A Clone of the Russian Wheat Aphid (*Diuraphis noxia*) as a Vector of the Barley Yellow Dwarf, Barley Stripe Mosaic, and Brome Mosaic Viruses

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ABSTRACT

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When *Diuraphis noxia* were given acquisition access feedings on plants infected with single isolates of the five strains of barley yellow dwarf virus (BYDV) and then confined on oats or barley test plants for inoculation, none of 643 plants became infected. When *D. noxia* were placed on barley stripe mosaic virus (BSMV)-infected plants and subsequently confined on barley test seedlings, none of 165 plants became infected. Only three of 302 barley seedlings developed symptoms typical of brome mosaic virus (BMV) infection following inoculation access feedings by aphids previously fed on BMV-infected sources. However, enzyme-linked immunosorbent assay showed 19 of these plants were positive for BMV, suggesting symptomless infections. When *D. noxia* were fed on barley doubly infected with BSMV and BMV, little difference in transmission efficiency was observed. No transmission of BSMV occurred, and only one of 88 plants became infected with BMV. When aphids were given acquisition access feedings on barley doubly infected with the PAV isolate of BYDV and BMV, three of 301 test plants became infected with PAV and one with BMV. When *D. noxia* were exposed to the Rhopalosiphum padi virus (RhPV) while feeding on plants infected with BYDV-PAV and BMV, 19 of 229 test plants became infected with PAV. Ultrastructural studies indicated that *D. noxia* exposed to RhPV by feeding on virus-contaminated plants or on virus-sucrose solutions through membranes acquired the virus and developed typical cytopathological symptoms. When all transmissions from all experiments were combined, *D. noxia* at densities of up to 50 aphids per plant transmitted BMV to 2.5% and BYDV-PAV to 2.8% of all plants infested with RhPV.

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), was first reported as a pest of small grains in southern Russia and the Mediterranean region. Heavy crop losses due to *D. noxia* infestations were reported in the Crimea in 1900 and 1912 (15), in Spain in 1945, and in Turkey in 1962 (24). Current interest in these insects began in 1978, when they spread to major wheat-growing regions of South Africa and caused severe yield losses and alarm among growers (27). By 1980, the aphid was established in central Mexico (11), and in March of 1986 it was first collected in Texas (1). *D. noxia* now infests at least 16 western states in the United States and three Canadian provinces (2,18).

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D. noxia is considered an important pest of wheat and barley throughout its range and is capable of surviving on at least 65 wild and cultivated graminaceous species in North America (18). In wheat and barley, chlorotic streaks caused by toxic aphid secretions spread along the leaf from points of aphid feeding. Leaves often curl, giving protection to feeding aphid colonies (4).

In addition to its importance as an insect pest, *D. noxia* has been reported to transmit several viruses that affect small grains. Reports by von Wechmar and co-workers in South Africa (23, 25,26) suggest that *D. noxia* may transmit barley yellow dwarf virus (BYDV) (22), barley stripe mosaic virus (BSMV) (3), and brome mosaic virus (BMV) (19), in combination with an aphid-infecting virus, Rhopalosiphum padi virus (RhPV) (13).

In 1986, a meeting of the New Pest Advisory Group of the Animal and Plant Health Inspection Service was convened to set research priorities for evaluating the threat to cereal production posed by the introduction of *D. noxia* to the United States. One of the priorities selected was to determine the importance of *D. noxia* as a vector of viruses affecting small grains. The objective of our research was to test the ability of a U.S. isolate of *D. noxia* to transmit isolates of BYDV, BSMV, and BMV common to cereal-growing regions of the United States. All phases of this study were conducted at the Foreign Disease-Weed Science Containment Facility at Fort Detrick, Frederick, Maryland (21).

MATERIALS AND METHODS

A colony of *D. noxia* was obtained from Mike Rose, Texas A&M University. The aphids had been isolated from wheat (*Triticum aestivum* L.) and were reared on wheat cv. Max or barley (*Hordeum vulgare* L. 'Luther'). Identification of our colony was confirmed by Manya Stoetzel, Systematic Entomology Laboratory, Agricultural Research Service, Beltsville, Maryland.

