Virus Research 159 (2011) 201–205

Contents lists available at ScienceDirect

Virus Research

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

The influence of virus-induced changes in plants on aphid vectors: Insights from luteovirus pathosystems

Nilsa A. Bosque-Pérez*, Sanford D. Eigenbrode

Department of Plant, Soil and Entomological Sciences, P.O. Box 442339, University of Idaho, Moscow, ID 83844-2339, USA

ARTICLE INFO

Article history:
Available online 28 April 2011

Keywords:
Rhopalosiphum padi
Myzus persicae
Barley yellow dwarf virus
Potato leafroll virus
Luteoviridae
Virus-induced plant volatiles

ABSTRACT

Plant virus infection can alter the suitability of host plants for their aphid vectors. Most reports indicate that virus-infected plants are superior hosts for vectors compared to virus-free plants with respect to vector growth rates, fecundity and longevity. Some aphid vectors respond preferentially to virus-infected plants compared to virus-free ones, while others avoid infected plants that are inferior hosts. Thus, it appears vectors can exploit changes in host plant quality associated with viral infection. Enhanced vector performance and preference for virus-infected plants might also be advantageous for viruses by promoting their spread and possibly enhancing their fitness. Our research has focused on two of the most important luteoviruses that infect wheat (*Barley yellow dwarf virus*), or potato (*Potato leafroll virus*), and their respective aphid vectors, the bird-cherry oat aphid, *Rhopalosiphum padi,* and the green peach aphid, *Myzus persicae.* The work has demonstrated that virus infection of host plants enhances the life history of vectors. Additionally, it has shown that virus infection alters the concentration and relative composition of volatile organic compounds in host plants, that apterae of each vector species settle preferentially on virus-infected plants, and that such responses are mediated by volatile organic compounds. The findings also indicate that plants respond heterogeneously to viral infection and as a result different plant parts change in attractiveness to vectors during infection and vector responses to virus-infected plants are dynamic. Such dynamic responses could enhance or reduce the probability of virus acquisition by individual aphids searching among plants. Finally, our work indicates that compared to non-viruliferous aphids, viruliferous ones are less or not responsive to virus-induced host plant volatiles. Changes in vector responsiveness to plants after vectors acquire virus could impact virus epidemiology by influencing virus spread. The potential implications of these findings for virus ecology and epidemiology are discussed.

1. Introduction

Insect-vectored plant viruses constrain crop production worldwide and seriously affect many important crops in the USA. Many of the most devastating viruses of crop plants around the world are vectored, often obligately, by insects. In such cases, the host plant, vector and virus become interdependent components of a complex pathosystem (Irwin and Thresh, 1990). The behavior of such pathosystems depends upon virus–plant, plant–vector, and virus–vector interactions. These interactions are biologically intricate and not always well understood, but once elucidated, provide scope for innovative management strategies aimed at reducing the impact of virus disease. In addition, studies of such interactions are important as the potentially complex ecological and evolutionary relationships amongst the three components in these pathosystems are likely to provide additional opportunities for basic discoveries with long-term potential for improving virus disease management (Jones, 2004; Lovisolo et al., 2003).

Current management tactics for aphid-vectored viruses target vector–plant interactions, relying in large part on monitoring and suppressing the vectors, thus reducing the frequency and severity of virus outbreaks (Perring et al., 1999). Although molecular and conventional breeding projects are pursuing virus resistance in many crops (McGrath et al., 1997; Barker and Waterhouse, 1999; Bosque-Pérez, 2000; Sivamani et al., 2002; Elomaa et al., 2008), the resulting varieties will not be grown universally and most will not have immunity to viruses, necessitating continuing vector management in the foreseeable future (Jones, 2004). Improved understanding of vector behavior can provide opportunities for innovative management. In particular, possible vector discrimination among host plants based on host infection status is understudied, despite the influence this could have on virus epidemiology (Irwin and Thresh, 1990).

Plant virus infection can alter the suitability of host plants for aphid vectors. Most published literature indicate that virus-

* Corresponding author. Tel.: +1 208 885 7544; fax: +1 208 885 7760.
E-mail addresses: nbosque@uidaho.edu (N.A. Bosque-Pérez), sanforde@uidaho.edu (S.D. Eigenbrode).

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.04.020

infected plants are superior to virus-free plants with respect to vector growth rates, reproduction and longevity (Kennedy, 1951; Baker, 1960; Maramorosch and Jensen, 1963; Miller and Coon, 1964; Gildow, 1980; Hodgson, 1981; Gildow, 1983; Araya and Foster, 1987; Fereres et al., 1989; Gildow, 1989; Costa et al., 1991; Quiroz et al., 1991; Castle and Berger, 1993; Blua et al., 1994; Fereres et al., 1999; Jiménez-Martínez et al., 2004a; Srinivasan et al., 2008), but opposite effects also occur (McIntyre et al., 1981; Ellsbury et al., 1985; Michels et al., 1994; Donaldson and Gratton, 2007; Jiménez-Martínez and Bosque-Pérez, 2009). Some aphid vectors respond preferentially to virus-infected plants compared to virus-free ones (Macias and Mink, 1969; Ajayi and Dewar, 1983; Eckel and Lampert, 1996; Castle et al., 1998; Jiménez-Martínez et al., 2004b; Srinivasan et al., 2006), while other vectors are deterred by infected plants that are inferior hosts (Blua and Perring, 1992). Thus, natural selection has apparently shaped vector behavior in response to virus-induced changes in host plant quality. Indeed, apparently adaptive vector responses seem to occur predominantly in pathosystems in which aphids and vectors are closely linked (Eigenbrode et al., 2002). Most examples of a coupling of enhanced vector performance and preference for virus-infected hosts occur in the persistently transmitted viruses of the *Luteoviridae*, whereas such effects have never been reported for non-persistently transmitted species of *Potyviridae* (Markkula and Laurema, 1964; Castle and Berger, 1993; Castle et al., 1998; Eigenbrode et al., 2002; Jiménez-Martínez and Bosque-Pérez, 2004; Donaldson and Gratton, 2007; Hodge and Powell, 2008). If enhanced vector performance on, and preference for, virus-infected plants promotes the spread of the virus, then virus and vector are potentially linked in a mutualistic interaction. The possibility has prompted speculation that some viral disease symptoms can be considered as mechanisms whereby the virus manipulates the vector via the shared host plant, thereby enhancing virus fitness (Blua and Perring, 1992; McElhany et al., 1995; Musser et al., 2003; Belliure et al., 2005, 2008; Hodge and Powell, 2008). Regardless of how these interactions between virus, plant, and vector have arisen, their existence presents an opportunity to discover mechanisms and disrupt them through deliberate manipulation to improve viral disease and vector management and thus have stimulated research by our and other research groups.

Our research has focused on two of the most important luteoviruses that infect wheat [*Barley yellow dwarf virus* (BYDV) (*Luteoviridae*: genus *Luteovirus*)], or potato [*Potato leafroll virus* (PLRV) (*Luteoviridae*: genus *Polerovirus*)], and their respective aphid vectors, the bird-cherry oat aphid, *Rhopalosiphum padi* (L.), and the green peach aphid, *Myzus persicae* (Sulzer). In addition to being a major staple food crop, wheat is a mainstay of agriculture in the USA and many countries around the world. BYDV causes the most widespread and economically important virus disease of cereals, including wheat, worldwide (Plumb, 1983; Irwin and Thresh, 1990). The BYDV disease causes stunting and reduces root growth, flowering, and plant vigor, resulting in reductions of grain yield and quality and making plants more vulnerable to other stresses (Irwin and Thresh, 1990; McKirdy et al., 2002). The causal virus is vectored by several aphid species and the specificity of these species varies with virus serotype (Irwin and Thresh, 1990). The serotype known as BYDV-PAV is vectored most efficiently by *R. padi*. BYDV disease management is mostly limited to methods that reduce virus transmission by aphids.

PLRV causes one of the most serious viral diseases of potato worldwide and is transmitted by several aphid species, most importantly *M. persicae* (Syller, 1996). PLRV is a major problem in potato production in the USA (Mowry et al., 2001; Alvarez et al., 2003). Severe PLRV infection can cause yield reductions of up to 40–70%, but tuber quality defects due to speckling or netting of infected tuber tissues ("net necrosis syndrome") are more commonly the cause of economic loss (Banttari et al., 1993). Potatoes intended for the fresh packing or processing industry are permitted to have a maximum of 5% stem-end discoloration of any kind, making any enhancement of this from net necrosis unacceptable (Nolte et al., 2000). Management of leafroll disease in potato is complicated because infected 'seed' tubers provide the primary source for subsequent spread of the virus by aphids. Consequently, aphid management is crucial, especially in 'seed' tuber potato production, and it is currently the main tactic for addressing PLRV problems in potato (Marsh et al., 1998, 2000; Mowry et al., 2001; Alvarez et al., 2003).

Preferential colonization of virus-infected plants by aphids has been attributed to the yellowing of infected tissues, which become more visually attractive to aphids (Macias and Mink, 1969; Ajayi and Dewar, 1983; Eckel and Lampert, 1996). Such preferences may involve aphid behavioral responses to virus-induced changes in plant quality such as amino acid content of phloem tissues (Markkula and Laurema, 1964; Ajayi, 1986; Blua et al., 1994), or soluble carbohydrate content in leaves (Jensen, 1972; Fereres et al., 1990). The effects of volatile organic compounds (VOC) produced by virus-infected plants on aphid vectors has been much less examined, despite evidence that VOC affect aphid host selection behavior (Nottingham et al., 1991; Pickett et al., 1992a; Pettersson et al., 1996; Visser et al., 1996) and that pathogen infection induces VOC production by plants (Preston et al., 1999; Cardoza et al., 2002). To fill some of these knowledge gaps, our work has focused on elucidating the responses of aphid vectors to virus-induced volatiles in plants using two luteovirus pathosystems as models. The main findings from our long-term studies are highlighted in this paper and published in detail elsewhere.

2. Studies on luteoviruses: key findings from model pathosystems

Several key findings have emerged from this research.

(1) The aphid vectors *R. padi* and *M. persicae* develop more rapidly, produce more offspring, or both, on their respective virus-infected hosts (Jiménez-Martínez et al., 2004a; Srinivasan et al., 2008).
(2) Apterae of each vector species settle preferentially on virus-infected host plants, and this discrimination occurs in darkness in the absence of visual cues (Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004b).
(3) This discrimination by apterae occurs even when aphids are prevented from contacting the leaf surface, demonstrating they are attracted or arrested by VOC in the vicinity of infected hosts (Jiménez-Martínez et al., 2004b; Srinivasan et al., 2006; Medina-Ortega et al., 2009).
(4) The VOC profile in the headspace of virus-infected plants differs substantially in overall concentration and relative composition compared to virus-free plants (Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004b; Srinivasan et al., 2006; Ngumbi et al., 2007; Werner et al., 2009).
(5) Some of the virus-induced volatiles (VIV) (those produced in higher concentrations by infected potato plants) are electrophysiologically and behaviorally active for *M. persicae*, but a blend of VIV is required to elicit aphid responses (Ngumbi et al., 2007, and unpublished).
(6) Individual VOC and blends that mimic the VIV in headspace of BYDV-infected wheat plants elicit a response by *R. padi* (Medina-Ortega et al., 2009).
(7) As disease progresses in each of these crop plants, aphid responses to virus-infected versus virus-free plants shift, with stronger arrestment or attraction occurring during intermediate (Werner et al., 2009) or later stages of infection (Medina Ortega, 2005).

(8) Headspace VOC profiles change during PLRV disease progression, likely accounting for the dynamic response of the aphids during disease progression (Werner et al., 2009).
(9) While nonviruliferous *R. padi* apterae respond preferentially to virus-infected wheat, viruliferous apterae do not (Medina-Ortega et al., 2009).
(10) Viruliferous *M. persicae* are less responsive to host VOC than nonviruliferous *M. persicae* (Werner, 2006, and unpublished).

Utilizing comparative studies with a BYDV-susceptible wheat genotype and a moderately virus-resistant transgenic wheat genotype that expresses the coat protein gene of the BYDV-PAV serotype, we have demonstrated that:

(11) Unlike for non-transgenic wheat, *R. padi* apterae do not respond preferentially to BYDV-infected transgenic compared to virus-free transgenic wheat plants (Jiménez-Martínez et al., 2004b; Medina-Ortega et al., 2009).
(12) The concentrations of VOC in BYDV-infected transgenic wheat plants are similar to those in virus-free ones (Jiménez-Martínez et al., 2004b), indicating that transgenic virus resistance in wheat can indirectly influence the production of VOC (Jiménez-Martínez et al., 2004b) and make plants less attractive to *R. padi* (Medina-Ortega et al., 2009).

3. Ecological and epidemiological implications and future research

While virus-infected plants have been shown to affect aphid vector behavior (Macias and Mink, 1969; Ajayi and Dewar, 1983; Blua and Perring, 1992; Eckel and Lampert, 1996, and other references cited above), our finding of the role of virus-induced plant volatiles in the PLRV-potato-*M. persicae* and BYDV-wheat-*R. padi* pathosystems (Eigenbrode et al., 2002; Jiménez-Martínez et al., 2004b; Ngumbi et al., 2007; Medina-Ortega et al., 2009; Werner et al., 2009) opens a new means of understanding host plant–virus–vector relationships. Discovering the mechanisms mediating such interactions is fundamental to understanding vector ecology and vector–virus co-evolution, and may serve as a basis for manipulating vectors to limit virus spread in crops. Clearly, the use of only one control practice is unlikely to keep crops virus-free and a combination of preventive and control measures should be employed to reduce economic losses (Heathcote, 1973; Perring et al., 1999).

As noted previously, enhanced vector reproduction on plants infected with persistently transmitted viruses coupled with attractiveness of these plants to vectors suggests a syndrome that may act to enhance virus spread within a host population. Models of the spread of plant pathogens that incorporate different vector responses to virus-infected and virus-free plants, however, suggest that orientation toward, or attraction to infected plants does not necessarily increase virus spread under most conditions (McElhany et al., 1995; Sisterson, 2008). Additional studies are required to understand the implications of these effects for virus ecology and epidemiology and disease management.

Our work (Werner et al., 2009) and that of others (Alvarez et al., 2007) has shown that VIV production differs within the plant in the PLRV-potato-*M. persicae* pathosystem. This within-plant heterogeneity may be related to the spatial and temporal dynamics of virus titer within the infected plant or to the relative importance of localized versus systemic responses of the plant to virus infection. The effect of this within-plant heterogeneity on aphid behavior may have implications for local spread of viruses. For example, if aphid responses and virus titer differ among plant parts, this could influence virus acquisition by individual aphids foraging within plants.

The focus of the work we have conducted thus far, has been on aphid responses to individual leaflets or leaves of plants with different infection status. These responses suggest but do not demonstrate that interplant movement rates will depend upon the infection status of plants. There is a need to study the responses of aphids to intact plants under greenhouse and field conditions that are more representative of grower's fields. In the field, between-plant movement by apterae is important for virus spread in both pathosystems (Hanafi et al., 1989; Irwin and Thresh, 1990; Badenhausser, 1994; Gourmet et al., 1994; Bailey et al., 1995; Syller, 1996; Thomas et al., 1997; Williams et al., 1998; Thackray et al., 2009). Moreover, movement of alatae also is important for generating new foci within fields and for the colonization of new fields (Irwin and Thresh, 1990). In potato, the principal source of PLRV inoculum is infected 'seed' tubers (Mowry, 2001) but immigrating infectious alatae also contribute to primary and secondary spread within fields. Primary infection foci for BYDV in wheat must be initiated by viruliferous immigrant aphids because the luteoviruses are not seed-borne (Irwin and Thresh, 1990; D'Arcy et al., 2002). Alatae and apterae that have been studied have similar responses to plant odors (Pickett et al., 1992b), but even different forms (gynoparae and virginoparae) can possess different olfactory peripheral sensitivity (Park et al., 2000) and may respond differently to VIV. The effects we have observed so far with apterae occur at close range (several cm). Settling by alatae can be influenced by close-range cues, including VOC such as short-chain fatty acids and sesquiterpenes that could be perceived by olfaction (Phelan and Miller, 1982). Alatae may also use olfaction for longer-range orientation during host finding (Nottingham et al., 1991) and so are theoretically capable of discriminating between virus-infected and virus-free plants at a distance. Preferential flight by aphids to BYDV-infected plants in a flight tunnel has been reported in an experiment that could not have excluded a role of VOC in the aphid response (Ajayi and Dewar, 1983). Thus, additional studies are required to clarify the role of VOC in influencing behavior of alates of both vector species.

Our work (Werner, 2006; Medina-Ortega et al., 2009) indicates that viruliferous aphids are somehow altered compared with nonviruliferous aphids in their responsiveness to host plant volatiles, or other plant attributes. For example, *M. persicae* reared continuously on *P. floridana* infected with PLRV was less likely to emigrate from the vicinity of potato leaflets, or controls, than were nonviruliferous aphids, although relative responses were similar. In contrast, when allowed only a 2-day acquisition access period on *P. floridana* viruliferous and nonviruliferous aphids behaved similarly (Werner, 2006). Changes in vector responsiveness to plants after these vectors acquire the virus would have potentially large effects on virus spread. Since this possibility has not been considered previously, models of virus spread as influenced by vector behavior have not included such effects (McElhany et al., 1995; Sisterson, 2008). Nonetheless, direct effects of plant viral pathogens on vector behavior could be important (as discussed but not modeled by McElhany et al., 1995). For example, if vectors are attracted to infected hosts only until virus acquisition occurs, this shift in aphid preference could accelerate virus spread (Medina-Ortega et al., 2009). Indeed, direct effects on behavior of vectors or intermediate hosts seem to be important in transmission dynamics of other types of pathogens and parasites (Poulin, 2000; Lèfevre and Thomas, 2008). Additional comparative studies between viruliferous and nonviruliferous aphids are on-going in our laboratories to further enhance our understanding of vector–plant–virus interactions.

Changes in aphid responses to infected plants after virus acquisition, variation in VIV emission and aphid responses among parts within plants, and changes in aphid responses to plants throughout the entire period of disease progress, potentially strongly influ-

ence how VIV contribute to spread of persistently transmitted plant viruses. Until these particulars are known, the role of VIV in spread of such viruses cannot be fully understood. As an illustration of the potential complexity, studies with non-persistently transmitted *Cucumber mosaic virus* (CMV) (*Bromoviridae*: genus *Cucumovirus*), infecting squash plants (*Cucurbita pepo* L.) have shown that aphids are initially attracted to VIV from CMV-infected plants, but subsequently disperse to colonize virus-free plants preferentially (Mauck et al., 2010). Since CMV can be acquired within a few seconds by a probing aphid, these behaviors can act to enhance the spread of this virus. A combination of more comprehensive behavioral bioassays, mesocosm experiments and modeling are required to elucidate these complex interactions.

In addition to the applied implications of our research, the fundamental discovery that VOC can mediate plant–virus–vector interactions contributes to a growing understanding of how responses by plants to biotic stresses can influence their ecology (Karban and Baldwin, 1997; Bleé, 1998; Hutcheson, 1998; Rhodes et al., 1999; Shinozaki and Yamaguchi-Shinozaki, 1999; Ryan, 2000; Walling, 2000; Kessler and Baldwin, 2002; Kessler and Halitschke, 2007). Furthermore, our finding that transgenic virus resistance in wheat can indirectly influence VOC production and alter vector responses to such plants opens new opportunities for the study of transgenic approaches to disease and vector management.

