Incidence of Yellows in Carrot and Lettuce and Characterization of Mycoplasmalike Organism Isolates in Oklahoma

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ABSTRACT

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Incidence of yellows was greater in lettuce (*Lactuca sativa*) than in carrots (*Daucus carota*) and greater at two eastern Oklahoma sites than at a site in central Oklahoma. Eight isolates of mycoplasmalike organisms were collected from carrots, lettuce, and daisy fleabane (*Erigeron strigosus*). These isolates were separated into four groups based on distinctly different symptomatology on aster (*Callistephus chinensis*). Six of these isolates (two groups) had symptomatology similar to that of aster yellows. Based on the symptomatology on celery (*Apium graveolens*), these six isolates fit the traditional criteria for aster yellows "Western strains." Mycoplasmalike organisms were detected by electron microscopy in representative plants infected with each isolate. Our data indicate occurrence of more than one mycoplasmalike organism in vegetable crops of Oklahoma.

Aster yellows (AY), an intensively studied plant disease of mycoplasmal etiology, affects approximately 350 different plant species belonging to 54 plant families (24). It is transmitted by and multiplies in the six-spotted (aster) leafhopper, *Macrosteles fascifrons* Stal. (18).

AY was first reported in carrot (*Daucus carota* L.) by Ivanoff and Ewart in 1944 in Texas (17). In 1940, Linn reported an average incidence of 5–6% of "yellows" in lettuce (*Lactuca sativa* L.) in New York with 70–80% incidence in some fields (22). The crop most susceptible to AY in Wisconsin is lettuce, for which reports of 100% losses in commercial fields have been published (24).

In 1945, AY was first reported in Oklahoma (25). The disease was diagnosed based on symptomatology in daisy fleabane (*Erigeron strigosus* Muhl. ex. Willd.) and lettuce. AY-like symptoms have frequently been observed in lettuce and carrot crops in eastern Oklahoma in subsequent years (K. E. Conway, B. Kahn, and J. Motes, *personal communication*). The aster leafhopper, *M. fascifrons*, is a common insect in the state. Recent emphasis on vegetable production in Oklahoma and knowledge of the presence of AY and its vector in the state led us to investigate the natural incidence of yellows in vegetable crops of Oklahoma and to characterize mycoplasmalike organism isolates collected from Oklahoma vegetable crops. Because our findings indicate the possibility that more than one mycoplasmalike organism is involved, the disease is herein referred to as "yellows." A preliminary report has been published (6).

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MATERIALS AND METHODS

Field studies. In 1985, three carrot cultivars (Danvers, Imperator, and Spartan Bonus) were planted at the Oklahoma State University (OSU) Vegetable Research Station (VRS) at Bixby and the OSU Plant Pathology Farm (PPF) at Stillwater. The plots were arranged in a randomized complete block design with four replications of a 10-m row. Planting dates were 15 April 1985 and 23 August 1985. Incidence of yellows symptoms was recorded approximately 100 days after planting.

During 1986–1987, the same carrot cultivars plus two lettuce cultivars (Great Lakes and Ithaca) were planted at the VRS at Bixby, at the PPF at Stillwater, and at the Wes Watkins Agriculture Research and Extension Center (WWAREC) at Lane. Each carrot cultivar was planted in four 10-m rows and each lettuce cultivar was planted in six 10-m rows. Planting was arranged in a randomized block design with four replications. The plots were planted on 30 April and 15 August 1986 and 25 April and 10 August 1987. Incidence of yellows symptoms was recorded at approximately 100 days after planting. On the same date, leafhoppers were collected by insect net sweeps, and numbers from a total of 10 sweeps were recorded. Approximately 100 representative leaf samples from mycoplasmalike organism diseased and healthy carrot and lettuce leaves and 15–20 leaf samples from potential alternate weed hosts were collected and evaluated by Dienes' stain (2), which is specific for mycoplasmalike organisms.

