Transmission of Bean Common Mosaic Virus by Cereal Aphids (Homoptera: Aphididae)

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ABSTRACT

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Bean common mosaic potyvirus (BCMV) is aphid-transmitted in a nonpersistent manner. BCMV can be subdivided into two serotypes, A and B. BCMV serotype A has been identified in Idaho as the cause of seed lot rejections and occasional crop failures in recent years. We suspected that cereal aphids could be important vectors of BCMV because they comprise a large proportion of airborne alate aphids in Idaho as measured by the Idaho suction trap survey system. Five species of cereal aphids—*Diuraphis noxia, Metopolophium dirhodum, Rhopalosiphum padi, Schizaphis graminum*, and *Sitobion avenae*—were tested along with *Myzus persicae* for efficiency of transmission of BCMV using timed acquisition probes. *D. noxia, Metopolophium dirhodum*, and *M. persicae* were tested in mass inoculation experiments, and winged *D. noxia* and *M. persicae* were tested further in an arena setting. All aphid species tested transmitted the virus in all three experiments except *D. noxia*, which appears to be a nonvector. Transmission efficiencies and average suction trap collections in bean production areas were combined to produce a potential vector index (PVI). Trap collection data for 1989–1992 showed a high correlation between the PVI and the number of hectares of seed beans rejected because of virus infection ($r^2 = 0.96$, $P = 0.018$). Prior to that (1985–1988), the correlation was poor in that far fewer hectares were rejected than predicted by the PVI.

Bean common mosaic potyvirus (BCMV) is seedborne and aphid-transmitted in a nonpersistent manner. It has been in Idaho for at least 70 yr (6). In epidemic years, serious losses have occurred because of direct crop injury and because of seed lot rejections by the Idaho Crop Improvement Association (6). BCMV serotype A (18) was distinguished from prevalent North American strains of BCMV (designated serotype B) on the basis of serology, biochemistry, and differential reactions on various diagnostic bean (*Phaseolus vulgaris* L.) cultivars (12,13). BCMV serotype A was first documented in Idaho after several cultivars resistant to the prevalent strain of BCMV in the region became infected in 1989 (7). Again, in 1991, several seed fields were rejected from certification because of BCMV (Idaho Crop Improvement Association, *personal communication*). A few Idaho fields experienced total crop failure because of blackroot (5), a lethal systemic vascular necrosis resulting from hypersensitivity of some dominant *I*-gene-bearing resistant bean cultivars to some BCMV serotype A strains (3).

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Aphid species that do not colonize beans should be considered as potential vectors of BCMV because BCMV is nonpersistently transmitted (10). Cereal aphids averaged about 54% of total aphids found in Idaho suction trap collections from 1985 to 1992 (University of Idaho *Aphid Flyer* newsletters, vols. 1–7, 1986–1992; S. E. Halbert, *unpublished*). *Diuraphis noxia* (Kurdjumov), the Russian wheat aphid, made up about 22% of the aphids collected between 1987, when it was first detected in Idaho, and 1992 (S. E. Halbert, *unpublished*). We suspected that cereal aphids may be important vectors of bean potyviruses because they comprise such a large proportion of our aphid samples. The objective of this research was to determine BCMV transmission efficiency of key cereal aphids that occur in Idaho.

MATERIALS AND METHODS

Virus isolate. In preliminary experiments, characterized laboratory isolates of BCMV strains (13) maintained by repeated mechanical inoculation were transmitted poorly or not at all by aphids. To ensure optimum aphid transmissibility, a field isolate of serotype A was collected at the University of Idaho Research & Extension Center, Kimberly, and maintained only by aphid transmission.

We increased the aphid-transmissible field isolate in the BCMV-susceptible cultivar Sutter Pink and tested symptomatic leaf tissue by indirect ELISA using a series of monoclonal antibodies (MAbs): POTY-1 (purchased from Agdia, Inc., Mishiwaka, IN); MAbs II-197, II-463, and I-2, serotype A-specific (developed at Prosser, WA); and MAb B5E5, serotype B-specific (developed at Braunschweig, Germany) (13). Fresh or air-dried leaf tissue was triturated 1:40 (w/v) in carbonate buffer (pH 9.6) containing 2% egg albumin, 0.2% polyvinylpyrrolidone, and 0.45% sodium diethyldithiocarbamate and tested as described earlier (13). The field isolate reacted strongly with serotype A-specific MAb I-2 but not at all with serotype B-specific MAb B5E5.

