High Incidence of Tobacco Streak Virus in Tobacco and Its Transmission by *Microcephalothrips abdominalis* and Pollen from *Ageratum houstonianum*

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ABSTRACT

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Incidence of tobacco streak virus (TSV) in an Australian tobacco crop at flowering was shown by enzyme-linked immunosorbent assay (ELISA) to be 58–59% in outside rows near weeds infected with TSV, decreasing to 10–18% in the 12th row inside the crop. The most prevalent dicotyledonous weed, *Ageratum houstonianum*, had a 50% incidence of infection and its flower heads were commonly infested with *Microcephalothrips abdominalis*. In transmission tests with either five or 10 adult thrips and pollen taken from the nearby *A. houstonianum*, TSV infection occurred in 32 of 45 tobacco, 11 of 12 cucumber, two of 12 *Nicotiana clevelandii*, and 18 of 22 *Chenopodium amaranticolor* test seedlings. Thrips-infested, flowering *A. houstonianum* appears to be responsible for this outbreak of TSV in subtropical Australia.

Although crops of tobacco (*Nicotiana tabacum* L.) in southeastern Queensland, Australia, are often infected by tobacco streak virus (TSV), the incidence is usually 1% or less (3). However, the virus is often more common in its many weed hosts in this area (4,8).

Transmission of TSV by *Frankliniella* sp. taken from the field-infected weed *Ambrosia polystachya* DC. in Brazil (2) or by a mixture of *Thrips tabaci* Lind. and *Frankliniella occidentalis* Pergande from *Melilotus alba* Medik. in Washington (5) has been reported. In Australia, it was recently found that under laboratory conditions, *T. tabaci* could transmit the Asclepias strain of TSV (As-TSV) only when either the thrips or the test plants were dusted with infective pollen before the thrips fed on the test plants (9). The mechanism of this transmission has not been determined unequivocally, but infectivity, ELISA, and immunogold labeling data suggest that TSV is present in anther debris external to the pollen cells as well as inside pollen (8).

Many thrips have a close association with their flowering hosts. *Thrips imaginis* Bagnall and *T. tabaci* feed on both leaves and pollen and can become externally contaminated with pollen (6,8). Because pollen from plants infected with TSV is usually shown by ELISA or infectivity tests to be heavily contaminated with TSV, carriage of pollen to new hosts by thrips could introduce the virus and result in infection via feeding wounds (8). This is a different mechanism of transmission from that of tomato spotted wilt virus, where the virus is acquired by nymphs during feeding on leaves and is transmitted only after a latent period of several days (7).

This paper describes an unusually severe outbreak of TSV in a tobacco crop in Queensland and experiments in which field-collected thrips and pollen were tested for their ability to cause TSV infection in tobacco and other test plants.

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MATERIALS AND METHODS

Field observations. These were made from February to May 1990 in a commercial crop of tobacco cv. ZZ100, which had been planted in late September–early October 1989 at Beerburrum in southeastern Queensland. The crop was divided in the middle by a grassy roadway infested with dicotyledonous weeds. The crop itself was clean-cultivated until a late stage. The farmer first reported vein necrosis symptoms in rows of tobacco adjacent to the weedy roadway in early November, but our observations began in early February. Observations on weed hosts continued until the end of May.

Testing of plants for TSV. Leaves and flowers from tobacco plants in the crop and weeds on the roadway were collected and tested for TSV by ELISA with antisera and methods described previously (9). Isolates of TSV were obtained by sap inoculation from tobacco and the weed *Ageratum houstonianum* Mill. to glasshouse-raised test plants.

Collection of pollen and thrips. Flowering plants were cut off and held in the laboratory with their stems in water for up to 3 days to allow pollen to mature and thrips to be extracted. Pollen from *A. houstonianum* was dislodged by gently brushing the flower heads over either tests plants or an open petri dish. Pollen collected in a dish from several plants of the one species was mixed before use in transmission tests. Adult thrips were collected on a sheet of white paper with a fine brush after pulling flowers apart under a stereo microscope and were either used for transmission tests or stored in alcohol for later identification.