Electron microscopy. Approximately 20 field-collected asymptomatic and symptomatic carrot and lettuce leaves, petioles, midribs, and stem samples per season were fixed and stained for observation by electron microscopy with established methods (14,15,27), except for the following modifications. After initial fixation in 6% glutaraldehyde, postfixation in osmium tetroxide (OsO_4), and dehydration in acetone, the tissue was infiltrated with Spurr's embedding medium (10 g of 4-vinylcyclohexene dioxide [resin], 0.4 g of 2-(dimethylamino)-ethanol [DMAE] [accelerator], 6 g of DER resin grade 736 [flexibilizer], and 26 g of nonenyl succinic anhydride [epoxy hardener]) (28) and propylene oxide (1:3 ratio) for 1 hr, 1:1 (Spurr's/propylene oxide) for 1 hr, and 3:1 (Spurr's/propylene oxide) for 1 hr. Vials were then uncapped and left in a desiccator overnight. To improve infiltration, the tissue was transferred to fresh Spurr's daily for 2–3 days without letting the Spurr's solidify (28). Blocks were polymerized at 60 C for 2–3 days and cured for at least 1 wk before sectioning. Thin sections were stained in 5% uranyl acetate for 30 min and in lead acetate

for 30 min. The sections were examined in a JEOL 100CX ASID transmission electron microscope. Healthy plant materials were treated similarly and used as controls.

Data analysis. Analysis of variance (ANOVA) was used to evaluate the 1985 disease incidence data. The 1986 and 1987 data were analyzed as a split-plot with locations, years, and seasons in the main plots, arranged in a randomized block design with cultivars in the subplots. *F* tests from the ANOVA procedure were used to test for interactions and main effects. ANOVA was performed separately on carrot and lettuce data. Comparisons among cultivars and seasons within each year and location and comparisons among locations and cultivars within each year and season were made with Duncan's multiple range test at the $P = 0.05$ level.

Isolate collection. Representative diseased carrot and lettuce plants from the trap plots, potted with field soil into 15-cm-diameter plastic pots, were sprayed with malathion and moved to the greenhouse for transmission studies. Three days after the insecticide spray, 30 third and fourth instar nymphs of *M. fascifrons* were allowed to feed on each infected plant in an individual (10.2 × 30.5 cm) cylindrical cage. After a 2-wk incubation period on barley (*Hordeum vulgare* L. 'Post'), the leafhoppers were allowed to feed on 7- to 9-day-old aster, *Callistephus chinensis* (L.) Nees (a short-term maintenance host), or 4-wk-old periwinkle, *Catharanthus roseus* (L.) G. Don, plants (a long-term maintenance host). The isolates were characterized based on the symptom expression on aster. Later transmission experiments involving isolates of interest were carried out with these asters (infected by mycoplasmalike organisms) as source plants.

Mycoplasmalike organism and vector maintenance. *M. fascifrons* was reared at 25–35 C in large wood and screen cages (60 × 60 × 60 cm) in the greenhouse. The insects were cultured on barley, a nonhost of AY mycoplasmalike organism (MLO). Colonies were monitored frequently for mycoplasmalike organism contamination by caging selected leafhoppers from the healthy colonies on aster seedlings and observing those asters for symptom development.

Transmission experiments. Insects carrying mycoplasmalike organisms were maintained on asters and barley. For transmission experiments, healthy insects were placed on yellows diseased source plants for 3–7 days and then transferred in groups to barley for a 2-wk incubation period. After the incubation period, 10–20 leafhoppers were placed on each test plant (7-day-old asters or 40-day-old celery [*Apium graveolens* L.]) for a 3- to 7-day inoculation access period. Inoculated plants were sprayed with malathion and moved to a greenhouse bench for observation of symptom development.

Experiments to characterize the mycoplasmalike organism isolates by symptomatology on aster and celery were carried out twice during the spring (25–30 C, 80% average RH) and twice during the fall (22–27 C, 75% average RH) in the greenhouse. These simultaneous transmissions allowed direct comparison of symptoms of each isolate under identical conditions. The success of transmission of mycoplasmalike organisms to celery and aster was confirmed by Dienes' staining and by back-inoculation to asters. Back-inoculation tests consisted of allowing healthy leafhoppers to feed on test plants after inoculation with the mycoplasmalike organism and then transferring the insects to healthy asters, which served as indicator plants to confirm transmission and infection.

RESULTS

Incidence of yellows in carrots. Although there was no significant difference in incidence of yellows in carrots in the three locations, the greatest incidence of yellows in carrots was at Bixby, followed by Lane and then Stillwater (Tables 1 and 2). The exceptions to this pattern were in spring 1987, when carrots at Stillwater showed the highest average incidence of yellows, followed by those at Bixby and then those at Lane, and in fall 1987, when carrots at Bixby had the highest incidence of yellows, followed by those at Stillwater and then those at Lane (Table 2).

