Screening of Wheat (*Triticum aestivum, T. turgidum*) and Peanut *(Arachis hypogea)* Cultivars for Susceptibility to an Oklahoma Strain of Aster Yellows Mycoplasmalike Organism

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Received: 1993 Jan 14; Revised: 1993 Mar 15

Aster yellows affects several graminaceous crop plants such as barley, oats and wheat, and other grasses. Several yellows diseases have been reported in legumes, including peanuts. Five cultivars each of wheat (*Triticum aestivum* and *T. turgidum*) and peanut (*Arachis hypogea*) commonly grown in Oklahoma, and wheat commonly grown in Canada, were screened for susceptibility to an Oklahoma strain of aster yellows mycoplasmalike organism (AY-OC 1). None of the tested cultivars of either wheat or peanut were infected by AY-OC 1 through aster leafhopper transmissions. The failure of AY-OC 1 to infect the Canadian wheat cultivars, which are natural hosts of aster yellows in Canada, suggests the possibile existence of different strains of aster yellows or of different leafhopper biotypes in the two locations.

INTRODUCTION

Aster yellows (AY), an intensively studied plant disease, affects more than 350 species in 54 plant families (*1*). It may cause devastating economic losses in agronomic and horticultural crops (*1*). The causal agent of AY is a non-culturable mycoplasmalike organism (MLO) that lacks a cell wall and is bound by a single unit membrane (*2*). AY is transmitted mainly by the six-spotted (aster) leafhopper, *Macrosteles quadrilineatus* (formerly known as *M. fascifrons* (Stal)), in which the MLO multiplies (*3*).

In 1960 Bantarri and Moore (*4*) first demonstrated the susceptibility of the family Graminae to AY by transmitting the disease agent (known until 1967 as the “aster yellows virus”), from field-infected barley plants, to and from greenhouse-grown barley, *Hordeum vulgare* L., cv. Vantage. In 1965, Chiykowski showed that twenty-four cultivars of barley were susceptible (*5*) and in 1991, twelve barley cultivars, currently grown in Canada, were found to be highly susceptible to AY MLO (*6*). The AY MLO was first transmitted to oats, *Avena sativa* L., in 1962 (*7*), and at least seven oat cultivars are now known to be susceptible to the Eastern strain (unable to infect celery) (*8-10*). Two strains of AY, a celery-infecting or Western strain and an Eastern strain, were transmitted experimentally to several cultivars of wheat, *Triticum aestivum* L. and *T. turgidum* Desf., by leafhoppers (*11*,*12*). Eight cultivars of *T. aestivum* (cvs. Cascade, Lemhi, Manitou, Selkirk, Soft White, and Thatcher), and three cultivars of *T. turgidum* (cvs. Pelissier, Ramsey, and Stewart) were susceptible to an Eastern AY MLO strain (*10-12*). Other experimental graminaceous hosts of AY were field brome (*Bromus arvensis* L.), annual canary grass (*Phalaris canariensis* L.), common rye grass (*Lolium multiforum* Lam.), chess brome (*Bromus secalinus* L.), needle grass (*Aristida adscensionis* L.), Indian grass (*Sorghastrum nutans* L.), little bluestem (*Andropogon scoparius* L.) and perennial ryegrass (*Lolium perenne* L.) (*13*). Except for one case in Finland (*14*), all natural AY MLO infections in cereals were reported from Canada.

Several MLOs have been reported to affect peanuts. Peanut proliferation was first reported in 1956 by Bergman in Indonesia (*15*). Peanut witches’-broom disease was reported in Taiwan by Yang and in Indonesia by Gourret and Triharso, and by Iwaki et al. (*15*). Phyllody of peanuts occurred in Thailand (*16*) and phyllody with proliferation was reported in India (*15*).

Wheat and peanuts are major crops in

Oklahoma. The prevalence of yellows disease in the state (*17-20*), the abundance of the aster leafhopper in Oklahoma (*19*), and reports of up to 65% yield losses in wheat due to AY in Canada (*12*, *21*), led us to investigate the susceptibility of Oklahoma-grown wheat and peanut cultivars to aster yellows. This paper reports the reactions of selected Oklahoma wheat and peanut cultivars to an aster yellows strain (AY-OC 1) collected in Oklahoma.

EXPERIMENTAL PROCEDURES

Oklahoma wheat and peanut cultivars. Wheat and peanut seeds were kindly provided by the following researchers: Wheat, *T. aestivum* cvs. Chisholm, Payne, TAM 101, Triumph 64 and Vona (R. M. Hunger, Oklahoma State University, Stillwater, OK) and cvs. Lemhi and Selkirk (S. Haber, Agriculture Canada, Winnipeg, Manitoba, Canada); ***T. turgidum*** cvs**.** Stewart, Ramsey and Thatcher (S. Haber, Manitoba, Canada); peanut, *A. hypogea* cvs**.** Florunner, Okrun, Pronto, Tamnut, and Toalson (H. A. Melouk, Oklahoma State University, Stillwater, OK). Aster (*Callistephis chinensis* L.) seed was obtained from Old's Seed Co, Madison, WI.