Acknowledgements

We are very grateful to our former and present students and to colleagues for their technical contributions to some of the research reviewed here and for insightful discussions and input: Juan Manuel Alvarez, Phil Berger, Hongian Ding, Laura Ingwell, Edgardo Jiménez-Martínez, Alex Karasev, Karla Medina-Ortega, Tom Mowry, Esther Ngumbi, Bryan Roosien, Babu Srinivasan, Brent Werner, and Robert Zemetra. Funding for this research was provided by USDA-NRI Competitive Grant No. 2003-35302-13354 and USDA-AFRI Competitive Grant No. 2009-65104-05730 to SDE and NBP, and by a grant from the NSF-Idaho EPSCoR Program and the National Science Foundation Cooperative Agreement no. EPS-9720634 to NBP. This is a publication of the Idaho Agricultural Experiment Station.

References

Ajayi, O., 1986. The effect of barley yellow dwarf virus on the amino acid composition of spring wheat. Ann. Appl. Biol. 108, 145–149.
Ajayi, O., Dewar, A.M., 1983. The effect of barley yellow dwarf virus on field populations of the cereal aphids. *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Biol. 103, 1–11.
Alvarez, A.E., Garzo, E., Verbeek, M., Vosman, B., Dicke, M., Tjallingii, W.F., 2007. Infection of potato plants with potato leafroll virus changes attraction and feeding behaviour of *Myzus persicae*. Entomol. Exp. Appl. 125, 135–144.
Alvarez, J.M., Stoltz, R.L., Baird, C.R., Sandvol, L.E., 2003. Potato insects and their management. In: Love, S., Stark, J. (Eds.), Potato Production Systems. University of Idaho Agricultural Communications, Moscow, ID, pp. 204–239.
Araya, J.E., Foster, J.E., 1987. Laboratory study on the effects of barley yellow dwarf virus on the life cycle of *Rhopalosiphum padi* (L.). J. Plant Dis. Protect. 94, 195–198.
Badenhausser, I., 1994. Spatial patterns of alate and apterous morphs of the *Brachycaudus helichrysi* (Homoptera: Aphididae) in sunflower fields. Environ. Entomol. 23, 1381–1390.
Bailey, S.M., Irwin, M.E., Kampmeier, G.E., Eastman, C.E., Hewings, A.D., 1995. Physical and biological perturbations: their effect on the movement of apterous *Rhopalosiphum padi* (Homoptera: Aphididae) and localized spread of barley yellow dwarf virus. Environ. Entomol. 24, 24–33.
Baker, P.F., 1960. Aphid behaviour on healthy and on yellow virus infected sugar beet. Ann. Appl. Biol. 48, 384–391.
Banttari, E., Ellis, P.J., Khurana, S.M.P., 1993. Management of diseases caused by viruses and virus-like pathogens. In: Rowe, R.C. (Ed.), Potato Health Managment. APS Press, St. Paul, Minessota, pp. 127–133.
Barker, H., Waterhouse, P.M., 1999. The development of resistance to luteoviruses mediated by host genes and pathogen-derived transgenes. In: Barker, H., Smith, H.G. (Eds.), The Luteoviridae. CABI Publishing, UK, pp. 169–210.
Belliure, B., Janssen, A., Sabelis, M.W., 2008. Herbivore benefits from vectoring plant virus through reduction of period of vulnerability to predation. Oecologia 156, 797–806.
Belliure, B., Janssen, A., Maris, P.C., Peters, D., Sabelis, M.W., 2005. Herbivore arthropods benefit from vectoring plant viruses. Ecol. Lett. 8, 70–79.
Bleé, E., 1998. Phytooxylipins and plant defense reactions. Prog. Lipid Res. 37, 33–72.
Blua, M.J., Perring, T.M., 1992. Effects of zucchini yellow mosaic virus on colonization and feeding behavior of *Aphis gossypii* (Homoptea: Aphididae) alatae. Environ. Entomol. 21, 578–585.
Blua, M.J., Perring, T.M., Madore, M.A., 1994. Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. J. Chem. Ecol. 20, 691–707.
Bosque-Pérez, N.A., 2000. Eight decades of maize streak virus research. Virus Res. 71, 107–121.
Cardoza, Y.J., Alborn, H.T., Tumlinson, J.H., 2002. In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. J. Chem. Ecol. 28.
Castle, S.J., Berger, P.H., 1993. Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus–vector relationship. Entomol. Exp. Appl. 69, 51–60.
Castle, S.J., Mowry, T.M., Berger, P.H., 1998. Differential settling by *Myzus persicae* (Homoptera: Aphididae) on various virus-infected host plants. Ann. Entomol. Soc. Am. 91, 661–667.
Costa, H.S., Brown, J.K., Byrne, D.N., 1991. Life history traits of the whitefly, *Bemisia tabaci* (Homjptera: Aleyrodidae) on six virus-infected or healthy plant species. Environ. Entomol. 20, 1102–1107.
D'Arcy, C.J., Domier, L.L. Mayo, M.A., 2002. Luteoviruses, ICTVdB Index of Viruses. ICTV, http://www.ictvdb,iacv,ac,uk/Ictv/fs_luteo.htm.
Donaldson, J.R., Gratton, C., 2007. Antagonistic effects of soybean viruses on soybean aphid performance. Environ. Entomol. 36, 918–925.
Eckel, R.V.W., Lampert, E.P., 1996. Relative attractiveness of tobacco etch virus-infected and healthy flue-cured tobacco plants to aphids. J. Econ. Entomol. 89, 1017–1027.
Eigenbrode, S.D., Ding, H., Shiel, P., Berger, P.H., 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. R. Soc. B 269, 455–460.
Ellsbury, M.M., Pratt, R.G., Knight, W.E., 1985. Effects of single and combined infection of arrowleaf clover with bean yellow mosaic-virus and a *Phytophthora* sp. on reproduction and colonization by pea aphids (Homoptera, Aphididae). Environ. Entomol. 14, 356–359.
Elomaa, P., Joensuu, J.J., Korpelainen, H., Makinen, K., Niklander-Teeri, V., Pirhonen, M., Seppanen, M.M., Teeri, T.H., Valkonen, J.P.T., 2008. Plant biotechnology for deeper understanding, wider use and further development of agricultural and horticultural crops. Agric. Food Sci. 17, 307–324.
Fereres, A., Araya, J.E., Housley, T.L., Foster, J.E., 1990. Carbohydrate composition of wheat infected with barley yellow dwarf virus. J. Plant Dis. Prot. 97, 600–608.
Fereres, A., Kampmeier, G.E., Irwin, M.E., 1999. Aphid attraction and preference for soybean and pepper plants infected with potyviridae. Ann. Entomol. Soc. Am. 92, 542–548.
Fereres, A., Lister, R.M., Araya, J.E., Foster, J.E., 1989. Development and reproduction of the English grain aphid (Homoptera: Aphididae) on wheat cultivars infected with barley yellow dwarf virus. Environ. Entomol. 18, 388–393.
Gildow, F.E., 1980. Increased production of alatae by aphids reared on oats infected with barley yellow dwarf virus. Ann. Entomol. Soc. Am. 73, 343–347.
Gildow, F.E., 1983. Influence of barley yellow dwarf virus-infected oats and barley on morphology of aphid vectors. Phytopathology 73, 1196–1199.
Gildow, F.E., 1989. Barley yellow dwarf-aphid vector interactions associated with virus transmission and vector specificity. In: Burnett, P.A. (Ed.), Barley Yellow Dwarf Virus, the Yellow Plague of Cereals. CIMMYT, Mexico, pp. 111–122.
Gourmet, C., Hewings, A.D., Kolb, F.L., Smyth, C.A., 1994. Effect of imidacloprid on nonflight movement of *Rhopalosiphum padi* and the subsequent spread of barley yellow dwarf virus. Plant Dis. 78, 1098–1101.
Hanafi, A., Radcliffe, E.B., Ragsdale, D.W., 1989. Spread and control of potato leafroll virus in Minnesota. J. Econ. Entomol. 82, 1201–1206.
Heathcote, G.D., 1973. Control of viruses spread by invertebrates to plants. In: Gibbs, A.J. (Ed.), Viruses and Invertebrates. Elsevier, Amsterdam, pp. 587–609.
Hodge, S., Powell, G., 2008. Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behavior and performance? Environ. Entomol. 37, 1573–1581.
Hodgson, C.J., 1981. Effects of infection with the cabbage black ringspot strain of turnip mosaic virus on turnip as a host to *Myzus persicae* and *Brevicoryne brassicae*. Ann. Appl. Biol. 98, 1–14.
Hutcheson, S.W., 1998. Current concepts of active defense in plants. Ann. Rev. Phytopathol. 36, 59–90.
Irwin, M.E., Thresh, J.M., 1990. Epidemiology of barley yellow dwarf: a study in ecological complexity. Ann. Rev. Phytopathol. 28, 393–424.
Jensen, S.G., 1972. Metabolism and carbohydrate composition in barley yellow dwarf virus-infected wheat. Phytopathology 62, 587–592.
Jiménez-Martínez, E.S., Bosque-Pérez, N.A., 2004. Variation in barley yellow dwarf virus transmission efficiency by *Rhopalosiphum padi* (Homoptera: Aphididae) after acquisition from transgenic and non-transformed wheat genotypes. J. Econ. Entomol. 97, 1790–1796.
Jiménez-Martínez, E.S., Bosque-Pérez, N.A., 2009. Life history of the bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae) on transgenic and non-transformed wheat challenged with Wheat streak mosaic virus. Entomol. Exp. Appl. 133, 19–26.

Jiménez-Martínez, E.S., Bosque-Pérez, N.A., Berger, P.H., Zemetra, R.S., 2004a. Life history of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae), on transgenic and untransformed wheat challenged with Barley yellow dwarf virus. J. Econ. Entomol. 97, 203–212.

Jiménez-Martínez, E.S., Bosque-Pérez, N.A., Berger, P.H., Zemetra, R.S., Ding, H., Eigenbrode, S., 2004b. Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to *Barley yellow dwarf virus*-infected transgenic and untransformed wheat. Environ. Entomol. 33, 1207–1216.

Jones, R.A.C., 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res. 100, 5–30.

Karban, R., Baldwin, I.T., 1997. Induced Responses to Herbivory. Chicago University Press, Chicago, IL.

Kennedy, J.S., 1951. Benefits to aphids from feeding on galled and virus-infected leaves. Nature 168, 825–826.

Kessler, A., Baldwin, I.T., 2002. Plant responses to insect herbivory: the emerging molecular analysis. Ann. Rev. Plant Biol. 53, 299–328.

Kessler, A., Halitschke, R., 2007. Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure. Curr. Opin. Plant Biol. 10, 409–414.

Lèfevre, T., Thomas, F., 2008. Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. Infect. Gen. Evol. 8, 504–519.

Lovisolo, O., Hull, R., Rösler, O., 2003. Coevolution of viruses with hosts and possible paleontology. Adv. Virus Res. 62, 325–379.

Macias, W., Mink, G.I., 1969. Preference of green peach aphids for virus-infected sugarbeet leaves. J. Econ. Entomol. 62, 28–29.

Maramorosch, K., Jensen, D.D., 1963. Harmful and beneficial effects of plant viruses in insects. Ann. Rev. Microbiol. 17, 495–530.

Markkula, M., Laurema, S., 1964. Changes in concentration of free amino acids in plants induced by virus diseases and the reproduction of aphids. Ann. Ag. Fenn. 3, 265–271.

Marsh, T.L., Huffaker, R.G., Long, G.E., 2000. Optimal control of vector–virus–plant interactions: the case of potato leafroll virus net necrosis. Am. J. Ag. Econ. 83, 556–569.

Marsh, T.L., Huffaker, R.G., Mittelhammer, R.C., Folwell, R.J., Long, G.E., Horton, D.R., Toba, H.H., 1998. Potato leafroll virus net necrosis: identifying pest management tradeoffs among inoculation interval, storage length and tuber weight. J. Econ. Entomol. 91, 923–932.

Mauck, K.E., Moraes, C.M.D., Mescher, M.C., 2010. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc. Natl. Acad. Sci. 107, 3600–3605.

McElhany, P., Real, L.A., Power, A.G., 1995. Vector preference and disease dynamics: a study of barley yellow dwarf virus. Ecology 76, 444–457.

McGrath, P.F., Vincent, J.R., Lei, C.-H., Pawlowski, W.P., Torbert, K.A., Gu, W., Kaeppler, H.F., Wan, Y., Lemaux, P.G., Rines, H.R., Somers, D.A., Larkins, B.A., Lister, R.M., 1997. Coat protein-mediated resistance to isolates of barley yellow dwarf in oats and barley. Eur. J. Plant Pathol. 103, 695–710.

McIntyre, J.L., Dodds, J.A., Hare, J.D., 1981. Effects of localized infections of *Nicotiana tabacum* by tobacco mosaic-virus on systemic resistance against diverse pathogens and an insect. Phytopathology 71, 297–301.

McKirdy, S.J., Jones, R.A.C., Nutter, F.W., 2002. Quantification of yield losses caused by Barley yellow dwarf virus in wheat and oats. Plant Dis. 86, 769–773.

Medina Ortega, K.J., 2005. *Rhopalosiphum padi* L. (Hemiptera: Aphididae) behavioral responses to headspace volatiles from *Barley yellow dwarf virus*-infected and non-infected transgenic and non-transformed wheat plants. M.S. Thesis, University of Idaho, Moscow, ID.

Medina-Ortega, K., Bosque-Pérez, N.A., Ngumbi, E., Jiménez-Martínez, E.S., Eigenbrode, S.D., 2009. *Rhopalosiphum padi* (Hemiptera: Aphididae) responses to volatile cues from Barley yellow dwarf virus-infected wheat. Environ. Entomol. 38, 836–845.

Michels, G.J., Rush, C.M., Whitaker-Deerberg, R.L., Heidel, G.B., 1994. Deleterious effects of wheat streak mosaic virus infection of winter wheat on greenbug (Homoptera: Aphididae) reproduction. Southwest. Entomol. 9, 109–113.

Miller, J.W., Coon, B.F., 1964. The effect of barley yellow dwarf virus on the biology of its vector the English grain aphid, *Macrosiphum granarium*. J. Econ. Entomol. 57, 970–974.

Mowry, T.M., 2001. Green peach aphid (Homoptera: Aphididae) action thresholds for controlling the spread of potato leafroll virus in Idaho. J. Econ. Entomol. 94, 1332–1339.

Mowry, T., Pike, K., Reed, G., Schreiber, A., Stoltz, B., 2001. Integrated pest management program for green peach aphid/potato leaf roll virus for Pacific northwest potatoes. http://www.wsu.edu/~potatoes/ipm.htm.

Musser, R.O., Hum-Musser, S.M., Felton, G.W., Gergerich, R.C., 2003. Increased larval growth and preference for virus-infected leaves by the Mexican bean beetle, *Epilachna varivestis* Mulsant, a plant virus vector. J. Ins. Behav. 16, 247–256.

Ngumbi, E., Eigenbrode, S.D., Bosque-Pérez, N.A., Ding, H., Rodriguez, A., 2007. *Myzus persicae* is arrested more by blends than by individual compounds elevated in headspace of PLRV-infected potato. J. Chem. Ecol. 33, 1733–1747.

Nolte, P., Sandvol, L., Mowry, T., Stoltz, R., Olsen, N., Geary, B., Whitworth, J., 2000. Potato leaf roll and net necrosis. University of Idaho Cooperative Extension Plant Disease Bulletin http://www.uidaho.edu/ag/plantdisease/pnniap.htm.

Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 1991. Behavioral and electrophysiological responses of aphids to host and non-host plant volatiles. J. Chem. Ecol. 17, 1231–1242.

Park, K.C., Elias, D., Donato, B., Hardie, J., 2000. Electroantennogram and behavioural responses of different forms of the bird cherry-oat aphid, *Rhopalosiphum padi*, to sex pheromone and a plant volatile. J. Ins. Physiol. 46, 597–604.

Perring, T.M., Gruenhagen, N.M., Farrar, C.A., 1999. Management of plant viral diseases through chemical control of insect vectors. Ann. Rev. Entomol. 44, 457–481.

Pettersson, J., Quiroz, A., Fahad, A.E., 1996. Aphid antixenosis mediated by volatiles in cereals. Acta Agric. Scand. 46, 135–140.

Phelan, P.L., Miller, J.R., 1982. Post-landing behavior of alate *Myzus persicae* as altered by (*E*)-β-farnesene and three carboxylic acids. Entomol. Exp. Appl. 32, 46–53.

Pickett, J.A., Pye, B.J., Wadhams, L.J., Woodccock, C.M., Campbell, C.A., 1992b. Potential applications of semiochemicals in aphid control. British Crop Protect. Conf. Monogr. 51, 29–33.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 1992a. The chemical ecology of aphids. Ann. Rev. Entomol. 37, 67–90.

Plumb, R.T., 1983. Barley yellow dwarf virus—a global problem. In: Plumb, R.T., Thresh, J.M. (Eds.), Plant Virus Epidemiology. Blackwell, London, pp. 185–198.

Poulin, R., 2000. Manipulation of host behaviour by parasites: a weakening paradigm? Proc. R. Soc. B 267, 787–792.

Preston, C.A., Lewandowski, C., Enyedi, A.J., Baldwin, I.T., 1999. Tobacco mosaic virus inoculation inhibits wound-induced jasmonic acid-mediated responses within but not between plants. Planta 209, 87–95.

Quiroz, C., Lister, R.M., Araya, J.E., Foster, J.E., 1991. Effect of symptom variants derived from the NY-MAV isolate of barley yellow dwarf virus on the life cycle of the English grain aphid (Homoptera: Aphididae) and on yield components in wheat and oats. J. Econ. Entomol. 84, 1920–1925.

Rhodes, J.D., Thain, J.F., Wildon, D.C., 1999. Evidence for physically distinct systemic signaling pathways in the wounded tomato plant. Ann. Bot. 84, 109–116.

Ryan, C.A., 2000. The systemin signaling pathway: differential activation of defensive genes. Biochim. Biophys. Acta 1477, 112–122.

Shinozaki, K., Yamaguchi-Shinozaki, K. (Eds.), 1999. Molecular Responses to Cold, Drought, Heat, and Salt Stress in Higher Plants. R.G. Landes Co., Austin, TX.

Sisterson, M.S., 2008. Effects of insect–vector preference for healthy or infected plants on pathogen spread: insights from a model. J. Econ. Entomol. 101, 1–8.

Sivamani, E., Brey, C.W., Talbert, L.E., Young, M.A., Dyer, W.E., Kaniewski, W.K., Qu, R., 2002. Resistance to wheat streak mosaic virus in transgenic wheat engineered with the viral coat protein gene. Transg. Resist. 11, 31–41.

Srinivasan, R., Alvarez, J.M., Bosque-Pérez, N.A., Eigenbrode, S.D., Novy, R.G., 2008. Effect of an alternate weed host, hairy nightshade, *Solanum sarrachoides* (Sendtner), on the biology of the two important Potato leafroll virus (Polerovirus: Luteoviridae) vectors, *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Aphididae: Homoptera). Environ. Entomol. 37, 592–600.

