Interactions between *Metopolophium festucae cerealium* (Hemiptera: Aphididae) and *Barley yellow dwarf virus* (BYDV-PAV)

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Abstract

Interactions between an invasive aphid, *Metopolophium festucae* (Theobald) subsp. *cerealium*, and *Barley yellow dwarf virus* (BYDV-PAV) were studied under laboratory conditions. *M. festucae cerealium* is an economic pest of wheat and barley that has recently been found in high population densities in wheat in the Pacific Northwest of the United States. BYDV-PAV is the most prevalent and injurious species of BYDV worldwide and in the Pacific Northwest. Although *M. festucae sensu stricto* (Theobald 1917) has been reported previously as a vector of some BYDV isolates, there is no confirmed transmission of BYDV by *M. festucae cerealium*. Two experiments examined the ability of *M. festucae cerealium* to transmit BYDV-PAV. The first used single aphids caged to indicator plants of a BYDV-susceptible winter wheat cultivar and the second used multiple aphids on each test plant. *M. festucae cerealium* did not transmit BYDV-PAV in either experiment, whereas transmission by a known BYDV vector, *Rhopalosiphum padi* L., was consistently high (≥93%). A third experiment compared the intrinsic growth rate, days until first reproduction and daily reproduction by *M. festucae cerealium* on sham-inoculated and BYDV-PAV-infected wheat, but detected no differences. The findings are reviewed in light published data on *M. festucae* species, BYDV transmission, and the potential pest status of this new invading aphid.

Key words: aphid, wheat, BYDV-PAV, vector

The aphid *Metopolophium festucae* (Theobald) subsp. *cerealium* has recently been detected in abundance on winter and spring wheat in inland Pacific Northwest United States (PNW): central Washington, northern Idaho, and Oregon (Halbert et al. 2013). A native to the United Kingdom, *M. festucae cerealium* colonizes cereal crops (oats, barley and wheat) and some of noncrop grasses (*Lolium perenne, L. multiflorum, Festuca rubra, F. pratensis, F. arundinacea, Dactylis glomerata; Dent and Wrettan 1986*). *M. festucae* sensu stricto was first reported in the United States in California in 1970 (Footit et al. 2006) and later as an intermittent pest on fescue in lawns in Boise, ID in the early 1990s (Halbert and Sandvol 1995, Halbert et al. 2013). *M. festucae cerealium* was first collected in suction traps in 1994 in Oregon at Corvallis, Madras, and Hermiston, although these collections were only reported informally (Halbert and Sandvol 1995). Recently, *M. festucae cerealium* appears to have transitioned to invasion dynamics. In 2011, high populations were detected in wheat fields in northern Oregon, central Washington state and northern Idaho (Halbert et al. 2013), and its abundance and prevalence have generally increased or remained high since then (Davis et al. 2014a, Eigenbrode et al. 2015). In many of these locations, *M. festucae cerealium* was more abundant than any other cereal aphid species. Direct feeding damage is associated with a characteristic red-pigmented spot on the leaf that eventually becomes necrotic, reducing the growth rate and the shoot and below-ground biomass of wheat, barley and other grassy hosts (Stroyan 1982, Dent 1985, Halbert and Voegtlin 1995, Davis et al. 2014b, S.E.S., unpublished data). The red staining and necrosis of the host plants are consistent with the presence of a salivary toxin, but this has not been examined for *M. festucae cerealium*.

In addition to this direct feeding injury, and based on previous studies with *M. festucae* (unspecified subspecies; Plumb 1995, Guglielmone and Caciagli 1996), *M. festucae cerealium* may be capable of acting as a vector of *Barley yellow dwarf virus* (BYDV). BYDV causes barley yellow dwarf, a serious disease of Poaceae (Gramineae) (Lister and Ranieri 1995) affecting small grain cereals worldwide. Yield losses of up to 46% in wheat, 25% in barley, and 15% in oat...
have been reported (Kaddachi et al. 2014). During a recent outbreak in Idaho, yield losses of 70–100% in fall planted wheat are attributed to BYDV (Marshall and Rashed 2014). Historically, the dominant species of BYDV encountered in the PNW is BYDV-PAV (Barley yellow dwarf virus-Padi avenae virus; a designation indicating the most efficient aphid vector for this species) (Hewings and Eastman 1995).