Transmission tests. Adult thrips collected from *A. houstonianum* flowers were placed in groups of either five or 10 on test plants, on which they were confined with a polystyrene cylindrical cage (75 mm high × 33 mm diameter) pushed into the soil. Some test plants were dusted with pollen from *A. houstonianum* before the thrips were introduced. After 24–30 hr, the cages were removed and the thrips were killed with omethoate. Symptoms on test plants were observed over 4 wk, and the plants were tested for TSV by ELISA.

In another treatment, the thrips from flowers of *A. houstonianum* were cleaned of most pollen with a fine brush and then caged on healthy cucumber cotyledons for 4–6 hr before caging on test plants. Additional tests were made by gently brushing flowers of *A. houstonianum* directly over test plants so that pollen and, occasionally, some thrips were dislodged onto them before caging. Control plants were caged either after

being dusted with pollen from which all thrips had been removed or without pollen or thrips.

Pollen drift tests. These were done by dislodging ripe pollen from flowers 0.3 m high over a smooth black surface in a 1.2 m/s horizontal air stream maintained by a fan. Movement of pollen was observed in horizontal light beams, and deposition along a 1.5-m was range examined with a stereo microscope.

RESULTS

Field observations. In November 1989, the farmer noticed leaf vein-banding necrosis symptoms in the crop and samples were diagnosed by Queensland Department of Primary Industry officers as being infected by TSV. When we observed the crop near maturity in February, we found that a severe epidemic had occurred. Counts of plants carrying leaves with dentate margins on a row nearest the roadway indicated 64% infection, and the sequence of leaves with narrow-toothed laminas began low on most infected plants, indicating early infection.

The incidence of infection in the crop was greater near the grassy roadway through the middle of the planting than in the rows far away from the roadway. Symptom counts and ELISA tests (Table 1) provided data consistent with the hypothesis that infection was associated with and spreading from dicotyledonous weed hosts growing on the roadway. A slightly increased incidence was also associated with other minor roadways through the crop that had relatively few weed hosts. Random counts of dicotyledonous weeds on the major roadway showed 84% were *A. houstonianum*, 9% were *Bidens pilosa* L., 2% were *Mitracarpus hirtus* DC., and <1% were *Hypochoeris radicata* L. ELISA tests on samples of these weeds found TSV infection in eight of 16 plants of *A. houstonianum*, four of 14 plants of *B. pilosa*, one of one *Conyza bonariensis* (L.) Cronq., and a composite sample of several *H. radicata* plants. Isolates of TSV were obtained from tobacco and *A. houstonianum* by sap inoculation to glasshouse test plants and their identity was confirmed by the development of typical TSV symptoms and by gel-diffusion serology.

Table 1. Incidence of tobacco streak virus in tobacco plants east and west of a weedy, grassy roadway running through the middle of the crop at Beerburrum, Queensland, Australia

Row no.	Infected plants [a] (%)	
	West of roadway	East of roadway
1	59	58
4	50	23
8	35	25
12	18	10
15[b]	19	24

[a] Twenty to 68 plants in each row were tested for TSV by ELISA.
[b] Row 15 was near a minor roadway that had fewer weeds than the main roadway.

Collection of pollen from weeds and tobacco. Flower heads of *A. houstonianum* produced mature pollen, particularly during a short peak of anther ripening. At this time, a sharp bump caused a thin white cloud of pollen to be dislodged. When dislodged at 0.3 m in a 1.2 m/s breeze, *A. houstonianum* pollen dispersed finely and sedimented from surface eddies evenly over the 1.5-m distance examined and continued beyond. In contrast, most tobacco pollen sedimented as clumps of up to 100 or more grains, usually at 0.2–0.4 m from the flower source. Pollen yield of *B. pilosa* was less than that of *A. houstonianum* and, like tobacco pollen, it was often clumped. *H. radicata* pollen was readily dislodged during flower harvesting and transport.

Incidence of thrips in tobacco and weeds. In February 1990, thrips were extracted from the flowers of the tobacco crop infected with TSV and the most common weeds growing on the grassy roadway in the middle of the crop. Twenty flowers, flower heads, or flower head panicles were collected and the adult thrips were counted and identified. When large numbers of thrips were found on a particular host, only 20% were mounted before identification.