Isolate characterization. The initial symptoms produced by the field isolate in differential bean cultivars were typical of those produced by the NL-8 strain of BCMV originally described by Drijfhout (3). Further characterization revealed two additional BCMV strains, the western strain (16) and an unidentified strain belonging to BCMV pathogroup VII (M. J. Silbernagel, *unpublished*). Once separated from each other and from the NL-8 strain by differential host passage, both the western and the unidentified strain reacted only with the serotype B-specific MAb. As expected, the NL-8 strain obtained from this mixture reacted only with the serotype A-specific MAb. This combination of three BCMV strains was isolated three different times over a period of 12 mo from our stock isolate, which was maintained by aphid transmission. Neither of the serotype B strains was detected serologically in subpropagations of the stock culture until they were separated and amplified in the appropriate bean cultivars, i.e., those susceptible to pathogroup VII but resistant to pathogroup III (3).

We designated the NL-8 strain isolated in this study as NL-8 Idaho. The pathogroup VII isolate will be reported later as US-10 in accordance with the identification system proposed earlier (4). The separated strains have been preserved at Prosser, Washington.

Aphid cultures. All aphid cultures came from Idaho. The *Myzus persicae* (Sulzer) culture was collected from potato (*Solanum tuberosum* L.). The *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius), and *D.*

noxia cultures came from wheat (*Triticum aestivum* L.); the Idaho *S. graminum* is not one of the described biotypes (8). The *Rhopalosiphum padi* (L.) culture was collected from *Prunus virginiana* L., and the *Metopolophium dirhodum* (Walker) culture was obtained from *Rosa* sp., both primary hosts. Cultures of *M. persicae, R. padi*, and *Metopolophium dirhodum* are known to be clonal. Others were started from field-collected colonies. Cereal aphids were propagated on wheat and barley (*Hordeum vulgare* L.), and the *M. persicae* culture was propagated on green pepper (*Capsicum annuum* L.) and *Datura stramonium* L. Voucher specimens are deposited at the University of Idaho.

Timed probe transmission experiments. Timed transmission experiments were conducted at the University of Idaho Parma Research & Extension Center. Aphids were starved for at least 1 hr prior to testing. They were allowed 15- to 60-sec acquisition feeding probes (11) on infected Sutter Pink bean leaves, after which they were transferred immediately to test seedlings of the same cultivar in the unifoliate leaf stage. One potentially infective aphid was caged on each test seedling for at least 5 hr (usually overnight). After transmission access, the plants were sprayed with bifenthrin (0.25 g a.i./L), placed in an aphid-free greenhouse, and fumigated with dichlorvos (0.03 g a.i./m^3). The greenhouse was fumigated regularly to prevent virus spread. Only nonwinged forms were used in timed transmissions to prevent accidental infections by escaping aphids. Experiments were done in five groups, one group per day, on 18–20 bean seedlings (depending on germination of beans) per aphid species per group, ensuring that aphid species would be tested uniformly. Plants were held in the greenhouse for at least 2 wk for observation of symptoms. Each plant was also tested by ELISA for BCMV infection.

Species tested in timed transmission experiments included *D. noxia, S. graminum, R. padi, Metopolophium dirhodum*, and *Sitobion avenae*. We tested the first three species from December 1991 to February 1992 and the latter two in May 1992, after *Metopolophium dirhodum* became available on rose. *M. persicae* was used as a standard in both sets of experiments.

Mass inoculations. Species tested by mass inoculation, also at the Parma Research & Extension Center, included *D. noxia* and *Metopolophium dirhodum*, with *M. persicae* included as a standard. Hundreds of aphids extracted from host plants with Berlése funnels were starved for at least 1 hr and then given 5 min of acquisition access to infected Sutter Pink bean leaves. They were then gently brushed onto healthy seedlings of the same cultivar and caged. We used at least 100 aphids per test seedling. Plants were sprayed, fumigated, and tested for virus infection as described above.