Collection and maintenance of AY MLO (AY-OC 1). Healthy aster leafhopper (*M. quadrilineatus*) colonies were initiated from adults obtained from C. E. Eastman (Illinois Natural History Survey, Champaign, IL). Leafhoppers were reared on barley (*H. vulgare* L. 'Post,' a cultivar resistant to AY MLO) in large wood and screen cages ($60 \times 60 \times 60$ cm) in the greenhouse at 22-30 °C. The colonies were frequently monitored for MLO contamination by caging selected leafhoppers from healthy colonies with aster seedlings and observing those asters for symptom development (*19*). An Oklahoma AY strain, AY-OC 1, was obtained in 1985 from a carrot plant grown in a plot at the Oklahoma State Vegetable Research Station, Bixby, OK (*19*). This strain was identified as AY by the symptomatology on aster and celery (*19*), serology (*19*), and Southern hybridization with an AY-specific DNA probe (*16*,*22*). The isolate was maintained in aster in the greenhouse by serial insect transfers using *M. quadrilineatus* (*19*).

Transmission experiments with wheat and peanut cultivars. In our transmission studies, leafhoppers (early instar nymphs) were caged in groups on infected (with AY-OC 1) aster plants for 3 to 7 days and then on healthy barley (1-2 wk old) for a 2-wk incubation period. A subset of these leafhoppers was then caged singly on 7-day-old healthy aster plants for 3 to 7 days. Inoculated plants were sprayed with malathion and moved to a greenhouse bench for observation of symptom development. Approximately 95% of these asters, inoculated by leafhoppers, expressed yellows symptoms 7 to 10 days after inoculation. Only batches of leafhoppers from which the subset individuals showed infectivity on aster were used in the transmission studies. All the transmission experiments were conducted in the greenhouse at 22-30 ºC.

For each cultivar in each experiment, 30 wheat seedlings (5/pot), or 30 peanut seedlings (3/pot), were exposed to AY MLO-exposed leafhoppers. Fifty AY-exposed leafhoppers were caged for 7 days on 7- to 8-day-old wheat seedlings, or on 21-to 23-day-old peanut seedlings. Three types of controls were included in each experiment: test plants caged with fifty non-exposed leafhoppers, test plants caged without leafhoppers, and aster plants (an indicator host) caged with fifty AY-exposed leafhoppers. After the inoculation access period (3 to 7 days), plants were sprayed with malathion and held to maturity in the greenhouse for symptom observation. Wheat screening experiments were replicated four times and peanut screening experiments twice. Transmission of MLOs to test plants and to aster controls was determined by symptomatology, Dienes' stain (*23*), electron microscopy, and back-inoculations to asters. Back-inoculations from representative wheat or peanut plants were conducted by caging 50 healthy leafhoppers on a pot with 2 to 3 exposed wheat or peanut plants for 7 days. The insects were transferred to barley for a 2-wk incubation period, and then caged on healthy aster indicator plants for 1 wk. The indicator plants were sprayed with malathion and held in the greenhouse to confirm transmission by symptom observation.

Electron microscopy. Samples of leaves, midribs, stems or petioles from symptomatic asters (positive controls), and selected asymptomatic wheat and peanut plants from transmission experiments, were fixed and

TABLE 1. Reaction of selected wheat and peanut cultivars to AY–OC 1, an Oklahoma strain of aster yellows mycoplasmalike organism.

Cultivars	NI[a] *expected* for an infection rate of 4%	NI / no. plants exposed to inoculative LH[b]	NI / no. plants exposed to healthy LH	NI / no.plants exposed to no LH	NI of aster plants / no. plants exposed to LH in B.I.[c]
WHEAT, Oklahoma					
T. aestivum					
Chisholm	4/120	0/120	0/120	0/120	0/52
Payne	4/120	0/120	0/120	0/120	0/50
TAM 101	4/120	0/120	0/120	0/120	0/45
Triumph	4/120	0/120	0/120	0/120	0/43
Vona	4/120	0/120	0/120	0/120	0/49
WHEAT, Canada					
T. aestivum					
Lemhi	4/120	0/120	0/120	0/120	0/47
Selkirk	4/120	0/120	0/120	0/120	0/44
T. turgidum					
Ramsey	4/120	0/120	0/120	0/120	0/47
Stewart	4/120	0/120	0/120	0/120	0/45
Thatcher	4/120	0/120	0/120	0/120	0/39
PEANUTS					
A. hypogea					
Florunner	2/60	0/60	0/60	0/60	0/26
Okrun	2/60	0/60	0/60	0/60	0/19
Pronto	2/60	0/60	0/60	0/60	0/26
Tamnut	2/60	0/60	0/60	0/60	0/20
Toalson	2/60	0/60	0/60	0/60	0/19
ASTER					
Callistephus chinensis[d]	5/125	120/125	0/125	0/125	204/211