Isolates of the five type strains of BYDV (22) were maintained in barley cv. Luther or oats (*Avena sativa* L. 'California Red'). The MI-3 isolate of BSMV (5) was obtained from Montana State University and maintained in infected seed of barley cv. Vantage. The BMV isolate was obtained from L. Lane and E. Ball, University of Nebraska, Lincoln, and was propagated in barley cv. Henry and wheat cv. Baart. The RhPV isolate was maintained in infected *Rhopalosiphum padi* (L.) colonies at Pennsylvania State University.

Barley yellow dwarf virus. The five type strains of BYDV were tested independently in direct transmission comparisons among *D. noxia* and their competent aphid vectors: PAV, *Rhopalosiphum padi*; MAV, *Sitobion avenae* (Fabricius); SGV, *Schizaphis graminum* (Rondani); RPV, *R. padi*; and RMV, *R. maidis* (Fitch). For all transmission

experiments, BYDV-infected leaf pieces from Luther barley or California Red oats were placed in 150-mm petri dishes and infested separately with competent vector species for a 48-hr acquisition access period. The remaining intact source plants, 25–30 days after inoculation, were then infested with *D. noxia* for a 7-day acquisition access period. Acquisition access feedings and inoculation access feedings were conducted between 18 and 22 C in a controlled temperature room.

Leaf pieces bearing 20 to 50 aphids (mixtures of apterae and various instars) were removed from the petri dishes or, in the case of *D. noxia*, cut from intact plants and placed into 2 × 29 cm, cellulose butyrate, tubular cages. The cages were inverted over one- or two-leaf test seedlings of Luther barley or California Red oats for 7-day inoculation access feeding periods. Seedlings were treated with insecticide (acephate or carbofuran) and allowed to grow in the glasshouse for 35 days at 20–25 C for observation and possible symptom expression. Transmission tests with each strain were replicated at least three times with 25 or more plants per replication with *D. noxia* and 10 plants per replication with each specific vector species.

Symptoms were recorded 20 and 35 days after inoculation access periods. After the 35-day readings, 1-g samples of young leaf tissue from individual test plants and control plants were harvested and assayed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (14).

Barley stripe mosaic virus. Vantage barley seed infected by the MI-3 isolate of BSMV (5) was germinated and grown in 10-cm clay pots for 25 days. Asymptomatic plants were rogued from the pots, and the remaining infected plants were infested with *D. noxia* for a 7-day acquisition access period. Leaf pieces bearing 20 to 100 aphids were cut from these source plants and placed into 2 × 29 cm tubular cages, which were then inverted over one- or two-leaf seedlings of barley cultivars Black Hulless, Henry, and Luther for a 7-day inoculation access period. As negative controls, *D. noxia* previously held for 7 days on healthy barley cv. Betzes were caged on barley test plants for a 7-day inoculation access period. Positive controls were either infected seedlings of Vantage barley or sap inoculation of Black Hulless barley and sweet corn (*Zea mays* L. subsp. *mays* 'Golden Bantam'), with 50 mM sodium phosphate buffered extracts from infected barley seedlings. Each experiment was conducted twice.

Following treatment with insecticides, the plants were held in the glasshouse at 20–25 C. After 25 days, 1-g samples of young leaf tissue from each pot were harvested and assayed by DAS-ELISA and immunosorbent electron microscopy (ISEM), generally following the protocols reported by Carroll et al (6) and Lister et al (20).

Brome mosaic virus. Source plants were young plants of Baart wheat and Henry barley inoculated with infectious BMV plant extracts 16–22 days before use. To test nonpersistent aphid transmission of BMV, symptom-bearing leaf pieces in 150-mm petri dishes were infested with nymphs and adults of *D. noxia* for an acquisition access period of 0.5–5.0 min. Aphids that had probed at least one time were transferred by camel hair brush in groups of 10 to healthy one- or two-leaf seedlings of Baart wheat and Henry barley for an inoculation access period of 72 hr. The same leaf pieces used as virus sources were then ground in 50 mM sodium phosphate buffer, pH 6, and the extract was rubbed onto leaves of wheat seedlings as a positive control. Four replications were conducted with six or seven test plants per replication.

