Virus Research xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Virus Research

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

Preference by a virus vector for infected plants is reversed after virus acquisition

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Potato leaf roll virus
Myzus persicae
Potato volatiles
Virus-induced plant volatiles
Plant–insect–virus-interactions
Disease spread

ABSTRACT

Pathogens and their vectors can interact either directly or indirectly via their shared hosts, with implications for the persistence and spread of the pathogen in host populations. For example, some plant viruses induce changes in host plants that cause the aphids that carry these viruses to settle preferentially on infected plants. Furthermore, relative preference by the vector for infected plants can change to a preference for noninfected plants after virus acquisition by the vector, as has recently been demonstrated in the wheat–*Rhopalosiphum padi*–*Barley yellow dwarf virus* pathosystem. Here we document a similar dynamic in the potato–*Myzus persicae* (Sulzer)–*Potato leaf roll virus* (PLRV) pathosystem. Specifically, in a dual choice bioassay, nonviruliferous apterous *M. persicae* settled preferentially on or near potato plants infected with PLRV relative to noninfected (sham-inoculated) control plants, whereas viruliferous *M. persicae* (carrying PLRV) preferentially settled on or near sham-inoculated potato plants relative to infected plants. The change in preference after virus acquisition also occurred in response to trapped headspace volatiles, and to synthetic mimics of headspace volatile blends from PLRV-infected and sham-inoculated potato plants. The change in preference we document should promote virus spread by increasing rates of virus acquisition and transmission by the vector.

1. Introduction

Pathogens and their vectors can interact either directly or indirectly via their shared hosts, with implications for the persistence and spread of the pathogen in host populations (Hurd, 2003; Lefèvre and Thomas, 2008). For example, some plant viruses induce changes in host plants that cause the aphids that can carry these viruses to settle preferentially on infected plants, with implications for virus spread (Castle et al., 1998; Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004; Srinivasan et al., 2006; Alvarez et al., 2007; Mauck et al., 2010; McMenemy et al., 2012). This type of phenomenon has been especially well documented for two pathosystems: the wheat–*Rhopalosiphum padi* (L.)–*Barley yellow dwarf virus* (Luteoviridae: Luteovirus) pathosystem (hereafter the BYDV system), and the potato–*Myzus persicae* (Sulzer)–*Potato leaf roll virus* (Luteoviridae: Polerovirus) pathosystem (hereafter the PLRV system) (Bosque-Pérez and Eigenbrode, 2011).

Furthermore, it has been recently shown that feeding preferences or feeding behavior of insect vectors of plant viruses can be altered after exposure to infected plants and acquisition of virus (Stafford et al., 2011; Ingwell et al., 2012; Shrestha et al., 2012; Moreno-Delafuente et al., 2013). In the BYDV system the vector, *R. padi*, preferentially settles on BYDV-infected wheat plants vs. noninfected, sham-inoculated controls before it has acquired the virus, but preferentially settles on noninfected plants after it has acquired the virus (Ingwell et al., 2012). This effect was observed in aphids that acquired the virus without contact with virus-infected plants by ingesting a liquid diet containing virus particles, demonstrating a direct effect of BYDV on its aphid vector. This dynamic preference by the vector should accelerate virus spread through a plant population (Roosien et al., 2013). The present study was conducted to examine if a similar change in vector preference occurs after virus acquisition in the PLRV system.

Green peach aphid, *M. persicae* is the principal vector of PLRV (Peters, 1987; Raman and Radcliffe, 1992), transmitting the virus in a persistent, circulative manner. Growth, fecundity and longevity of *M. persicae* are greater when they feed on PLRV-infected potato plants than noninfected potato plants (Castle and Berger, 1993) and *M. persicae* apterae preferentially settle on PLRV-infected plants compared with sham-inoculated controls (Castle et al., 1998; Eigenbrode et al., 2002). Preferential settling by *M. persicae* on PLRV-infected plants is mediated by olfactory cues, at least within the first 12–24 h of exposure (Eigenbrode et al., 2002; Ngumbi et al., 2007). The volatile organic compound (VOC) blends and aphid responses to them are dynamic, changing with disease progression

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http://dx.doi.org/10.1016/j.virusres.2013.11.005

(Werner et al., 2009) and the age at which plants are inoculated (Rajabaskar et al., 2013b). The phenomenon occurs in different potato varieties (Rajabaskar et al., 2013a), and in wild *Solanum sarrachoides* (Srinivasan et al., 2006) and *S. nigrum* (Ngumbi et al., unpublished).

Prior research on PLRV effects on vector behavior has used nonviruliferous *M. persicae* in bioassays, but an assessment of the preferences of viruliferous aphids is critical to understanding plant–vector–virus-interactions and their implications for disease spread. If vector preference changes after virus acquisition, rates of pathogen spread will increase (Ingwell et al., 2012; Roosien et al., 2013). Here we compare the behavioral responses of viruliferous and nonviruliferous *M. persicae* to PLRV-infected and sham-inoculated potato plants. We also compare responses by viruliferous and nonviruliferous *M. persicae* to blends of VOC trapped from headspace of PLRV-infected and sham-inoculated plants, and to synthetic blends that mimic these blends. Our objective was to establish whether a change in settling preference by aphids occurs after virus acquisition in a pathosystem other than the BYDV system and to delineate the cues to which the aphids are responding.