Members of the barley/Cereal yellow dwarf virus complex, including BYDV-PAV are transmitted in a persistent, circulative, and non-propagative manner by at least 23 species of aphids in 15 different genera (Blackman et al. 1990, Halbert and Voegtlin 1993). The principal and most studied vectors of BYDV are Rhopalosiphum padi, Sitobion avenae, Schizaphis graminum, R. maidis, and m. dirhodum, a congener of M. festucae cerealium. Three other Metopolophium species that affect cereal crops (Stroyan 1982, Halbert and Voegtlin 1995) are reported vectors of BYDV: M. albidum, M. frisicoma, and M. festucae (Jedlinski 1981, Blackman et al. 1990). Whether M. festucae cerealium is a BYDV vector is unknown. The changing taxonomic status of M. festucae may have hampered interpretation of previous virus/vector studies. M. festucae was originally separated into two distinct species (Theobald 1917; Hille Ris Lambers 1947, 1966), which were later designated subspecies: M. festucae sensu stricto and M. festucae cerealium (Stroyan 1982). In all studies evaluating transmission efficacy of M. festucae for BYDV, the M. festucae subspecies has not been specified. These studies have found equivocal evidence for transmission of BYDV. Transmission of BYDV-PAV by M. festucae was reported as 0% (Plumb 1974) and 2% (Plumb 1990). Among the 52 individuals of M. festucae caught in a suction trap during the autumn periods over 20 yr in Pozzuolo del Friuli, Italy, none of 12 individuals tested by enzyme linked immunosorbent assay (ELISA) was positive for BYDV-PAV, Macrosiphum avenae virus (MAV), Rhopalosiphum padi virus (RPV) or Rhopalosiphum maidis virus (RMV) (Cocenceo et al. 2009). On the other hand, M. festucae is a competent vector for some BYDV species based on controlled studies (Plumb 1990, 1993; Guglielmone and Caciagli 1996). Unfortunately, as the subspecies was not identified in these prior reports, the vector status of M. festucae cerealium remains uncertain.

The relationship between insect vectors and plant viruses is additionally complicated by the effects of virus-infected plants on aphid performance and behavior (Bosque-Pérez and Eigenbrode 2011). Virus infection can alter plant physiology and biochemistry and as a result, suitability for aphids (Fereres et al. 1989; Eigenbrode et al. 2002; Fiebig et al. 2004; Jiménez-Martínez and Bosque-Pérez 2004, 2009; Bosque-Pérez and Eigenbrode 2011). Most studies show positive effects of BYDV-infected plants on vector reproduction (Araya and Foster 1987, Fereres et al. 1989, Irwin and Thresh 1990, Quiroz et al. 1991, Power and Gray 1995, Jiménez-Martínez et al. 2004a, Davis et al. 2015), but Fiebig et al. (2004) found the opposite effect. Virus infection can also produce varying effects on the performance of nonvectors (Hara and Dodds 1987, Lin et al. 2008, Belliure et al. 2010, Van Mollen et al. 2012, Nachappa et al. 2013, Pan et al. 2013). Whether or not M. festucae cerealium is a vector of BYDV, effects of BYDV-infected host plants on its reproduction could have economic importance wherever M. festucae cerealium is a pest.

Despite the recent high population densities of M. festucae cerealium in wheat in the PNW, neither its ability to transmit BYDV nor its responses to BYDV-infected plants have been examined. The main objectives of this study were to 1) assess the ability of M. festucae cerealium to transmit BYDV-PAV, the most severe and prevalent species of BYDV in the world and in the PNW; and 2) determine the effect of BYDV-infected wheat on reproductive fitness of M. festucae cerealium as compared with noninoculated control plants.