Arena test. Summers et al (17) reported that *D. noxia* transmitted beet mosaic virus only when alatae were released in an arena and given unrestricted access to indicator plants and an inoculum source. To determine whether the same pattern might apply to BCMV, we duplicated the methods of Summers et al (17). We released 25 alate aphids into arenas containing one BCMV source plant surrounded by six healthy Sutter Pink bean seedlings. Arenas had slanted glass tops installed about 40 cm above 41 × 46 cm floors. After 3 days, test plants were removed, sprayed, fumigated, and tested for virus as described above. Only *D. noxia* and *M. persicae* (included as a standard) were tested in the arenas. The experiment was replicated three times, using one arena for each species per replicate. The six arenas were arranged in a randomized complete block design in an insectary room at the Parma Research & Extension Center that was set at 20 C and 14 hr light/10 hr dark.

Idaho suction trap survey system. The Idaho suction trap survey system was established in 1985 (9). Traps are 8 m high and built according to the design by Allison and Pike (1). Trap collections reflect aphid flight activity within a radius of about 30 km of the traps. Collection of long-distance (>100 km) migrants is probably rare (9).

Collections from traps located in Idaho bean production areas (Parma, Caldwell, and Allendale in Canyon County; Mountain Home in Elmore County; Kimberly in Twin Falls County; and Burley in Cassia County) were used for comparisons with laboratory virus transmission data. At least three traps were operating in any given year. Log_{10} transformed averages, used because of variability in trap counts, were calculated for each species for each year according to the following formula: Antilog $\{[\Sigma \log_{10}(x+1)]/n\}-1$, where x is the number of specimens of a given species collected in a suction trap prior to 1 August and n is the number of traps in operation.

A potential vector index (PVI), similar to the infectivity index for barley yellow dwarf virus (14) but using laboratory transmission efficiency data, was calculated for each species tested for each year (1985–1992) using the $\log_{10}$ transformed average suction trap collections prior to 1 August and transmission percentages from timed probe transmission experiments.

RESULTS

Timed probe transmission experiments. Results of the timed probes showed that all species except *D. noxia* transmitted BCMV (Table 1). Transmission rates for cereal aphids spanned a range of 3.0–21.9%. *S. graminum* was the most efficient cereal aphid vector. Transmission rates for *M. persicae* were 53 and 47% in the two groups of tests, for a mean of 50%.

Mass inoculations. In the mass transmission experiments, both *M. persicae* and *Metopolophium dirhodum* transmitted BCMV to all the indicator plants (four of four plants and eight of eight plants, respectively). *D. noxia* never transmitted the virus (none of 10 plants).

Arena test. In the arena experiment, *M. persicae* transmitted BCMV to 14 of the 18 plants, whereas *D. noxia* never transmitted the virus.

Suction trap survey data. Log_{10} transformed mean trap collections indicated fluctuations in populations exceeding an order of magnitude over time for most of the cereal aphid species tested (Table 2). The contribution of each species to the total cereal aphids collected also varied

Table 1. Bean common mosaic virus transmission efficiency of major Idaho cereal aphids and *Myzus persicae*

Species	Percent transmission	No. of aphids tested
Diuraphis noxia	0.0	96
Metopolophium dirhodum	3.0	100
Rhopalosiphum padi	9.3	96
Schizaphis graminum	21.9	96
Sitobion avenae	5.0	100
Myzus persicae	50.0	195

Table 2. Log_{10} transformed mean[a] number of aphids per suction trap for five species of cereal aphids and *Myzus persicae* collected before 1 August in the bean production areas of Idaho, 1985–1992

Species	Mean number of aphids per suction trap							
	1985	1986	1987	1988	1989	1990	1991	1992
Diuraphis noxia	0	0	1	2,306	86	1,354	384	2,350
Metopolophium dirhodum	101	482	400	37	483	76	164	135
Rhopalosiphum padi	148	71	48	14	189	33	88	107
Schizaphis graminum	658	639	362	260	232	17	113	32
Sitobion avenae	77	146	64	38	60	17	9	10
Myzus persicae	8	6	1	7	2	3	8	1

[a] Antilog $\{[\Sigma \log_{10}(x+1)]/n\}-1$, where x is the number of specimens of a given species collected in each suction trap in bean production areas prior to 1 August for each year and n is the number of suction traps in operation.