2. Materials and methods

2.1. Aphids

The *M. persicae* colonies used in these experiments were derived from one originally established in the early 1990s at the University of Idaho Research and Extension Center in Parma, Idaho. The colony was established and is maintained on PLRV-free *Physalis floridana* Rybd. Plants and aphids are kept in an environmental chamber at 22 ± 2 °C, 40–60% r.h. and a 16:8 L:D photoperiod. Late-instar aphids from this colony used in bioassays are hereafter referred to as nonviruliferous aphids. A second colony was established using aphids from the nonviruliferous colony and maintained on PLRV-infected *P. floridana* under identical conditions. Aphids from this colony used in bioassays are hereafter referred to as viruliferous aphids.

2.2. Test plants

Certified virus-free potato seedlings (cultivar Russet Burbank) were grown from tissue culture from the University of Idaho Potato Nuclear Seed Program. Seedlings were transplanted into 10-cm^2 pots filled with Sunshine Mix No. 1 (Sun Gro Horticulture Canada, Vancouver, Canada) and grown in a greenhouse at 22 ± 2 °C with supplementary lighting to achieve a 16:8 L:D. Potato plants were inoculated with PLRV by placing 10 aphids from the viruliferous colony in a clip cage on a single leaflet for a 5-d inoculation access period. Sham-inoculated plants were treated in the same manner using aphids from the nonviruliferous colony, and the plants were therefore not infected with virus. Plants were used for bioassays 4 wks after virus inoculation or sham inoculation. The PLRV infection status of aphid colonies and potato plants (before and after bioassays) were confirmed using double antibody sandwich enzyme-linked immunosorbent assay following the protocol of Agdia (Elkhart, IN, USA) (Clark and Adams, 1977).

2.3. Aphid preference for PLRV-infected vs. sham-inoculated potato plants

Dual-choice bioassays were employed to assess preferential settling by viruliferous and nonviruliferous *M. persicae* in response to foliage of PLRV-infected and sham-inoculated potato plants. The bioassay was conducted in the greenhouse at 22 ± 2 °C. A bioassay arena was constructed from a 200-mm long × 50-mm diam. polystyrene tube. The tube was positioned horizontally and had a 20-mm circular hole on its upper side, through which aphids could be released into the arena. The tube was ventilated with a small screened window (2 cm diam.). Two potato plants (PLRV-infected and sham-inoculated) were positioned on opposite sides of the arena and a single leaflet from each, still attached to the plant, was placed inside the tube near one end and anchored in place with a soft polyurethane foam stopper. The leaflets were not touching but were close to each other within the arena so that aphids could readily move between them. For an individual test, 30 apterous (wingless) *M. persicae* were placed into the arena directly onto one of the leaflets through the hole on upper side of the arena, which was then closed tightly with a cork plug. Equal numbers of replicates were conducted with aphids released onto leaflets from PLRV-infected plants and leaflets from sham-inoculated plants. Movement rates from PLRV-infected to sham-inoculated plants and *vice versa* were measured but the effect of release point was not tested. Replicates were conducted using either viruliferous or nonviruliferous aphids within a single experiment. There were 15 replicates for all treatment combinations conducted over two days. The number of aphids settling on either side of the tube relative to its midpoint, whether on the tube wall or on the leaflet, was counted after 12 h. The effect of plant infection status (PLRV-inoculated vs. sham-inoculated), aphid status (viruliferous vs. nonviruliferous), and their interactions were examined using a generalized linear model with a binomial distribution, logit link function (PROC GENMOD, SAS, 2010).

2.4. Headspace volatile collection and analysis

Within two days of completing the bioassay, volatiles were trapped from the headspace of six test plants from each treatment (PLRV-infected and sham-inoculated). Collections were performed in the greenhouse where the bioassay had been conducted between 0800 and 1400 h. To trap the volatiles, a single plant was enclosed in a glass chamber through which carbon filtered air was drawn at 300 cm^3/min, exiting through a trap charged with Super-Q adsorbent resin. Traps were eluted with methylene chloride and the elutant was standardized to 200 μl. One microliter of each sample was injected into a Hewlett Packard 6890 gas chromatograph coupled to a Hewlett Packard 5973 Mass Selective Detector (Agilent Technologies, Palo Alto, CA). The column (HP-5; 30 m × 0.25 mm ID) was held at 40 °C for 2 min and then increased to 250 °C at 10 °C/min and held for 10 min using the method of Eigenbrode et al. (2002). Peaks were identified by comparison with spectra and retention times of authentic standards, others were identified based on spectral matches with the National Institute of Standards and Technology (NIST) mass spectra library, presence of diagnostic ions, and published Kovats indices. Each peak was quantified and standardized to ng/g plant fresh weight/h, based on response factors for compound classes, as determined from standard curves of authentic standards for each compound class. The total concentration of VOC trapped from headspace (ng/g plant fresh weight/h) was compared between the two plant treatments. In a separate analysis, VOC were grouped into four classes (monoterpene, sesquiterpene, green leaf volatiles and aldehydes) and concentrations within classes compared between the two plant treatments. Concentrations were analyzed by two-way ANOVA using PROC GLM (SAS, 2010).

2.5. Headspace VOC and headspace blend mimics for bioassay

To assess *M. persicae* responses to headspace VOC, the trapped VOC from all six plants in each treatment (PLRV-infected and

Please cite this article in press as: Rajabaskar, D., et al., Preference by a virus vector for infected plants is reversed after virus acquisition. Virus Res. (2013), http://dx.doi.org/10.1016/j.virusres.2013.11.005