Journal of the Arkansas Academy of Science

Volume 36 Article 41

1982

Host Reactions and Physical Properties of Four Isolates of Tobacco Ringspot Virus

Lisa Smith
University of Arkansas, Fayetteville

James M. McGuire
University of Arkansas, Fayetteville

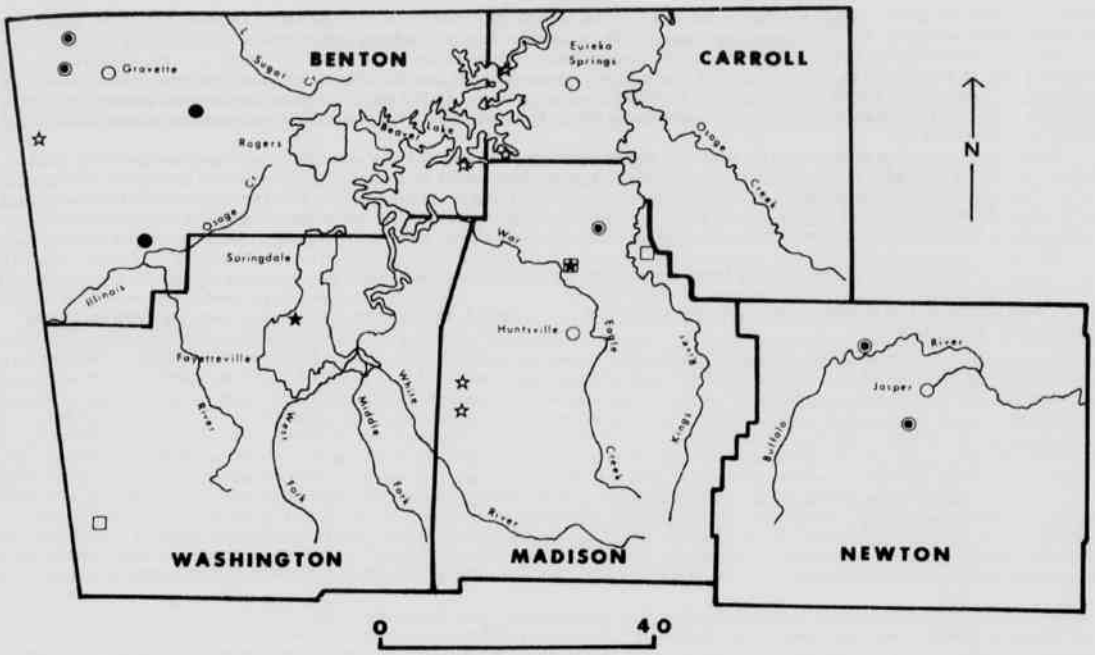

Figure. Distribution of troglobitic asellids in northwest Arkansas. *Caecidotea ancyla* (□), *C. antricola* (●), *C. steevesi* (★), *C. stiladactyla* (☆), *Caecidotea* sp. (◉). Collection sites reported by W. C. Welbourn are not shown.

HOST REACTIONS AND PHYSICAL PROPERTIES OF FOUR ISOLATES OF TOBACCO RINGSPOT VIRUS

Tobacco ringspot virus (TRSV) affects herbaceous and woody plants causing such symptoms as ring patterns, apical distortion and wilt, dwarfing, and chlorotic or necrotic symptoms (McLean, 1960, 1962; Stace-Smith, 1970). The virus is readily transmitted through sap inoculation and has a wide host range (Stace-Smith, 1970). It occurs in areas where *Xiphinema americanum*, the most common vector of TRSV, is prevalent (McGuire and Wickizer, 1980). Tobacco ringspot (TRSV) causes significant diseases of such plants as blueberry, watermelon and other curcurbits, and soybean in Arkansas, and the virus can be isolated from these plants (McGuire and Wickizer, 1980; Stace-Smith, 1970). The objectives of this greenhouse study were to determine how certain plants are affected by different isolates of TRSV and to compare the physical properties of the isolates.