Table 3. Comparison of cereal aphid and *Myzus persicae* potential vector indices[a] with bean seed hectares rejected for certification by the Idaho Crop Improvement Association because of bean common mosaic virus infection, 1985–1992

Year	Cereal aphid index[b]	*M. persicae* index	No. of hectares rejected
1985	165	4	0.0
1986	168	3	18.8
1987	99	1	20.0
1988	61	4	0.0
1989	86	1	218.4
1990	10	2	10.0
1991	39	4	69.6
1992	22	1	0.0

[a]The potential vector index (PVI) was calculated for each species by multiplying Antilog $\{[\Sigma \log_{10}(x+1)]/n\}-1$ by percent transmission obtained in laboratory experiments, where x is the number of specimens of a given species collected in each suction trap in bean production areas prior to 1 August for each year and n is the number of suction traps in operation.

[b]The sum of PVIs for *Metopolophium dirhodum*, *Rhopalosiphum padi*, *Schizaphis graminum*, and *Sitobion avenae*; *Diuraphis noxia* is a nonvector and therefore is not included.

widely. Very few *M. persicae* were collected in any year.

PVI. The sum of the PVIs for the five cereal aphid species tested was compared with the number of bean seed hectares rejected because of BCMV infection (Table 3). The correlation was poor when all 8 yr (1985–1992) were considered ($r^2 = 0.0004$, $P = 0.96$) but very good ($r^2 = 0.96$, $P = 0.018$) when only 1989–1992 were used.

DISCUSSION

The lack of correlation between hectares rejected and the PVI prior to 1989 may have occurred because BCMV serotype A was not present in commercial bean fields in Idaho before 1989 (7). The relatively low number of seed lot rejections prior to 1989 suggests that inoculum level rather than aphid vector pressure was restricting spread of BCMV prior to the establishment of BCMV serotype A.

Even though *M. persicae* was the most efficient vector of BCMV, it is probably not the most important vector in bean production areas of Idaho. Data on aphids collected before 1 August, the time period in which initial BCMV infections may cause yield losses, showed that major cereal aphid species were far more numerous than *M. persicae* (Table 2). The *M. persicae* PVI was consistently low, and there was no correlation between the PVI and the number of hectares rejected ($r^2 = 0.03$ for 4 yr [1989–1992] and 0.11 for 8 yr [1985–1992]). Thus, because *M. persicae* usually comprises such a small percentage of the Idaho trap collections in bean production areas, we suspect it contributes little to virus incidence despite its efficiency.

High numbers of *D. noxia* occurred in 1988, 1990, and 1992. Numbers of trapped *D. noxia* were particularly high in Parma in 1988 and 1990 (16,423 and 13,283, respectively). Very few hectares of seed beans were rejected in those years, suggesting that in the field as well as the laboratory, *D. noxia* is not an important vector of BCMV.

Our results indicate that *D. noxia* is a nonvector of BCMV. The explanation for this is unknown. *D. noxia* appears to probe readily on bean, making a simple behavioral explanation unlikely. Powell (15) showed that aphids were much more apt to transmit potyviruses if cells were punctured during probing than if they were not. This mechanism would explain a poor vector but not a nonvector. Lack of transmission may be the result of interactions between the virus and potential binding sites on the stylets and/or plant chemicals that regulate release of bound virus particles (2). Alternatively, saliva of *D. noxia* is toxic to host plants and may also be toxic to the virus. Possible *D. noxia* toxicity symptoms were observed on several mass-inoculated bean plants.

It is of interest to note that aphid colonies (*M. persicae* and *S. graminum*) used to maintain our field culture of BCMV by mass inoculation perpetuated the mixture of three strains. Infected plants obtained from individual aphid transmissions were used as source plants in subsequent transmission experiments; thus, single aphids as well as mass inoculation maintained the mixed infection. Unfortunately, source plants were selected for vigor and symptom expression, and the vectors that infected the plants were not recorded. Consequently, it is impossible to determine whether all vector species transmitted the mixture intact.