Tests for persistent transmission of BMV were conducted by allowing *D. noxia* acquisition access periods of 24 or 72 hr on BMV-infected source leaves in petri dishes. The leaf pieces, each bearing 20 to 50 aphids, were handled as previously described. To reduce the likelihood of mechanical inoculation, aphids were allowed to walk from the source plant leaf pieces onto test seedlings, so that there was minimal physical abrasion. After 72 hr, the cages were removed, and the seedlings were treated with either carbofuran or acephate. Test plants were allowed to grow in a glasshouse for 28–35 days. The test was repeated three times with seven to 14 plants per test.

Plants were evaluated for symptom expression after 35 days. Leaf samples were removed for back-assay by sap inoculation (as described above) onto healthy seedlings and DAS-ELISA (8,10) using a polyclonal rabbit antiserum to BMV. Those seedlings shown to be infected by positive back-assay or ELISA were used for inoculum for the next series of aphid transmission experiments. Three sequential experimental series were conducted with each series consisting of at least three replications. In a final experiment, *D. noxia* were given a 7-day acquisition access period on intact, BMV-infected (by sap inoculation) Henry barley plants and were then caged in groups of 50–100 aphids on 35 healthy Henry barley test seedlings.

Multiple virus combinations. The ability of *D. noxia* to transmit virus from plants infected with different combinations of BYDV, BSMV, and BMV was tested. In the first experiment, eight seedlings of Henry and Luther barley were mechanically inoculated with BMV and then inoculated 3 days later with the PAV isolate of BYDV by *R. padi*. Other plants were inoculated either with BMV or BYDV-PAV for use as controls. Following fumigation with DDVP (O,O-dimethyl-2,2-dichlorovinyl phosphate) to kill aphids, all plants were maintained in a glasshouse at 18–23 C to observe symptom development. One month later, one plant of each cultivar was infested with several hundred *R. padi* infected with RhPV (13). Seven days later the plants were fumigated. These plants and the remaining six source plants without RhPV were then each infested with 400–500 *D. noxia*. Leaf pieces containing 20–25 aphids then were removed from the source plants at 7 days, 17 days, and 26 days and caged on Luther barley seedlings for a 7-day inoculation access period. After the aphids were killed, the plants were placed in the glasshouse and observed over 35 days for symptom development. Virus-infected source plants were tested by ELISA and aphid transmission (*R. padi*) to verify isolate identity. Test seedlings developing symptoms were tested by ELISA to verify infection. A random sample of plants without symptoms also was tested by ELISA.

Acquisition of BYDV-PAV, BMV, and RhPV by *D. noxia* was studied in another set of experiments utilizing Barsoy barley plants infected with PAV + BMV prepared as above. In one treatment, aphids were injected with 0.02 μl of a 10-μg/ml solution of RhPV immediately prior to confining them on BYDV-PAV and BMV source plants for an acquisition access period. Aphids for another treatment were injected with 0.02 μl of a 3-mg/ml solution of BMV purified by sucrose density gradient. A third group of aphids was allowed to feed 24 hr on stretched Parafilm membranes containing 3 mg/ml of BMV in 20% sucrose. Injected aphids, membrane-fed aphids, and untreated healthy aphids were then given acquisition access periods of 6, 9, or 12 days on Barsoy barley infected with PAV, BMV, PAV + BMV, or on healthy plants as controls. Aphids of each treatment then were given a 7-day inoculation access period on 7-day-old barley seedlings. Plants were sprayed with acephate and observed in the glasshouse for 35 days for symptom development. Viral symptoms were recorded, and infections were verified by ELISA.

In a third experiment, we used Barsoy barley source plants infected singly with PAV, BMV, and BSMV, or doubly with PAV + BMV and BMV + BSMV. Healthy plants served as controls. In addition, one set of source plants for each treatment was infested with several hundred RhPV-infected *R. padi* as a means of introducing RhPV into the virus complex 7 days before placement of *D. noxia* onto these source plants for possible acquisition. Three acquisition access periods (7, 14, and 21 days) and three *D. noxia* densities (1, 10, and 25 aphids per plant) were tested with Luther

barley seedlings. *D. noxia* were allowed a 7-day inoculation access period on test plants. The plants were observed for 30 days for symptom development. Test plants were tested by ELISA for BYDV-PAV and BMV.