A seasonal difference in incidence of yellows was recorded in carrot plots during all three study years, with the fall crops generally sustaining greater disease than spring crops. Carrots at Bixby during fall 1985 and at Bixby and Lane during fall 1986 and 1987 had higher ($P = 0.05$) incidence of disease than during spring 1985, 1986, and 1987 at the same locations. However, at Stillwater there was a higher ($P = 0.05$) incidence of yellows in spring 1985 than in fall 1985. No significant difference was observed between seasons in 1986 at Stillwater (Table 2).

There were a few differences in incidence of yellows among carrot cultivars. No differences were observed among carrot cultivars in either season at Stillwater during 1985 and 1986, at Bixby during 1986, or at Lane during 1986 and 1987 (Tables 1 and 2). However, variability in incidence of disease within the cultivars was observed at Bixby and Stillwater in 1987. For example, during spring 1987, cv. Danvers at Stillwater showed higher ($P = 0.05$) incidence of yellows than cvs. Imperator and Spartan Bonus. In the fall of the same year, Danvers showed the lowest incidence of disease (Table 2). Other cultivar differences were noted in Bixby during both seasons of 1987 (Table 2).

Incidence of yellows in lettuce. Incidence of yellows in lettuce was different at the three locations, being significantly greater ($P = 0.05$) at Bixby and Lane than at Stillwater. In 1986, lettuce plots at Bixby and Lane had 46.8 and 46.7% disease incidence, respectively, whereas lettuce plots in Stillwater had less than 5%. However, during spring 1987, the highest ($P = 0.05$) incidence of yellows in lettuce was at Stillwater, with no disease at Bixby or Lane. In fall 1987, there were no significant differences in the incidence of yellows in lettuce at Lane, Stillwater, or Bixby.

Seasonal differences in incidence of yellows were inconsistent. Incidence in lettuce at all three locations was higher ($P = 0.05$) during spring 1986 than during fall 1986, but this seasonal trend was reversed in 1987 with higher ($P = 0.05$) incidence at all three locations in fall than in spring 1987 (Table 3).

There was no difference among lettuce cultivars in 1986 or 1987 in any of the three locations (Table 3).

Leafhopper populations. Leafhopper numbers in trap plots of carrot and lettuce were higher in fall than in spring of any given year and generally higher in 1986 than in 1987 (Fig. 1). Among the locations, the greatest number of leafhoppers was collected at Bixby, followed by Lane and Stillwater. While most of the collected leafhoppers appeared to be *M. fascifrons*, they were not identified individually. No mycoplasmalike organisms were transmitted from these field-trapped leafhoppers to greenhouse-grown asters.

Detection with Dienes' stain and electron microscopy. When Dienes' stain was used for the detection of mycoplasmalike organisms, the phloem of the healthy sections remained unstained. However, in phloem tissue of stem, petiole, midrib, and root infected with a mycoplasmalike organism, several groups of cells were stained blue. Xylem appeared turquoise blue and cortex stained light blue. All of the symptomatic test plants showed a positive reaction with Dienes' staining, whereas the plants infected with either fungi or bacteria gave negative results. Several weed samples (mare's tail and pigweed) also tested positive.

In electron microscopy studies, no mycoplasmalike organisms were seen in sections from healthy control samples. However, pleomorphic, 70- to 800-nm-diameter unit membrane-bound mycoplasmalike organisms were observed at ×7,200 in the phloem regions of the infected plants. At higher magnification (×100,000), electron-dense ribosomelike granules and DNA-like materials were present.

Characterization of isolates on aster, celery, and periwinkle. *Aster yellows from Oklahoma carrot 1 (AY-OC 1).* An isolate of AY obtained from carrot was one of the more prevalent isolates

collected in our study. Three other isolates, designated AY-OC 3, AY-OC 4, and AY-OC 5, collected over a period of 4 yr (1985–1989) showed symptoms similar to those of AY-OC 1 on aster and periwinkle. AY-OC 1 was maintained on aster and periwinkle in the greenhouse and was transmitted to celery for evaluation of symptoms. In all three of these hosts, symptoms were typical of the Western strains of AY (19,20). In aster, AY-OC 1 caused vein clearing in immature leaves, followed by vein clearing in other developing leaves, chlorosis of the leaves, elongation of the petioles and internodes, and proliferation of lateral shoots from the axils of mature leaves. Severe stunting was observed in aster infected within 3 wk of germination. Flowers showed phyllody and virescence. Several infected plants showed proliferation from the flowers (stigma, style, and ovary were replaced by shoots) (Fig. 2A). Necrosis of the axillary shoots was followed by the eventual death of the plant. Symptoms of AY-OC 1 on periwinkle included yellowing, leaf size reduction, proliferation, phyllody, and virescence. Also observed were elongation of internodes, twisting and intertwining of the stems, elongation of flower stalks, reduction of flower size, and flower necrosis. In several cases, proliferation from virescent flowers occurred. The symptoms of AY-OC 1 in celery were vein clearing, yellowing of the leaves, and stunting of the plant. Symptom appearance in celery at 45 days after inoculation characterized AY-OC 1 as an AY Western strain (19).

