden Delicious caused the highest damage (4.75 ± 0.25) while those reared on Shaki and Red Delicious induced the lowest leaf curling (1.50 ± 0.64 and 1.75 ± 0.47, respectively). Although, damage level on Starkrimson, Granny



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Smith, Golden Smoothee, and Boshghabi was similar, but the most leaf-curling was observed on Boshghabi. No significant difference was found for the aphid abundance among the cultivars tested ($F = 2.000$; df = 6, 21; $P > 0.05$) (Table 1). However, Golden Delicious and Golden Smoothee had the highest number of apterous aphids as opposed to Red Delicious and Shaki, on which the lowest numbers of apterous aphids were recorded.

**Table 1** Damage ratings and population density of rosy apple aphid *Dysaphis plantaginea* on seven apples cultivars under greenhouse conditions.

| Apple cultivars | Aphid damage rating | Aphid density rating |
|---|---|---|
| Shaki | 1.50 ± 0.64 c | 1.75 ± 0.47 a |
| Red Delicious | 1.75 ± 0.47 c | 1.50 ± 0.28 a |
| Starkrimson | 2.75 ± 1.10 bc | 2.25 ± 0.47 a |
| Granny Smith | 3.50 ± 0.64 b | 2.50 ± 0.28 a |
| Golden Smoothee | 3.75 ± 0.94 ab | 2.75 ± 0.25 a |
| Boshghabi | 4.25 ± 0.47 ab | 2.50 ± 0.28 a |
| Golden Delicious | 4.75 ± 0.25 a | 2.75 ± 0.25 a |

Means in a column followed by the same letters are not significantly different ($P \geq 0.05$; SNK's test).

## Field studies

Rosy apple aphid abundance was significantly different on the cultivars tested ($F = 2.855$; df = 6, 28; $P < 0.05$). The lowest and the highest number of aphids per leaf were observed on Shaki (0.80 ± 0.20) and Golden Delicious (2.60 ± 0.24), respectively. Abundance of aphids on Starkrimson, Red Delicious, Granny Smith, Boshghabi, and Golden Smoothee was comparable, but the least number was on Starkrimson. Also, the damage level by rosy apple aphid was significantly different among the tested apple cultivars ($F = 4.917$; df = 6, 28; $P < 0.05$). The least curling was observed on Shaki (1.40 ± 0.40), while the most deformation was detected on Boshghabi (4.25 ± 0.51) (Table 2).

**Table 2** Damage ratings and population density of rosy apple aphid *Dysaphis plantaginea* on seven apples cultivars under field conditions.

| Apple cultivars | Aphid damage rating | Aphid density rating |
|---|---|---|
| Shaki | 1.40 ± 0.40 c | 0.80 ± 0.20 b |
| Red Delicious | 1.80 ± 0.37 bc | 1.40 ± 0.51 ab |
| Starkrimson | 2.00 ± 0.54 bc | 1.20 ± 0.37 ab |
| Granny Smith | 3.00 ± 0.44 ab | 2.00 ± 0.44 ab |
| Golden Smoothee | 3.20 ± 0.49 ab | 2.40 ± 0.40 ab |
| Boshghabi | 4.25 ± 0.51 a | 2.00 ± 0.44 ab |
| Golden Delicious | 4.20 ± 0.37 a | 2.60 ± 0.24 a |

Means in a column followed by the same letters are not significantly different ($P \geq 0.05$; SNK's test).

There was positive correlation between damage in greenhouse and field conditions ($R^2 = 0.957$; df = 7; $P < 0.001$). In addition, the estimated correlation between aphid abundance and damage level in field were high ($R^2 = 0.947$; df = 7; $P < 0.001$).

## Discussion

In the present study, it was revealed that apple cultivars strongly affected rosy apple aphid population and that the damage level on different cultivars was not similar. The low population densities of rosy apple aphid on Shaki resulted in lower pest damage, indicating that the aphid could not successfully build up large populations on this cultivar. In contrast, abundance of rosy apple aphid reared on Golden Delicious was highest among the cultivars tested. This high population increased curling of leaves. The leaf curling can be considered as an indicator for cultivars' susceptibility to aphids (Kindler and Springer, 1991). Evidence exists that differences in susceptibility vary among cultivars within the same species (Qubbaj *et al.,* 2005). For instance, Miñarro and Dapena (2007) demonstrated that Gala was the most