[a] NI=number of infections detected by symptomatology on all plants, and electron microscopy and back-inoculations to indicator plants (asters) on a subset of the plants.
[b] LH=leafhoppers; B.I.=back-inoculation.
[c] Back-inoculation tests: aster plants (indicators) were caged with leafhoppers which previously had been caged with test plants; see text.
[d] Aster plants (*C. chinensis*), a known host of AY–OC 1, were used as a positive control.

stained for examination using a JEOL 100CX ASID transmission electron microscope as described previously (*19*). Representative samples from plants in all three experimental groups were treated similarly.

RESULTS and DISCUSSION

Screening of wheat and peanut cultivars for susceptibility to AY-OC 1. None of the wheat or peanut plants in any of our experiments developed symptoms of yellows disease (Table 1). A total of 95.5% of the aster (positive) controls showed symptoms. All test plants, except for positive control aster plants, reacted negatively with Dienes' stain. In electron microscopy studies, MLOs were observed in the phloem cells of all the Symptomatic asters tested but were not detected in samples of representative AY-exposed wheat and peanut plants. No yellows symptoms were observed on the indicator asters in back-inoculation experiments from representative wheat and peanut plants exposed to inoculative leafhoppers. Back-inoculation attempts from symptomatic aster controls gave positive results. Symptomatology, Dienes' staining, electron microscopy, and back-inoculation suggest that the wheat and peanut cultivars tested are not susceptible to AY-OC 1. Successful detection of low levels of infection is clearly determined by the number of test plants in the experiment. Chiykowski (*12*) used 50 test plants of each cultivar of *T. aestivum* and *T. turgidum* to demonstrate a low level of infection (4%) with the Western strain of AY. In our experiments the total number of test plants of each cultivar (120 wheat seedlings and 60 peanut seedlings) was sufficient to detect an infection level as low as 0.8% (wheat) or 1.6% (peanut).

The consistent detection of MLOs in

the control aster plants confirms that representative leafhoppers were inoculative and that the transmission procedures were suitable for aster. Containment regulations precluded our testing the reaction of any Canadian AY strains on either Canadian or Oklahoma wheat cultivars, or the reaction of AY-OC 1 on Canadian-grown wheat cultivars, under our conditions. Thus the efficacy of our transmission procedures for infecting wheat could not be confirmed. However, survival of the leafhoppers, on wheat and peanut plants for over 3 weeks indicated that they were feeding on these plants. Five wheat cultivars, reported susceptible to AY in Canada (*12*) were not infected by AY-OC 1 in our experiments. This suggests the possibility that there are differences in AY strains or leafhopper biotypes in Canada and Oklahoma.

Although peanut has been reported to be susceptible to unidentified MLOs (*15,24*), AY MLO has not been specifically reported in peanut. Lack of transmission of AY-OC 1 to Oklahoma peanut cultivars suggests that these cultivars, are not susceptible to this AY MLO strain. Additional testing of AY-MLO strains and peanut cultivars would have to be done to determine the general susceptibility of peanuts to infections with the AY MLO.

We have tested several of the cultivars of wheat and peanut commonly grown in Oklahoma, using a prevalent strain of AY, and have seen no infection. We conclude that these cultivars are not susceptible to AY-OC 1. However, our previous work indicated the presence of more than one strain of yellows agents, including AY, in Oklahoma (*16-19,22*). Chiykowski reported significantly higher infection rates in cereals with non-celery-infecting (Eastern) strains of AY than with celery-infecting (Western) strains (*12*). While AY-OC 1 has been characterized as a western strain on the basis of symptomatology (*19*) and DNA hybridization (*16*), another Oklahoma AY isolate, which resembled a western strain in symptomatology (*19*), was classified with the eastern types by DNA homology (*16*). The other Oklahoma MLO isolates have not yet been tested for pathogenicity in wheat or peanut. In addition, only a limited number of each species was tested in our experiments. Thus, although failure to obtain AY infection in the cultivars tested suggests the non susceptibility of wheat and peanut plants to AY-OC 1, we cannot conclude that yellows diseases will never pose a significant problem in these crops in Oklahoma. New, agronomically superior cultivars regularly replace older cultivars; their susceptibility to yellows type diseases may be different. Damage to wheat and peanut crops in other geographical areas by yellows agents justifies continued vigilance for susceptibility in Oklahoma.

ACKNOWLEDGMENTS

We thank Amy James for maintenance of plants, and R.M. Hunger, H.A. Melouk, and S. Haber for supplying seeds. This is Journal Article No. 6404, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma.

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