Mycoplasmalike organism from Oklahoma carrot (MLO-OC). MLO-OC was different from the other carrot isolates (AY-OC 1, AY-OC 3, AY-OC 4, and AY-OC 5) and from AY as reported in the literature (5–7,10, 18,19). Symptoms on asters included mild vein clearing, slight yellowing (less than with AY-OC 1), elongation of petioles, proliferation, virescence, and phyllody (Fig. 2B). Less stunting of early-infected plants was seen than with AY-OC 1. Periwinkle infected with MLO-OC showed slight reduction of leaf and flower size and yellowing (but less pronounced than with AY-OC 1). The MLO-OC-infected celery showed vein clearing, yellowing, and stunting. Symptoms appeared between 40 and 45 days after inoculation in celery that were similar to the reaction to the AY Western strain.

Aster yellows from Oklahoma lettuce (AY-OL 1 and AY-OL 2). Isolates collected from lettuce produced symptoms similar to those of AY-OC 1 on aster but showed more pronounced etiolation and elongation than AY-OC 1. Petioles assumed an upright position as described for the AY Eastern strain (18,19) compared with the lateral growth of healthy asters. Phyllody, virescence, and stunting were more typical of the Western strain of AY (Fig. 2C). Infected periwinkle showed reduced leaf size (more pronounced than with AY-OC 1 and MLO-OC) and yellowing (but less than with the carrot isolates). Flowers showed phyllody and virescence, and, in severe cases, proliferation of shoots arose from virescent flowers. Sometimes flowers were absent. The symptomatology and 48-day incubation period in celery were typical of AY Western strain. AY-OL 1 and AY-OL 2 could not be distinguished from each other based on symptomatology. AY-OL is also one of the more prevalent isolates collected in our study.

Mycoplasmalike organism from Oklahoma daisy fleabane (MLO-OF). The symptom expression by the isolate from daisy fleabane on aster and periwinkle was distinctly different from that of the Eastern strain of AY, the Western strain of AY, and all other OK MLO isolates. On aster, this isolate produced neither vein clearing nor chlorosis on leaves, but leaves became narrow and tapered. Elongation of internodes and virescence and phyllody of floral structures were observed, but no proliferation was seen (Fig. 2D). MLO-OF showed less yellowing and stunting in periwinkles than did the other MLO isolates. Neither virescence nor phyllody was observed in this host, although flowers were slightly smaller than those of healthy controls. On celery, MLO-OF caused vein clearing

Table 1. Incidence of yellows in carrot in trap plots at Bixby and Stillwater, OK, during 1985[y]

Season	Cultivar	Location	
		Bixby	Stillwater
Spring	Danvers	34.2 a[z]	5.1 a
	Imperator	17.4 a	4.4 a
	Spartan Bonus	21.9 a	4.4 a
Fall	Danvers	17.6 a	1.2 a
	Imperator	36.9 a	1.2 a
	Spartan Bonus	26.3 a	1.8 a

[y] Location × season and location × cultivar interactions were nonsignificant; season × cultivar and location × season × cultivar interactions were significant at $P = 0.05$.
[z] Values are the means of four replications and when followed by the same letter within the column and season are nonsignificant ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Incidence of yellows in carrot in trap plots at Bixby, Lane, and Stillwater, OK, during 1986–1987[y]

Season	Cultivar	1986			1987		
		Bixby	Lane	Stillwater	Bixby	Lane	Stillwater
Spring	Danvers	15.0 a[z]	4.1 a	0.7 a	0.0 a	0.0 a	4.0 a
	Imperator	11.8 a	3.5 a	0.9 a	0.7 a	0.0 a	3.6 ab
	Spartan Bonus	11.7 a	4.7 a	1.2 a	4.8 a	0.0 a	0.8 b
Fall	Danvers	24.3 a	10.8 a	1.0 a	2.4 b	4.8 b	1.2 b
	Imperator	24.3 a	8.1 a	0.9 a	10.9 a	8.1 a	9.7 a
	Spartan Bonus	20.2 a	7.9 a	1.1 a	10.6 a	8.0 a	12.7 a

