Grasses as a Reservoir of Barley Yellow Dwarf Virus in Indiana

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ABSTRACT

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About 50% of the grass samples collected in Indiana during the summer of 1980 were infected with barley yellow dwarf virus (BYDV) according to enzyme-linked immunosorbent assay. The isolates distinguished by the tests resembled PAV (nonspecifically transmitted by *Rhopalosiphum padi* and *Macrosiphum avenae*), MAV (specifically transmitted by *M. avenae*), or RPV (specifically transmitted by *R. padi*). Results indicated that there is a large reservoir of BYDV in perennial grasses in Indiana but that this may not be the most important source of inoculum for spread to cereals. For example, RPV-like isolates were the most prevalent type in grasses at the Purdue University Agronomy Farm, but samples from winter wheat and spring oats collected there were infected mostly with PAV-like isolates. In addition, grasses around a plot where wheat artificially infected with a PAV-like isolate had been grown for several seasons showed very few PAV-like infections. Other possible sources of BYDV, including corn (*Zea mays*), need investigation in relation to epidemiology in cereals.

Soft red winter wheat is the third most important arable crop in Indiana, accounting for about 450,000 ha annually. It is sown from mid-September to mid-October, overwinters as young tillered plants, and is harvested in July. Barley yellow dwarf virus (BYDV) infection is a regular economic problem in the crop, to a degree depending on cultivar and season. However, the epidemiology of BYDV in Indiana, as elsewhere in the Midwest, is poorly understood. The virus is exclusively aphid-borne and must be introduced by viruliferous aphids moving into the crop. Whether such aphids usually come from adjacent or distant virus sources is not known, nor is the significance of various possible hosts as reservoirs of BYDV for spread to the cereal crop.

A major reason for this lack of information has been the difficulty of identifying BYDV infections by aphid transmission tests, especially because of the existence of several vector-specific

viruses, referred to as isolates by Rochow (eg, 16). Recently, however, the successful application of isolate-specific, enzyme-linked immunosorbent assay (ELISA) tests (9,10,17,19) has greatly simplified identification.

We report here on a survey of the prevalence of BYDV isolates in grasses at the Purdue University Agronomy Farm and elsewhere in Indiana. Some tests of cereal samples from the Purdue Farm were also done for comparison with the situation found in the grasses.

MATERIALS AND METHODS

Virus isolates. The isolates of BYDV used as standards in the ELISA tests were of the MAV, RPV, and PAV type—ie, respectively transmitted specifically by *Macrosiphum avenae* Fab., transmitted specifically by *Rhopalosiphum padi* L., and transmitted nonspecifically by *R. padi* and *M. avenae* (16). Three (MAV, RPV, and PAV) were authentic isolates kindly supplied by W. F. Rochow in infected leaves of *Avena byzantina* Koch cv. Coast Black. A fourth, PAV-like isolate (P-PAV) was isolated in Indiana by J. E. Foster as representing a typical wheat isolate (J. E. Foster, *personal communication*) and maintained in the greenhouse at Purdue on *A. sativa* L. cv. Clintland 64. All samples were stored frozen as harvested leaves at −80 C.

Antisera. Antiserum to P-PAV was made at Purdue in 1979 by injecting rabbits with partially purified virus preparations made by the method of Rochow et al (18); antisera to the MAV and RPV isolates were those used previously (10).

ELISA procedure. Partially purified immunoglobulins (Ig's) were prepared by precipitation by half-saturation with

ammonium sulfate or sodium sulfate, followed by resuspension in phosphate-buffered saline (PBS), pH 7.4, and dialysis against two 1-L lots of PBS.

The Ig's at 1 mg of protein per milliliter ($A_{280} = 1.4$) were conjugated with alkaline phosphatase (Type VII, Sigma Chemical Co., St. Louis, MO) at an enzyme/Ig ratio of 5:3 (w/w) by treatment with 0.06% glutaraldehyde (4 hr at room temperature) and were stored at 4 C with an equal volume of bovine serum albumin at 10 mg/ml containing 0.04% sodium azide.

