Response of Burley Tobacco Cultivars and Certain *Nicotiana* spp. to Alfalfa Mosaic Virus Infection

E. C. TEDFORD, Research Specialist, and M. T. NIELSEN, Associate Professor, Department of Agronomy, University of Kentucky, Lexington, KY 40546

ABSTRACT

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Thirteen burley tobacco (*Nicotiana tabacum* L.) cultivars were inoculated separately with two alfalfa mosaic virus (AMV) isolates collected in Kentucky and, in a different greenhouse experiment, with three AMV isolates obtained elsewhere. Isolate means for symptom expression were not significantly different, but cultivar means for this trait did vary. The cultivar Tn 86 had the severest symptom ratings, and all cultivars had some symptoms of AMV infection and showed positive ELISA reactions. In an evaluation of 15 *Nicotiana* spp. inoculated with one AMV isolate, several developed no symptoms of AMV infection but had positive ELISA, whereas only *N. debneyi* had no symptoms and a negative ELISA reading.

Alfalfa mosaic virus (AMV) is a complex, RNA-containing virus that is prevalent worldwide (6). It has an extensive host range that includes 305 species in 47 families (11) and has been reported as an important pathogen of beans (18,20–22), potato (3,15), celery (17), pepper (2), and clover (8,12). Although numerous *Nicotiana* spp. may serve as hosts of AMV (9,16,19), reports of natural infections in field-grown tobacco have been limited (13,16).

Infection of tobacco with AMV most likely results from aphid transmission of the virus from other host species, given the extent of aphid infestations in tobacco, although seed transmission of AMV has been shown in other crops (7,11). In recent years we have observed an increase in the number of cases of AMV infection of Kentucky-grown burley tobacco. Several factors may be responsible for this increase. These include increased acreage of crops (particularly alfalfa) that may serve as AMV hosts, greater and more persistent aphid infestations, and the introduction and use of new burley tobacco cultivars. These factors would correspond to a greater reservoir of AMV, increased opportunities for virus transmission, and, perhaps, a more severe response to infection. The current study was designed mainly to assess the latter component: cultivar variation for response to AMV infection. The specific objectives were to evaluate symptom expression and virus replication (ELISA response) following AMV infection of a number of cultivars of burley tobacco, to determine cultivar responses to infection with different isolates of AMV, and to evaluate selected *Nicotiana* spp. for AMV resistance.

MATERIALS AND METHODS

Two experiments with different AMV isolates, but with the same 13 burley tobacco cultivars, were performed. For both experiments plants were grown in Jiffy-7 peat pellets and kept in a growth chamber at 28 C with a 12-hr photoperiod of combined fluorescent and incandescent light (50 μE m^{-2}sec^{-1}) for approximately 30 days. Plants were then moved to the greenhouse and transplanted into plastic pots, 8.5 cm in diameter, containing steam sterilized soil mixture (soil:sand:peat; 2:2:1 v/v/v). Plants were fertilized once a week with 20-20-20 Peters fertilizer. Approximately 6 wk after seeding, plants were inoculated with AMV. The sources of the AMV

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isolates used were: AMV-f, donated by O. W. Barnett (Clemson University), originally from R. W. Fulton (1); AMV-Medic, a South Australian isolate collected by O. W. Barnett; AMV-12, donated by S. A. Ghabrial (University of Kentucky); and AMV-1 and AMV-10, which the authors collected from tobacco grown in Kentucky. To provide inoculum, all isolates were increased in *N. tabacum* cv. Burley 21 grown in the greenhouse. Three weeks after inoculation of Burley 21, systemically infected leaf tissue for each isolate was separately triturated in 0.05 M $Na_2HPO_4KH_2PO_4$ buffer (pH 7.5), at a rate of 1 g of tissue per 5 ml of buffer, and used as inoculum. Two grams of Carborundum (320 grit) was added to each 100 ml of inoculum, and one leaf of each plant was mechanically inoculated using cheesecloth dipped in inoculum.

In the first experiment, isolates AMV-1 and AMV-10 were used, and in the second experiment isolates AMV-12, AMV-f, and AMV-Medic were used. The first experiment was conducted three times, with three replications the first two times and five replications the last time. The second experiment was repeated once with four replications both times. Both experiments were conducted using a split-plot experimental design with AMV isolates as whole plots and cultivars as subplots.

In a third experiment, seed of 15 *Nicotiana* spp., selected to represent most sections of the genus, were germinated in a steam-pasteurized soil mixture and were maintained in a growth chamber for 6 wk. Seedlings were transplanted into individual plastic pots (8.5 cm in diameter) containing sterile soil mixture and allowed to grow for another 3 wk under the greenhouse conditions described above. Plants were inoculated as described below using isolate AMV-1. The experimental design was a randomized complete block design with 4 replications, and the experiment was conducted twice.