[y] Location × cultivar, year × season, and location × year × season × cultivar interactions were nonsignificant; location × year × cultivar and location × season × cultivar interactions were significant at $P = 0.05$; season × cultivar and year × season × cultivar interactions were significant at $P = 0.001$; and location × year, location × season, year × cultivar, and location × year × season interactions were significant at $P = 0.0001$.
[z] Values are the means of four replications and when followed by the same letter within the column and season are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 3. Incidence of yellows in lettuce in trap plots at Bixby, Lane, and Stillwater, OK, during 1986–1987[y]

Season	Cultivar	1986			1987		
		Bixby	Lane	Stillwater	Bixby	Lane	Stillwater
Spring	Great Lakes	58.8 a[z]	62.2 a	4.9 a	0.0 a	0.0 a	7.9 a
	Ithaca	62.8 a	48.5 a	4.7 a	4.1 a	0.0 a	6.6 a
	Great lakes	48.2 a	49.7 a	2.8 a	36.3 a	56.3 a	46.9 a
Fall	Ithaca	45.5 a	43.6 a	2.1 a	39.2 a	56.3 a	72.0 a

[y] Year × cultivar, season × cultivar, location × season × cultivar, location × year × season, location × year × cultivar, and year × season × cultivar interactions were nonsignificant; location × cultivar and location × year × season × cultivar interactions were significant at $P = 0.05$; location × season interaction was significant at $P = 0.0015$; and location × year and year × season interactions were significant at $P = 0.0001$.
[z] Values are the means of four replications and when followed by the same letter within the column and season are not significantly different according to Duncan's multiple range test ($P = 0.05$).

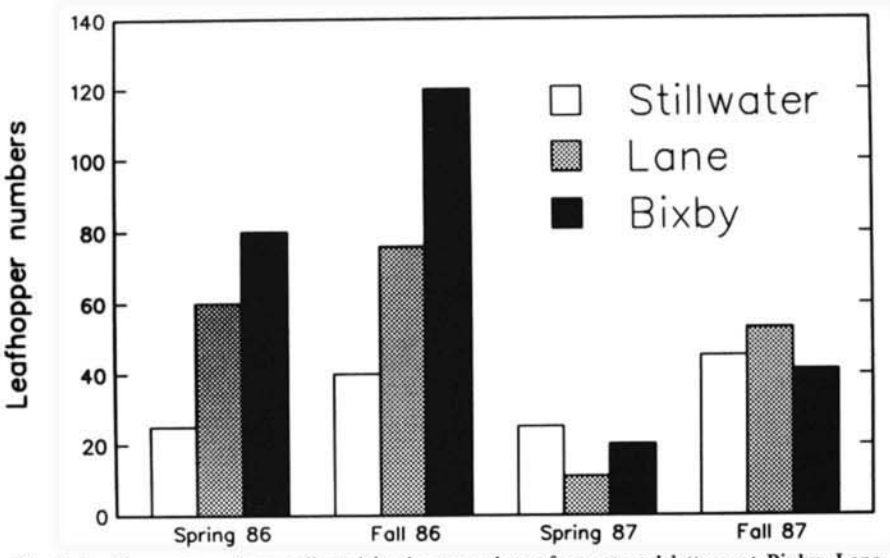

Fig. 1. Leafhopper numbers collected in the trap plots of carrot and lettuce at Bixby, Lane, and Stillwater during spring and fall of 1986 and 1987.