Srinivasan, R., Alvarez, J.M., Eigenbrode, S.D., Bosque-Pérez, N.A., 2006. Influence of hairy nightshade *Solanum sarrachoides* (Sendtner) and *Potato leafroll virus* (Luteoviridae: Polerovirus) on the host preference of *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Environ. Entomol. 35, 546–553.

Syller, J., 1996. Potato leafroll virus (PLRV): its transmission and control. Integr. Pest Mngt. Rev. 1, 217–227.

Thackray, D.J., Diggle, A.J., Jones, R.A.C., 2009. BYDV PREDICTOR: a simulation model to predict aphid arrival, epidemics of Barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean-type environment. Plant Pathol. 58, 186–202.

Thomas, P.E., Kaniewski, W.K., Lawson, E.C., 1997. Reduced field spread of potato leafroll virus in potatoes transformed with the Potato leafroll virus coat protein gene. Plant Dis. 81, 1447–1453.

Visser, J.H., Piron, P.G.M., Hardie, J., 1996. The aphids' peripheral perception of plant volatiles. Entomol. Exp. Appl. 80, 35–38.

Walling, L.L., 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19, 195–216.

Werner, B.J., 2006. Behavioral responses of the green peach aphid to virus-induced volatiles produced by potatoes infected with potato leafroll virus. M.S. Thesis, University of Idaho, Moscow, ID.

Werner, B.J., Mowry, T.M., Bosque-Pérez, N.A., Ding, H., Eigenbrode, S.D., 2009. Changes in green peach aphid responses to Potato leafroll virus-induced volatiles emitted during disease progression. Environ. Entomol. 38, 1429–1438.

Williams, I.S., Dewar, A.M., Dixon, A.F.G., 1998. The influence of size and duration of aphid infestation on host plant quality, and its effect on sugar beet yellowing virus epidemiology. Entomol. Exp. Appl. 89, 25–33.

Functional Ecology

British Ecological Society

Functional Ecology 2012 doi: 10.1111/j.1365-2435.2012.02026.x

REVIEW

Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses

Kerry Mauck[1], Nilsa A. Bosque-Pérez[2], Sanford D. Eigenbrode[2], Consuelo M. De Moraes[1] and Mark C. Mescher*[,1]

[1]*Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania, 16802 USA; and*
[2]*Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, Idaho, 83844-2339 USA*

Summary

1. Vector-borne pathogens and parasites can induce changes in the phenotypes of their hosts that influence the frequency and nature of host–vector interactions and hence transmission, as documented by both empirical and theoretical studies. To the extent that implications for transmission play a significant role in shaping the evolution of parasite effects on host phenotypes, we may hypothesize that parasites exhibiting similar transmission mechanisms – and thus profiting from similar patterns of interaction among hosts and vectors – will have correspondingly similar effects on relevant host traits. Here, we explore this hypothesis through a survey and synthesis of literature on interactions among plant viruses, their hosts, and insect vectors.

2. Insect-vectored plant viruses that differ in their modes of transmission benefit from different patterns of interaction among host plants and vectors. The transmission of persistently transmitted (PT) viruses requires that vectors feed on an infected host for a sustained period to acquire and circulate (and sometimes replicate) virions, then disperse to a new, healthy host. In contrast, non-persistently transmitted (NPT) viruses are effectively transmitted when vectors briefly probe infected hosts, acquiring virions, then rapidly disperse.

3. Based on these observations, and empirical evidence from our previous work, we hypothesized that PT and NPT viruses will exhibit different effects on aspects of host phenotypes that mediate vector attraction to, arrestment on and dispersal from infected plants. Specifically, we predicted that both PT and NPT viruses would tend to enhance vector attraction to infected hosts, but that they would have contrasting effects on vector settling and feeding preferences and on vector performance, with PT viruses tending to improve host quality for vectors and promote long-term feeding and NPT viruses tending to reduce plant quality and promote rapid dispersal.

4. We evaluated these hypotheses through an analysis of existing literature and found patterns broadly consistent with our expectations. This literature synthesis, together with evidence from other disease systems, suggests that transmission mechanisms may indeed be an important factor influencing the manipulative strategies of vector-borne pathogens, with significant implications for managing viral diseases in agriculture and understanding their impacts on natural plant communities.

Key-words: acquisition access period, adaptive manipulation, aphids, non-persistently transmitted virus, persistently transmitted virus, plant volatiles, thrips, vector behaviour, vector performance, whiteflies

Introduction

Vector-borne pathogens and other parasites often alter the traits of their hosts in ways that influence the frequency and nature of interactions between hosts and vectors (Roy & Raguso 1997; Ebbert & Nault 2001; Eigenbrode *et al.* 2002; Hurd 2003; Maris *et al.* 2004; Belliure *et al.* 2005; Lacroix *et al.* 2005; Lefévre *et al.* 2006; Mauck, De Moraes & Mescher 2010; Bosque-Pérez & Eigenbrode 2011). These parasite-induced changes in host phenotype often

*Correspondence author. E-mail: mcmescher@psu.edu

have significant implications for parasite transmission (Lefévre & Thomas 2008) and can also impact broader community dynamics (Lefévre *et al.* 2006; Wood *et al.* 2007; Lefévre & Thomas 2008). Some pathogen-induced effects are sufficiently complex to be obvious cases of manipulation (e.g. parasitic rust fungi that induce the production by hosts of pseudoflowers that facilitate fungal transmission by insect pollinators) (Roy & Raguso 1997). In contrast, it can be difficult to determine the adaptive status of less dramatic effects that might plausibly be construed as mere by-products of infection (e.g. up-regulation in elm trees infected with Dutch Elm Disease of sesquiterpene emissions that are attractive to bark beetle vectors) (McLeod *et al.* 2005). Nevertheless, it seems certain that natural selection will rarely be indifferent to pathogen effects that have significant implications for transmission (Anderson & May 1991; Poulin 2010). That is, in the absence of strong countervailing factors, selection should favour pathogen effects on host–vector interactions that are conducive to transmission. Consequently, we may expect that most pathogen-induced changes in host phenotypes will have positive (or neutral) effects on transmission by vectors. We might also predict that pathogens which exhibit similar modes of transmission – and therefore presumably benefit from similar patterns of host–vector interaction – will exhibit significant convergence in their effects on host phenotypes (Mauck, De Moraes & Mescher 2010); however, this hypothesis has received little attention to date.

We have recently investigated the effects of several plant viruses on the quality of host plants as a resource for aphid vectors and on the host-derived cues presented to foraging aphids (Eigenbrode *et al.* 2002; Jiménez-Martínez *et al.* 2004a,b; Srinivasan *et al.* 2006; Medina-Ortega *et al.* 2009; Werner *et al.* 2009; Mauck, De Moraes & Mescher 2010). The results of these studies suggest an interesting pattern of variation among viruses that differ in their mode of transmission: we, and other groups, have documented increased aphid performance on plants infected by persistently transmitted (PT) viruses (Montllor & Gildow 1986; Castle & Berger 1993; Jiménez-Martínez *et al.* 2004a), which, as discussed below, require sustained aphid feeding for effective transmission. In contrast, we found that aphid performance was strongly reduced on plants infected by the non-persistently transmitted (NPT) *Cucumber Mosaic virus* (CMV, *Cucumovirus*, Bromoviridae) (Mauck, De Moraes & Mescher 2010), which is quickly acquired during aphid feeding and benefits from rapid vector dispersal. Interestingly, aphids exhibited preferential attraction to the olfactory cues of plants infected by both persistently and NPT viruses compared with healthy controls (Eigenbrode *et al.* 2002; Jiménez-Martínez *et al.* 2004b; Srinivasan *et al.* 2006; Medina-Ortega *et al.* 2009; Werner *et al.* 2009; Mauck, De Moraes & Mescher 2010) – in the case of CMV, this attraction to infected plants, despite their decreased quality as a resource for aphids, appeared to result from the increased emission from these plants of an olfactory blend otherwise similar to that of healthy plants. Taken together, these findings suggest a pattern consistent with the hypothesis that the mode of transmission should be a major factor shaping the effects of plant viruses – and other vector-borne parasites – on aspects of the host phenotype that influence interactions with vectors (Mauck, De Moraes & Mescher 2010). However, this recent work has addressed far too few systems to allow us to draw general conclusions. Therefore, in this paper, we analyse the broader literature addressing virus effects on host plant phenotypes, to explore whether the pattern we observe holds more generally and to test the hypotheses that (i) pathogen effects on host phenotypes will generally be conducive to transmission by vectors and (ii) pathogens with similar modes of transmission will also exhibit similarity in their effects on aspects of the host phenotype (e.g. nutritional and defence chemistry, olfactory and visual cues) that influence the frequency and nature of interactions between hosts and vectors.

Background and specific predictions:

PATHOGEN EFFECTS ON HOST–VECTOR INTERACTIONS:

Insects are among the most important vectors of both plant and animal pathogens, and most insects have specific adaptations for host finding and feeding that can potentially be exploited to facilitate transmission of parasites from infected to healthy hosts (Bernasconi *et al.* 1998; Dicke 2000; Park & Hardie 2003; Birkett *et al.* 2004; Powell, Tosh & Hardie 2006; Fereres & Moreno 2009). Among plant pathogens, viruses represent a widespread and diverse group, and the transmission of many plant viruses is obligately dependent on insect vectors (e.g. aphids, whiteflies, planthoppers, leafhoppers, mealybugs, thrips and beetles). Thus, the presence and abundance of insect vectors, and the preferences and movements of vectors in relation to infected and healthy plants, are theoretically important regulators of virus spread (McElhany, Real & Power 1995; Madden, Jeger & van den Bosch 2000; Power 2008; Sisterson 2008). However, these specific interactions including transmission dynamics remain understudied (Malmstrom, Melcher & Bosque-Pérez 2011), especially in comparison with insect-vectored animal parasites.

In this study, we generate several predictions regarding the optimal patterns of vector behaviour that favour the spread of the major classes of plant viruses according to their transmission mode and then explore these predictions through a synthesis of available literature. Specifically, we focus on several discrete aspects of the host–vector interaction that contribute to effective transmission (Fig. 1). For example, vectors must first approach an infected host (*attraction*), establish contact with the host leading to pathogen acquisition, usually through trophic interactions (*settling* and *feeding*), and finally *disperse* from the host and carry the pathogen to new, non-infected hosts (Fig. 1).

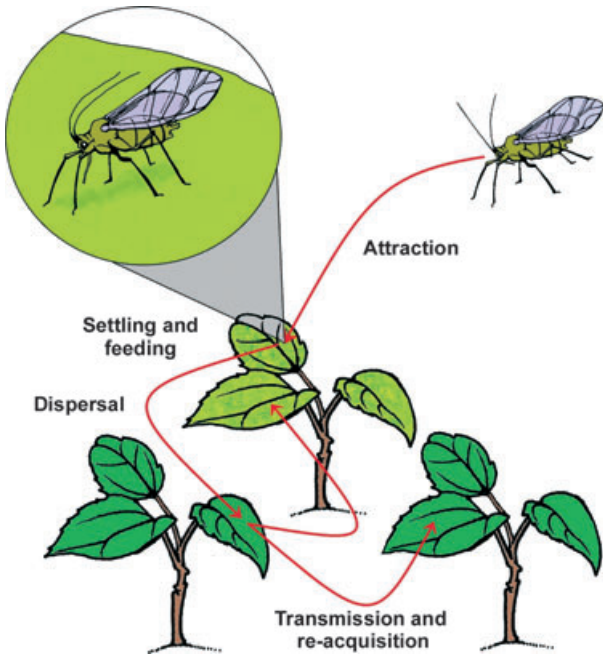

Fig. 1. Patterns of vector behaviour in relation to infected and healthy hosts. In this schematic, the vector is an alate aphid, and the pathogen is a virus (infected plant is discoloured and yellow), but the principles illustrated apply broadly. In order for transmission to occur, the vector must locate and orient toward the infected plant (attraction), which is mediated by chemical cues (volatile organic molecules detectable by vectors) and visual cues (spectral reflectance changes associated with infection). Once the infected host is located, the vector must interact trophically with the host to acquire the pathogen (probing, biting or sustained feeding). The duration and nature of trophic interactions may be influenced by infection-induced changes in host defences, host nutritional status, or morphological or chemical attributes. After acquiring the pathogen, the vector must disperse to a susceptible host for transmission to occur. Pathogen-induced changes in the cues mediating host selection behaviour can influence dispersal in the short term, and effects of infected hosts on vector performance (development, reproduction and survival) can influence dispersal and transmission in the long term. Some pathogens can be inoculated multiple times following acquisition, and some must be re-acquired to achieve transmission to multiple hosts by a single vector.

The specific predictions about pathogen effects on these interactions presented below are informed by our knowledge of the biochemical mechanisms by which different viruses are transmitted by various insect vectors and theoretical work on the epidemiology of insect-transmitted plant viruses.

PREDICTIONS FOR VIRUS EFFECTS ON PLANT–VECTOR INTERACTIONS

Insect-borne plant viruses exhibit transmission mechanisms ranging from transient associations with vector mouthparts to long-lasting physiological associations, including replication within both the vector and the host plant (Nault 1997; Ng & Falk 2006; Hogenhout *et al*. 2008). NPT viruses (also called 'stylet-borne' viruses) occupy one end of this spectrum: they are generally retained within the vector for only a short time and are transmitted most efficiently when insects acquire virions during brief probes of plant epidermal cells and then quickly move on to probe another host or a non-infected portion of the same plant (advancing spread) (Martín *et al*. 1997; Ng & Falk 2006). The initiation of feeding within the phloem (the long-term feeding site for many insect vectors of plant viruses) thus reduces the probability of successful transmission of NPT viruses, as virions are expelled into the same plant from which they are acquired and are not re-acquired from the phloem during long-term feeding (Wang & Ghabrial 2002). As a result, arrestment of vectors on infected plants will often reduce viral spread and fitness. In contrast, PT viruses are acquired by vectors, usually from phloem vascular tissues, during longer periods of feeding (often for several hours or days), and infected vectors typically remain competent (able to transmit) throughout the remaining life of the insect. A further distinction can be drawn between *persistent-circulative* viruses, which circulate within the insect body during a latent period and are retained within vector salivary glands but do not replicate within the vector (e.g. aphid-transmitted viruses in the family Luteoviridae), and *persistent-propagative* viruses that replicate to varying degrees in both vectors and plants – these are generally associated with vectors other than aphids, including whiteflies, thrips and leafhoppers (Sylvester 1980; Hogenhout *et al*. 2008). *Semi-persistently transmitted* (SPT) *viruses* are non-circulative and bind to the insect foregut (e.g. beetle and leafhopper vectors) or to specific portions of the stylet (e.g., *Cauliflower mosaic virus*) (Uzest *et al*. 2007). These viruses, like NPT viruses, can also be acquired quickly, but longer-term feeding (such as sustained feeding in the phloem in the case of an aphid vector) generally increases the probability of virus acquisition (Palacios *et al*. 2002). The vectors of semi-persistent viruses usually remain competent for several hours or days following acquisition of virions (Ng & Perry 2004). The varying requirements for vector feeding and dispersal between non-persistent and persistent virus transmission mechanisms yield a number of hypotheses regarding optimal patterns (relative to virus fitness) of vector attraction to, settling and feeding on, and dispersal from virus-infected hosts:

Vector attraction

Because insect vectors must interact with the infected host (probe or feed) to become viruliferous, we hypothesize that – regardless of transmission mechanism – viruses that increase the apparency or attractiveness of their hosts to vectors will exhibit increased transmission, especially when infected plants are rare. At high frequencies, vector attraction to infected plants may become self-defeating (unless the behaviour of infected and non-infected vectors differ, e.g. Medina-Ortega *et al*. 2009). However, virus presence within a host population often begins with a single-infected

plant, and consequently a very low ratio of infected to healthy plants (Power 1991; McElhany, Real & Power 1995). The rate at which the epidemic expands from this starting point can determine pathogen fitness, given that hosts may not be susceptible for an entire season, vectors may be transiently present, and host susceptibility is influenced by other pathogen infections (Thresh 1974; Syller 2011). Additionally, in many cases, the frequency of infection may rarely reach levels at which increased apparency to vectors is disfavoured (e.g. Power 1991; Raybould *et al.* 1999). *Thus, our first prediction (P1) is that PT, SPT and NPT viruses will influence host-derived cues in ways that have positive (or neutral, but rarely negative) effects on vector attraction.*

Vector settling and feeding

Persistently transmitted viruses are acquired during sustained feeding bouts – and sometimes only through continued feeding by immature life stages of the vector [e.g. only larval forms of thrips (Thysanoptera) acquire *Tomato spotted wilt virus* (TSWV, *Tospovirus,* Bunyaviridae), which they later transmit in the mobile adult stage] (Whitfield, Ullman & German 2005). Preferential initiation of extended feeding (or preferential oviposition) by vectors on hosts infected with PT viruses should therefore increase the probability of vectors becoming competent. In contrast, the acquisition of NPT viruses should be favoured by a reduction in the likelihood of long-term feeding (as these virions are often lost from the insect mouthparts during sustained feeding) (Martín *et al.* 1997; Ng & Falk 2006). *Thus, our second prediction (P2) is that PT and SPT viruses will have positive (or neutral) effects on vector settling and long-term feeding on virus-infected hosts, while NPT viruses will tend to have negative effects on these behaviours.*

Vector performance

Even where sustained feeding is essential for the acquisition of PT viruses, a dispersal mechanism must also exist to allow virus spread following acquisition. Enhanced reproduction by vectors and/or accelerated juvenile development on virus-infected plants may lead to crowding, accelerated use of host resources, and eventual dispersal of large numbers of competent vectors carrying virions (Gildow 1980, 1983; Zhang, Holt & Colvin 2000; Müller, Williams & Hardie 2001). This suggests that PT and SPT viruses may benefit by enhancing host plant quality for vectors. In contrast, NPT viruses are spread when vectors briefly probe infected plants (acquiring virions) and then disperse prior to initiating long-term feeding. Consequently, non-persistent viruses might be expected to reduce host quality or, more importantly, palatability, thus resulting in rapid vector dispersal following virus acquisition. *Thus, our third prediction (P3) is that PT and SPT viruses will have positive effects on vector survival, fecundity or longevity, and NPT viruses will have negative effects on one or more of these parameters.*

SUPPORT FROM PAST THEORETICAL WORK

Theoretical support for these predictions is provided by a handful of studies that have explored the epidemic dynamics of PT, SPT and NPT viruses in the context of the key vector behaviours of attraction; settling and feeding; and dispersal (or overall activity). For example, McElhany, Real & Power (1995) examined the disease dynamics of viruses with low persistence (i.e. NPT viruses) and high persistence [in this case, *Barley yellow dwarf virus* (BYDV, *Luteovirus*, Luteoviridae) was modelled explicitly] through simple analytical models whose parameters addressed disease prevalence, vector preference for diseased plants, disease persistence within the vector, the spatial structure of disease spread, and global vs. local vector movements. Our vector-attraction prediction (P1) finds support in their conclusion that preferential vector visits to diseased plants will enhance pathogen spread at a low prevalence of infected plants. Vector preference for infected plants was predicted to eventually slow the spread of the virus, as the number of infected plants increases, because vectors are more likely to repeatedly visit infected hosts. However, recent work by Medina-Ortega *et al.* (2009) suggests that the preferential attraction of aphid vectors to BYDV-infected plants documented previously (Jiménez-Martínez *et al.* 2004b) does not hold for aphids that are already carrying the virus. Thus, vector preferences may be dynamic and contingent on previous exposure to virus-infected hosts or direct effects of the virus on the vector. In contrast to results for a PT virus, the McElhany *et al.* model predicted that vector preference for diseased plants enhances disease spread at both low and intermediate levels of disease prevalence when the pathogen has low persistence. The difference in the outcomes modelled for PT and NPT viruses largely reflects the fact that continual re-acquisition of the pathogen is necessary for the spread of a NPT virus.