Isolates used in this experiment were designated 1) Type - taken from watermelon; 2) Jersey - isolated from the Jersey variety of blueberry; 3) Collins - isolated from the Collins variety of blueberry; and 4) Soybean - isolated from soybean with bud blight symptoms. Cucumber served as the maintenance host from which infective sap was obtained. Virus inocula were prepared by grinding infected cucumber leaves showing chlorotic mottle symptoms in .01M phosphate buffer, pH 7.2. Plants were sprinkled with fine mesh carborundum and inoculated by rubbing with cotton swabs soaked in the infective sap.

Symptom and host range studies were carried out during June and July, 1981. Test plants grown in 7.5 cm clay plots included: cucumber, eggplant, Gomphrena, lupine, pinto bean, soybean, squash, sunflower, tobacco, topcrop bean, and zinnia. Plants were inoculated in the cotyledonary or primary leaf stage, except for Gomphrena, lupine and tobacco. They were inoculated when three or four leaves had expanded.

The physical properties longevity in vitro, dilution end point, and thermal inactivation point were tested. Sap was extracted from 1 g of infected cucumber in 5 ml of phosphate buffer for each isolate and each property to be tested. For each isolate, four half-leaves of blackeye cowpea and two cucumber in the cotyledonary stage were dusted with carborundum and inoculated per treatment.

During the longevity in vitro experiment, sap was tested at the time of extraction, and after being incubated for 4, 7, 9 and 11 days at approximately 25 °C. Dilutions of infective crude sap of 1:100, 1:1000, 1:2000, 1:4000, 1:8000, 1:16,000 and 1:32,000 were tested. Each of the four isolates was heated at 50°, 55°, 60°, 65°, and 70°C for 10 minutes to test thermal inactivation.

Test plants inoculated and their reaction to each isolate are presented in Table 1. The most commonly observed symptoms were chlorotic mottling and ring patterns. Symptom types varied greatly between hosts. On some hosts, such as cucumber and sunflower, all isolates produced

similar symptoms. On others, such as eggplant, lupine and soybeans, symptoms varied for different isolates. In general, type (from watermelon) caused the mildest symptoms; whereas the soybean isolate was the most severe. The two blueberry isolates produced similar symptoms in all hosts.

Isolates varied somewhat in physical properties. All remained infective in plant sap held at 25° C for 9 days, but only the soybean isolate was active after 11 days (Table 2). Titer of the latter appeared to be greater after 11 days than titer of the other isolates at 9 days. Dilution end points ranged from between 1:8000 and 1:16,000 for Type and Jersey to between 1:16,000 and 1:32,000 for Soybean and greater than 1:32,000 for Collins (Table 3). Three isolates were thermally inactivated at 60° C, but heating at 65° C for 10 minutes was required to inactivate the Jersey blueberry isolate (Table 4).

Variations in symptoms produced on the same host species by different isolates of TRSV and differences in physical properties show that the four isolates are somewhat different biologically. The soybean isolate had a much more drastic effect on several hosts than the other three strains. While the soybean isolate remained infective for the longest period of time in the longevity in vitro experiments, Collins blueberry TRSV can be diluted more than the other isolates and still produce symptoms. Jersey blueberry TRSV can withstand higher temperatures than the other strains tested. It should be taken into consideration that since the strains were isolated from different sources, this could be the basis for the dissimilarity in results.

Published with approval of the Director, Arkansas Agricultural Experiment Station.

Table 1. Host reaction to four isolates of tobacco ringspot virus following mechanical inoculation.

