Virus Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Virus Research

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

A non-persistently transmitted-virus induces a pull–push strategy in its aphid vector to optimize transmission and spread

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Aphis gossypii
Cucumber mosaic virus
Non-persistent virus transmission
Feeding behaviour
Electrical penetration graph
Disease epidemics

ABSTRACT

Plant viruses are known to modify the behaviour of their insect vectors, both directly and indirectly, generally adapting to each type of virus–vector relationship in a way that enhances transmission efficiency. Here, we report results of three different studies showing how a virus transmitted in a non-persistent (NP) manner (*Cucumber mosaic virus*; CMV, *Cucumovirus*) can induce changes in its host plant, cucumber (*Cucumis sativus* cv. Marumba) that modifies the behaviour of its aphid vector (*Aphis gossypii* Glover; Hemiptera: Aphididae) in a way that enhances virus transmission and spread non-viruliferous aphids changed their alighting, settling and probing behaviour activities over time when exposed to CMV-infected and mock-inoculated cucumber plants. Aphids exhibited no preference to migrate from CMV-infected to mock-inoculated plants at short time intervals (1, 10 and 30 min after release), but showed a clear shift in preference to migrate from CMV-infected to mock-inoculated plants 60 min after release. Our free-choice preference assays showed that *A. gossypii* alates preferred CMV-infected over mock-inoculated plants at an early stage (30 min), but this behaviour was reverted at a later stage and aphids preferred to settle and reproduce on mock-inoculated plants. The electrical penetration graph (EPG) technique revealed a sharp change in aphid probing behaviour over time when exposed to CMV-infected plants. At the beginning (first 15 min) aphid vectors dramatically increased the number of short superficial probes and intracellular punctures when exposed to CMV-infected plants. At a later stage (second hour of recording) aphids diminished their feeding on CMV-infected plants as indicated by much less time spent in phloem salivation and ingestion (E1 and E2). This particular probing behaviour including an early increase in the number of short superficial probes and intracellular punctures followed by a phloem feeding deterrence is known to enhance the transmission efficiency of viruses transmitted in a NP manner. We conclude that CMV induces specific changes in a plant host that modify the alighting, settling and probing behaviour of its main vector *A. gossypii*, leading to optimum transmission and spread of the virus. Our findings should be considered when modelling the spread of viruses transmitted in a NP manner.

1. Introduction

Plant viruses as obligate parasites, need to move from host to host to survive. Although other virus transmission ways are possible, vectors transmit most of the known plant viruses. Insects, particularly hemipterans with piercing-sucking mouthparts (aphids, whiteflies and leafhoppers mainly), are by far the most frequent and efficient vectors of plant viruses (Nault, 1997; Hogenhout et al., 2008). For this reason the knowledge on insect behaviour and dispersal is of key importance to understand virus epidemiology. The behaviour of insect vectors can be altered by vector-borne-viruses such that the frequency and nature of the virus–vector interaction is modified to enhance virus transmission and spread (Eigenbrode et al., 2002; Bosque-Pérez and Eigenbrode, 2011; Mauck et al., 2012; Shrestha et al., 2012; van Molken et al., 2012; Moreno-Delafuente et al., 2013; Huot et al., 2013).

Recently, the "Vector Manipulation Hypothesis" (VMH) has been proposed to explain the strategies plant pathogens use to enhance their own spread by altering the behaviour of their insect vectors (Ingwell et al., 2012). According to the VMH, plant pathogens can influence the behaviour and fitness of their insect vectors in two different ways: directly (mediated by the presence of the virus in the vector's body) and indirectly (mediated by changes occurring in the plant as a consequence of infection). It is expected that most virus-induced changes in plants have positive (or neutral) effects on transmission by vectors and that viruses showing similar virus–vector relationships share similar effects on vector behaviour in a way that transmission efficiency is optimized (Mauck et al., 2012).

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0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.12.012

Aphid behaviour and virus–vector interactions determine how the virus is transmitted as well as the efficiency of a given aphid species to transmit the virus (Gray and Banerjee, 1999). Thus, persistently-transmitted viruses (PT) (and some semipersistent viruses – SP) are usually transmitted by colonizing aphid species, which need to reach and feed from the phloem to acquire and inoculate de virus effectively. PT viruses have a very specific relationship with their vectors representing a narrow range of species able to transmit them (Gildow and Gray, 1993; Gray and Gildow, 2003). On the other hand, viruses transmitted in a non-persistent (NP) manner, which represent the majority of aphid-borne transmitted viruses, are vectored by many non-colonising aphid species during brief intracellular stylet punctures in superficial plant tissues. Long feeding probes are known to reduce their transmission efficiency (Ng and Falk, 2006). The relationship of viruses transmitted in a NP manner with their aphid vectors is not as specific and intimate as for PT viruses and interactions in these pathosystems are likely limited to indirect effects through the host plant (Nault, 1997; Mauck et al., 2010).

PT virus-infected plants tend to be more attractive and/or arrestant to aphid vectors than healthy ones (Castle et al., 1998; Eigenbrode et al., 2002; Jimenez-Martínez et al., 2004a; Srinivasan et al., 2006; Medina-Ortega et al., 2009; Bosque-Pérez and Eigenbrode, 2011). Moreover, vectors feeding on PT virus-infected plants often have greater nymphal survival, adult fecundity, longevity and/or increased growth rate (Fereres et al., 1989; Castle and Berger, 1993; Jiménez-Martínez et al., 2004b; Srinivasan and Alvarez, 2008).