RhPV infection treatments with *D. noxia*. In two of three experiments involving RhPV (experiments 1 and 3), source plants infected with BYDV-PAV, BMV, or BSMV were infested with several hundred RhPV-infected *R. padi* from chronically infected colonies maintained at Pennsylvania State University. RhPV-infected aphids were allowed to feed on source plants for 7 days. These source plants were fumigated to kill the aphids, washed in tap water, and then used 24 or 48 hr later to initiate acquisition access by *D. noxia*. RhPV is not known to infect plants; however, aphids feeding on contaminated plants can acquire RhPV (13). To determine whether *D. noxia* could acquire and be infected by RhPV after feeding on RhPV-contaminated plants, healthy *D. noxia* and *Schizaphis graminum* (as a control) were reared 21 days on plants infested with RhPV-infected *R. padi*. Aphids were then tested for RhPV infection by ISEM (13). In one experiment, healthy *D. noxia* were given a 24-hr acquisition access to RhPV through stretched Parafilm membranes containing 50 μg/ml of purified RhPV in 20% sucrose immediately prior to initiating the acquisition access on source plants infected with BYDV-PAV and BMV. To determine whether *D. noxia* could become infected with RhPV by feeding through membranes, healthy *D. noxia* and *R. padi* were fed 24 hr on 25 μg/ml RhPV and then fixed and embedded for transmission electron microscopic (TEM) examination as previously described (13).

RESULTS

Barley yellow dwarf virus transmission tests. None of 643 oat or barley test seedlings infested with about 13,000 *D. noxia* that had previously fed on plants infected with the five BYDV strains developed symptoms of virus infection (Table 1). ELISA tests of 131 of these plants verified the lack of BYDV infection. By comparison, 119 of 178 test plants fed on by the competent BYDV vectors, as positive controls, became infected (9).

Barley stripe mosaic virus transmission tests. None of the 165 barley seedlings fed on by about 3,000 *D. noxia* previously given feeding access to BSMV-infected barley became infected (Table 2). Mechanical transmissions from BSMV-infected plants used as virus sources for acquisition access feedings resulted in diagnostic BSMV symptoms in barley and sweet corn test plants. These observations were confirmed by ELISA and ISEM tests (Table 2). ELISA and ISEM observations for BSMV in

Table 1. Comparison of transmissibility of five vector-specific isolates of barley yellow dwarf virus (BYDV) by four aphid vectors and *Diuraphis noxia*

BYDV isolate	Specific aphid vector	Plants infected/plants inoculated[a]	
		Specific aphid vector	*D. noxia*
RPV	*Rhopalosiphum padi*[b]	29/41	0/82
PAV	*R. padi*	34/43	0/105
RMV	*R. maidis*	20/34	0/132
MAV	*Sitobion avenae*	26/29	0/88
SGV	*Schizaphis graminum*	10/31	0/236

[a]Natural vectors given a 48-hr acquisition access period on detached BYDV-source leaves and a 7-day inoculation feeding period on healthy test seedlings of oats cv. California Red, or barley cvs. Henry and Luther. *Diuraphis noxia* given a 7-day acquisition feeding on intact plants and a 7-day inoculation feeding on healthy seedlings.
[b]BYDV type strains originally obtained from W. F. Rochow, Cornell University, and maintained in oats cv. Coast Black at Pennsylvania State University with clones of natural aphid vectors.

Table 2. Efficiency of barley stripe mosaic virus (BSMV) transmission by mechanical inoculation, seed transmission, and *Diuraphis noxia* fed on three barley cultivars

Transmission method[a] Test cultivar	Infection data[b]	DAS-ELISA results[c]	ISEM results[d]
D. noxia			
Barley cv. Black Hulless	0/49	0/12	0/5
Barley cv. Luther	0/96	0/23	0/2
Barley cv. Henry	0/20	0/4	0/1
Mechanical			
Barley cv. Black Hulless	15/15	3/3	1/1
Sweet corn cv. Golden Bantam	3/6	3/3	1/1
Seed			
Barley cv. Vantage	28/33	4/4	1/1
Seedling controls			
Barley cv. Luther	0/9	0/2	NT
Barley cv. Henry	0/12	0/2	0/2
Sweet corn cv. Golden Bantam	0/4	0/2	NT
Seed control			
Barley cv. Betzes	0/8	0/2	0/1