Methods for ELISA were essentially as described by Clark and Adams (3). Wells in polystyrene micro-ELISA plates (1.233-29; Dynatech Laboratories, Inc., Alexandria, VA 22314) were coated by incubating 250 µl of unlabelled Ig diluted in 0.05 M sodium carbonate, pH 9.6, at 36 C in each well for 3 hr. Peripheral wells were not used but were kept filled with water to help stabilize the temperature. Plates were covered and put in plastic bags containing a moist paper towel. After incubation, they were rinsed three times with PBS containing 0.05% Tween (PBS-Tween).

Antigen preparations, ie, cereal or grass extracts (see below) in PBS containing 0.05% Tween 20 and 2% polyvinylpyrrolidone (PVP, MW 40,000; Sigma), were then incubated similarly for 6 hr at 37 C in the coated, rinsed wells to react with the bound Ig. After further rinsing, diluted enzyme-conjugated Ig was added to react with bound antigen during a further incubation overnight at 6 C. The overnight incubation of conjugate at 6 C, combined with a 6-hr incubation with antigen at 37 C, gave lower background values than the reverse combination and allowed easier visual discrimination between positive and negative reactions. Similarly, to reduce background the conjugate was cross-absorbed (10) by dilution in a buffer (PBS-Tween-PVP) extract of healthy Clintland 64 oat leaf tissue (1:4, w/v).

Finally, unreacted conjugate was rinsed away, and specific antibody-antigen reactions were assessed by adding 250 µl *p*-nitrophenyl phosphate at 1 mg/ml in 10% diethanolamine buffer, pH 9.8. Reactions were stopped after 45–60 min by adding 50 µl of 3 M sodium hydroxide per well. Assay was by visual observation of the yellow nitrophenolate, based on a "plus" and "minus" classification, and by reading absorbances

(A_{405}) of well contents directly or after dilution with water. Control reactions with extracts from healthy leaves were low—usually less than 0.1. Those reactions giving obvious yellow color or absorbance equal to or greater than twice the average for healthy control samples in the same experiment were regarded as positive. All tests were duplicated, and the results were highly reproducible. Based on the results of preliminary tests, MAV and RPV coating and conjugate Ig's were used at 2.5 and 5 μg/ml, respectively, and PAV coating and conjugate Ig's were used at 10 and 2.5 μg/ml, respectively.

Extraction procedures. Buffer extracts of grass or cereal samples, all collected during May, June, and July 1980, were made by one of the two following procedures, which gave similar results.

In the first procedure, 1 g of leaf, frozen in liquid nitrogen, was pulverized to a powder in a mortar, then ground further in 2 ml of 0.1 M phosphate, pH 7.0. The product was squeezed through cheesecloth, and 2 ml of PBS/Tween/PVP was added.

In the second and most commonly used procedure, the leaf powder prepared by being pulverized in liquid nitrogen was transferred to a tube with 2 ml of 0.1 M phosphate, pH 7.0, plus 2 ml of PBS/Tween/PVP. This mixture was then blended in a Polytron homogenizer (Brinkman Instruments, Westbury, NY) (15 sec at speed setting 5 with generator PT-10-ST), and the extracts were squeezed through cheesecloth.

Purdue Farm survey. The grasses sampled at the Purdue Farm were growing in strips 50 cm to 2 m broad, bordering the pathways surrounding each field. Samples were removed at regular intervals (approximately 80 m) at each side of every accessible path. Thus, the frequency of sampling was fairly even over the farm (Fig. 1). Each sample was taken from what appeared to be the most prevalent grass species in each sampling area. Yellowed and stunted samples were selected preferentially, but these were rare and the great majority of samples showed no obvious symptoms.

Samples, removed with an auger, consisted of cores about 10 cm in diameter and 10 cm deep. After being sprayed with malathion to kill aphids, each core was transplanted to a 12-cm-diameter pot containing potting soil and maintained in an insect-free greenhouse. For ELISA testing, 1-g lots of leaves were cut from each sample 1 or 2 wk after collection, and buffer extracts (1:4, w/v) were tested with the MAV, PAV, and RPV antisera as described above. Each test was duplicated, and the few doubtful results obtained were checked by repeat tests using fresh leaf samples. Later, the grass species predominating in each pot were identified.