Host	Type	Symptoms[a] caused by Isolates[b] Collins	Jersey	Soybean
cucumber	cm	cm	cm	cm
eggplant	ns	ns	ld,cm	ld,cr
Gomphrena	ld	cm	cm	cr,cm
lupine	ncm	nll,bb	nll,bb	nll,bb
pinto bean	cll,nll	vn,cll,nll	vn,cll,nll	nll
soybean	cm	cll,bb	cll,bb	nll,bb
squash	cm	ns	cm	ns
sunflower	ncr	ncr	ncr	ncr
tobacco	ncr	cr	cr	cr
topcrop bean	cm,bb	cm,bb	cm,bb	nll,bb
zinnia	ns	ns	ns	ns

[a] Symptoms are: bb = bud blight; cm = chlorotic mottle (ncm=mild); cr = chlorotic ringspot (ncr = mild); cll = chlorotic local lesions; ld = leaf distortion; nll = necrotic local lesions; vn = veinal necrosis; ns = no symptoms.

[b] Type = from watermelon; Collins = from Collins blueberry; Jersey = from Jersey blueberry; Soybean = from soybean with bud blight.

Table 2. Longevity in vitro of four isolates of tobacco ringspot virus in cucumber sap at room temperature (approximately 25° C).

Isolate[b]	Indicator	Days after sap extracted[a] 0	4	7	9	11
Type -	cucumber	+[c]	+	+	+	-
	cowpea	>100[d]	5-15	5-15	1-5	-
Jersey -	cucumber	+	+	+	+	-
	cowpea	50-100	5-15	5-15	1-5	-
Collins -	cucumber	+	+	+	+	-
	cowpea	>100	5-15	5-15	1-5	-
Soybean -	cucumber	+	+	+	+	+
	cowpea	50-100	15-35	15-35	15-35	5-15

[a] sap extracted from 1 g infected cucumber leaves in 5 ml .01 M phosphate buffer, pH 7.2 (equivalent to 1:10 dilution)

[b] see Table 1 for isolate source.

[c] + = symptoms produced; - = no symptoms.

[d] number of necrotic local lesions per half leaf of cowpea.

Table 3. Dilution end point of four isolates of tobacco ringspot[a].

Dilution[a]	Lesions[b] produced by Isolates[c] on cowpea Type	Jersey	Collins	Soybean
1:100	15-35	5-15	15-35	15-35
1:1000	1-5	5-15	15-35	15-35
1:2000	1-5	5-15	15-35	15-35
1:4000	1-5	5-15	5-15	5-15
1:8000	0(+)[d]	1-5	1-5	1-5
1:16,000	0	0	1-5	0(+)[d]
1:32,000	0	0	0(+)[d]	0

[a] sap from infected cucumber diluted in .01 M phosphate buffer, pH 7.2

[b] number of local lesions per half leaf of cowpea.

[c] see Table 1 for isolate source.

[d] no symptoms in cowpea, but cucumber was infected.

Table 4. Thermal inactivation points of four isolates of tobacco ringspot virus.

Isolate[a]	Indicator	Temperatures 50°	55°	60°	65°
Type	cucumber	+[b]	+	-	-
	cowpea	>100[c]	35-50	-	-
Jersey	cucumber	+	+	+	-
	cowpea	>100	35-50	1-5	-
Collins	cucumber	+	+	-	-
	cowpea	>100	15-35	-	-
Soybean	cucumber	+	+	-	-
	cowpea	>100	15-35	-	-

LITERATURE CITED

MCGUIRE, J. M., and S. L. WICKIZER. 1980. Occurrence of necrotic ringspot of blueberry in Arkansas. Arkansas Farm Res. 29(4):6.

MCLEAN, D. M. 1960. Diseases caused by tobacco ringspot virus in the lower Rio Grande Valley of Texas. Plant Dis. Rep. 44:738-741.

MCLEAN, D. M. 1962. Differentiation of tobacco ringspot and tomato ringspot viruses by experimental host reactions. Plant Dis. Rep. 46:877-881.

STACE-SMITH, R. 1970. Tobacco ringspot virus. CMI/AAB-Descriptions of Plant Viruses No. 17:4p.

LISA SMITH and JAMES M. MCGUIRE, Dept. of Plant Pathology, PS-217, University of Arkansas, Fayetteville, Arkansas 72701.