Prediction P1 is also consistent with models developed by Sisterson (2008), which separate the preference parameter into 'orientation preference' (attraction based on odour or visual cues) and 'feeding preference' (arrestment on infected or healthy plants). These models indicate that a significant orientation preference for infected plants increases the rate of pathogen spread when infected plant densities are low. For NPT pathogens, orientation preference for infected plants increases the likelihood of initiating an epidemic – indeed, if vectors prefer healthy plants, an epidemic fails to ensue. Sisterson's models furthermore suggest that the loss of preference for infected plants observed for viruliferous vectors (Medina-Ortega *et al.* 2009) may prevent reduction in the rate of virus spread as the prevalence of infected hosts rises (particularly for PT viruses). Finally, these models indicate that the relative effects of vector

preference on virus spread diminish with increasing vector population density.

The models of McElhany *et al*. and Sisterson are informative, but lack parameters to describe additional aspects important to virus spread, such as immigration and emigration in the field, vector reproduction, vector removal (mortality), plant resistance, and vector aggregation. Theoretical work by Madden, Jeger & van den Bosch (2000) incorporated some of these missing parameters and additionally focused on the epidemic progression of four virus transmission types that we described above: non-persistent, persistent-circulative, persistent-propagative, and semi-persistent. Both our vector attraction prediction (P1) and vector settling and feeding prediction (P2) are supported by this work, which suggests that small changes in vector activity can have large effects on the spread of NPT viruses (as might be expected given their short acquisition and retention times), but only limited impacts on the spread of PT viruses (Madden, Jeger & van den Bosch 2000). The activity and movement of vector insects is influenced by plant characteristics that are subject to alteration by virus infection, including odour, taste, defence status and nutritional quality (Powell, Tosh & Hardie 2006). Our vector performance prediction (P3) also finds support in the theoretical demonstration by Madden, Jeger & van den Bosch (2000) that changes in vector mortality can have large effects on PT propagative viruses (because vectors remain competent for life), but comparatively small effects on NPT viruses. Additionally, changes in vector reproduction, immigration and emigration were found to have differential effects on the four virus classes, with the spread of NPT viruses being little affected by changes in these parameters, while the spread of PT viruses is more susceptible to fluctuations, as might be expected for viruses that can be inoculated multiple times without re-acquisition.

Concurrent work by Zhang, Holt & Colvin (2000) incorporated vector aggregation because of settling and feeding preferences for virus-infected plants and increased reproduction on infected plants into a model fitted to field data on the spread of a PT virus, *African cassava mosaic virus* (ACMV, *Begomovirus,* Geminiviridae), by its whitefly vector. Both P1 and P2 are supported by this work; field data on virus spread were best explained if the model included whitefly aggregation on infected plants. And the authors suggested that, in systems in which infected plants are superior hosts for vectors (as is the case for the whitefly–ACMV–cassava system), preferential aggregation on virus-infected plants will lead to over-crowding and increased dispersal of viruliferous vectors to new susceptible host populations (key elements of our vector-performance prediction).

Evaluation of predictions

To test our three predictions relating to vector attraction, vector settling and feeding behaviour, and vector performance, we evaluated the available literature addressing each of these three virus–host–vector interactions. Only a few virus–host combinations have been comprehensively studied with respect to the overall effects of infection on plant–insect interactions (Bosque-Pérez & Eigenbrode 2011): The persistent-circulative *Potato leafroll virus* (PLRV, *Polerovirus*, Luteoviridae) has been shown (in multiple hosts) to induce odour cues that are attractive and/or arrestant to aphid vectors and may also induce contact or taste cues that favour vector settling (Eigenbrode *et al*. 2002; Srinivasan *et al*. 2006; Ngumbi *et al*. 2007; Werner *et al*. 2009). Aphid performance studies on potato suggest that crowding is a mechanism leading to dispersal, as PLRV-infected plants are better hosts for aphid vectors (Castle & Berger 1993). Other studies have shown a similar pattern for BYDV, another persistent-circulative virus (Montllor & Gildow 1986; Jiménez-Martínez *et al*. 2004a,b; Medina-Ortega *et al*. 2009). In contrast, a recent study by Mauck, De Moraes & Mescher (2010) reported a very different pattern for the NPT CMV. As in the PLRV and BYDV pathosystems, odours from CMV-infected host plants (*Cucurbita pepo*, squash) are more attractive to aphid vectors than those of sham-infected control plants; but instead of settling on the infected plants, aphids rapidly disperse after initial probes of plant tissue – during which virions are acquired (Powell *et al*. 1999; Powell, Tosh & Hardie 2006). The higher rates of aphid dispersal following contact with CMV-infected hosts are likely mediated by reduced host quality, because vector populations also increase more slowly on virus-infected plants. These results suggest that NPT viruses like CMV may deceive vectors into visiting lower-quality hosts, thus increasing virus acquisition and subsequent dispersal and virus spread (Mauck, De Moraes & Mescher 2010).

Beyond the work just discussed, a larger body of research has touched on specific host-mediated (and direct) effects of plant viruses on vector behaviour and performance, including studies documenting vector orientation, settling and feeding preferences; dispersal; nymphal development; adult fecundity; and overall survival. Our analyses explored this broader literature to evaluate our three predictions about the effects of PT and NPT viruses on vector attraction, settling and feeding behaviour, and performance.

LITERATURE SELECTION

Full details of literature selection (search terms, inclusion criteria and analysis) are provided in the supporting information for this review. Briefly, we conducted a Google Scholar search based on relevant search terms to identify literature, followed by further analyses to find additional publications that cited, or were cited by, the papers identified in our initial search. Ultimately, we identified 55 papers that examined some aspect of insect vector attraction, settling and feeding, or performance in relation to infected and healthy plants. We then parsed each study into individual experiments, each addressing a single virus

strain or isolate by host species (or cultivar) by vector interaction (224 experiments total) (Tables S1–S3; references for all papers used in the analyses are included in the supporting information). Each of the 224 experiments identified was categorized as pertaining to one of the three types of virus–host–vector effects addressed by our predictions, and results of each were categorized based on outcomes. For experiments measuring attraction or settling and feeding, results were categorized as demonstrating a vector preference for virus-infected plants, for healthy plants, or no preference. For assays of vector performance, results were categorized as indicating a positive, neutral or negative effect on vector performance. Tabulated results were evaluated for departure from an expected even distribution of effects (positive/neutral/negative or virus-infected/no-preference/healthy) using chi-square tests (see figure captions). To examine the distribution of experiments among different plant virus lineages in the categories of vector performance and vector settling and feeding preference, results were also tabulated (Table 1).

RESULTS FOR PREDICTION P1: VECTORS ARE ATTRACTED TO VIRUS-INFECTED PLANTS

We identified 30 experiments bearing on this prediction and their outcomes indicate that PT viruses consistently induce changes that enhance visitation by vectors relative to healthy hosts (Fig. 2; Table S1); however, all of the studies exploring this interaction in PT viruses focused on the Luteoviridae. For NPT viruses, only four studies were found that tested attraction preferences independent of settling or feeding preferences: Mauck, De Moraes & Mescher (2010) explicitly tested attraction based on volatile cues for two aphid vectors of CMV (*Aphis gossypii* and *Myzus persicae*) and found that winged and wingless morphs of both of these vectors preferred the cues of virus-infected plants. Eigenbrode *et al.* (2002) tested emigration over time of *M. persicae* from the headspace above plants infected with *Potato virus Y* (PVY, *Potyvirus*, Potyviridae) and found that emigration did not differ between infected and healthy plants for the first 30 min, but in the period between 30 and 50 min fewer aphids emigrated from infected plants relative to healthy plants. Fereres, Kampmeier & Irwin (1999) also examined PVY as well as the related *Soybean mosaic virus* (SMV, *Potyvirus*, Potyviridae) and found no preference based on visual changes in plant phenotype. Finally, Eckel, Randi & Lampert (1996) performed field evaluations of vector attraction to tobacco plants infected with *Tobacco etch virus* (TEV, *Potyvirus*, Potyviridae) relative to healthy plants and had consistently higher vector trap catches near TEV-infected plants.

Thus, despite few studies addressing NPT viruses and limited taxonomic diversity in studies of PT viruses, the available data suggest that virus infection of host plants either has no effect on visitation rates by vectors or enhances visitation through elicitation of a more attractive host phenotype. This is consistent with expectations from the theoretical work described above that vector attraction to virus-infected plants is generally beneficial for virus transmission given that a high initial rate of virus spread is often important for establishing infections and avoiding competition (Thresh 1974; Syller 2011). Moreover, while the number of available studies is limited, it is notable that no experiments reported virus infection-induced host changes that resulted in lower rates of visitation by vectors. This pattern seems unlikely to be an artefact of publication bias, as all three outcomes are of scientific and practical interest. We therefore view the overall pattern observed as likely reflecting selection against virus genotypes that reduce host attractiveness to vectors and at least consistent with the presence of virus adaptations that enhance vector attraction. More experiments are needed that tease apart the effects of viruses on plant size and plant odour cues, which both have implications for plant apparency to vectors. Common vectors, such as aphids, initially choose hosts largely based on visual cues while flying (using the contrast of a green plant on a dark soil background) because of their poor ability to orient or change direction (Webster 2012). Dwarfing of plants by virus infection may reduce plant apparency, while virus-induced change in plant colour e.g. yellowing may enhance visual attractiveness. In contrast to flying aphids, walking aphids make extensive use of volatile cues to locate a proper host and can discriminate among odour blends while doing so (Webster 2012). Viruses may therefore compensate for unavoidable pathological effects on plant size (and reduced apparency) by enhancing or altering odour cues that could function

Table 1. Distribution of experiments by viral families among the three categories for vector performance and for settling and feeding preference

		Effect on vector performance			Settling and feeding preference		
Virus taxon	Genome	Positive	Neutral	Negative	Infected	No Pref.	Healthy
Bunyaviridae	Negative/ambisense segmented RNA	8	6	1	13	0	0
Luteoviridae	Positive sense single stranded RNA	20	6	4	18	8	2
Geminiviridae	Single stranded circular DNA	16	5	12	3	1	0
Closteroviridae	Single stranded RNA	5	2	0	6	1	0
Comoviridae	Single stranded RNA	1	0	0	1	0	0
Sobemovirus	Single stranded RNA	1	0	0	1	0	0

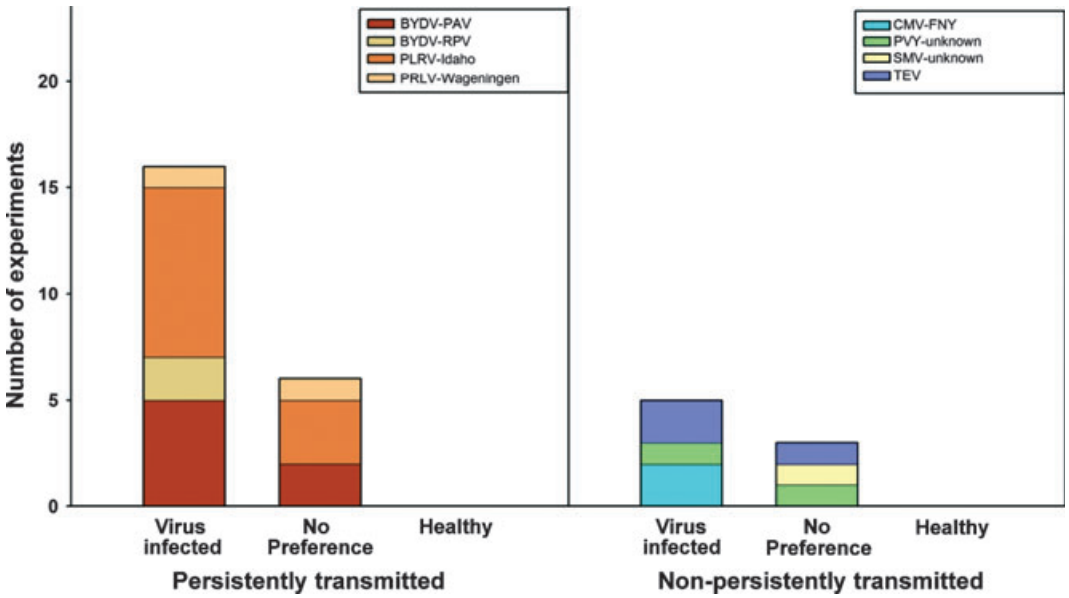

Fig. 2. Effects of viruses on vector attraction preference by transmission type. Chi-square analysis was conducted for results within each transmission type to determine whether the distribution of three possible outcomes (attraction to healthy plants, infected plants, or no discrimination) deviated from the null expectation of equal probability. Outcomes for the persistently transmitted (PT) circulative and semi-PT virus types significantly deviated from the null (χ^2 = 17·836, d.f. = 2, P = 0·0001) and were marginally non-significant for non-persistently transmitted (NPT) viruses (χ^2 = 4·769, d.f. = 2, P = 0·0921). The sample size for the NPT category was extremely low for this analysis, but a two-way analysis (preference for virus-infected + no preference vs. preference for healthy) was significant (χ^2 = 8·00, d.f. = 1, P = 0·0047). The legend indicates the pathosystem used for each experiment (see Table S1 for complete identifiers).

to attract vectors moving from plant to plant or over soil.

RESULTS FOR PREDICTION P2: VECTORS PREFER TO SETTLE AND FEED ON PLANTS INFECTED WITH PT VIRUSES

For PT viruses, and the few SPT viruses that have been examined (BPMV, SBMV, and BYV in Figs 3 and 4, Tables S2 and S3), our analyses suggest that vectors frequently show a preference for settling on virus-infected plants over healthy plants (Fig. 3; Table S2). As noted above, settling is critical for the acquisition of PT viruses because vectors must engage in sustained feeding to acquire them. This is certainly true for PT viruses that are confined to vascular tissues (e.g. phloem-borne Luteoviridae) (Hogenhout *et al.* 2008) but also for most of those more widely distributed within the plant, including the Geminiviridae and others (Bosque-Pérez 2000; Ng & Falk 2006). Although the number of experiments with NPT viruses is again limited (20 of 74 total experiments), the pattern revealed by these experiments contrasts sharply with that seen for PT viruses (Fig. 3). Most experiments addressing NPT viruses detected either a preference for settling on healthy plants or no settling preference, with only two experiments describing preferential settling on infected plants (Fig. 3). Again, this pattern is consistent with our prediction given the non-persistent mode of transmission for these viruses, where virions are acquired during brief probes of surface tissues and can be lost from the mouthparts if the vector commences long-term feeding (Martín *et al.* 1997; Wang & Ghabrial 2002).

RESULTS FOR PREDICTION P3: VECTORS PERFORM BETTER ON PLANTS INFECTED WITH PT VIRUSES AND WORSE ON PLANTS INFECTED WITH NPT VIRUSES

Our survey of available experiments indicates that PT and SPT viruses have largely positive effects on vector performance (survival, fecundity, or longevity) (Fig. 4; Table S3). While feeding on virus-infected plants is necessary to acquire the pathogen, dispersal is also necessary to induce vectors to move off of the virus-infected host *after* becoming viruliferous. For many vector species (e.g. aphids and whiteflies), crowding and consequent resource depletion lead to dispersal via several mechanisms (reviewed in Müller, Williams & Hardie 2001). Increasing vector performance may therefore be adaptive for persistent-circulative viruses (and SPT viruses) if vectors are more likely to arrest on virus-infected plants, acquire virions through sustained feeding, and reproduce at high rates, leading to rapid dispersal of virus-bearing vectors. The trend toward positive effects on vector performance is present even among PT viruses that replicate within the vector (e.g. TSWV isolates) (Fig. 4). Given that these viruses are thought to be derived from genera that are ancestrally pathogenic to insects and other animals (reviewed in Hogenhout *et al.* 2008), both positive and neutral effects on vectors can be construed as evidence of viral adaptation shaped by the mode of transmission.

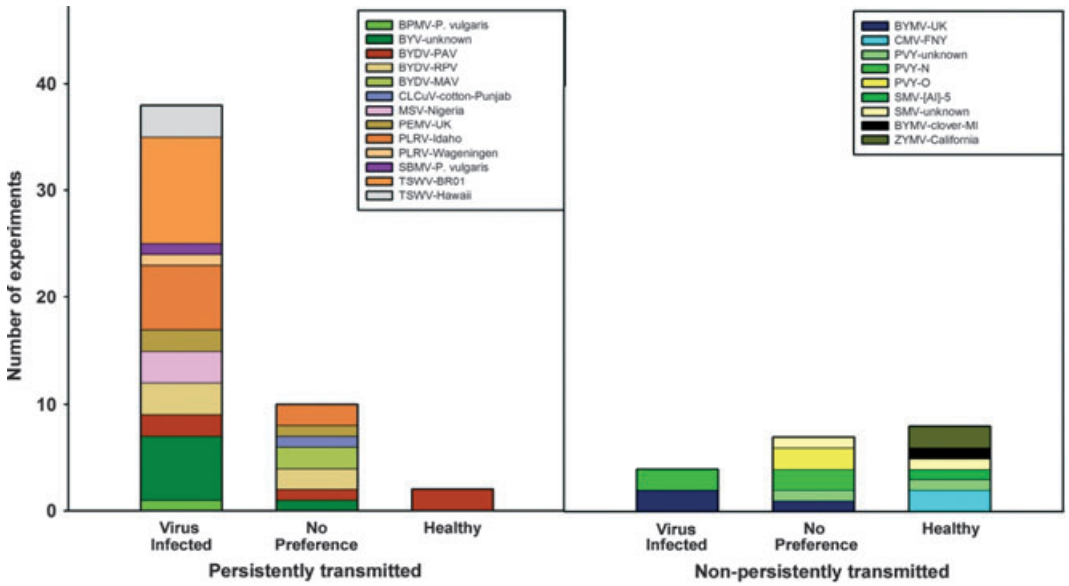

Fig. 3. Effects of viruses on vector settling and feeding behaviour by transmission type. Chi-square analysis was conducted for results within each transmission type to determine whether the distribution of three possible outcomes (settling preference for healthy, virus infected, or no discrimination) deviated from the null expectation of equal probability. Outcomes for the persistently transmitted (PT) and semi-PT virus types deviated from the null (χ^2 = 49·778, d.f. = 2, P < 0·0001) and for non-persistently transmitted (NPT) viruses did not (χ^2 = 0·695, d.f. = 2, P = 0·7064). The sample size for the NPT category was extremely low for this analysis, but a two-way analysis, (no preference + healthy preference vs. virus-infected preference) was significant (χ^2 = 5·00, d.f. = 1, P = 0·0253). The legend indicates the pathosystem used for each experiment (see Table S2 for complete identifiers).