Some samples of winter wheat (*Triticum aestivum* L.) and spring oats growing in the two rectangular plots indicated in field 11 (Fig. 1) were collected. The eastern half of the northern plot (NE 11) contained individual plants of each of a large number of wheat selections under test in the Purdue cereal breeding program for their reaction to BYDV (P-PAV isolate). Each had been inoculated by exposure to viruliferous *R. padi* in the greenhouse before being transplanted in the fall of 1979 for comparison with control plants treated similarly but not infested. Because a similar procedure had been used in each of the previous 3 yr, P-PAV inoculum had been present for several growing seasons. The western half of this plot (NW 11) contained 1-m rows of wheat breeding lines planted in a drill directly in the field but not artificially infested with aphids. The southern plot (S 11) was planted with a filler crop of spring oats in 1980. Samples collected from each of these plots during the survey period were tested by ELISA as described above.

The area around these plots in field 11 was filled with long-established perennial grasses. Samples were collected from this area, both close to the plots (Table 1, "field 11") and farther afield (Fig. 1; Table 1, "general survey").

Samples were also collected in the only two other cereal plantings available for sampling at the Purdue Farm during 1980. These were field 41 (winter wheat) and field 63 (spring oats) (Fig. 1).

Surveys of other Indiana sites. Some tests were also done with grasses sampled randomly from established (at least 10-yr) lawns throughout the Lafayette area.

Grasses from in and near four orchards in the state were also sampled at random and tested. These sites were near Indianapolis (Marion County), Michigan City (LaPorte County), and Vincennes (Knox County) in central, northern, and southern Indiana, respectively.

RESULTS

Characteristics of ELISA reactions. Clear-cut positive ELISA values of five to 12 times background were obtained with extracts from the standard MAV, RPV, and P-PAV samples tested at a fourfold dilution. However, ELISA values for extracts from field-collected grass samples varied over the period of the survey from about 12 times background for undiluted extracts from samples
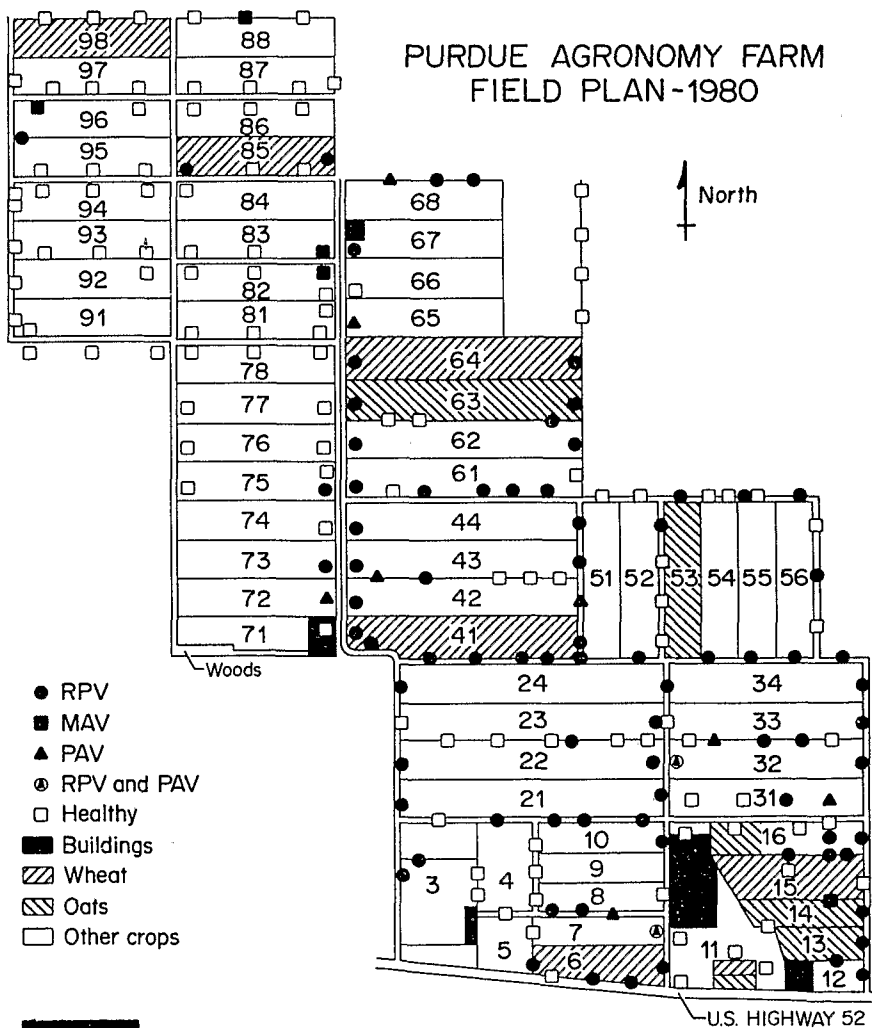