[a]For all aphid transmission experiments, *D. noxia* were given 7-day acquisition access periods followed by 7-day inoculation access periods on healthy indicator barley test seedlings. Controls were either healthy seedlings of barley or sweet corn or ground whole barley seeds known to be free of BSMV.
[b]Number of plants infected/number of plants tested.
[c]Number of samples testing positive by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (3× mean A_{405} for healthy plants)/total number of ELISA samples.
[d]Number of plants testing positive by immunosorbent electron microscopy (ISEM)/total number of ISEM samples. Positive control (not shown) consisted of a preparation of partially purified BSMV, MI-3 isolate, at 726 μg/ml. The number of BSMV particles counted in one representative 5-mm square of an electron micrograph at 40,000× was 134. NT = not tested.

Table 3. Brome mosaic virus (BMV) transmission experiments with the Russian wheat aphid, *Diuraphis noxia*

Expt. no.	Virus source[a]	Acquisition access period[b]	Aphids/plant	No. reps	Plant reactions[c]	Infectivity assay[d]	ELISA[e]
1	Wheat cv. Baart	0.5–5 min	0	4	0/25	0/25	0/25
2	Wheat cv. Baart	1 day	20–50	3	0/19	5/19	7/19
3	Wheat cv. Baart	3 days	20–50	3	0/42	1/42	1/42
4	Barley cv. Henry	3 days	20–50	2	0/35	0/35	10/35
5	Barley cv. Henry	3 days	20–100	2	0/32	0/32	0/32
6	Barley cv. Henry	7 days	>50	2	1/35	1/35	1/35

[a]Virus source plants for experiments 1–3 and 6 were mechanically inoculated with BMV 20–24 days before use; virus source plants for experiments 4 and 5 consisted of positive assay plants from experiments 3 and 4, respectively.
[b]Acquisition access period as indicated; inoculation access period of 3 days for expts. 1–5 and 7 days for expt. 6.
[c]Number of plants with brome mosaic symptoms/number tested.
[d]Number of barley cv. Henry plants giving positive BMV symptoms by sap transmission/number assayed.
[e]Number of plants testing positive by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (A_{405} absorbance value greater than the mean of the healthy controls + four standard deviations)/number tested.

plants fed on by *D. noxia* were negative. None of the 33 plants maintained as healthy controls became infected.

Brome mosaic virus transmission tests. Only one of 188 test plants developed mosaic symptoms typical of BMV infection (Table 3) when they were infested with 20–50 aphids previously fed on BMV-infected source plants for 1–7 days. When these 188 test plants were assayed, however, BMV was recovered from seven plants by mechanical inoculation bioassay and detected in 19 plants by DAS-ELISA. No transmission was detected following acquisition access feedings of 0.5–5.0 min.

As noted in Table 3, apparent transmission of BMV, as verified by bioassay and ELISA, was inconsistent. For example, in experiments 2 and 3, in which aphids were given a 3-day acquisition access period on BMV sources, the bioassay data (6 of 61 test plants infected) and ELISA data (8 of 61 test plants infected) were in close agreement. In experiment 4, however, bioassays did not indicate any of 35 plants positive for BMV, although 10 of these plants tested positive by ELISA. Subsequently, no test plant infections occurred in experiment 5, and only 1 of 35 plants became infected in experiment 6 when aphids were given a longer acquisition access feeding of 7 days.

Although all but one test plant exposed to *D. noxia* previously exposed to BMV-infected plants remained symptomless, virus was detected by ELISA in 10% of the 188 plants tested. The range of absorbance values at 405 nm for the 10 positive plants in experiment 4 was 0.107–0.519. For six positive BMV controls from mechanically inoculated tissues showing obvious symptoms, the range was 0.327–1.241; for the six healthy control barley plants, the absorbance values ranged from 0.036 to 0.053. The range of values for the 10 test plants was well above the value of 0.066 selected as the positive threshold (average healthy plant value + four standard deviations). These repeated transmission tests, verified by ELISA done over several months, indicated that *D. noxia* could transmit BMV. The efficiency of transmission was low, and the basis for this inefficient transmission was unclear.