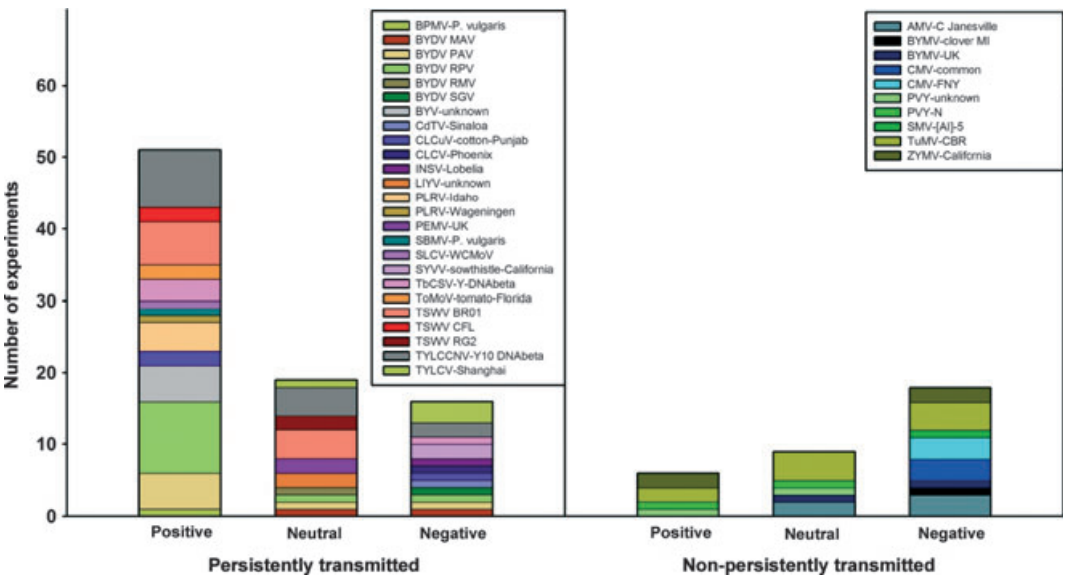

Fig. 4. Effects of viruses on vector performance by transmission type and virus × host × vector experiments. Chi-square analysis was conducted for results within each transmission type to determine whether the distribution of three possible outcomes (positive, neutral, or negative effect of infection of the host plant with a virus on the vector) deviated from the null expectation of equal probability. Outcomes for both transmission types deviated from the null [persistently transmitted (PT) χ^2 = 25·103, d.f. = 2, P < 0·0001, non-persistently transmitted (NPT) χ^2 = 7·091, d.f. = 2, P = 0·0289]. The legend indicates the pathosystem used for each experiment (see Table S3 for complete identifiers).

In contrast to our results for PT viruses, most experiments examining the performance of vectors on hosts infected with NPT viruses reported negative host-mediated effects on vectors (Fig. 4; Table S3). This suggests that NPT viruses tend to alter host chemistry or morphology in ways that reduce the likelihood of vectors colonizing infected plants. As discussed above, NPT viruses are inoculated most efficiently when vectors disperse shortly after acquiring virions rather than initiating sustained feeding. Moreover, reduced host quality is likely to increase vector

activity (movement among plants) which theoretical models generally predict to be beneficial for the spread of NPT viruses (Madden, Jeger & van den Bosch 2000).

PHYLOGENETIC PATTERNS IN VIRUS EFFECTS ON HOSTS

On the whole, our analyses support all three of our predictions, indicating that the mode of viral transmission plays a significant role in shaping virus effects on host–vector interactions. However, a significant limitation of our analyses is the existence of limited variation in transmission mechanisms within virus groups. Thus, viruses within a given taxon are not independent of one another, as their shared evolutionary history might plausibly explain their similarity in both transmission mechanism and effects on host phenotypes without the latter necessarily being caused by the former. Nevertheless, our analyses clearly indicate that individual virus taxa typically exhibit effects on host phenotypes consistent with those we would predict to be compatible with their mode of transmission. Moreover, convergence in the effects on host–vector interaction of viruses from multiple lineages that share a common transmission mechanism suggests that such effects are adaptive (Thomas, Adamo & Moore 2005), or at least that they have been shaped by selective constraints acting against the evolution of traits that are incompatible with that mode of transmission.

For PT viruses, a sufficient number of studies exist to allow us to examine patterns in the effects induced by different virus families (Table 1) that vary in their genomic organization (as indicated in the table), host-use strategies (e.g. replication site within the cell), and use of vectors as additional sites of replication (discussed below). Thus, we can examine whether families with similar transmission characteristics but otherwise different biological characteristics (and evolutionary origins) converge on a similar strategy in terms of virus-induced effects on vector feeding and performance. With regard to effects on vector performance (the interaction represented by the greatest number of studies), the Bunyaviridae, which replicate in their vectors and plant hosts, have largely positive effects (eight experiments) or neutral effects (six experiments) with only one instance of negative effects on the vector (DeAngelis, Sether & Rossignol 1993). These studies are all very similar in methodology, using laboratory assays to examine the survival and growth rate of thrips vectors on excised tissue. Notably, the study by Belliure *et al*. (2005) also demonstrates that the positive effect of virus infection in the host counteracts defences induced in response to damage by the vector (increasing performance), and the study by Wijkamp, Goldbach & Peters (1996) demonstrates that replication within the vector does not negatively affect performance, at least for one common isolate of TSWV. The Luteoviridae, persistent-circulative viruses that do not replicate in the vector, likewise show largely positive effects (20 experiments) or neutral effects (six experiments) on vector performance, with only four experiments revealing negative effects. Again, these studies employed laboratory or greenhouse assays, generally with whole plants, where researchers measured the growth rates of aphid vectors over time (either individually or as populations), and so results are comparable across studies. This group also has good representation of different strains in combination with different cultivars and species of host plants (Table S3). For geminiviridae, which are largely persistent circulative but for which some evidence exists of replication in the vector (Hogenhout *et al*. 2008), most experiments were represented among the positive-effect (16) and neutral-effect (5) categories, with the negative-effects category having 12 experiments. Experiments in this group also consist of laboratory- or greenhouse-based assays measuring vector fecundity and development. In addition, the SPT viruses examined (Closteroviridae, Comoviridae, *Sobemovirus*) also exhibited the pattern expected for viruses that benefit from longer acquisition access periods. These groups also showed similar patterns with respect to vector settling and feeding preferences. A few experiments measuring settling and feeding preference used whole plant assays (e.g. Maris *et al*. 2004), with the majority measuring preference in arenas presenting equal amounts of infected and healthy tissue (usually still attached to the plant, e.g., Castle, Mowry & Berger 1998; Eigenbrode *et al*. 2002; Srinivasan *et al*. 2006; and others, but also with detached leaves, e.g., Hodge & Powell 2008). Some experiments assessed preference by performing electrical penetration graphing to examine fine-scale feeding behaviour (e.g. Alvarez *et al*. 2007). All of these methods are common and effective ways to assess the relative palatability of tissue to an insect herbivore. Tabulation of results shows that across all PT and SPT groups, vectors exhibited a settling preference for healthy plants in only 2/54 instances, and no preference in only 10 instances (Table 1). Only single-stranded positive sense RNA viruses [Luteoviridae (PT), Bromoviridae (NPT), and Potyviridae (NPT)] have been tested with regard to virus effects on host attractiveness to vectors (Fig. 2), so there is insufficient diversity within this group to perform the same examination of attractiveness by virus classification within either transmission type.

Conclusions

Our analysis of the existing literature indicates that plant viruses exhibiting different transmission mechanisms tend to exhibit corresponding effects on vector attraction; settling and feeding; and performance. Consistent with our prediction regarding vector attraction (P1), we found that vectors are generally attracted to virus-infected hosts over non-infected hosts via virus-induced changes in host-derived cues (Fig. 2, Table S1), a preference which should act to increase the rate of virus spread. Our prediction regarding vector settling and feeding (P2) was that viruses that require sustained

feeding to be acquired by vectors (PT viruses and SPT viruses) should primarily induce phenotypes that stimulate vector settling and feeding on infected plants. Again, we found that vectors prefer to settle on plants infected with PT viruses, while NPT viruses generally either have no effect on vector settling and feeding or cause infected plants to be less preferred than healthy plants (Fig. 3, Table S2). These settling preferences are in line with our findings regarding virus effects on vector performance (P3). We found that PT viruses typically increased host quality for vectors, resulting in increased vector survival, fecundity or longevity (Fig. 4, Table S3). In contrast, NPT viruses more often decreased or had no effect on host quality.

Our analyses furthermore reveal consistent patterns of congruence between transmission mechanism and virus effects on host phenotype across highly divergent taxonomic groups of viruses (Table 1). Convergent evolution of similar phenotypic traits among unrelated taxa is commonly invoked as evidence for adaptation, and similarity in the effects induced in hosts by parasites that are transmitted in a similar manner has previously been treated as evidence of adaptive manipulation in animal parasite systems (Poulin 1995, 2010; Thomas, Adamo & Moore 2005). In a review of animal parasites vectored by blood-feeding insects, Lefévre & Thomas (2008) noted that highly divergent parasites (viruses, bacteria, *Plasmodium* spp., trypanosomes, and nematodes) induce specific behavioural changes in their vectors that alter key behaviours (e.g. vector biting rate and/or feeding duration) in ways that increases parasite transmission. Furthermore, host attractiveness is also increased relative to non-infected individuals within and across several of these groups (Lefévre & Thomas 2008). Thus, among animal-infecting parasites vectored by blood-feeding insects (a shared transmission mechanism), there is considerable convergence in adaptive manipulation strategies. To date, we have limited knowledge about the effects of plant viruses (or other plant pathogens) on host–vector interactions, but the analyses presented here, together with past findings from animal disease systems, suggest that transmission mechanisms may indeed be an important factor shaping their evolution.

Future directions

While our findings suggest that transmission mechanism is a key factor shaping virus effects on host phenotypes that mediate interactions with insect vectors, there is a great deal more to be known about the evolutionary processes that give rise to the observed patterns and about the ecological significance of these effects not only for plant–vector interactions and disease transmission, but also for broader community and ecosystem-level processes. Here, we suggest several goals for future research that build upon our current findings:

EXPAND THE NUMBER AND DIVERSITY OF PATHOSYSTEMS EXAMINED WITH REGARD TO ALL THREE ASPECTS OF VIRUS–HOST–VECTOR INTERACTIONS

Only a few pathosystems have been examined with regard to all three types of vector–host interactions described in Figs 2–4. Thus, while our survey reveals broad patterns among viruses with different transmission mechanisms, we cannot determine whether each of the viruses featured in our data set conforms to each of our predictions for the virus–host–vector interactions important for transmission. NPT viruses in particular are poorly characterized with regard to their effects on vectors, potentially because they are thought to have relatively limited interactions with vectors (although recent research has demonstrated that some of these pathogens have very specific and complex interactions with vector mouthparts, reviewed in Brault *et al.* 2010). However, these viruses are both abundant (about 42% of all insect-vectored viruses are NPT viruses) (Hogenhout *et al.* 2008) and extremely important from an economic perspective, because their rapid transmission strategy makes chemical control of vectors largely ineffective in monocultures of susceptible hosts where insecticides may instead stimulate increased movement and probing by vectors before they are killed (Roberts *et al.* 1993; Perring, Gruenhagen & Farrar 1999). It may also be useful to compare and contrast the effects of insect-vectored viruses with vertically transmitted or mechanically transmitted viruses that do not rely on insect vectors and thus would not be expected to induce similar changes in plant chemistry (e.g. as in Castle & Berger 1993; Castle, Mowry & Berger 1998; Eigenbrode *et al.* 2002). Additionally, of the studies that have been performed across both NPT and PT virus groups, few provide insight into the mechanisms by which plant viruses actually influence important aspects of vector biology and behaviour. One notable, very recent exception is a paper demonstrating that *Tomato yellow leaf curl China virus* (see Fig. 4), which, when infecting a tobacco host plant along with its satellite virus, suppresses a key phytohormone (jasmonic acid) that mediates plant defence against the vector (Zhang *et al.* 2012). Other recent work on CMV has demonstrated, using mutant viruses, that a virus-derived protein is capable of modifying phytohormones that mediate interactions between vectors and host plants (Lewsey *et al.* 2010). Therefore, in addition to expanding the scope of research to include more virus species across multiple taxonomic groups, future studies should attempt to characterize the molecular and biochemical changes that explain observed patterns. Additional work examining different isolates and strains of viruses in combination with multiple hosts and vectors would also inform our understanding of the genetic basis for pathogen effects and would provide information on the frequency with which such effects

are likely to occur given the host and vector ranges of different strains (e.g. Stumpf & Kennedy 2005, 2007).

DETERMINE HOW VIRAL MANIPULATION OF VECTOR BEHAVIOUR INFLUENCES VIRUS EPIDEMICS AND THE IMPLICATIONS FOR MODELS OF VIRUS SPREAD

Although models of plant virus spread have included parameters representing vector preference and performance (Jeger *et al*. 2004; Jeger, Madden & van den Bosch 2009), these models are not inclusive of the wide range of effects that have been observed in experimental studies. More comprehensive models will potentially reveal new (and perhaps non-intuitive) epidemiological outcomes of direct and indirect effects of viruses on vectors. Studies that also examine the behavioural and performance patterns described here, along with virus spread, on scales larger than laboratory performance and choice tests would provide empirical data to support efforts to model the effects of viruses on vectors in real-world situations. These models have clear applications for managing viruses in agricultural systems in addition to aiding our understanding of the diversity of selection pressures shaping virus evolution.

EXAMINE VIRUS–HOST–VECTOR INTERACTIONS IN NATURAL PLANT COMMUNITIES

The great majority of existing studies of virus–host–vector interactions have been performed using agriculturally relevant pathosystems (Tables S1–S3). Natural plant communities harbour an incredible diversity of plant viruses (Wisler & Norris 2005; Roossinck *et al*. 2010), many of which are cryptic species that do not cause the typical visual symptoms that we associate with well-studied agricultural pest species (for a discussion of different virus lifestyles see Roossinck 2010). Although we are just beginning to understand the diversity of viruses found in natural plant communities and how they fit into the existing taxonomic framework (Roossinck *et al*. 2010), it is likely that virus-induced changes in plant chemistry also influence interactions with vectors for these newly discovered virus species. Viruses that do not cause outward visual symptoms of disease may nevertheless cause subtle changes in the production and emission of volatile organic compounds that are important cues for vector insects, or changes in nutrients and defence chemistry that influence vector feeding.

EXAMINE GENERALIST PATHOGENS AND COMPLEX SELECTION ENVIRONMENTS

Some viruses, such as certain strains of CMV, can infect a large number of hosts within certain plant groups or families with varying degrees of infection success and severity, while other species are restricted to a limited number of hosts within one or two plant families and usually are well adapted to these hosts. Similarly, many multi-host (generalist) pathogens can be transmitted by more than one (and often many) vector species, with varying degrees of efficiency. Thus, the selection pressure on generalist pathogens may be complex, favouring variants which cause changes that are likely to positively influence vector behaviour (in terms of viral fitness) across a range of vector species (e.g. a general increase in volatile emissions to increase plant apparency, as is seen for the CMV-squash pathosystem). The ability of viral variants to influence host plant chemistry may also vary from host-to-host, so landscapes that are patchy with regard to optimal hosts may influence the frequency of 'manipulative' genotypes, or may select for variants that are specialized in influencing plant chemistry for the most abundant susceptible host. More studies are needed that employ both generalist and specialist viruses derived from different geographical areas and host backgrounds. Although our data set includes generalists, there is insufficient information to determine whether these viruses (e.g. CMV) are able to alter hosts in ways that benefit transmission across multiple host species or families. This work would provide a better understanding of how complex selection environments influence the maintenance of viral variants capable of manipulating hosts.

DETERMINE HOW VIRUS-INDUCED CHANGES IN HOST PHENOTYPE INFLUENCE (AND ARE INFLUENCED BY) OTHER PATHOGENS AND NON-VECTOR ORGANISMS

The role of other organisms, such as pathogens, non-vector herbivores, and vector natural enemies, is important to consider in studying virus effects on host behaviour in both natural and managed systems (Malmstrom, Melcher & Bosque-Pérez 2011). These organisms are important regulators of host-plant and vector populations and can also have significant impacts on vector behaviour (Chau & Mackauer 1997; Schmidt *et al*. 2003). For instance, parasitic wasps that attack aphids have been shown to induce vector dispersal and drastically increase the spread of a NPT virus to susceptible hosts adjacent to a focal infection (Hodge, Hardie & Powell 2010), but the same result was not observed for wasps attacking aphids transmitting BYDV, a PT virus (Smyrnioudis *et al*. 2001). Interactions among pathogens within a host – particularly among co-infecting viruses – may also influence virus–host–vector interactions and selection on viruses. Co-infections can sometimes result in synergistic effects that increase the transmission of both pathogens (Murphy & Kyle 1995), but are also sometimes incompatible, resulting in premature host death (Wang *et al*. 2004) or reduced titres of one of the pathogens because of competition (Power 1996). Even though other viral and non-viral pathogens, non-vector herbivores, and vector natural enemies are often abundant and ubiquitous components of plant communities affected by viruses, their roles in influencing virus spread – and selection pressure on viruses to manipulate host chemistry and vector behaviour – remain largely unexplored.

INCORPORATE THE EFFECTS OF ABIOTIC FACTORS ON HOST PLANT–VIRUS–VECTOR INTERACTIONS

Simultaneous abiotic and biotic stresses certainly influence chemically mediated multi-trophic interactions in natural settings. The phenotype of a host plant depends upon abiotic factors including light, soil nutrient and water availability, temperature regimes, and CO_2 concentrations. For example, such factors are known to influence host-derived cues that have been shown to mediate virus–vector interactions, such as the emission of volatile organic compounds from plants (Holopainen & Gershenzon 2010). Less is known about how co-occurring stress factors, including virus infections, influence plant responses to abiotic factors. The strength of direct and indirect effects of viruses on their vectors, and the evolution and maintenance of viral variants that produce these effects, will depend in part upon climatic and edaphic factors. Global changes in CO_2, ozone and temperature could alter aspects of host chemistry that are influenced by virus infection, such as emissions of volatiles from plants and the accumulation of metabolites in plant tissues (Yuan *et al.* 2009), with implications for vector behaviour and the epidemiology of vector-borne viruses. Furthermore, climate change and other impacts of human activity, such as the introduction of non-native plants, are also hypothesized to influence virus epidemiology by promoting the movement of vectors, plants and pathogens into new areas, resulting in increased incidence of novel virus–host interactions (Canto, Aranda & Fereres 2009). Emerging viral pathogens may initially be maladapted to new host environments, so these novel interactions could present a unique opportunity to track (in nature) whether there is selection for variants that alter host phenotypes in ways that preserve or enhance the probability of transmission by local or introduced vectors.

The synthesis of literature presented here thus provides a starting point for many additional avenues of research pertaining to insect vector behaviour and virus adaptation, including the elucidation of mechanisms of viral manipulation, the frequency of manipulations in natural communities, the role of patchy host plant distributions and multiple vectors, interactions involving non-vector organisms and their effects on vector behaviour and virus spread, and the influence of abiotic factors. Such work will have significant implications both for the management of plant viral diseases in agriculture and for our understanding of the ecological effects of viruses and other manipulative parasites on the functioning of natural systems.