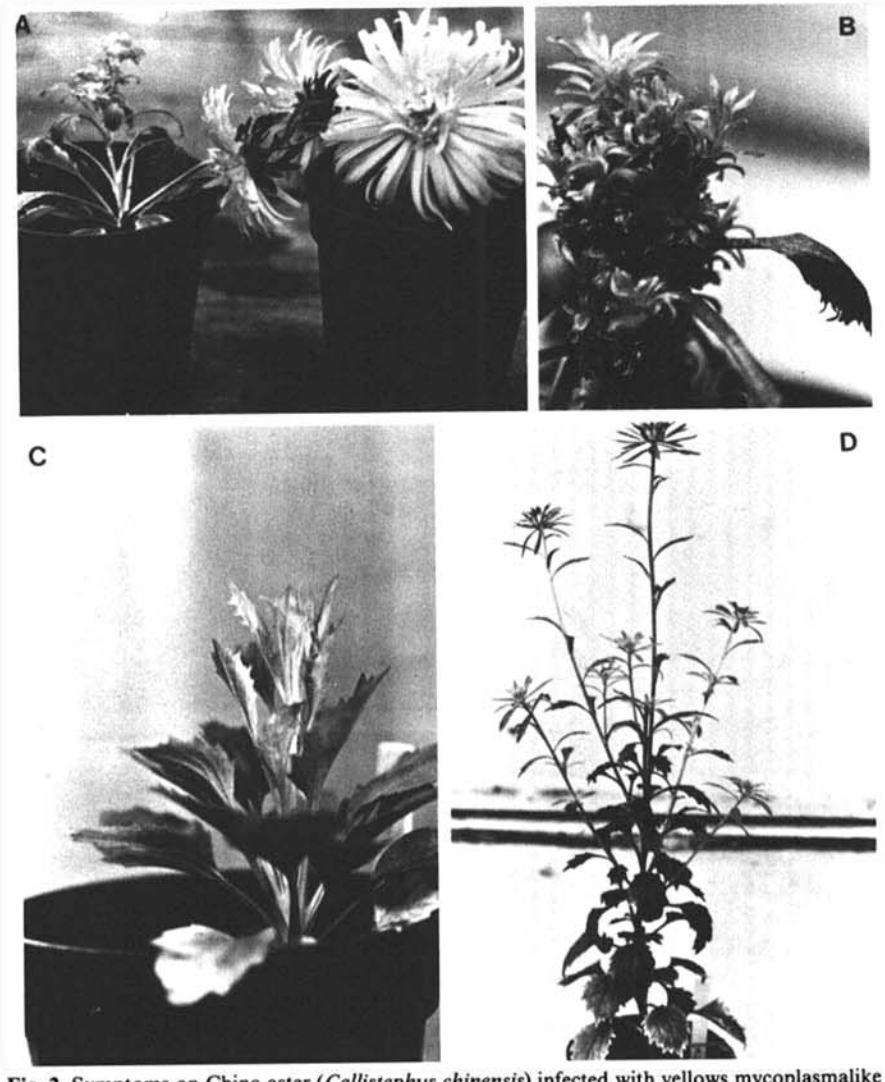

Fig. 2. Symptoms on China aster (*Callistephus chinensis*) infected with yellows mycoplasmalike organism isolates: **(A)** aster yellows mycoplasmalike organism isolate from carrot (AY-OC 1) producing yellowing, stunting, phyllody and virescence (left), and healthy aster with normal flowers (right); **(B)** mycoplasmalike organism isolate from Oklahoma carrot 2 (MLO-OC) producing yellowing, stunting, phyllody, and proliferation; **(C)** aster yellows mycoplasmalike organism isolate from lettuce (AY-OL) producing stunting, phyllody, virescence, and upright growth of the petioles; and **(D)** mycoplasmalike organism isolate from Oklahoma daisy fleabane (MLO-OF), producing tapered and narrow leaves, elongated internodes, and virescent flowers but no yellowing.

and stunting. Production of symptoms at 43 days after inoculation in celery showed similarity with the AY Western strain.

DISCUSSION

The high incidence of MLO disease in carrots grown at VRS (1985) and in carrots and lettuce plots at VRS and WWAREC (spring and fall 1986) may have been attributable to high primary inoculum in weed (pigweed, mare's tail) and vegetable hosts (overwintered carrots and lettuce) surrounding the trap plots at those locations. Bixby and Lane are predominantly vegetable-growing areas. Volunteer lettuce and carrots and some dicotyledonous weeds adjacent to the trap plots may serve as sources of inoculum at these research station locations. Stillwater, located in a non-vegetable-growing region, may not have as much primary inoculum. Hagel and Landis described AY in overwintering perennials in eastern Washington (11). AY also overwinters in Minnesota in certain weed hosts including horseweed, volunteer parsnip, and dandelion (23,24). Leafhoppers in Wisconsin overwintered as eggs in grasses, winter grains, and perennial hosts, and during the spring, the adult population was concentrated on these hosts. With maturation of spring hosts, these leafhoppers, along with the adult migrants, dispersed to summer plant species (22). Harvey and Schroeder reported that newly hatched nymphs became infective by feeding on the overwintering weeds and grains infected with AY (13).