Fig. 1. Plan of Purdue Agronomy Farm, showing location of cereal crops in 1980 and sites sampled for barley yellow dwarf in grasses. Scale bar = 300 m.

Table 1. Barley yellow dwarf virus (BYDV) infections detected by enzyme-linked immunosorbent assay in samples of grasses and cereals from various locations in Indiana

Location	Host	No. tested/infected	Percentage infected	BYDV type detected			
				RPV	PAV	MAV	RPV + PAV
Purdue Farm general survey	*Poa pratensis*	138/75	54	71	3	1	0
	Festuca arundinacea	29/6	21	2	1	3	0
	P. pratensis + *F. arundinacea*	16/7	44	2	4	0	1
	P. pratensis + other species[a]	3/2	...	2	0	0	0
	F. arundinacea + other species	1/0	...	0	0	0	0
	Other species	15/5	33	1	0	2	2
	Total	202/95	47	78	8	6	3
Purdue Farm field 11	*P. pratensis*	30/9	30	9	0	0	0
	P. pratensis + *F. arundinacea*	8/7	88	7	0	0	0
	P. pratensis + other species	2/1	...	0	1	0	0
	Other species	1/1	...	0	1	0	0
	Spring oats	54/41	76	6	31	3	1
	Winter wheat	14/14	100	0	9	0	5
Purdue Farm field 41	Winter wheat	6/5	83	2	3	0	0
Purdue Farm field 63	Spring oats	26/9	35	1	8	0	0
Lafayette area (lawns)	Grasses[b]	83/47	57	39	5	0	3
Indianapolis (NE)	Grasses	14/3	21	0	3	0	0
Indianapolis (SW)	Grasses	7/6	86	1	4	0	1
Michigan City	Grasses	7/7	100	0	7	0	0
Vincennes	Grasses	9/6	67	5	0	0	1

[a] Annuals: *Bromus commutatis* Schrad., *B. japonicus* Thunb., *B. tectorum* L., and *Setaria viridis* (L.) Beauv.; perennials: *B. inermis* Leyss., *Dactylis glomerata* L., *Festuca rubra* L., *Phleum pratense* L., and *Poa compressa* L. Infections were detected in *B. tectorum, F. rubra,* and *P. pratense.*
[b] Various species, mainly *Poa pratensis.*

collected in mid-June to little more than two times background for some samples collected in late July. This effect was associated with reduction in vegetative growth during warm and dry conditions. By contrast, ELISA values for field-collected cereal samples tended to increase through the survey period, ranging from about five times background for June samples to 10–12 times background for July samples, which included some samples consisting of dry senescent leaves.

Table 2 illustrates the serological relationship as determined by ELISA among the BYDV isolates used as standards during the survey. Neither MAV nor P-PAV Ig's detected the RPV antigen, a result consistent with previous findings of nonrelationship between RPV and the two other isolates (1). The P-PAV antigen reacted with MAV Ig, but not as strongly as with the homologous P-PAV Ig. However, the P-PAV Ig did not give positive reactions with the MAV antigen. Similarly, heterologous reactions occurred with most, but not all, of the PAV-like antigens found during the survey and the MAV Ig, but they were always less intense than the "homologous" reactions with P-PAV Ig.