Acknowledgements

We are grateful to numerous colleagues for stimulating discussions on the subjects addressed in this paper. Funding for our research is provided by a NSF DDIG (DEB-1011122) and a USDA-AFRI NIFA Predoctoral Fellowship (2011-67011-30655) to KEM, a USDA-CSREES-NRI grant to MCM and CDM (2008-35302-04577), a USDA-AFRI NIFA Competitive Grant No. 2009-65104-05730 to SDE and NBP and a USDA-AFRI NIFA competitive grant 2011-68002-30191 to SDE.

References

Alvarez, A.E., Garzo, E., Verbeek, M., Vosman, B., Dicke, M. & Tjallingii, W.F. (2007) Infection of potato plants with *potato leafroll virus* changes attraction and feeding behaviour of *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **125**, 135–144.

Anderson, R.M. & May, R.M. (1991) *Infectious Diseases of Humans*. Oxford University Press, Oxford, UK.

Belliure, B., Janssen, A., Maris, P.C., Peters, D. & Sabelis, M.W. (2005) Herbivore arthropods benefit from vectoring plant viruses. *Ecology Letters*, **8**, 70–79.

Bernasconi, M., Turlings, T., Ambrosetti, L., Bassetti, P. & Dorn, S. (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, **87**, 133–142.

Birkett, M.A., Agelopoulos, N., Jensen, K.M.V., Jespersen, J.B., Pickett, J. A., Prijs, H.J., Thomas, G., Trapman, J.J., Wadhams, L.J. & Woodcock, C.M. (2004) The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. *Medical and Veterinary Entomology*, **18**, 313–322.

Bosque-Pérez, N.A. (2000) Eight decades of *maize streak virus* research. *Virus Research*, **71**, 107–121.

Bosque-Pérez, N.A. & Eigenbrode, S.D. (2011) The influence of virus-induced changes in plants on aphid vectors, insights from luteovirus pathosystems. *Virus Research*, **159**, 201–205.

Brault, V., Uzest, M., Monsion, B., Jacquot, E. & Blanc, S. (2010) Aphids as transport devices for plant viruses. *Comptes Rendus Biologies*, **333**, 524–538.

Canto, T., Aranda, M.A. & Fereres, A. (2009) Climate change effects on physiology and population processes of hosts and vectors that influence the spread of Hemipteran-borne plant viruses. *Global Change Biology*, **15**, 1884–1894.

Castle, S.J. & Berger, P.H. (1993) Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. *Entomologia Experimentalis et Applicata*, **69**, 51–60.

Castle, S.J., Mowry, T.M. & Berger, P.H. (1998) Differential settling by *Myzus persicae* (Homoptera, Aphididae) on various virus-infected host plants. *Annals of the Entomological Society of America*, **91**, 661–667.

Chau, A. & Mackauer, M. (1997) Dropping of pea aphids from feeding site, a consequence of parasitism by the wasp, *Monoctonus paulensis*. *Entomologia Experimentalis et Applicata*, **83**, 247–252.

DeAngelis, J.D., Sether, D.M. & Rossignol, P.A. (1993) Survival, development, and reproduction in western flower thrips (Thysanoptera, Thripidae) exposed to impatiens necrotic spot virus. *Environmental Entomology*, **22**, 1308–1312.

Dicke, M. (2000) Chemical ecology of host-plant selection by herbivorous arthropods, a multitrophic perspective. *Biochemical Systematics and Ecology*, **28**, 601–617.

Ebbert, M.A. & Nault, L.R. (2001) Survival in *Dalbulus* leafhopper vectors improves after exposure to maize stunting pathogens. *Entomologia Experimentalis et Applicata*, **100**, 311–324.

Eckel, W., Randi, V. & Lampert, E.P. (1996) Relative attractiveness of *Tobacco etch virus*-infected and healthy flue-cured tobacco plants to aphids (Homoptera, Aphididae). *Journal of Economic Entomology*, **89**, 1017–1027.

Eigenbrode, S.D., Ding, H., Shiel, P. & Berger, P.H. (2002) Volatiles from potato plants infected with *Potato leafroll virus* attract and arrest the virus vector, *Myzus persicae* (Homoptera, Aphididae). *Proceedings of the Royal Society of London. Series B*, **269**, 455–460.

Fereres, A., Kampmeier, G.E. & Irwin, M.E. (1999) Aphid attraction and preference for soybean and pepper plants infected with potyviridae. *Annals of the Entomological Society of America*, **92**, 542–548.

Fereres, A. & Moreno, A. (2009) Behavioral aspects influencing plant virus transmission by homopteran insects. *Virus Research*, **141**, 158–168.

Gildow, F.E. (1980) Increased production of alatae by aphids reared on oats infected with *Barley yellow dwarf virus*. *Annals of the Entomological Society of America*, **73**, 343–347.

Gildow, F.E. (1983) Influence of *Barley yellow dwarf virus*-infected oats and barley on morphology of aphid vectors. *Phytopathology*, **73**, 1196–1199.

Hodge, S., Hardie, J. & Powell, G. (2010) Parasitoids aid dispersal of a non-persistently transmitted plant virus by disturbing the aphid vector. *Agricultural and Forest Entomology*, **13**, 83–88.

Hodge, S. & Powell, G. (2008) Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behavior and performance. *Environmental Entomology*, **37**, 1573–1581.

Hogenhout, S., Ammar, E., Whitfield, A. & Redinbaugh, M.G. (2008) Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*, **46**, 327–359.

Holopainen, A.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, **15**, 176–184.

Hurd, H. (2003) Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology*, **48**, 141–161.

Jeger, M.J., Madden, L.V. & van den Bosch, F. (2009) The effect of transmission route on plant virus epidemic development and disease control. *Journal of Theoretical Biology*, **258**, 198–207.

Jeger, M.J., Holt, J., van den Bosch, F. & Madden, V. (2004) Epidemiology of insect-transmitted plant viruses, modeling disease dynamics and control interventions. *Physiological Entomology*, **29**, 291–304.

Jiménez-Martínez, E.S., Bosque-Pérez, N.A., Berger, P.H. & Zemetra, R.S. (2004a) Life history of the bird cherry-oat aphid, *Rhopalosipum padi* (Homoptera, Aphididae), on transgenic and untransformed wheat challenged with *Barley yellow dwarf virus*. *Journal of Economic Entomology*, **97**, 203–212.

Jiménez-Martínez, E.S., Bosque-Pérez, N.A., Berger, P.H., Zemetra, R.S., Ding, H. & Eigenbrode, S.D. (2004b) Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera, Aphididae) to *Barley yellow dwarf virus*-infected transgenic and untransformed wheat. *Environmental Entomology*, **33**, 1207–1216.

Lacroix, R., Mukabana, W.R., Gouagna, L.C. & Koella, J.C. (2005) Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biology*, **3**, e298.

Lefévre, T. & Thomas, F. (2008) Behind the scene, something else is pulling the strings, emphasizing parasitic manipulation in vector-borne diseases. *Infection, Genetics and Evolution*, **8**, 504–519.

Lefévre, T., Koella, J.C., Renaud, F., Hurd, H., Biron, D.G. & Thomas, F. (2006) New prospects for research on manipulation of insect vectors by pathogens. *PLoS Pathogens*, **2**, e72.

Lewsey, M.G., Murphy, A.M., MacLean, D., Dalchau, N., Westwood, J. H., Macaulay, K., Bennett, M.H., Moulin, M., Hanke, D.E., Powell, G., Smith, A.G. & Carr, J.P. (2010) Disruption of two defensive signaling pathways by a viral RNA silencing suppressor. *Molecular Plant-Microbe Interactions*, **23**, 835–845.

Madden, L.V., Jeger, M.J. & van den Bosch, F. (2000) A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. *Phytopathology*, **90**, 576–594.

Malmstrom, C.M., Melcher, U. & Bosque-Pérez, N.A. (2011) The expanding field of plant virus ecology, Historical foundations, knowledge gaps, and research directions. *Virus Research*, **159**, 84–94.

Maris, P.C., Joosten, N.N., Goldbach, R.W. & Peters, D. (2004) *Tomato spotted wilt virus* infection improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathology*, **94**, 706–711.

Martín, B., Collar, J.L., Tjallingii, W.F. & Fereres, A. (1997) Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *Journal of General Virology*, **78**, 2701–2705.

Mauck, K.E., De Moraes, C.M. & Mescher, M.C. (2010) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *Proceedings of the National Academy of Sciences*, **107**, 3600–3605.

McElhany, P., Real, L.A. & Power, A.G. (1995) Vector preference and disease dynamics: a study of *Barley Yellow Dwarf Virus*. *Ecology*, **76**, 444–457.

McLeod, G., Gries, R., von Reuß, S.H., Rahe, J.E., McIntosh, R., König, W.A. & Gries, G. (2005) The pathogen causing Dutch elm disease makes host trees attract insect vectors. *Proceedings of the Royal Society of London. Series B*, **272**, 2499–2503.

Medina-Ortega, K.J., Bosque-Perez, N.A., Ngumbi, E., Jiménez-Martínez, E.S. & Eigenbrode, S.D. (2009) *Rhopalosiphum padi* (Hemiptera, Aphididae) responses to volatile cues from *Barley yellow dwarf virus*–infected wheat. *Environmental Entomology*, **38**, 836–845.

Montllor, C.B. & Gildow, F.E. (1986) Feeding responses of two grain aphids to *Barley yellow dwarf virus*-infected oats. *Entomologia Experimentalis et Applicata*, **42**, 63–69.

Müller, C.B., Williams, I.S. & Hardie, J. (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology*, **26**, 330–340.

Murphy, J.F. & Kyle, M.M. (1995) Alleviation of restricted systemic spread of pepper mottle potyvirus in *Capsicum annuum* cv. Avelar by coinfection with a Cucumovirus. *Phytopathology*, **85**, 561–566.

Nault, L.R. (1997) Arthropod transmission of plant viruses, a new synthesis. *Annals of the Entomological Society of America*, **90**, 521–541.

Ng, J.C.K. & Falk, B. (2006) Virus-vector interactions mediating non-persistent and semi-persistent transmission of plant viruses. *Annual Review of Phytopathology*, **44**, 183–212.

Ng, J.C.K. & Perry, K.L. (2004) Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, **5**, 505–511.

Ngumbi, E., Eigenbrode, S.D., Bosque-Pérez, N.A., Ding, H. & Rodriguez, A. (2007) *Myzus persicae* is arrested more by blends than by individual compounds elevated in headspace of PLRV-infected potato. *Journal of Chemical Ecology*, **33**, 1733–1747.

Palacios, I., Drucker, M., Blanc, S., Leite, S., Moreno, A. & Fereres, A. (2002) *Cauliflower mosaic virus* is preferentially acquired from the phloem by its aphid vectors. *Journal of General Virology*, **83**, 3163–3171.

Park, K.C. & Hardie, J. (2003) Electroantennogram responses of aphid nymphs to plant volatiles. *Physiological Entomology*, **28**, 215–220.

Perring, T., Gruenhagen, N. & Farrar, C. (1999) Management of plant viral diseases through chemical control of insect vectors. *Annual Review of Entomology*, **44**, 457–481.

Poulin, R. (1995) "Adaptive" change in the behaviour of parasitized animals, a critical review. *International Journal for Parasitology*, **25**, 1371–1383.

Poulin, R. (2010) Parasite manipulation of host behavior, an update and frequently asked questions. *Advances in the Study of Behavior Vol. 41* (ed. H.J. Brockmann), pp. 151–186. Academic Press, Burlington.

Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids, behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309–330.

Powell, G., Maniar, S.P., Pickett, J.A. & Hardie, J. (1999) Aphid responses to non-host epicuticular lipids. *Entomologia Experimentalis et Applicata*, **91**, 115–123.

Power, A.G. (1991) Virus spread and vector dynamics in genetically diverse plant populations. *Ecology*, **72**, 232–241.

Power, A.G. (1996) Competition between viruses in a complex plant-pathogen. *Ecology*, **77**, 1004–1010.

Power, A.G. (2008) Community ecology of plant viruses. *Plant Virus Evolution* (ed. M.J. Roossinck), pp. 15–26. Springer-Verlag, Berlin, Heidelberg.

Raybould, A.F., Maskell, L.C., Edwards, M.L., Cooper, J.I. & Gray, A.J. (1999) The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. *New Phytologist*, **141**, 265–275.

Roberts, J.M.F., Hodgson, C.J., Jackai, L.E.N., Thottappilly, G. & Singh, S.R. (1993) Interaction between two synthetic pyrethroids and the spread of two non-persistent viruses in cowpea. *The Annals of Applied Biology*, **122**, 57–67.

Roossinck, M.J. (2010) Lifestyles of plant viruses. *Philosophical Transactions of the Royal Society, Series B*, **365**, 1899–1905.

Roossinck, M.J., Saha, P., Wiley, G.B., Quan, J., White, J.D., Lai, H., Chavarría, F., Shen, G. & Roe, B.A. (2010) Ecogenomics, using massively parallel pyrosequencing to understand virus ecology. *Molecular Ecology*, **19** (Suppl. 1), 81–88.

Roy, B.A. & Raguso, R.A. (1997) Olfactory versus visual cues in a floral mimicry system. *Oecologia*, **109**, 414–426.

Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M. & Tscharntke, T. (2003) Relative importance of predators and parasitoids for cereal aphid control. *Proceedings of the Royal Society of London. Series B*, **270**, 1905–1909.

Sisterson, M.S. (2008) Effects of insect-vector preference for healthy or infected plants on pathogen spread, insights from a model. *Journal of Economic Entomology*, **101**, 1–8.

Smyrnioudis, I.N., Harrington, R., Clark, S.J. & Katis, N. (2001) The effect of natural enemies on the spread of barley yellow dwarf virus (BYDV) by *Rhopalosiphum padi* (Hemiptera, Aphididae). *Bulletin of Entomological Research*, **91**, 301–306.

Srinivasan, R., Alvarez, J.M., Eigenbrode, S.D. & Bosque-Pérez, N.A. (2006) Influence of hairy nightshade *Solanum sarrachoides* (Sendtner) and *Potato leafroll virus* (Luteoviridae, *Polerovirus*) on the host preference of *Myzus persicae* (Sulzer) (Homoptera, Aphididae). *Environmental Entomology*, **35**, 546–553.

Stumpf, C.F. & Kennedy, G.G. (2005) Effects of *Tomato spotted wilt virus* (TSWV) isolates, host plants, and temperature on survival, size, and development time of *Frankliniella fusca*. *Entomologia Experimentalis et Applicata*, **114**, 215–225.

Stumpf, C.F. & Kennedy, G.G. (2007) Effects of *Tomato spotted wilt virus* isolates, host plants, and temperature on survival, size, and development time of *Frankliniella occidentalis*. *Entomologia Experimentalis et Applicata*, **123**, 139–147.

Syller, J. (2012) Facilitative and antagonistic interactions between plant viruses in mixed infections. *Molecular Plant Pathology*, **13**, 204–216.
Sylvester, E. (1980) Circulative and propagative virus transmission by aphids. *Annual Review of Entomology*, **25**, 257–286.
Thomas, F., Adamo, S. & Moore, J. (2005) Parasitic manipulation, where are we and where should we go? *Behavioral Processes*, **68**, 185–199.
Thresh, J.M. (1974) Temporal patterns of virus spread. *Annual Review of Phytopathology*, **12**, 111–128.
Uzest, M., Gargani, D., Drucker, M., Hébrard, E., Garzo, E., Candresse, T., Fereres, A. & Blanc, S. (2007) A protein key to plant virus transmission at the tip of the insect vector stylet. *Proceedings of the National Academy of Sciences*, **104**, 17959–17964.
Wang, R. & Ghabrial, S. (2002) Effect of aphid behavior on efficiency of transmission of *Soybean mosaic virus* by the soybean-colonizing aphid, *Aphis glycines*. *Plant Disease*, **86**, 1260–1264.
Wang, Y., Gaba, V., Yang, J., Palukaitis, P. & Gal-On, A. (2004) Characterization of synergy between *Cucumber mosaic virus* and Potyviruses in cucurbit hosts. *Phytopathology*, **92**, 51–58.
Webster, B. (2012) The role of olfaction in aphid host location. *Physiological Entomology*, **37**, 10–18.
Werner, B.J., Mowry, T.M., Bosque-Pérez, N.A., Ding, H. & Eigenbrode, S.D. (2009) Changes in green peach aphid responses to *Potato leafroll virus*-induced volatiles emitted during disease progression. *Environmental Entomology*, **38**, 1429–1438.
Whitfield, A.E., Ullman, D.E. & German, T.L. (2005) Tospovirus-thrips interactions. *Annual Review of Phytopathology*, **43**, 459–489.
Wijkamp, I., Goldbach, R. & Peters, D. (1996) Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. *Entomologia Experimentalis et Applicata*, **81**, 285–292.
Williams, C.T. (1995) Effects of plant age, leaf age and virus yellows infection on the population dynamics of *Myzus persicae* (Homoptera, Aphididae) on sugarbeet in field plots. *Bulletin of Entomological Research*, **85**, 557–567.
Wisler, G. & Norris, R. (2005) Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science*, **53**, 914–917.
Wood, C.L., Byers, J.E., Cottingham, K.L., Altman, R., Donahue, M.J. & Blakeslee, M.H. (2007) Parasites alter community structure. *Proceedings of the National Academy of Sciences*, **104**, 9335–9339.
Yuan, J.S., Himanen, S., Holopainen, J.K., Chen, F. & Stewart, C.N.J. (2009) Smelling climate change: plant volatile organic compounds in changing environments. *Trends in Ecology and Evolution*, **24**, 323–331.
Zhang, X.S., Holt, J. & Colvin, J. (2000) A general model of plant-virus disease infection incorporating vector aggregation. *Plant Pathology*, **49**, 435–444.
Zhang, T., Luan, J., Qi, J., Huang, C., Li, M., Zhou, X. & Liu, S. (2012) Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Molecular Ecology*, **21**, 1294–1304.

Received 28 January 2012; accepted 22 May 2012
Handling Editor: Charles Fox

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Detailed methods for literature selection and analysis.
Table S1. References used to construct Fig. 2. virus identifiers, and pathosystems described in each reference.
Table S2. References used to construct Fig. 3. virus identifiers, and pathosystems described in each reference.
Table S3. References used to construct Fig. 4. virus identifiers, and pathosystems described in each reference.
Data S2. References used to construct Supplementary Tables 1–3 and Figs 2–4 in main article.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ANIMAL BEHAVIOUR
ECOLOGY
PLANT SCIENCES
VIROLOGY

Received
22 May 2012

Accepted
31 July 2012

Published
15 August 2012

Correspondence and requests for materials should be addressed to N.A.B.P. (nbosque@uidaho.edu)

Plant viruses alter insect behavior to enhance their spread

Laura L. Ingwell, Sanford D. Eigenbrode & Nilsa A. Bosque-Pérez

Department of Plant, Soil, and Entomological Sciences, P.O. Box 442339, University of Idaho, Moscow, ID 83844-2339, USA.

Pathogens and parasites can induce changes in host or vector behavior that enhance their transmission. In plant systems, such effects are largely restricted to vectors, because they are mobile and may exhibit preferences dependent upon plant host infection status. Here we report the first evidence that acquisition of a plant virus directly alters host selection behavior by its insect vector. We show that the aphid *Rhopalosiphum padi*, after acquiring *Barley yellow dwarf virus* (BYDV) during *in vitro* feeding, prefers noninfected wheat plants, while noninfective aphids also fed *in vitro* prefer BYDV-infected plants. This behavioral change should promote pathogen spread since noninfective vector preference for infected plants will promote acquisition, while infective vector preference for noninfected hosts will promote transmission. We propose the "Vector Manipulation Hypothesis" to explain the evolution of strategies in plant pathogens to enhance their spread to new hosts. Our findings have implications for disease and vector management.