A second factor contributing to the high yellows incidence during these years may be strong wind currents which carry yellows-inoculative leafhoppers to the eastern Oklahoma locations of Bixby and Lane from the southern United States during the spring and summer months. Winds influence the direction and dispersal of *M. fascifrons*; Chiykowski and Chapman associated the movement of spring air masses with the migration of leafhoppers from southern to northern United States and Canada (1). Breeding sources of the migratory populations of aster leafhoppers were found in the winter grain fields of western Louisiana, western Arkansas, northeastern Oklahoma, eastern Kansas, southwestern Missouri, and northern Texas (1,4). The leafhoppers infected with AY were trapped at eastern Arkansas locations during their spring flight from the southern United States (J. Dale, *personal communication*). Bixby and Lane are in the path of leafhopper spring migration, whereas Stillwater lies 120 km to the west. We observed fewer leafhoppers in spring and fall 1986 at Stillwater than at the other two locations.

Patterns of disease incidence of yellows in lettuce and carrots in all three locations were different in 1987 than in

the other study years. Yellows incidence was lower at Bixby and Lane in 1987 than in the other test years. There are several possible reasons. Although the summer of 1987 was unusually dry, heavy spring rains in that year washed out the trap plots, and the replanted plots were in the field for only 2 mo instead of the usual 3.5 mo. Short plant growth periods caused by delayed planting may have influenced the amount of leafhopper feeding in these plots. *M. fascifrons*, found in abundance on irrigated lands in Washington (12) and California (9), took flight from grasses and weeds as they dried up, and moved to creek bottoms or irrigated fields (26). Chiykowski and Chapman suggested that drought conditions may have accounted for the low numbers of migratory leafhoppers in the panhandle regions of Texas and Oklahoma in 1955 (1). Thus, a second possible explanation for low incidence of yellows at Bixby and Lane in 1987 was the scant rainfall during that summer, which might have adversely affected leafhoppers and/or weed plants serving as inoculum sources. Our data did show fewer leafhoppers collected in 1987 than in 1986. During both seasons in 1987, carrot and lettuce at Stillwater showed slightly higher incidence of yellows than in the previous 2 yr. At Stillwater during fall 1987, irrigated winter wheat surrounding our trap plots might have served as a shelter and breeding host for aster leafhoppers during the dry summer months of that year. Additionally, the trap plots planted in the three previous years might have contributed to increasing primary inoculum in the weeds adjacent to the OSU PPF at Stillwater.

The seasonal differences in yellows incidence in all three locations may be attributable to low numbers of leafhoppers and low primary inoculum levels in the spring months. Most of the weed hosts, infected with yellows, that serve as primary inoculum for the trap plots are annuals that die during the winter. This would result in lower primary inoculum in spring, whereas by the fall season, primary inoculum may have built up in weeds and in leafhoppers that fed on the infected plants. The smaller leafhopper populations observed in our trap plots during spring (as compared with fall) correspond with yellows incidence. Very few aster leafhoppers were found during winters in Wisconsin and Canada (1,26). A general conclusion regarding yellows incidence at three locations is suggested by the 1985 and 1986 data, which indicate that under normal rainfall conditions in a given year, vegetables at Bixby sustain the highest yellows incidence, followed by those at Lane and Stillwater. This observation is also consistent with leafhopper collection data, with insect numbers greatest at Bixby and lowest at Stillwater.

The lack of differences among cultivars reflects our choice of lettuce and carrot cultivars. The lettuce cvs. Great Lakes and Ithaca and carrot cvs. Danvers, Imperator, and Spartan Bonus were found to be susceptible to AY (26). Because our primary objective was to record the natural incidence of AY, we selected cultivars that were highly susceptible to the disease.

When comparing combinations of factors in the experimental design, several two-factor and all three-factor interactions were significant, while the four-factor interaction was not (Table 2). Significant two- and three-factor interactions suggest a lack of consistent patterns among the means for one factor or set of factors when compared for two combinations of the remaining factors. A given two-factor interaction that is significant when considered overall, may, when considered with certain combinations of other factors, be significant in some but nonsignificant in others. For example, during 1986 and 1987, a year × cultivar (two-factor) interaction ($P = 0.001$) was observed for the percentage of yellows incidence in carrots (Table 2). Examining the six combinations of location and season, the year × cultivar interaction was found to be significant for only two of these location and season combinations ($P = 0.028$–0.666): fall planting at Bixby and fall planting at Stillwater. Interpretations based on this analysis must be made with caution.