For NP viruses, studies describing vector attraction and feeding preferences and/or fitness on infected plants are more limited. Recent work by Mauck et al. (2010) with *Cucumber mosaic virus* (CMV, *Bromoviridae*: *Cucumovirus*) showed that winged and wingless morphs of *Aphis gossypii* (Glover) and *Myzus persicae* (Sulzer) are initially attracted by the volatile organic compounds emitted by CMV infected squash plants. However, some time after landing they prefer non-infected plants. Furthermore Eckel (1990), showed that tobacco plants infected with *Tobacco etch virus* (TEV, *Potyviridae*: *Potyvirus*) were more attractive to alighting aphids than non-infected plants. Furthermore, studies conducted with *Soybean mosaic virus* (SMV, *Potyviridae*: *Potyvirus*) showed that *Rhopalosiphum maidis* (Fitch) remained longer on non-infected than on SMV-infected soybean plants before taking off, although *M. persicae* exhibited no preference (Fereres et al., 1999).

The insect's choice to colonize a plant is a complex process involving different stimuli and responses. To find and identify feeding sites when searching for their host plants, phloem-feeding insects follow a series of events that culminate in sustainable phloem sap ingestion if plants are recognized as acceptable (Powell et al., 2006). During their pre-alighting phase, aphids are guided by visual and olfactory cues coming from plants that may act as potentially acceptable hosts. It seems that most aphids species are specially attracted to yellow or yellow-green, which is thought to indicate a favourable nutritional status in terms of soluble nitrogen (Kennedy et al., 1961; Moericke, 1969; Fereres et al., 1999; Döring et al., 2009). Virus infection may produce similar changes in canopy colour as well as in plant volatile compounds emission that favours aphid attraction and landing on the crop (Ajayi and Dewar, 1983; Eigenbrode et al., 2002). That is the case of some NP viruses that promote alate aphid vector attraction to the chlorotic symptoms and the odour emissions emitted by infected plants (Macias and Mink, 1969; Mauck et al., 2010). This attraction of aphid vectors to infected plants may have important consequences on virus spread as models on NP virus transmission show that the number of plants visited per day is a key variable driving virus epidemics (Madden et al., 1990, Madden et al., 2000).

Once aphids land on a plant they make consecutive superficial probes and use gustatory cues to discriminate between host and non-host plants by means of consecutive intracellular stylet punctures. Then, stylets penetrate deeper through the intercellular spaces to reach the vascular bundle and penetrate the phloem sieve elements where they remain feeding for long periods of passive sap ingestion (Fereres and Moreno, 2009). All the above mentioned stylet penetration behaviours can be monitored using the Electrical Penetration Graph (EPG) technique, which records signal waveforms reflecting different insect activities (Tjallingii, 1988). Using EPGs, it has been possible to study the host selection process of pierce-sucking insects as well as the characterization of insect stylet activities associated to the transmission of plant viruses. It is well known that intracellular stylet punctures visualized as potential drops (pd) during brief probes in epidermal and mesophyll cells are responsible of the acquisition and inoculation of NP-viruses (Powell et al., 1995; Martín et al., 1997; Powell, 2005). The number of short probes and number of intracellular stylet punctures (pd) have a positive correlation with the acquisition and subsequent transmission of NP viruses (Collar et al., 1997; Collar and Fereres, 1998). In other words, aphids that make a larger number of brief superficial probes and intracellular stylet punctures transmit NP-viruses with higher efficiency.

Here, we describe a series of time-controlled preference and probing behaviour studies using CMV infected cucumber plants and the aphid vector, *A. gossypii*. A combination of free-choice preference assays conducted in test arenas under semi-field conditions and the EPG technique allowed us to evaluate the indirect effects of CMV infection on the alighting, settling and probing behaviour of non-viruliferous aphids on cucumber (*Cucumis sativus* cv. Marumba) plants. Our results indicate that aphid response to CMV-infected plants at different time intervals have significant implications in the transmission, spread and epidemiology of viruses transmitted in a NP manner and should be considered to construct or tune up existing simulation models and patterns of spread that describe virus disease epidemics.

2. Materials and methods

2.1. Biological material: aphid colonies, plants and virus isolates

A single virginiparous aptarae collected from melon in Almería, Spain, in 1998 was used to initiate a virus-free laboratory culture of *A. gossypii*. This colony was reared on melon plants (*Cucumis melo* L. cv. Primal) for several generations in rearing cages in environmental growth chambers at a 23:18 °C temperature (D:N), photoperiod of 16:8 h (L:D) and 60–80% RH. For each experiment, newly emerged alates (24–72 h after last moult) were collected with an aspirator from the top of the rearing cages on the same day and time in which the experiments were started. We assumed that alate aphids at the top of the cage had terminated their migratory flight phase. Cucumber plants were germinated in 12 cm diameter pots using a mixture of equal parts of vermiculite (No. 3, Asfaltex S.A., Barcelona, Spain) and soil substrate (Kekilla Iberica, Almería, Spain). They were watered three times a week and a nutritional complex of 20-20-20 (N:P:K) Nutrichem 60 fertilizer (Miller Chemical & Fertilizer Corp., PE, USA) was added to the irrigation water in a proportion of 0.25 g/l dosage. Cucumber plants were mechanically inoculated with CMV (isolate M06) (Diaz et al., 2003) obtained from a melon crop in 1996 in Tarragona Spain, and kindly provided by Dr. E. Moriones (EELM-CSIC, Spain) Plants were inoculated 2 weeks after sowing at the 1-true leaf stage and used 4 weeks post-inoculation as viral sources (6-leaf stage). Mock-inoculated cucumber plants (rubbed only with buffer solution) of the same growth stage were used as non-infected controls. All plants were

Please cite this article in press as: Carmo-Sousa, M., et al., A non-persistently transmitted-virus induces a pull–push strategy in its aphid vector to optimize transmission and spread. Virus Res. (2014), http://dx.doi.org/10.1016/j.virusres.2013.12.012