Pathogenic and parasitic organisms interact with their hosts on a variety of cellular and organismal levels that potentially cause changes in host behavior leading to enhanced transmission[1–5]. This phenomenon led to the emergence of the "Host Manipulation Hypothesis" (HMH)[6]. The HMH and its synonyms the adaptive manipulation[7] and behavioral manipulation[8] hypotheses posit that natural selection on the parasite or pathogen has favored the capacity to elicit host behavior that enhances their transmission. Although examination of the HMH has progressed from descriptive studies to investigations of the mechanisms through which parasites affect host behavior and their consequences for parasite spread[9,10], the field remains predominantly focused on animal pathosystems.

Pathogens or parasites can influence the behavior not only of their primary hosts, but also of their vectors. Arthropods are important vectors of both animal and plant pathogens, transmitting thousands of species of pathogens, including viruses, bacteria, phytoplasmas, trypanosomes and Plasmodia[2,11]. The effects of pathogens on vector biology and behavior have been documented in several pathosystems, including those associated with important human diseases such as malaria, leishmaniasis and sleeping sickness[2,5]. The observed changes in vector behavior include those related to pathogen transmission. For example, mosquitoes infected with the malaria parasite exhibit increased biting frequency and increased attraction to humans infected with the gametocytes of the parasite compared to noninfected humans[1,3].

In contrast to animal pathosystems, plant pathosystems have been less well studied for evidence of host or vector manipulation by pathogens[12]. While animal pathogens can alter the behavior of both hosts and vectors in ways that increase frequency of host-host or host-vector encounters[2,4,5], in plant pathosystems the host is sessile, so the potential for behavioral manipulation is restricted to the vector, the mobile component in these systems. Furthermore, unlike animal pathogens most plant pathogens, including the majority of plant viruses, do not replicate within the vector, so these vectors are not pathogen hosts, *sensu stricto*.

We previously demonstrated that *Barley yellow dwarf virus* (BYDV) infecting wheat and *Potato leafroll virus* (PLRV) infecting potato indirectly induce changes in the host selection behavior of their respective principal aphid vectors, *Rhopalosiphum padi* and *Myzus persicae*[13–16]. We also have shown that plants infected with these viruses have altered volatile organic compound profiles that elicit greater settling of or arrestment by their noninfective vectors[13,14,16,17]. Luteoviruses (viruses in the family Luteoviridae), including BYDV and PLRV are persistently transmitted. They are ingested and pass through the midgut or hindgut into the hemocoel, eventually associating with the accessory salivary glands of the vector[18]. These viruses rely almost exclusively on insect vectors for transmission and require sustained feeding by a vector for their successful acquisition and transmission[19]. After acquisition, the insect remains a vector for life. Although they do not replicate within the vector, persistently-transmitted viruses interact with the vector at the cellular level during movement among tissues and organs[20], with the potential to directly alter vector physiology and behavior.

Preferential settling by vectors onto infected plants, as occurs for BYDV and PLRV, could contribute to enhanced pathogen spread. Models indicate that a preference for infected plants will accelerate pathogen spread, but only when infected plants are rare, not when they are prevalent in a plant population[21]. Conditional vector preference, however, could enhance pathogen spread regardless of the prevalence of infected plants. Specifically, if noninfective vectors prefer infected plants thereby promoting acquisition, and infective vectors prefer noninfected hosts promoting transmission, overall spread would be accelerated. The possibility of conditional vector preference for pathogen-infected plants has hardly been examined despite its potential importance. Changes in vector behavior that occur after feeding on virus-infected plants could be attributed to direct effects of the acquired virus on the vector, but such direct effects are difficult to distinguish from indirect ones associated with feeding on virus-infected plants. Here we test the hypothesis that a change in host plant selection behavior by an insect vector is the direct result of virus acquisition by the vector. We provide the first experimental evidence that acquisition of a plant virus through *in vitro* feeding, which eliminates indirect effects of an infected plant host, directly alters subsequent host plant selection behavior of its vector. These findings enhance our understanding of how plant viruses spread to new hosts, with implications for disease and vector management.

Results

We first examined host plant selection preferences of infective (reared on virus-infected plants) and noninfective (reared on virus-free plants) *R. padi*. In dual-choice bioassays using an arena in a platform[22] (Fig. 1) infective or noninfective insects were allowed to select BYDV-infected or sham-inoculated wheat plants as their hosts. Sham-inoculated plants are noninfected plants previously fed upon by noninfective aphids and are utilized in our bioassays to account for potential aphid feeding-induced changes in plants[23]. Infective and noninfective insects were tested simultaneously in separate platforms. Each platform contained a leaf from each plant treatment, BYDV-infected or sham-inoculated, onto which aphids could settle and feed throughout the bioassay. We compared the responses of infective and noninfective aphids by examining the proportion of aphids that settled on BYDV-infected or sham-inoculated plants every 12 h for 72 h. A 72-h time period is sufficiently long for virus acquisition by noninfective aphids to occur when exposed to BYDV-infected plants, while a 12-h time period is unlikely to result in noninfective aphids becoming infective due to the latent period of the virus[24,25]. We therefore compared aphid responses at the first 12-h observation, and after 72 h when responses were pooled over time. The 12-h observation occurs before additional virus acquisition was expected while the 72-h comparison is more powerful statistically and incorporates a time period more meaningful for transmission dynamics in the field. Noninfective aphids significantly preferred to settle on BYDV-infected wheat compared to infective aphids at the first 12-h observation point (generalized linear model; $\chi^2 = 3.12$, $p = 0.0774$, marginally significant) (Fig. 2a, Supplementary Table S1a) and throughout the duration of the experiment (generalized linear model; $\chi^2 = 19.33$, $p < 0.0001$) (Fig. 2b, Supplementary Table S2a). In contrast, infective aphids significantly preferred to settle on sham-inoculated wheat

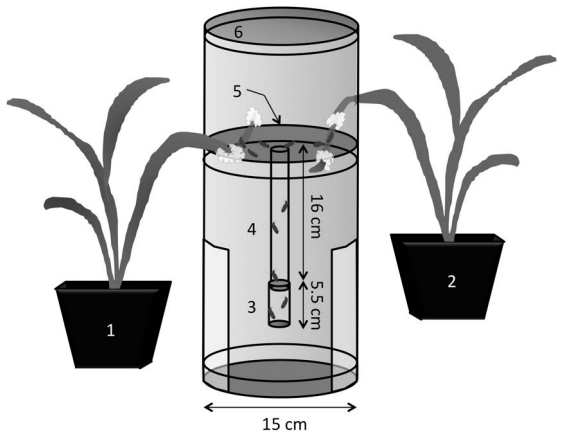

Figure 1 | Diagrammatic illustration of the dual-choice bioassay arena used in experiments. Adapted from Castle et al.[22]. 1, BYDV-infected wheat; 2, sham-inoculated wheat; 3, vial (5.5 x2.5 cm; Lx D) initially containing 50 aphids; 4, tube (16x2.5 cm; LxD); 5, platform (15 cm; D); 6, lid enclosing the arena.

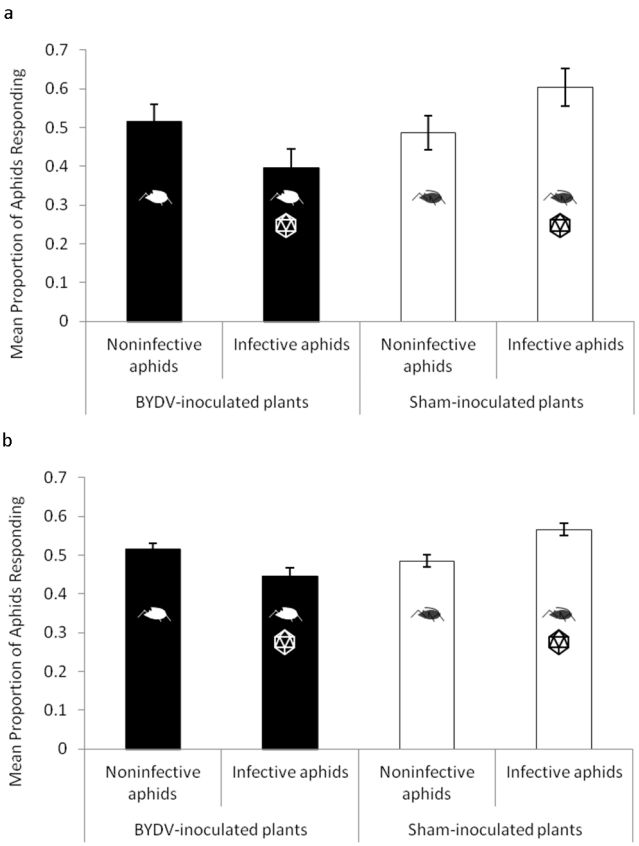

Figure 2 | Mean proportion of infective and noninfective aphids responding in a dual-choice bioassay examining host plant selection preferences to BYDV-infected and sham-inoculated wheat (noninfected plants previously fed upon by noninfective aphids) as influenced by indirect effects of feeding on virus-infected plants. Each replicate ($n = 12$) consisted of one arena with noninfective aphids paired with one arena of infective aphids, randomized in a complete block design over time. Statistical analyses compared the response of infective and noninfective aphids to the BYDV-infected or sham-inoculated plant treatment. (a) Aphid responses at the first observation point made 12 h after release. Noninfective aphids preferred BYDV-infected wheat compared to infective aphids (generalized linear model; $\chi^2 = 3.12$, $p = 0.0774$, marginally significant). Infective aphids preferred sham-inoculated plants compared to noninfective aphids (generalized linear model; $\chi^2 = 3.12$, $p = 0.0774$, marginally significant). (b) Aphid responses pooled over time (6 observations). Noninfective aphids significantly preferred BYDV-infected wheat compared to infective aphids (generalized linear model; $\chi^2 = 19.33$, $p < 0.0001$). Infective aphids significantly preferred sham-inoculated plants compared to noninfective aphids (generalized linear model; $\chi^2 = 20.14$, $p < 0.0001$). Data are means ± SE following logit transformation. Errors bars are s.e.m.

compared to noninfective aphids at the first observation point (generalized linear model; $\chi^2 = 3.12$, $p = 0.0774$, marginally significant) (Fig. 2a, Supplementary Table S1b) and throughout the duration of the experiment (generalized linear model; $\chi^2 = 20.14$, $p < 0.0001$) (Fig. 2b, Supplementary Table S2b). The time at which the observations were made was not a significant factor when examining the response to BYDV-infected wheat (generalized linear model; $\chi^2 = 4.96$, $p = 0.4203$) (Supplementary Table S2a) or sham-inoculated wheat (generalized linear model; $\chi^2 = 2.15$, $p = 0.8282$) (Supplementary Table S2b). The results suggest that virus acquisition changes vector host plant selection behavior to favor noninfected plants rather than infected plants.

These behavioral changes could result either from direct effects of acquired virus particles on the aphid, or from insect exposure to cues from virus-infected host plants. To isolate potential direct effects of virus acquisition on the vector we conducted a similar experiment using *in vitro* feeding to obtain infective and noninfective aphids. Insects were first reared on virus-free plants and subsequently transferred to membrane feeding chambers[26] (Fig. 3) that contained artificial phloem with either purified BYDV particles or no virus. Host plant selection preferences of infective and noninfective insects were examined every 12 h for 72 h using an arena as described above. Observation time was not a significant factor when examining the response to BYDV-infected wheat (generalized linear model; $\chi^2 = 2.41$, $p = 0.7906$) (Supplementary Table S2c) or sham-inoculated wheat (generalized linear model; $\chi^2 = 3.66$, $p = 0.5995$) (Supplementary Table S2d). We present the results of the aphid responses at the first 12-h observation point as well as the responses pooled over time. Noninfective aphids significantly preferred BYDV-infected wheat compared to infective aphids at the first observation point (generalized linear model; $\chi^2 = 4.24$, $p = 0.0394$) (Fig. 4a, Supplementary Table S1c), and throughout the duration of the experiment (generalized linear model; $\chi^2 = 16.18$, $p < 0.0001$) (Fig. 4b, Supplementary Table S2c). Similar to the patterns obtained using aphids that acquired virus from plants, infective aphids significantly preferred sham-inoculated wheat compared to noninfective aphids at the first observation point (generalized linear model; $\chi^2 = 5.64$, $p = 0.0176$) (Fig. 4a, Supplementary Table S1d), and throughout the duration of the experiment (generalized linear model; $\chi^2 = 16.32$, $p < 0.0001$) (Fig. 4b, Supplementary Table S2d).

Results from RT-PCR tests verified that our inoculation and acquisition methods were successful (See Supplementary Figure S1). All plants used in the dual choice tests were tested via RT-PCR immediately after the bioassays. Sham-inoculated plants remained virus-free and infected plants remained BYDV-infected, indicating that during the bioassays (72 h) the plant treatments were stable, despite being exposed to potential feeding by infective aphids. Tests of aphids using RT-PCR revealed that infective aphids remained BYDV-infective subsequent to the bioassay, while 25% of noninfective aphids acquired BYDV during the 72-h bioassay when they had access to BYDV-infected plants in the bioassay arena. Although the bioassay design unavoidably results in virus acquisition by some noninfective aphids, the result is a more conservative test of our hypothesis since within-bioassay virus acquisition should tend to diminish detectable differences between the aphid treatments.

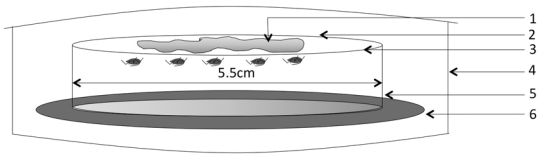

Figure 3 | Diagrammatic illustration of a membrane feeding chamber. 1, artificial diet solution (100 µL); 2, upper layer of Parafilm®; 3, bottom layer of Parafilm®; 4, humid chamber; 5, petri dish (5.5 cm; D); 6, moist filter paper.

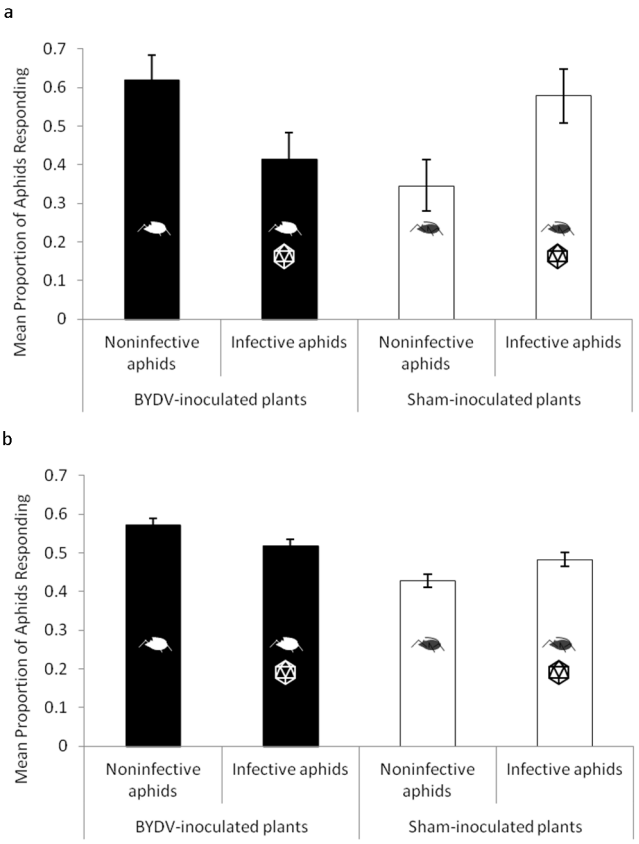

Figure 4 | Mean proportion of infective and noninfective aphids responding in a dual-choice bioassay examining host plant selection preferences to BYDV-infected and sham-inoculated wheat plants as influenced by direct effects of virus acquisition following membrane feeding. Each replicate (n = 12) consisted of one arena with noninfective aphids paired with one arena of infective aphids, randomized in a complete block design over time. Statistical analyses compared the response of infective and noninfective aphids to the BYDV-infected or sham-inoculated plant treatment. (a) Aphid responses at the first observation point made 12 h after release. Noninfective aphids significantly preferred BYDV-infected wheat compared to infective aphids (generalized linear model; $\chi^2 = 4.24$, $p = 0.0394$). Infective aphids significantly preferred sham-inoculated wheat compared to noninfective aphids (generalized linear model; $\chi^2 = 5.64$, $p = 0.0176$). (b) Aphid responses pooled over time (6 observations). Noninfective aphids significantly preferred BYDV-infected wheat compared to infective aphids (generalized linear model; $\chi^2 = 16.18$, $p < 0.0001$). Infective aphids significantly preferred sham-inoculated wheat compared to noninfective aphids (generalized linear model; $\chi^2 = 16.32$, $p < 0.0001$). Data are means ± SE following logit transformation. Errors bars are s.e.m.

Furthermore, the aphid responses after 72 h in the bioassay arena are consistent with the preferences observed after 12 h, during which time noninfective aphids almost certainly remained noninfective[24,25]. The lack of BYDV infection of the sham-inoculated plants after 72 h of exposure to initially noninfective aphids in an arena with BYDV-infected plants also indicates that these aphids did not become infective during the bioassay.

Discussion

Assays utilizing membrane-fed infective aphids yielded results similar to those obtained using aphids that acquired BYDV from infected plants, confirming our hypothesis that changes in host plant selection by the vector are mediated by direct effects of virus acquisition, rather than indirect effects of feeding on infected host plants.

Direct effects of virus acquisition on the vector host plant selection behavior in a manner that will promote the spread of the virus is consistent with an evolved strategy in the pathogen of manipulation of its vector. We propose the "Vector Manipulation Hypothesis" (VMH) to explain the evolution of strategies in plant pathogens that enhance their spread to new hosts through their effects on mobile vectors. Selection should favor both direct and indirect mechanisms producing such effects. Vectors that feed on virus-infected host plants exhibit faster growth rates, higher fecundity, greater longevity and/or enhanced production of alate forms of the vector[27–33], which can lead to increased virus spread and are typically attributed to indirect effects of virus infection on host quality. Virus- infection-mediated alterations of the host plant's secondary chemistry can affect vector behavior. Evidence for such indirect effects of pathogens on vector behavior continues to accumulate and is consistent with the VMH[13–16,34–36]. We provide the first evidence for a direct effect of a plant virus on its vector consistent with the VMH, specifically by influencing the vector's host selection behavior to maximize pathogen spread. In our model pathosystem, noninfective vectors are attracted to virus-infected host plants, which is beneficial as it increases vector fitness[23]. After virus acquisition virus vector preferences shift to noninfected hosts, maximizing pathogen transmission potential by promoting the movement of infective aphids onto non-infected host plants. Our results offer a specific example of a plant virus directly manipulating its vector in a manner that is likely to maximize pathogen transmission potential between hosts, providing support for the VMH.