Materials and Methods

Aphids

A virus-free colony of R. padi was established from individuals collected near Moscow, ID (46° 43’ 54” N, 116° 59’ 50” W), in 2001 (Idaho clone) and maintained in environmental growth chambers (Mod-1-36VLX, Percival Scientific, Perry, IA) at 20 ± 3°C and a photoperiod of 16:8 (L:D) h on barley cultivar Sprinter. Virus-free colonies of M. festucae cerealium were established from neonates deposited by asexual virginigal specimens collected via sweep net from winter wheat fields near Moscow, ID in May 2013. Colonies are maintained in 60 by 60 by 60 cm³ mesh enclosures (BugDorm 2120, Mega View Science Co. Ltd., Taichung, Taiwan) containing potted wheat (cultivar Alturas) and kept in a greenhouse with temperature mean 24.0, min. 16.6 and max. 30.5°C, ambient relative humidity (near 60%), and a photoperiod of 14:10 (L:D) h.

Source of Virus

BYDV was obtained from a colony of R. padi kept on Sprinter barley plants infected with a Washington state isolate of BYDV-PAV. Chamber model and conditions were identical to those used for the virus-free R. padi colony. To prevent cross-contamination between the colonies, they were maintained in separate buildings.

BYDV Transmission by M. festucae cerealium

To increase the chances of detecting BYDV transmission by M. festucae cerealium, two types of test were used to evaluate BYDV transmission by M. festucae cerealium. In the first of these, individual viruliferous aphids were confined on a leaf of the test plants. In the second, host plants were infested with groups of viruliferous aphids. In both types of experiments, transmission by M. festucae cerealium was compared with transmission by R. padi, the most efficient known vector of BYDV-PAV (Sadeghi et al. 1997, Jiménez-Martínez and Bosque-Pérez 2009). For all experiments, the samples were tested using a double-antibody sandwich (DAS)-ELISA following the manufacturer’s protocol (SRA 27500, Agdia Inc., Elkhart, IN). Buffers were also purchased from Agdia and are described in the protocol provided by the manufacturer.

Single-Aphid Test

A BYDV-susceptible winter wheat cultivar (Lambert) was used for virus source plants and for indicator plants during the experiment. Seeds for source plants were planted in 10.2-cm-diameter plastic pots filled with soil mixture (6:1 ratio Sunshine mixture #1, SunGro Horticulture, Bellevue, WA) and sand. After germination (~5 d postplanting), plants were kept in a growth chamber at 20 ± 3°C and a photoperiod of 16:8 L:D h. Plants were fertilized every 2 wk with soluble N–P–K (20:20:20). Fourth-instar nymphs and adult apterous, viruliferous R. padi were used to inoculate source plants when plants reached the two- to three-leaf stage. Ten aphids were confined on a single leaf in a cage made of dialysis tubing (~2.5 cm in length by 1 cm in diameter; Spectrum Lab. Inc., Rancho Dominguez, CA) on 12 healthy plants for a 72-h inoculation access period (IAP). The cage was secured to the plant and sealed with foam stoppers at both ends to provide ventilation. After 72 h, the cage was removed and all aphids were killed by hand. Plants were then kept in an environmental growth chamber at 20 ± 3°C and a photoperiod of 16:8: L:D for a 28-d virus incubation period. At this point, fully expanded leaves on each source plant were selected for virus acquisition by R. padi and M. festucae cerealium.

To test BYDV transmission, M. festucae cerealium or R. padi were confined within dialysis tubing cages on source plants. Aphids...
were left on the plants for a 72-h virus acquisition period (AAP). Aphids were then transferred individually to two- to three-leaf stage seedlings (indicator plants) for a 72-h IAP. There were 48 replications (i.e., indicator plants) for M. festucae cerealum and 29 for R. padi, the latter serving as a positive control. After the IAP, aphids were removed and killed by hand. Six additional plants were not exposed to any aphids, serving as negative healthy controls. Healthy and indicator plants were placed in a growth chamber under the conditions described above for 35 d, after which they were tested for BYDV infection status using ELISA.

Multiple Aphid Whole-Plant Test
This test was carried out in a controlled growth chamber under the same conditions as described above. Winter wheat (cultivar Lambert) was used for both source plants and indicator plants. To generate source plants, four wheat seedlings were established in each of six 10.2-cm plastic pots (with similar soil and maintenance as described above). When plants reached the one-leaf stage, five apterous, viruliferous R. padi were transferred to each plant. After a 72-h virus IAP, the aphids were killed with a soap solution and the plants were left for 3 wk of virus incubation period in the growth chamber (18:6 [L:D] h, 22 ± 2°C, and 50 ± 5% RH). A leaf sample was then taken and stored at −4°C until testing with ELISA for infection status. This test was conducted twice in succession, and R. padi was used as a positive control for the second of these.