Serial transmission of the mixture occurred despite the fact that the culture reacted only with the serotype A-specific MAb. Although the two serotype B strains could not be detected in tissue extracts by the serotype B-specific MAb, both were readily transmitted from mixed culture to differential cultivars by rub-inoculation. It was not clear how the presence or absence of any of the strains affected aphid transmissibility of the mixture.

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LITERATURE CITED

1. Allison, D., and Pike, K. S. 1988. An inexpensive suction trap and its use in an aphid monitoring network. J. Agric. Entomol. 5:103-107.
2. Boiteau, G., and Singh, R. P. 1991. Difference in the transmission of PVY^O to potato and tobacco by the potato aphid, *Macrosiphum euphorbiae* (Thomas): Effects of probing duration and frequency. Can. Entomol. 123:869-874.
3. Drijfhout, E. 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Agric. Res. Rep. 872. PUDOC, Wageningen, Netherlands.
4. Drijfhout, E., Silbernagel, M. J., and Burke, D. W. 1978. Differentiation of strains of bean common mosaic virus. Neth. J. Plant Pathol. 84:13-26.
5. Forster, R. L., and Meyers, J. R. 1992. The bean common mosaic virus situation in Idaho in 1991. Annu. Rep. Bean Improvement Coop. 35:58-59.
6. Forster, R. L., Meyers, J. R., and Berger, P. H. 1978. Bean common mosaic virus. Pac. Northwest Ext. Bull. 358.
7. Forster, R. L., Meyers, J. R., Mink, G. I., and Silbernagel, M. J. 1991. NL-8 strain of bean common mosaic virus in Idaho bean seed fields. Plant Dis. 75:537.
8. Halbert, S. E., Connelly, B. J., Lister, R. M., Klein, R. E., and Bishop, G. W. 1992. Vector specificity of barley yellow dwarf virus serotypes and variants in southwestern Idaho. Ann. Appl. Biol. 121:123-132.
9. Halbert, S. E., Connelly, B. J., and Sandvol, L. E. 1990. Suction trapping of aphids in western North America (emphasis on Idaho). Acta Phytopathol. Entomol. Hung. 25:411-422.
10. Halbert, S. E., Irwin, M. E., and Goodman, R. M. 1981. Alate aphid (Homoptera: Aphididae) species and their relative importance as field vectors of soybean mosaic virus. Ann. Appl. Biol. 97:1-9.
11. Harris, K. F. 1977. An ingestion-egestion hypothesis of noncirculative virus transmission. Pages 165-220 in: Aphids as Virus Vectors. K. F. Harris and K. Maramorosch, eds. Academic Press, New York.
12. McKern, N. M., Mink, G. I., Barnett, O. W., Mishra, A., Whittaker, L. A., Silbernagel, M. J., Ward, C. W., and Shukla, D. D. 1992. Isolates of bean common mosaic virus comprising two distinct potyviruses. Phytopathology 82:923-929.
13. Mink, G. I., and Silbernagel, M. J. 1992. Serological and biological relationships among viruses in the bean common mosaic virus subgroup. Arch. Virol. Suppl. 5:397-406.
14. Plumb, R. T., Lennon, E., and Gutteridge, R. A. 1982. Barley yellow dwarf virus (BYDV). Pages 195-198 in: Rothamsted Exp. Stn. Rep. 1981 Pt. I.
15. Powell, G. 1991. Cell membrane punctures during epidermal penetrations by aphids: Consequences for the transmission of two potyviruses. Ann. Appl. Biol. 119:313-321.
16. Skotland, C. B., and Bush, D. W. 1961. A seed-borne bean virus of wide host range. Phytopathology 51:565-568.
17. Summers, C. G., Newton, A. S., Jr., Kirk, M., and Temple, R. 1990. Transmission of beet yellows and beet mosaic viruses by noncolonizing aphid vectors. J. Econ. Entomol. 83:2448-2451.
18. Wang, W.-y. 1983. Serology of bean common mosaic virus strains. M.S. thesis. Washington State University, Pullman.