Transmissions from mixed virus infections. In the first experiment, there was no transmission of any virus to any of 113 plants infested with over 2,300 *D. noxia* fed previously for 7–26 days on source plants infected with BYDV-PAV and/or BMV without exposure to RhPV (Table 4). When *D. noxia* were given an acquisition access period of 7 days on BYDV-PAV + BMV sources infested with RhPV and then were confined for inoculation on test plants at a density of 20 aphids per plant, none of 86 plants became infected. However, when the acquisition access period was extended to 17 or 26 days, 7 of 30 and 12 of 37 plants became infected with BYDV-PAV (treatment 4, combined data of three feeding times). These positive BYDV-PAV transmissions were verified by recovery transmission bioassays with *R. padi* to California Red oats and by ELISA. None of 27 plants fed on by approximately 500 *D. noxia* previously given acquisition access feeds on BYDV-PAV sources for 7 days became infected.

A total of six of 187 test plants fed on by *D. noxia* exposed to BYDV-PAV at approximately 20 aphids per plant became infected with BYDV-PAV in the second mixed infection experiment (Table 4). Three transmissions from aphids injected with RhPV prior to acquisition access feeding on BYDV-PAV sources (treatment 2) occurred, and three transmissions resulted when aphids fed on BYDV-PAV + BMV sources without exposure to RhPV (treatment 3). Only two of 144 plants fed on by aphids exposed to BMV sources became infected with BMV (treatments 3 and 5). None of 80 aphids injected with 60 ng of purified BMV transmitted the virus.

None of 313 barley test seedings fed on by over 2,800 *D. noxia* exposed to sources infected with BYDV-PAV became infected in experiment 3 (Table 4, treatments 1–4). Very slight differences were observed among treatments receiving 1, 10, or 25 aphids per plant, or for treatments in which aphids were given acquisition access feeds for 7, 14, or 21 days. Therefore, all data for aphid densities and acquisition access times were combined in experiment 3. One of 234 plants fed on by *D. noxia* exposed to BMV-infected sources became infected with BMV (treatment 5).

The presence of BSMV in a mixed infection with BMV or in conjunction with RhPV did not enhance the probability of successful transmission of BSMV. None of 51, 45, 24, and 64 test plants exposed to *D. noxia* fed previously for 7–14 days on source plants containing BSMV, BSMV + RhPV, BSMV + BMV, or BSMV + BMV + RhPV, respectively, became infected with BSMV. These tests represented inoculation access feedings by over 1,800 aphids at densities of 1–25 per plant. One plant in the BSMV + BMV + RhPV treatment fed on by 25 aphids following a 14-day acquisition access period on BMV became BMV infected.

Verification of RhPV acquisition by *D. noxia*. Results of ISEM tests of *D. noxia* and *Schizaphis graminum* reared for 21 days on plants infested with RhPV-infected *R. padi* verified that *D. noxia* could acquire RhPV by this method. When grids for ISEM incubated on extracts from 10 aphids each were examined, RhPV was readily seen on all five grids prepared from *S. graminum* and on three of eight grids prepared from *D. noxia* reared with infected aphids, but none appeared on three grids each from *D. noxia* or *S. graminum* reared on healthy barley. In a second test, RhPV was detected by ISEM on all four grids prepared from extracts containing five aphids each of *D. noxia* confined for 8 days on plants infested with RhPV-infected *R. padi*. In another test, healthy *D. noxia*, *R. padi*, and *S. graminum* were fed through membranes 24 hr on 25 μg/ml RhPV and then reared 7 days on healthy barley before examination by ISEM. RhPV was readily observed covering grids incubated on extracts prepared from five aphids each on three of four grids from *D. noxia*, four of four from *R. padi*, and on two of two from *S. graminum*. Virus was not detected on any of three grids prepared from healthy aphid colonies.