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susceptible apple cultivar and Florina was the resistant one to rosy apple aphid. Also, they showed that GoldRush and Galarina cultivars were not only tolerant to apple scab *Venturia inaequalis* (Cooke) but also to rosy apple aphid and did not exhibit typical leaf-rolls. Our observations indicated that the population growth of rosy apple aphid on Shaki was restricted. This incident could be described by biological features of the aphid, having more immature mortality and lower female fertility. Angeli and Simoni (2006) demonstrated that Querina Florina and Golden Orange resistance is mostly as a result of a low fecundity rates and a high antixenotic effect on the immature stages of *D. plantaginea* and a moderately high antixenotic effect on rosy apple aphid adults. Insect resistant plants may influence pests by decreasing their survivorship or prolonging their developmental time that makes possible the exposure of them to the natural enemies (Verkerk *et al.,* 1998). Furthermore, differences in host plant quality and sap composition or presence of different phenolic compounds in apple cultivars can play an important role in resistance to aphids (Piccinelli *et al.,* 1995; Dixon, 1998). Also, Marchetti *et al.* (2009) demonstrated that aphids on Florina cultivar needed a longer period before the first probe and did not show signs of entire phloem ingestion. It indicates that surface and phloem factors are responsible for the cultivar resistance to the aphids.

In the current study, different results were observed in the field and greenhouse conditions. The damage level followed by aphid density was higher in greenhouse compared to field conditions. Miñarro and Dapena (2007) believe that greenhouse-grown plants due to their thinner leaves could be susceptible to pests. The large population of aphids might also be as a result of controlled conditions of greenhouse. Normally, in the greenhouse lack of limiting factors (natural enemies or unfavorable environmental conditions) lead to increased populations. For example, one of the effective natural enemies of the rosy apple aphid is *Adalia bipunctata* (L.) (Col.: Coccinellidae) that successfully reduces the increased populations of the aphid early in the spring (Wyss *et al.,* 1999 a, b). Environmental factors (such as temperature, relative humidity and photoperiod) could be cited as another reason for the observed differences. Dry and warm conditions lead to rapid growth of the aphids. Emission of plant volatiles increase in drought stress and these volatiles have an important role in aphid distribution (Quiroz *et al.,* 1999; Blommers *et al.,* 2004; Vallat *et al.,* 2005). Although, damage level and abundance of rosy apple aphid in greenhouse were different from those obtained under field conditions, there was positive correlation between damage in greenhouse and field studies. As well, aphid abundance was positively correlated with damage in field, indicating that with more population on the leaves; more damage would be expected.

In summary, the results obtained show that Shaki was the unsuitable (moderately resistant) cultivar to rosy apple aphid and Golden Delicious was the most suitable (susceptible) cultivar to the aphid among the cultivars tested. Use of resistant cultivars can be an effective strategy to manage the population of rosy apple aphid thereby reducing the use of insecticides in the apple orchards. However, the resistance level of Shaki cultivar, reported here, is not sufficient to achieve adequate control of this aphid pest. Therefore, further research is required to investigate the potential of this cultivar in combination with other control strategies especially biological control in the integrated management of rosy apple aphid.

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# ارزیابی مقاومت هفت رقم سیب نسبت به شته آردآلود سیب در شرایط گلخانه و مزرعه

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**چکیده:** شته آردآلود سیب، *Dysaphis plantaginea* (Passerini)، از آفات کلیدی سیب و دارای پراکنش جهانی است. مقاومت گیاه میزبان یکی از اجزای اساسی مدیریت تلفیقی این آفت می‌باشد. در این مطالعه مقاومت هفت رقم سیب شامل Starkrimson، Granny Smith، Golden Delicious، Red Delicious، Golden Smoothee، Boshghabi و Shaki نسبت به این آفت در شرایط گلخانه و مزرعه بررسی کردیم. میزان خسارت شته آردآلود سیب ۲۱ روز پس از آلودگی در شرایط گلخانه و مزرعه، در بین ارقام مورد مطالعه تفاوت معنی‌داری داشت. حداقل پیچیدگی برگ روی رقم Shaki و بیشترین آن روی رقم Golden Delicious مشاهده شد. همچنین کمترین و بیشترین تعداد شته‌ها به‌ترتیب روی ارقام Shaki و Golden Delicious دیده شد. خسارت شته آردآلود سیب در مزرعه و گلخانه همبستگی مثبت و معنی‌داری داشت. نتایج ما نشان می‌دهد که در میان ارقام مختلف مورد مطالعه، رقم Shaki از بیشترین میزان مقاومت نسبت به شته آردآلود سیب برخوردار بود و می‌تواند در مدیریت تلفیقی این آفت مورد توجه قرار گیرد.

**واژگان کلیدی**: شته مومی سیب، سطح خسارت، *Malus domestica*، فراوانی شته، مقاومت گیاهان به آفات

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