In this study, a total of eight mycoplasmalike organism isolates were collected and characterized from three geographical locations in Oklahoma. Because of the nonculturable nature of the pathogen, AY MLO isolates have traditionally been characterized on the basis of symptomatology on aster, periwinkle, celery, and other hosts (16,18,19). For these studies, traditional methods were used because antisera and DNA probes against AY MLO were either unavailable or in developmental stages. The presence of mycoplasmalike organisms was confirmed by Dienes' stain and electron microscopy (3,16). One isolate from carrot, MLO-OC, differed from the other carrot isolates in symptomatology on test plants and did not react with antiserum specific for AY-OC 1. Two isolates from lettuce, AY-OL 1 and AY-OL 2 (AY-OL group), were different from both carrot groups and from a daisy fleabane isolate (MLO-OF), although they did react positively with anti-AY-OC 1 serum. The AY-OC 1 group and AY-OL group isolates were collected during all study years from all three geographical areas, suggesting prevalence of these two isolates in these locations in Oklahoma. However, AY-OC 1 group isolates were found more frequently than AY-OL isolates. MLO-OC was collected only from Bixby, whereas MLO-OF was collected only from the Stillwater area.

Historically, two strains of AY MLO were differentiated on the basis of host range and vector specificity (19). The California or Western strain readily infects celery with symptoms appearing 40 days after inoculation and is transmitted by 25 leafhopper species (18,19). The Eastern strain produces symptoms 115 days after inoculation in celery and is transmitted by only one leafhopper species (19). Both strains are transmitted by *M. fascifrons* (20). In this study, all eight MLO isolates were transmitted to celery by the aster leafhopper. All eight produced symptomatology on celery consistent with those of Western strains, but only six of these produced typical AY symptoms on aster and periwinkle.

Carrots and lettuce that had symptoms of yellows may have been carrying one, two, or more different mycoplasmalike organisms. Our isolates AY-OC 1 and MLO-OC were originally collected from the same infected carrot plant (6). It is possible that mixed infections could occur with more than one type of mycoplasmalike organism coexisting in the same plant (8,20); in Wisconsin, Lee found more than one AY strain in an aster plant from the field (26), and Granados (10) reported three strains of AY in one periwinkle in a greenhouse in Wisconsin. All four groups of isolates (AY-OC 1, MLO-OC, AY-OL, and MLO-OF) were transmitted by the aster leafhopper and all produced symptoms in aster, periwinkle, and celery. Positive reaction with Dienes' staining and electron microscopy studies showing the presence of mycoplasmalike organisms in the phloem regions of the plants infected with four groups of isolates suggest that the symptoms were caused by mycoplasmalike organisms (3). Neither Dienes' stain nor electron microscopy could distinguish between mycoplasmalike organisms. Serological tests with anti-AY-OC 1 serum were positive with AY-OC 1, AY-OC 3, AY-OC 4, AY-OL 1, and AY-OL 2 (5,7). These findings are consistent with a report of Lee and Davis (23) that AY-OC 1 hybridized with their AY-DNA probe but inconsistent with the lack of homology between AY-OL-DNA and their AY-DNA probe. The positive reaction of AY-OL with OK AY-OC 1 antiserum and negative reaction with the AY-DNA probe of Lee and Davis could be explained by strain differences in DNA sequences other than those encoding the epitopes recognized by the antiserum. Thus, AY-OC 1 and AY-OL may represent different strains of AY. MLO-OC and MLO- OF did not react with anti-AY-OC 1 serum, nor did they hybridize with the DNA probe of Lee and Davis (21). These data, coupled with the differences in symptomatology, lead us to suspect that MLO-OC and MLO-OF may be mycoplasmalike organisms other than AY.

Our experiments indicated that al-

though yellows incidence can be inconsistent from year to year and from one location to another, the disease can be devastating in lettuce and carrot crops in Oklahoma. Most of the yellows disease was caused by AY-OC 1 (carrots) and AY-OL (lettuce). AY-OC 1 is a strain of AY MLO based on serological data and DNA homology; AY-OL is serologically related to AY-OC 1. However, other mycoplasmalike organisms may also occur in Oklahoma vegetables and weeds. These mycoplasmalike organisms have a potential to occur in high frequencies in vegetable crops and may cause economic damage. The occurrence and severity of AY/yellows disease should be considered in future plans for development of vegetable production areas in Oklahoma.

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