After the virus incubation period, approximately 100 M. festucae cerealum (third and/or fourth nymph instars) were placed on each of the source plants for a 72-h virus AAP. Eight M. festucae cerealum individuals (third or fourth-instar nymphs or wingless adults) were transferred from the source plants to indicator plants (2-wk-old seedlings planted three to four per 1-liter pot) for a 72-h virus IAP. Twelve pots (replications) were used in each experiment, with a total of 43 and 48 wheat plants in the first and second experiment, respectively. During the IAP, plants were held in 60 by 60 by 60 cm$^3$ mesh enclosures (BugDorm 2120, Mega View Science Co. Ltd.) under the same growth chamber conditions as described above. After the IAP, aphids were killed with soapy water and the plants were placed in a growth chamber (conditions as above) for a 21-d virus incubation period. Afterwards, a leaf sample was taken from each of the indicator plants and stored at −4°C until testing with ELISA for infection status. In each experiment, one wheat plant (cultivar Lambert), caged separately in the growth chamber, served as negative healthy control.

Reproduction of M. festucae cerealum on BYDV-PAV-Infected and Sham-Inoculated Plants
The experiment was conducted in a greenhouse (14:10 [L:D] h, 33°C, and 50 ± 5% RH). Each of thirty 1-liter pots was planted with three seeds of spring wheat (cultivar ‘Kelse’). Fourteen days after planting, all but the largest wheat plant in each pot were removed. On the following day, each plant was inoculated with BYDV-PAV using the methods of Jiménez-Martínez et al. (2004a,b). Inoculation was accomplished by caging 10 viruliferous R. padi from BYDV-infected barley (cultivar ‘Sprinter’) onto each plant for a 96-h IAP. Sham-inoculated plants (n = 30) received the same treatment except the R. padi had been reared on virus-free barley. After 96 h (an additional 24 h was used to ensure infection), both the viruliferous and nonviruliferous R. padi aphid were removed by hand from both sham-inoculated and BYDV-inoculated plants. Pots were then positioned randomly on the greenhouse bench.

Thirty-five days after germination (20 d after virus inoculation), three apterous M. festucae cerealum adults were caged onto a leaf of each plant in a single clip cage. The cages were checked daily until a nymph was produced, which became the foundress. Foundresses were monitored daily to record the time of first reproduction and daily reproduction thereafter. Nymphs were removed after each recording. The intrinsic rate of increase was calculated using the method of Wyatt and White (1977) as follows:

$$r_m = 74\left(\log (M_d)/d\right),$$

where $M_d$ is the number nymphs produced by a foundress and $d$ is the number of days after the birth date of the foundress until the first nymph was produced.

Statistical Analyses
In the single aphid, clip cage test for transmission by M. festucae cerealum, the percentage of virus-infected plants (percent transmission) was calculated by dividing the total number of plants that were positive based on ELISA by the total number of plants tested. In this experiment, 13 R. padi repetitions were eliminated prior to calculating the percentage of infected plants because of mortality during the IAP. In the multiple-aphid, whole-plant tests, each pot (with 3–4 plants) was considered a single replicate and transmission percentages were calculated among repetitions. In the experiment to test effects of BYDV-infected plants on M. festucae cerealum reproduction, a two-sampled t-test was used to compare the estimated $R_m$ and the days to first reproduction for aphids in the two treatments (BYDV-inoculated and sham-inoculated), with a criterion for significance of $\alpha = 0.5$ (performed using Minitab 17 statistical software).