According to previous reports (10,19), heterologous reactions between the authentic (Cornell) type PAV antigen and MAV sera in ELISA tests are relatively weak compared with reactions using type PAV and its antiserum. This effect was confirmed in our tests (Table 2), which also showed that the P-PAV Ig reacted less efficiently with type PAV

Table 2. Homologous and heterologous reactions of barley yellow dwarf virus (BYVD) preparations in enzyme-linked immunosorbent assay[a]

Antigen	Immunoglobulin		
	MAV	P-PAV	RPV
	Experiment 1		
MAV	1.64	0	0.22
P-PAV	1.89	2.20	0
RPV	0	0	1.37
	Experiment 2		
MAV	1.12	0.04	...[b]
P-PAV	1.38	2.24	...
PAV	0.24	0.80	...

[a] Values obtained in reactions of extract from oat infected with the MAV, P-PAV, PAV, or RPV isolates of BYDV, using the immunoglobulin indicated as coat and conjugate. Values for control extracts from healthy plants have been subtracted.
[b] Not tested.

than with P-PAV antigen, suggesting that the P-PAV differs from the type PAV in its degree of relationship with type MAV.

This and the fact that some of the PAV-like isolates from grass samples reacted with MAV Ig less strongly than P-PAV, or not at all, suggests that a range of PAV-like isolates occurs in the state. This is consistent with the situation found in surveys elsewhere (Rochow, *personal communication*).

Purdue Farm surveys. Of a total of 202 samples tested in the systematic general survey of grasses at the Purdue Farm, 95 indexed positive for BYDV (Table 1). Infections were distributed fairly uniformly over the area surveyed (Fig. 1). *Poa pratensis* L. (Kentucky bluegrass) and *Festuca arundinacea* Schreb. (tall fescue), the two most commonly occurring perennial grasses, frequently indexed positive; however, BYDV was

identified in several other species, including annuals and perennials (Table 1). In a few cases, the source of virus in a test sample was unknown because the samples included mixtures of grass species. Isolates of the RPV type occurred in 81 of the 95 infected samples. Eleven samples contained PAV-like isolates and six contained MAV-like isolates.

Early in the survey, six of the artificially infected wheat plants selected at random in the NE 11 area were sampled for ELISA. Each indexed positive only for BYDV of the PAV type. During June and July, some of the noninfested wheat plants in the NW 11 area showed symptoms suggesting BYDV infection; 14 of these plants, selected at random and representing about one-half of those seen, assayed positive for BYDV of the PAV type, and five also

indexed positive for RPV. Similarly, 41 of 54 oat plants showing leaf chlorosis, browning, and reddening found scattered throughout the S 11 plot were positive for BYDV, mostly PAV-type (Table 1).

The remainder of field 11 was in grass (mostly *P. pratensis* and *F. arundinaceae*), and most of the infections in 41 samples collected at random around and within about 2 m of the cereal plots were of the RPV type (Table 1).

Fourteen of 32 cereal samples collected near the edges of field 41 (winter wheat) and field 63 (oats) indexed as positive. The PAV-type predominated (11 samples), but RPV was present in three samples. No samples contained both isolates together or virus of the MAV type (Table 1).

Samples from other locations in Indiana. A total of 120 randomly selected, symptomless grass samples was collected from various locations in Indiana (Table 1). Over half of those from established lawns in older gardens in the Lafayette area indexed positive. Also, of the remaining 37 samples collected from other sites, again more than half indexed as positive for BYDV. Most isolates, especially from the Lafayette area, were of the RPV type, but in three locations the PAV type predominated.

DISCUSSION

The results indicated that BYDV was common and widespread in perennial grasses on the Purdue Farm. Moreover, the fact that BYDV was found frequently even in the superficial survey of other areas indicates that it was common and widespread in grasses elsewhere in Indiana. Because of the specificity of ELISA in tests for BYDV (19), the antisera used probably detected only isolates of the MAV, RPV, and PAV types, and BYDV may thus be even more common than the survey indicated. At the Purdue Farm, RPV was the predominant virus type detected in grasses; overall, however, the predominating isolate varied from one area to another.