Three weeks after inoculation, plants were visually inspected and rated for symptom expression using a 0–3 scale: 0 = no symptoms present; 1 = <25%, 2 = 25–50%, and 3 = >50% of the total leaf area with mosaic.

Detection of virus replication in inoculated plants was accomplished by direct (double antibody sandwich) DAS ELISA (4) 3 wk after inoculation. The antiserum was provided by S. A. Ghabrial (University of Kentucky). Negative and positive controls consisted of extract from noninoculated burley tobacco plants and dry leaf tissue from the original AMV isolates, respectively. Reactions were measured spectrophotometrically at 405 nm using a Titertek Multiskan ELISA plate reader. Plants were considered positive for virus if the A_{405} reading exceeded the mean of healthy control plants by at least three standard deviations.

To partially characterize the two isolates (AMV-1 and AMV-10) collected in Kentucky, six differential host species were inoculated separately for each virus. The species, *Cucumis sativus* L. cv. National Pickling, *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Phaseolus vulgaris* L. cv. Bountiful, *Vigna unguiculata* (L.) Walp. cv. Blackeye, and *Capsicum annuum* L. cv. Yolo Wonder, were grown and evaluated in the greenhouse. The type and degree of symptoms of AMV infection were recorded.

RESULTS

Analyses of variance (not shown) for the first experiment indicated no significant differences between isolates AMV-1 and AMV-10 for symptom development. On the other hand, differences among cultivars for symptom expression were highly significant ($P = 0.01$), but there was no significant ($P = 0.05$) cultivar by isolate interactions for this same trait. Cultivar means for symptom rating (means across the two isolates) were 0.6 or less for all cultivars except Tn 86, which had mosaic symptoms on nearly 50% of its total leaf area (Table 1). Mean symptom rating for Burley 21 was significantly greater than that of three of the remaining 11 cultivars. Nearly all plants of the 13 cultivars had positive ELISA reactions 3 wk after inoculation with AMV. There was no substantial difference between AMV-1 and AMV-10 for the total number of plants that were ELISA positive.

Inoculation of the six differential host species with AMV-1 and AMV-10 resulted in very similar symptom development in the host species for both isolates. Thus, based on this test, there appeared to be no differences between AMV-1 and AMV-10.

Table 1. Cultivar means for symptom rating and proportion of infected plants based on ELISA reaction following infection with two isolates of alfalfa mosaic virus (AMV)

	AMV Isolate		
Cultivar	AMV-1	AMV-10	Mean symptom rating[a]
Burley 21	11/11[b]	11/11	0.6 b
Burley 37	10/11	11/11	0.5 bc
KY 9	11/11	11/11	0.1 c
KY 10	10/11	10/10	0.2 c
KY 12	11/11	11/11	0.2 bc
KY 14 × L8	11/11	9/10	0.4 bc
KY 15	11/11	10/10	0.5 bc
KY 16	11/11	10/11	0.4 bc
KY 17	11/11	11/11	0.3 bc
KY 14	8/11	10/11	0.1 c
TN 86	11/11	10/10	1.9 a
VA 509	9/9	11/11	0.4 bc
VA 528	11/11	10/10	0.3 bc
Healthy control	0/11	0/11	0
Virus control	11/11	11/11	...

[a] Symptom ratings are averaged across isolates AMV-1 and AMV-10. Means followed by the same letter were not significantly different ($LSD_{0.05}$).
[b] Number of plants which tested ELISA-positive/total number of plants.

Table 2. Symptom ratings and ELISA reactions for 16 *Nicotiana* species infected with a single alfalfa mosaic virus isolate (AMV-1)

Species	ELISA[a]	Symptoms
N. stocktonii (Brandegee)	8/8 a[b]	2.8 a
N. tabacum (L.)	8/8 a	2.6 ab
cv. Tennessee 86	8/8 a	2.6 ab
cv. T.I. 1406	8/8 a	1.9 bc
N. benthamiana (Domin)	8/8 a	1.9 bc
N. megalosiphon (Heurck & Mueller)	8/8 a	1.9 bc
N. rosulata (S. Moore) Domin	7/8 a	1.8 c
N. umbratica (Burbidge)	8/8 a	1.3 cd
N. acuminta v. *multiflora* (Graham) Hooker	8/8 a	0.8 de
N. nudicaulis (Watson)	7/8 a	0.8 de
N. tomentosiformis (Goodspeed)	7/8 a	0.8 de
N. longiflora (Cavanilles)	8/8 a	0.4 e
N. knightiana (Goodspeed)	2/8 c	0.4 e
N. undulata (Ruiz & Pavon)	8/8 a	0.3 e
N. debneyi (Domin)	0/8 d	0 e
N. cavicola (Burbidge)	8/8 a	0 e
N. acaulis (Spegazzini)	1/8 cd	0 e
N. africana (Merxmuller)	4/8 b	0 e

[a] Number plants which tested ELISA-positive/total tested.
[b] Values within a column followed by the same letter were not significantly different ($LSD_{0.05}$).