Results supportive of the VMH also have been reported from work on nonpersistently-transmitted plant viruses examining effects on noninfective vector behavior. Non-persistently transmitted viruses bind transiently to insect mouthparts[20] and interactions in these pathosystems are likely limited to indirect effects on vectors. Recent work with the non-persistently transmitted *Cucumber mosaic virus* (CMV), which is acquired rapidly during aphid feeding and benefits from rapid vector dispersal, showed that aphids are initially attracted to volatile organic compounds from CMV-infected squash plants, but subsequently prefer to colonize noninfected plants[34]. Attraction to CMV-infected plants appears to be mediated by their increased emission of volatile organic compounds similar to those from healthy plants. Since CMV can be acquired within a few seconds by an aphid probing on an infected plant, these behaviors can act to enhance virus spread[34] and illustrate manipulation of an insect vector by the virus. Interestingly, in addition to manipulating vectors, CMV also may manipulate defensive signaling pathways in plants that could result in enhanced vector survival[37].

Our findings highlight the ecological and evolutionary significance of vector manipulation by pathogens and parasites. Effects like those we document for a plant virus, consistent with the VMH, may be widespread since direct and indirect mechanisms that enhance the spread of plant viruses should be favored by natural selection. Furthermore, similar patterns in behavioral changes among vectors of other plant pathogens, such as bacteria and phytoplasmas, which are limited to sessile plant hosts, might also occur. Although our results do not address the specific cellular and molecular mechanisms mediating direct plant virus effects on their vectors, they offer strong quantitative evidence for the VMH, providing a foundation upon which to base further studies of pathogen-mediated manipulation of their vectors and the identification of underlying mechanisms. The evolution of host-vector interactions has recently been suggested to be in part, mediated by virus transmission mechanisms[38] underlying the importance of studying such interactions. Greater understanding of host plant-virus-vector interactions has the potential to improve management of vectors and plant diseases in agricultural settings and enhance our understanding of the role plant viruses play in natural settings[39], including their effects on ecological processes at the community and ecosystem levels[38].

Methods

Virus maintenance and insect rearing. The model system for our study was the wheat-*R. padi*-BYDV pathosystem. BYDV is exclusively transmitted among *Poaceae* hosts by aphids, including *R. padi*, in a persistent circulative manner and the virus does not replicate within the vector[40,41]. A Washington State isolate of BYDV-PAV maintained by mass transfer of *R. padi,* the bird-cherry oat aphid, on cv. Sprinter barley plants was used to inoculate wheat plants[23]. *Rophalosiphum padi* is the most efficient vector of the BYDV-PAV serotype[40]. Both the virus and an infective colony of *R. padi* are maintained at the University of Idaho (UI) Agricultural Biotechnology Laboratory. Aphids were originally obtained from Washington State University and are kept virus-infective through serial transfer[23]. A noninfective colony of *R. padi* was derived from the infective colony and is maintained at the UI Manis Entomological Laboratory. Infective and noninfective aphid colonies are reared on Sprinter barley in environmental chambers (20±2°C; 16 h light photoperiod). Aphids from each colony are examined on a regular basis using RT-PCR tests to ensure that the respective colonies remain virus-free or BYDV-PAV infected (see Supplementary Methods).

Plant rearing and inoculation. Winter wheat cv. Lambert was used for all bioassays. Seeds were planted at a density of one per pot in 10.2 cm^2 plastic pots. Pots were filled with a mixture of 6:1:0.02 ratio of Sunshine mix #1: sand: Osmocote®, placed on trays in an environmental chamber (20±2°C; 16 h light photoperiod) and bottom watered. After germination, plants were fertilized using a soluble N-P-K fertilizer (15:30:15) biweekly.

Plant inoculations were done at the 2–3 leaf stage (14–16 days after planting). BYDV-infected plants were obtained by caging 10 adult aphids from the infective colony per plant for a 72 h virus inoculation access period[23]. Cages consisted of a 4-cm long piece of 23 mm dialysis tubing (14.6 mm D, Spectra/Por®) sealed on both ends with a foam stopper. Since BYDV is exclusively insect-transmitted, all BYDV-infected plants are fed-upon by aphids. Insect feeding may induce resistance in plants and potentially affect the response of insects subsequently exposed to such plants[23]. To account for such potential confounding effects sham-inoculated plants were produced and served as virus-free controls. Sham-inoculation was conducted by caging 10 adult aphids from the noninfective colony per plant for 72 h[23].

Infective and noninfective aphid handling. To examine effects of plant virus acquisition from infected plants on host selection behavior, apterous aphids (fourth nymphal stage to early adults) originated from the respective infective and noninfective colonies. Previous research in this pathosystem has focused on aptera[14,15,23,25]. While alates are important vectors for long-distance dispersal events, apterous aphid behavior can be used to predict severity of epidemics within a field once the virus and vector are established[21]. Future studies will examine alate behavior in response to BYDV-infection. Aphids for each treatment were individually removed from plants using a number 3 camel's hair brush and placed into vials (2.3x5.5 cm; DxH). Fifty aphids were placed per vial. Vials were capped and aphids starved for one hour prior to the bioassay. A total of 600 aphids of each treatment (infective and noninfective) were tested among 12 replicates of the dual-choice bioassay described below.

Aphids for the experiment to assess the direct effects of virus acquisition originated from the noninfective *R. padi* colony. Tests were conducted using membrane feeding chambers modified after Trębicki et al.[26] (Fig. 3), containing artificial diet as described by Ramsey and Jander[42]. After preparation the diet solution was sieved using a bacteria-proof filter (0.2 μm cellulose acetate). To set up membrane feeding chambers, the bottom halves of glass petri dishes (5.5 cm; D) were first sterilized under UV light for 10 min. Aphids (fourth nymphal stage to early adults) were collected from colony plants using a number 3 camel's hair brush and placed in the petri dishes, 35 aphids per dish. Parafilm®, sterilized with 70% ethanol, was used as membrane material. After placing the aphids in the petri dish, the dish was immediately sealed with a layer of Parafilm® stretched tightly across the dish top. After all dishes were sealed with the first Parafilm® layer 100 μL of artificial diet was pipetted onto the membrane and a second layer of Parafilm® was stretched tightly to sandwich the diet[26]. The diet was then spread across the surface of the membrane by applying pressure to the top layer with a fingertip. Dishes were placed in a tray with moistened filter paper and the tray covered with cling wrap and placed inside an environmental chamber (20±2°C; 16 h light photoperiod) for 24 h. Non-infective aphids were fed on an amino acid and sucrose diet solution. To obtain infective aphids, insects were fed on the same diet solution that was infused with purified BYDV at a concentration of 100 μg/mL. Virus was purified following a method adapted from Hammond et al.[43], and obtained from Dr. Alex Karasev, UI PSES Department. After a 24-h feeding period, aphids were transferred into a vial as described above, starved for one hour and released in a bioassay arena (see below). A total of 840 aphids of each treatment were placed in membrane chambers, 600 were tested among 12 replicates of the dual-choice bioassay described below. The remaining aphids were stored in 70% ethanol at −20°C to verify their status (infective vs. noninfective). Virus-infection status of plants and aphids was determined using RT-PCR (See Supplementary Methods and Supplementary Figures S1–S2).

We recognize that purified virus may contain phloem proteins. Such proteins have been reported to occur *in vivo*, and were recently reported to play a role in virus transmission[44]. Additional studies are required to determine if a virus-plant protein complex is present *in vitro* and if such a complex could contribute to behavioral changes in vectors.

Bioassays to assess aphid preferences. Dual-choice bioassays were performed 40–46 days after plant inoculation, utilizing an arena adapted from Castle et al.[22] (Fig. 1). The base of the arena was glued into the lid portion of a 15 cm D petri dish. The platform of the arena consisted of the inverted bottom of the petri dish with a 2.5 cm D hole cut in the center. A clear plastic tube (16x2.5 cm; LxD) was inserted into the bottom of the dish and secured with glue. The arena was wrapped in a heavy weight mylar frame (30.5x46.1 cm; WxL) to add stability to the structure. Holes were cut in the mylar, four (2 cm; D) equally spaced around the top and two (8x8 cm^2) in the bottom to access the arena. One leaf still attached to the plant from each treatment (BYDV-infected and sham-inoculated) was inserted through holes on either side of the arena and held in place with a cotton seal. A vial (5.5x2.5 cm; LxD) containing 50 aphids, starved for one hour, was inserted into the bottom of the plastic tube leading to the arena. Apterous infective and noninfective aphids were released simultaneously into separate arenas. Aphids crawled up the tube and emerged onto a platform with one leaf from each treatment on either side (3 cm on either side of where aphids entered the arena). Aphids were able to settle on, feed and move between the two leaves. Aphids were released at the start of a dark period and monitored every 12 h (alternating dark and light times) for a 72-h period. The number of aphids on each leaf was counted at each observation, using a red light when monitoring during the dark cycles[14]. Assays were conducted in a growth room (14±3°C; 12 h light photoperiod). One replicate consisted of an arena containing infective aphids paired with another arena containing noninfective aphids, constituting a single block. Twelve replicates were performed across time in a randomized complete block repeated measures design.

Data analysis. The proportion of aphids responding to either the BYDV-infected or sham-inoculated plant treatment was compared using a generalized linear model assuming a binomial distribution and logit transformation (SAS, Proc Genmod). Logit transformation was performed to stabilize the variance and meet the assumptions of normality for analysis. Aphids not located on either plant leaf in an arena were considered non responsive and excluded from the analysis. The partial model examined the main effects of replicate (block; $n = 12$) and aphid treatment (infective or noninfective). The analysis was conducted separately four times, once for each plant treatment (BYDV-infected or sham-inoculated) for the indirect effects experiment (aphids reared on noninfected plants or virus-infected plants) (Supplemental Table S1a-b) and the direct effects experiment (aphids fed on membrane chambers with or without virus) (Supplemental Table S1c-d). The full model examined the main effects of replicate, aphid treatment and time ($n = 6$) assuming a compound symmetric correlation. The time variable examined observations made at 12, 24, 36, 48, 60 and 72 h after release using a repeated measures design. Observations made at 12, 36, and 60 h were recorded in the dark. Light and dark observations were examined with the model separately and no significant interactions were observed, thus results were pooled in the overall analysis (Supplemental Table S2). All statistical tests (likelihood ratio χ^2) were carried out at the alpha = 0.05 level of significance.

1. Koella, J. C., Sorensen, F. L. & Anderson, R. A. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **265**, 763–768 (1998).
2. Hurd, H. Manipulation of medically important insect vectors by their parasites. *Annu. Rev. Entomol.* **48**, 141–161 (2003).
3. Lacroix, R., Mukabana, W. R., Gouagna, L. C. & Koella, J. C. Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biol* **3**, e298 (2005).
4. Vyas, A., Kim, S. K., Giacomini, N., Boothroyd, J. C. & Sapolsky, R. M. Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 6442–6447 (2007).
5. Lefèvre, T. & Thomas, F. Behind the scene, something else is pulling the strings: Emphasizing parasitic manipulation in vector-borne diseases. *Infect., Genet. Evol.* **8**, 504–519 (2008).
6. Holmes, J. C. & Bethel, W. M. in *Behavioral Aspects of Parasite Transmission* (eds Canning, E. U. & Wright, C. A.) 123149– (Academic Press, 1972).
7. Poulin, R. Manipulation of host behaviour by parasites: a weakening paradigm? *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **267**, 787–792 (2000).
8. Thomas, F., Adamo, S. & Moore, J. Parasitic manipulation: where are we and where should we go? *Behav. Processes* **68**, 185–199 (2005).
9. Poulin, R. "Adaptive" changes in the behaviour of parasitized animals: A critical review. *Int. J. Parasitol.* **25**, 1371–1383 (1995).
10. Moore, J. & Gotelli, N. J. in *Parasitism and Host Behaviour* (eds Barnard, C. J. & Behnke, J. M.) 193–233 (Taylor & Francis Ltd., 1990).
11. Gray, S. M. & Banerjee, N. Mechanisms of arthropod transmission of plant and animal viruses. *Microbiol. Mol. Biol. Rev.* **63**, 128–148 (1999).
12. Bosque-Pérez, N. A. & Eigenbrode, S. The influence of virus-induced changes in plants on aphid vectors: Insights from luteovirus pathosystems. *Virus Res* **159**, 201–205 (2011).
13. Eigenbrode, S. D., Ding, H., Shiel, P. & Berger, P. H. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **269**, 455–460 (2002).
14. Jiménez-Martínez, E. S. *et al.* Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to *Barley yellow dwarf virus*-infected transgenic and untransformed wheat. *Environ. Entomol.* **33**, 1207–1216 (2004).
15. Medina Ortega, K. J., Bosque-Pérez, N. A., Ngumbi, E., Jiménez-Martínez, E. S. & Eigenbrode, S. D. *Rhopalosiphum padi* (Hemiptera: Aphididae) responses to volatile cues from *Barley yellow dwarf virus*-infected wheat. *Environ. Entomol.* **38**, 836–845 (2009).
16. Werner, B. J., Mowry, T. M., Bosque-Pérez, N. A., Ding, H. & Eigenbrode, S. Changes in green peach aphid responses to potato leafroll virus-induced volatiles emitted during disease progression. *Environ. Entomol.* **38**, 1429–1438 (2009).
17. Ngumbi, E., Eigenbrode, S. D., Bosque-Pérez, N. A., Ding, H. & Rodriguez, A. *Myzus persicae* is arrested more by blends than by individual compounds elevated in headspace of PLRV-infected potato. *J. Chem. Ecol.* **33**, 1733–1747 (2007).
18. Gildow, F. Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. *Phytopathology* **83**, 270–277 (1993).
19. Sylvester, E. S. Circulative and propagative virus transmission by aphids. *Annu. Rev. Entomol.* **25**, 257–286 (1980).
20. Nault, L. R. Arthropod transmission of plant viruses: A new synthesis. *Ann. Entomol. Soc. Am.* **90**, 521–541 (1997).
21. McElhany, P., Real, L. A. & Power, A. G. Vector preference and disease dynamics: A study of *Barley yellow dwarf virus*. *Ecology* **76**, 444–457 (1995).
22. Castle, S. J., Mowry, T. M. & Berger, P. H. Differential settling by *Myzus persicae* (Homoptera: Aphididae) on various virus infected host plants. *Ann. Entomol. Soc. Am.* **91**, 661–667 (1998).
23. Jiménez-Martínez, E. S., Bosque-Pérez, N. A., Berger, P. H. & Zemetra, R. S. Life history of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae) on transgenic and untransformed wheat challenged with *Barley yellow dwarf virus*. *J. Econ. Entomol.* **97**, 203–212 (2004).
24. Gray, S. M., Power, A. G., Smith, D. M., Seaman, A. J. & Altman, N. S. Aphid transmission of *Barley yellow dwarf virus*: acquisition access periods and virus concentration requirements. *Phytopathology* **81**, 539–545 (1991).
25. Jiménez- Martínez, E. S. & Bosque-Pérez, N. A. Variation in *Barley yellow dwarf virus* transmission efficiency by *Rhopalosiphum padi* (Homoptera: Aphididae) after acquisition from transgenic and non-transformed wheat genotypes. *J. Econ. Entomol.* **97**, 1790–1796 (2004).
26. Trębicki, P., Harding, R. M. & Powell, K. S. Anti-metabolic effects of *Galanthus nivalis* agglutinin and wheat germ agglutinin on nymphal stages of the common brown leafhopper using a novel artificial diet system. *Entomol. Exp. Appl.* **131**, 99–105 (2009).
27. Kennedy, J. S. Benefits to aphids from feeding on galled and virus-infected leaves. *Nature* **168**, 825–826 (1951).
28. Baker, P. F. Aphid behavior on healthy and on yellow virus infected sugar beet. *Ann. Appl. Biol.* **48**, 384–391 (1960).
29. Gildow, F. E. Increased production of alatae by aphids reared on oats infected with *Barley yellow dwarf virus*. *Ann. Entomol. Soc. Am.* **73**, 343–347 (1980).
30. Hodgson, C. J. Effects of infection with the cabbage black ringspot strain of turnip mosaic virus on turnip as a host to *Myzus persicae* and *Brevicoryne brassicae*. *Ann. Appl. Biol.* **98**, 1–14 (1981).
31. Ajayi, O. & Dewar, A. M. The effect of *Barley yellow dwarf virus* on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. *Ann. Appl. Biol.* **103**, 1–11 (1983).
32. Araya, J. E. & Foster, J. E. Laboratory study on the effects of *Barley yellow dwarf virus* on the life cycle of *Rhopalosiphum padi* (L.). *J. Plant Dis. Protect.* **94**, 195–198 (1987).
33. Fereres, A. & Moreno, A. Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Res.* **141**, 158–168 (2009).
34. Mauck, K. E., De Moraes, C. M. & Mescher, M. C. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 3600–3605 (2010).
35. Mann, R. S. *et al.* Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. *PLoS Path.* **8**, e1002610 (2012).
36. Stafford, C. A., Walker, G. P. & Ullman, D. E. Infection with a plant virus modifies vector feeding behavior. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 9350–9355 (2011).
37. Ziebell, H. *et al.* Cucumber mosaic virus and its 2b RNA silencing suppressor modify plant-aphid interactions in tobacco. *Sci. Rep.* **1**, 187; DOI: 10.1038/srep00187 (2011)
38. Mauck, K. E., Bosque-Pérez, N. A., Eigenbrode, S., De Moraes, C. M. & Mescher, M. C. Transmission mechanisms shape pathogen effects on host-vector interactions: evidence from plant viruses. *Funct. Ecol.* DOI:10.1111/j.1365–2435.2012.02026.x. (2012).
39. Malmstrom, C., Melcher, U. & Bosque-Pérez, N. A. The expanding field of plant virus ecology: Historical foundations, knowledge gaps, and research directions. *Virus Res.* **159**, 84–94 (2011).
40. Gildow, F. E., Frank, J., Bingham, D. & Powell, C. Barley yellow dwarf viruses in small grains in Pennsylvania: isolate identification, distribution, and vector efficiency. *Plant Dis.* **71**, 922–926 (1987).
41. Power, A. G. Virus spread and vector dynamics in genetically diverse plant populations. *Ecology* **72**, 232–241 (1991).

42. Ramsey, J. S. & Jander, G. Testing nicotine tolerance in aphids using an artificial diet experiment. *JoVE*, e701 (2008).
43. Hammond, J., Lister, R. M. & Foster, J. E. Purification, identity and some properties of an isolate of *Barley yellow dwarf virus* from Indiana. *J. Gen. Virol.* **64**, 667–676 (1983).
44. Bencharki, B. *et al*. Phloem protein partners of *Cucurbit aphid borne yellows virus*: Possible involvement of phloem proteins in virus transmission by aphids. *Mol. Plant. Microbe Interact.* **23**, 799–810 (2010).

Acknowledgements

Our research was supported by USDA-AFRI award 2009-6510405730. We thank A. Busch, S. Eid, E. Kmieciak, J. Knerr, A. Poplawsky, P. Trębicki, and L. Unger for technical assistance, A. Karasev for providing purified BYDV, and B. Price for input on statistical analysis.

Author contributions

N.B.P., L.L.I., and S.D.E. conceived and designed research; L.L.I. performed research and analyzed data; L.L.I., N.B.P., and S.D.E. interpreted results and wrote the paper.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Ingwell, L.L., Eigenbrode, S.D. & Bosque-Pérez, N.A. Plant viruses alter insect behavior to enhance their spread. *Sci. Rep.* **2**, 578; DOI:10.1038/srep00578 (2012).