The proportion of infected grass samples detected was high (around 50%), as has also been true of surveys elsewhere—eg, 70% in grass fields in Scotland (8), 70 and 86% in pastures in Northern Ireland (5), and 90% in England (6). Whether such sources of virus are significant in the infection of spring and winter cereals depends on whether viruliferous aphids move readily between these crops and grasses. If so, it would be expected that the virus isolates from grass and cereals would be of a similar type. But the survey results indicated that the 1980 winter wheat and spring oat crops at the Purdue Farm were infected mostly by BYDV of the PAV type, even when surrounded by perennial grasses infected by the RPV type. It is of particular interest also that the grasses around the cereal plots in field 11 at the Purdue Farm, where wheat inoculated with PAV-type BYDV had been grown for several seasons, showed very few PAV-type infections but that the PAV type was common in infections in the adjacent oat plot.

Taken together, these observations suggest that although aphid movement from the infected wheat plants to oats may have caused spread of BYDV, movement of viruliferous aphids from grasses to adjacent cereals and from cereals to the adjacent grasses was limited in the 1979–1980 cereal growing season. The virus reservoir in grasses in this location, therefore, need not be the major source of BYDV for spread directly to cereals. This suggestion is consistent with observations made of aphid populations and the occurrence of different types of BYDV in cereals and annual and perennial grasses elsewhere (eg, Europe [14], the northeastern United States [21], and Canada [7,22]), which have indicated that local grasses do not necessarily play the major role in BYDV epidemiology.

However, in the absence of similar survey results for Indiana for years prior to 1979–1980, it is not possible to judge whether this situation is typical. Also, virus spread from a reservoir in grasses need not be frequent for it to be significant in virus epidemiology. Again, the presence of aphid vector species on grasses depends on host efficiency and attractiveness. For example, *P. pratensis*, the most prevalent species in our surveys, is reported to be a poor host for *R. padi*, whereas *F. arundinaceae* is listed as an efficient host (4). Climatic conditions affect the multiplication of aphids on grasses and could also affect migration to and from cereals. The situation regarding virus spread could thus vary widely among areas and from year to year (15,17). Surveys of both BYDV and aphids need to be conducted in grasses and cereals for several years in Indiana before firm conclusions can be reached regarding the role of local grasses in BYDV epidemiology. Moreover, a complete investigation would require the inclusion of tests for other types of BYDV (16,20).

Spring outbreaks of BYDV could result from spread from local sources or from airborne viruliferous aphids carried over a long distance by southern winds (7,12,13,24). Infection would thus occur because of the settling of alate viruliferous aphids carried from far afield directly onto the cereal crop, and the isolate type would be fortuitous.

The origin of autumn infection, which is the more damaging to winter wheat, is also unclear. It may also be caused by migrations from the south, although such late-season migrations have not been observed in Indiana. Aphids feeding on infected volunteer cereals or on infected grasses at the end of the summer and moving into winter crops could induce part of the infection. Stoner (23) established that BYDV can easily and consistently be transmitted and recovered from several corn (*Zea mays* L.) cultivars by *R. padi*. Large populations of *R. padi* including alatae have been observed on corn during autumn at the Purdue Farm (J. E. Foster, *personal communication*). Thus, it is possible that corn, the major agricultural crop in Indiana, could play a part in the autumn infection of winter wheat. This hypothesis is consistent with the recommendation of Moreau and Lapierre (11) that volunteer corn and corn straw should be eliminated before the autumn sowings of cereals in France. Recent observations in eastern Washington State (2) have also implicated corn as an oversummering host of BYDV and its aphid vectors.

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Host Range of the Columbia Root-Knot Nematode

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ABSTRACT

O'Bannon, J. H., Santo, G. S., and Nyczepir, A. P. 1982. Host range of the Columbia root-knot nematode. Plant Disease 66:1045-1048.

The Columbia root-knot nematode, *Meloidogyne chitwoodi,* a severe pest of potato in the Pacific Northwest, reproduced on 53 of 68 plant species tested under greenhouse conditions. Both monocotyledonous and dicotyledonous plant species were good hosts, indicating that *M. chitwoodi* has a wide host range. Principal crops used in rotation with potato in the Pacific Northwest include a poor host (alfalfa), but cereals such as barley, corn, and wheat were good hosts for this nematode.