To verify that RhPV was infecting *D. noxia*, and to determine the tissues

Table 4. Virus transmission by healthy or Rhopalosiphum padi virus (RhPV)-exposed *Diuraphis noxia* given acquisition access feeds on barley singly or doubly infected with the PAV isolate of barley yellow dwarf virus (BYDV) and brome mosaic virus (BMV)

Treatment no. and virus sources[a]	BYDV-PAV transmission[b]			BMV transmission[b]		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
1. BYDV-PAV	0/27	0/37	0/117	ND[c]	ND	ND
2. BYDV-PAV + RhPV	ND	3/40	0/42	ND	ND	ND
3. BYDV-PAV + BMV	0/113	3/100	0/88	0/113	1/100	0/88
4. BYDV-PAV + BMV + RhPV	19/153	0/10	0/66	0/153	0/10	0/66
5. BMV	ND	ND	ND	ND	1/34	1/80

[a] Infection of source plants with BYDV-PAV and BMV was verified by symptom development and enzyme-linked immunosorbent assay (ELISA) prior to initiating acquisition access feedings. Positive transmissions were verified by bioassay with *R. padi* and ELISA.

[b] *Rhopalosiphum padi* controls that were fed in parallel on BYDV-PAV source plants transmitted BYDV-PAV to 28 of 28 plants tested. Acquisition access feedings ranged from 7 to 26 days. Inoculation access feedings were 7 days for all experiments. Aphid densities used for inoculation access feedings varied from 1 to 25 aphids per plant among experiments and treatments. For RhPV treatments, source plants were infested with several hundred RhPV-infected *R. padi* for 7 days prior to exposure to *D. noxia* for acquisition access feedings (expts. 1 and 3), or *D. noxia* test aphids were injected with purified RhPV 24 hr before acquisition access feeding on source plants (expt. 2). Few differences were observed among treatments for acquisition access time or aphid densities; therefore, data for subtreatments within an experiment have been combined by acquisition virus source in expt. 3.

[c] Not done.

involved, aphids were fed through membranes on RhPV and then prepared as ultrathin sections for examination by TEM. In the first experiment, healthy *D. noxia* and *R. padi* were fed 24 hr on 3 mg/ml of RhPV in 20% sucrose, then maintained for 5 days on healthy barley leaves before fixation for TEM. When sections were examined, RhPV was observed infecting midgut and hindgut tissues in five of five *D. noxia* and two of five *R. padi*. In a second similar experiment, *D. noxia* fed 24 hr on 25 μg/ml of RhPV were examined 3 and 14 days later. RhPV was detected in the lumen of the midgut and hindgut, verifying virus acquisition, but was not observed infecting the cytoplasm of the one aphid examined from the 3-day treatment. However, when five aphids were examined from the 14-day treatment, RhPV infected the gut tissue of all aphids. The cytopathology observed was similar to that described for *R. padi* (13). Virus particles were numerous in the midgut and hindgut epithelial cell cytoplasm and in the gut lumen. When the accessory and principal salivary glands were examined, however, no virus was detected in any of the five aphids from the 14-day experiment. Thus, the Texas clone of *D. noxia* is susceptible to the Illinois isolate of RhPV and is capable of acquiring virus through membranes and from infested plants.

DISCUSSION

Our experiments with a Texas clone of *D. noxia* and North American isolates of BYDV, BMV, and BSMV were unable to duplicate earlier reports from South Africa (23,25,26) implicating *D. noxia* as an important vector of these viruses. Differences between our results and those of von Wechmar and Rybicki (26) may be explained by differences among geographically separated aphid clones, differences in virus strains, or by differences in methods used. No evidence is currently available to compare South African and North American aphid clones or virus isolates. Our primary objective was to determine whether or not the Texas clone of *D. noxia* could efficiently transmit any of the small grain viruses tested, all of which are common to cereal-growing regions of North America. For this reason, we used well-characterized and widely available virus isolates with a single common clone of aphid.

Studies in South Africa utilized RhPV-infected *D. noxia* given acquisition access feeds on plants infected with mixed combinations of viruses (25,26). Most of our experiments were conducted with virus-free healthy *D. noxia* and source plants infected with single virus strains. For example, each type-isolate of BYDV was tested individually, and comparisons were made between efficient natural vectors and *D. noxia*. Inoculation access feedings were done using large numbers of aphids (25–50 per plant) of all instars. Under these conditions, we found no evidence that our healthy clone of *D. noxia* could transmit single isolates of BYDV.