Results
BYDV Transmission by M. festucae cerealum
In the single-aphid clip cage test, transmission of BYDV-PAV was only detected for R. padi (93%), whereas there was no transmission by M. festucae cerealum (Table 1). Similarly, in the test using multiple aphids on whole plants, transmission of BYDV-PAV was only detected for R. padi (100%, second experiment), whereas there was no transmission by M. festucae cerealum in either experiment (Table 1).

| Table 1. Transmission of BYDV-PAV by R. padi and M. festucae cerealum using a multiple-aphid whole-plant test and a single-aphid caged test |
|-----------------|-----------------|-----------------|
| Aphid species   | R. padi         | M. festucae cerealum |
|-----------------|-----------------|-----------------|
| Multiple-aphid whole-plant test | NS | 0/12 |
| NI/NT first experiment | 6/6 | 0/12 |
| NI/NT second experiment | 6/6 | 0/24 |
| Total NI/NT | 12/12 | 0/36 |
| % Transmission | 100% | 0% |
| Single-aphid caged test | 15/16 | 0/48 |
| NI/NT | 93% | 0% |

NI/NT, number of infested plants/number of tested plants; NS, not studied.
Reproduction of *M. festucae cerealium* on BYDV-PAV-Infected and Sham-Inoculated Plants

In both the sham- and virus-infected treatments, 10 of 15 foundresses lived to double the time required for reproduction, allowing calculation of \( R_m \) by the method of Wyatt and White (1977). In the sham treatment one outlier was removed because no new nymphs were produced from days 13 to 27, reducing the \( n \) for that treatment to 9. The mean intrinsic growth rates and days to first reproduction by *M. festucae cerealium* did not differ significantly between BYDV-PAV-infected and sham-inoculated plants. Mean daily reproduction was similar for *M. festucae cerealium* on BYDV-PAV-infected plants and sham-inoculated plants (Fig. 1).

Discussion

*Metopolophium festucae cerealium* is an invasive pest in the PNW (Halbert et al. 2013), with the potential to spread to cereal crops in other parts of the United States. The expanding range to more than 105 sample sites in central Washington and northern Idaho, and the growth in its populations from 2011 to 2013 (Halbert et al. 2013, Eigenbrode et al. 2015, S.E.S. unpublished data) suggest its potential importance as a pest is increasing. Continued monitoring and experimental studies like this one are merited to assess the type of threat it poses to cereal production systems. Ongoing work is documenting the levels of direct feeding injury that *M. festucae cerealium* is capable of inflicting on the crop.

We have demonstrated that a colony of *M. festucae cerealium* established from specimens collected in the PNW is unable to transmit BYDV-PAV. This is consistent with low to zero transmission of several BYDV serotypes by *M. festucae* (subspecies undetermined) previously reported (Plumb 1974, 1990, 1995; Halbert and Voegtlin 1995; D’Arcy and Burnett 1995; Guglielmone and Caciagli 1996; Coceano et al. 2009). Some researchers (Stroyan 1982, Hand 1989, Blackman et al. 1990, Halbert and Voegtlin 1995) have suggested that the reported transmission of BYDV by *M. festucae* (subspecies unspecified) implies transmission by *M. festucae cerealium*. Our study indicates that this inference may be unjustified.

We also detected no effects of BYDV-PAV infection in wheat on the intrinsic growth rate, the average days until first reproduction, or daily reproduction by *M. festucae cerealium* reared on the plants. This result is consistent with several studies in which virus infection has neutral effects on both vectors and nonvectors (Mowry 1994, Lin et al. 2008, Mauck et al. 2012, Van Molken et al. 2012, Nachappa et al. 2013, Pan et al. 2013, Kersch-Becker and Thaler 2014).

In summary, our results indicate that *M. festucae cerealium*, although potentially a threat due to its direct injury to wheat plants, will likely not pose a risk as a virus vector, or because its populations are amplified on BYDV-PAV-infected wheat. Additional work is indicated to determine whether *M. festucae cerealium* can transmit other B/CYDV species, such as MAV for which *M. festucae* (unspecified subspecies) is a vector (Plumb 1974, 1990, 1995), or SGV, which occurs in perennial grasses of the PNW (Ingwell and Bosque-Pérez 2015). *Metopolophium festucae cerealium* has been collected from perennial grasses such as *Alopecurus pratensis* L. in the PNW (S.E.S. unpublished data), which could serve as a reservoir for BYDV serotypes.

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