No significant differences were found in the second experiment among the three virus isolates AMV-12, AMV-Medic, and AMV-f for their mean symptom expression in the burley tobacco cultivars. As in the first experiment, there was no significant isolate by genotype interaction for symptom expression, but a highly significant cultivar effect was found. Again, Tn 86 had the highest mean symptom rating; however, the rating for Tn 86 was not significantly greater than those obtained for Ky 14 and Ky 16. All of the remaining cultivars had symptom ratings that were 1.0 or less. No significant differences were found among the cultivars for the number of plants that were detected as ELISA positive. The percentages of plants across all cultivars that were ELISA positive for the three isolates were 89%, 97%, and 83% for AMV-12, AMV-Medic, and AMV-f, respectively.

Four of the *Nicotiana* spp. inoculated with AMV-1 developed no symptoms of the virus infection (Table 2). However, six other species had symptom ratings that were not significantly different from the zero ratings for *N. debneyi, N. cavicola, N. acaulis*, and *N. africana*. One species, *N. stocktonii*, appeared more sensitive to AMV infection than all other *Nicotiana* spp. tested except *N. tabacum* cv. Tn 86. Three species, *N. knightiana, N. debneyi*, and *N. acaulis*, tested ELISA positive on 25% or less of their total plants, and for one of these species (*N. debneyi*) no AMV was detected by ELISA in the apical region of any of the plants tested.

DISCUSSION

The lack of significant differences due to isolate effects in the first two experiments suggested that the isolates used in each of these experiments were similar. Since both AMV-1 and AMV-10 were collected from tobacco grown in Kentucky, it is possible that these two isolates are identical. However, in the second experiment the three isolates were obtained from different sources, yet symptom development in the burley tobacco cultivars was similar for all three isolates. Strains of AMV that cause varied symptoms in other plant species have been noted (20), but there is little, if any, evidence that different AMV strains cause differential genotypic responses in tobacco. The lack of isolate variation as evidenced by the criteria used in the current study could be explained partially by an apparent lack of genetic variation among the burley cultivars for their response to the different AMV strains, although it is also likely that the AMV isolates tested were the same strain. Further testing of additional AMV strains would be needed to fully assess the extent of variation for tobacco host reaction to variation in AMV.

In the first two experiments, the cultivar Tn 86 was more sensitive to all AMV strains than nearly all other cultivars evaluated. Tn 86 was released in 1986 (14) and has been grown on 5–7% of the total burley tobacco acreage since its release. Considering the relatively low symptom ratings for the other cultivars, the authors' notice in recent years of a small increase of AMV infections in burley tobacco may be attributed to the increased use of Tn 86. A trait that distinguishes Tn 86 from the other cultivars tested is that it possesses resistance to tobacco etch and tobacco vein mottling viruses. This resistance was derived from T.I. 1406 (also known as Virgin A mutant). Although symptom ratings for Tn 86 and T.I. 1406 were similar in the third experiment, additional evaluations of cultivars or breeding lines resistant to tobacco etch and tobacco vein mottling viruses are needed to determine the extent of the relationship between that resistance and sensitivity to AMV infection.

The combination of low symptom ratings and AMV multiplication in most of the burley tobacco cultivars evaluated suggests that all of the cultivars are susceptible, but it also suggests that estimates of the extent of AMV infection in field-grown burley tobacco may be low. Thus, only a small percentage of AMV-infected plants may actually be visually diagnosed as infected. None of the cultivars tested were symptomless, but other factors, including environmental conditions, time of infection, etc., could affect the extent of symptom expression. Although AMV infection of burley tobacco may be more prevalent than is now estimated, there is, to our knowledge, no information on the effects of AMV infection on yield and chemical composition of burley tobacco.

The *Nicotiana* spp. were inoculated with only AMV-1 because of the lack of isolate effects in the first two experiments. Four of the species developed no symptoms, but only one of these, *N. debneyi*, had no detectable AMV multiplication in the sampled apical leaf. *Nicotiana debneyi* may be a source of resistance to AMV, and, furthermore, this species has served previously as a source of disease resistance for the improvement of tobacco (5). However, Silber and Heggestad (16) reported severe symptoms on *N. debneyi* inoculated with an AMV strain isolated from tobacco grown in Wisconsin. *Nicotiana acaulis* may also be a source of AMV resistance, since no symptoms were observed on any plants of this species, and only one of eight plants tested positive by ELISA.

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