An undescribed root-knot nematode was found attacking potato (*Solanum tuberosum* L.) in the Pacific Northwest in 1977-1978 (9). The new species was described as *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, and Finley (4) and given the common name "Columbia root-knot nematode." It is presently known in Idaho, Oregon, Washington, northern California, and Nevada (6). Economically, potato is the major crop most severely damaged by *M. chitwoodi,* but it can reproduce and is pathogenic on oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.), crops that are used in rotation with potato (8,9).

The northern root-knot nematode, *M. hapla,* which also attacks potatoes, is limited to broad-leaf plants (2). Because of the need for effective rotation and weed control, a study was made to determine crop and weed hosts of *M. chitwoodi.*

MATERIALS AND METHODS

Several host range tests were conducted in a greenhouse for 2 yr. Four or five

seeds, later thinned to one or two seedlings or a single seed piece, were either pregerminated or planted directly into 10-cm plastic pots containing loamy sand (82.3% sand, 14.8% silt, 2.9% clay) fumigated with methyl bromide. Pots were randomized in five replicates on greenhouse benches, and plants were inoculated after 2- or 3-wk growth with 3,000-4,000 *M. chitwoodi* eggs in a 25-ml water suspension added to the roots of plants in each pot. Inoculum came from infected tomatoes (*Lycopersicon esculentum* Mill. 'Rutgers') maintained in a greenhouse. Eggs were extracted from roots by the sodium hypochlorite method (5).

Greenhouse temperatures were maintained at 20-25 C, which is favorable for nematode infection and reproduction (7). Plants were watered daily and fertilized with Hoagland's nutrient solution bimonthly. Tests were terminated after 50 days, and the roots were carefully removed and washed free of soil. To measure host susceptibility and nematode reproduction, roots were soaked (15 min) in a phloxine B solution (1) (150 mg/L) and examined with a dissecting microscope. After examination, nematode eggs were extracted (5) and brought to volume (100 ml), and a 1-ml aliquot sample was counted. Roots were oven-dried and weighed, and the number of eggs per gram of dry root was calculated. Plants were rated as to relative degree of nematode reproduction based on egg masses and eggs per gram of oven-dry root. Ratings were 0 = no reproduction, nonhost; 0.1-1 = trace reproduction,

<100 eggs per gram of root, very poor host; 1.1-2 = light reproduction, 100-1,000 eggs, poor host; 2.1-3 = moderate reproduction, >1,000-10,000 eggs, moderate host; 3.1-4 = high reproduction, >10,000-100,000 eggs, good host; 4+ = very high reproduction, >100,000 eggs, very good host.

Because several plants observed in our studies are grown in a rotation program, we tested several varieties within a species to ascertain the possible existence of resistant germ plasm or nematode-tolerant cultivars.

RESULTS AND DISCUSSION

M. chitwoodi was found to infect and reproduce on 53 of 68 plant species tested, indicating a wide host range (Table 1). Unlike *M. hapla* (2), many of the Gramineae as well as many dicotyledonous species were good host of *M. chitwoodi.* Because alfalfa is often used in the rotation, several *Medicago sativa* cultivars were tested and found to be either nonhosts or poor hosts. Most alfalfa varieties are susceptible to *M. hapla,* and several cereals susceptible to *M. chitwoodi* were also used in the rotation. Therefore, this information is of value to growers when practicing rotation with potatoes and alerts field and extension personnel to the necessity of species identification between *M. chitwoodi* and *M. hapla.*

In the field, galls and egg masses produced by *M. chitwoodi* are often difficult to see on roots of most host plants, even with the aid of a hand lens. To see egg masses better, roots should be stained with phloxine B, which stains the masses red for rapid identification (1). This can be done in the field and also serves as an excellent teaching example for the grower.

To aid interested persons in developing diagnostic evaluation for species separation of suspicious sites, specific hosts for *M. chitwoodi* or *M. hapla* should be used. Wheat cv. Nugaines, a host of *M. chitwoodi,* and pepper cv. California Wonder, a host of *M. hapla,*