When combinations of viruses were inoculated into source plants, and when healthy or RhPV-exposed *D. noxia* were given acquisition access feeds for relatively long periods on source plants containing virus combinations, some BYDV transmission was observed. The PAV isolate of BYDV was transmitted with low efficiency from plants multiply infected with BYDV-PAV and BMV by healthy and RhPV-exposed *D. noxia* (1 and 8.3%, respectively). When healthy and RhPV-exposed aphids were compared directly in the same experiments for their ability to transmit BYDV-PAV from singly infected plants, only RhPV-exposed *D. noxia* transmitted BYDV-PAV (3.6%), and all aphids that transmitted the virus had been given acquisition access feeds longer than 7 days. Ultrastructural observations indicated that *D. noxia* could be infected with the Illinois isolate of RhPV with subsequent breakdown of alimentary canal tissues. Our results suggest that pathologic, RhPV-induced, physiological and cytological alterations of the aphid may enhance the probability of BYDV transmission. Unfortunately, BYDV-PAV transmission among treatments and among experiments was not consistent.

These observations are consistent with the hypothesis that BYDV-PAV transmission is a chance occurrence enhanced by abnormal metabolism and is not regulated by virus-specific mechanisms similar to those described for other BYDV vectors (12). Our results concerning the poor success of *D. noxia* as a BYDV vector substantiate a recent report by Halbert et al (16) describing results of transmission studies of BYDV by trap-collected *D. noxia* in the northwestern United States. Only one aphid of over 2,000 individuals tested over a 4-yr period transmitted a single SGV isolate of BYDV. Subsequent transmission of this isolate by *D. noxia* could not be repeated in studies using over 800 aphids, even though it was readily transmitted by *S. graminum*, its natural vector.

We did not observe transmission of the MI-3 strain of BSMV with healthy or RhPV-exposed *D. noxia*, either from singly infected sources or from combinations with BMV. This finding agrees with the results of Chiko (7), who found no evidence of insect transmission of BSMV in an earlier study. This virus is transmitted through barley seed, ovules, and pollen and by sap inoculation (3). We observed no changes in test plant growth patterns up to 32 days after initiation of inoculation with 20–100 aphids per plant. Von Wechmar (25) reported that young barley leaves infected by BSMV via aphid transmission exhibited sudden brittle death, so sudden there was no color transition from green to yellow. We did not observe such symptoms, nor were ELISA or ISEM assays positive.

Although BMV is readily transmitted by sap inoculation, no aerial vector had been linked to BMV transmission before 1981, when von Wechmar and Rybicki (26) reported South African isolates of BMV to be seedborne and readily transmitted by *D. noxia* and *R. padi*. The South African BMV isolate, when transmitted in a nonpersistent manner, was reported to produce low titer infections, with severe yellowing of leaves, sometimes accompanied by streaking and death of young leaves. Jilaveanu (17) reported BMV transmission by *R. padi* in Romania. We were unable to observe BMV transmission using 0.5- to 5-min acquisition access periods conducive to nonpersistent transmission. We did observe sporadic BMV transmission following acquisition access feedings of 24 hr or more. The severe yellowing symptoms described above were never observed.

Microinjection of infectious purified BMV (60 ng/aphid) into 80 *D. noxia* and the subsequent feeding of these injected aphids on test barley plants resulted in no infections. Although only one of 188 test plants fed on by BMV-fed aphids in one series of experiments developed symptoms, BMV was detected by ELISA in 10% of the plants. This apparent rate of transmission was not duplicated in a second set of experiments in which only 0.5% of 732 test plants were infected by aphids fed previously on singly or doubly infected BMV sources. The presence of the RhPV aphid virus had no effect on BMV transmission. These apparent inconsistencies in BMV transmission, symptom development, and ELISA detection are difficult to explain, and further work will be necessary to fully understand the potential BMV–*D. noxia* vector relationship.

Our controlled studies with the Texas clone of *D. noxia* showed that this aphid species was not able to transmit BSMV under any conditions. However, with long acquisition access feedings and high aphid densities, BMV was erratically transmitted in an inefficient manner; and under certain conditions the PAV isolate of BYDV was occasionally transmitted. Given the high aphid densities achieved under some field conditions, even inefficient vectors like *D. noxia* could become a significant factor in virus spread and survival. The role of RhPV in potentially enhancing BYDV-PAV transmission is counterbalanced by its potential debilitating effects on aphid longevity and fecundity. Field surveys on the distribution of RhPV throughout the range of *D. noxia* will be necessary to ascertain this aphid's importance in this regard.

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