Only *A. houstonianum* and *H. radicata* had numerous thrips, and in each case, a different thrips species was predominant. Of the 153 adult thrips from 20 flower head panicles of *A. houstonianum*, three were of an unidentified species, while the other 150 were similar, and all of the 20% mounted were identified as *Microcephalothrips abdominalis* (Crawford). Of the 189 adult thrips from *H. radicata*, all were similar, and all of the 20% mounted were identified as *Ceratothrips frici* (Uzel). Of the weeds with small numbers of thrips, *M. hirtus* had three *Anaphothrips obscurus* (Muller) per 20 flower heads, whereas tobacco had one *Frankliniella schultzei* (Trybom), one *Chirothrips manicatus* (Haliday), and one probable *M. abdominalis* from 20 flowers. No thrips were obtained from 20 flower heads of *C. bonariensis* or *Richardia brasiliensis* Gomes or 20 flowers of *Crotalaria lanceolata* E. Meyer or *C. pallida* Aiton. Further sampling from *A. houstonianum* at nearby localities over 4 mo confirmed a consistent, predominant association with *M. abdominalis*.

Association of thrips and pollen. Undisturbed thrips on *A. houstonianum* flowers and those extracted by carefully breaking flower heads apart had only zero to five pollen grains adhering to them. In contrast, thrips dropped onto leaves that had been dusted with pollen of *A. houstonianum* became heavily contaminated with pollen, and the normally dark-colored adults took on a white, mealy appearance. Often, more than 100 grains of the small spiny pollen adhered to a single thrips.

Transmission of TSV by thrips. The results of transmission tests with *M. abdominalis* obtained from *A. houstonianum* plants at Beerburrum are given in Table 2. When pollen and either five or 10 thrips were placed on each test plant, 17–100% of the test plants became infected. When pollen and only a few

Table 2. Results of transmission tests with *Microcephalothrips abdominalis* and pollen from tobacco streak virus-infected *Ageratum houstonianum* growing near the tobacco crop at Beerburrum, Queensland, Australia[a]

Treatment[b]	Test plant	Test plants infected[c] (no.)	
		Symptoms	ELISA
Pollen and five thrips	Tobacco Xanthi	18/25	18/25
	Cucumber	4/5	4/5
Pollen and 10 thrips	Tobacco Xanthi	14/20	14/20
	Nicotiana clevelandii	NT[d]	2/12
	Cucumber	7/7	7/7
	Chenopodium amaranticolor	NT	18/22
Pollen and a few thrips direct from *Ageratum*[e]	Tobacco Xanthi	3/8	3/8
	Cucumber	NT	4/6
10 thrips directly from *A. houstonianum* flowers	Tobacco Xanthi	0/4	0/4
	C. amaranticolor	NT	2/4
	Cucumber	NT	1/2
Five thrips (pollen removed)	Tobacco Xanthi	0/12	0/12
Pollen (thrips removed)	Tobacco Xanthi	0/14	0/14
Nil (controls)	Tobacco Xanthi	0/20	0/20
	C. amaranticolor	0/10	0/10
	Cucumber	0/10	0/10
	N. clevelandii	NT	0/5

[a] Data from tests made with four collections of thrips and pollen have been combined.
[b] Per test plant.
[c] Number of infected test plants over total number of test plants used.
[d] Not tested.
[e] *A. houstonianum* flowers were brushed over the test plants providing pollen and a few thrips.

uncounted thrips were brushed onto each test plant, 38–67% of the test plants became infected. When 10 thrips were taken from *A. houstonianum* flowers and placed directly on test plants without added pollen, 0–50% of test plants became infected. Thrips cleaned of pollen by gentle brushing, and pollen in the absence of thrips, did not cause TSV infection, and untreated control plants remained healthy.

No distinction could be made by host symptoms or gel-diffusion serology among isolates of TSV obtained by thrips transmission involving *A. houstonianum* pollen, by sap transmission from the tobacco crop, or from a naturally infected plant of *Gomphocarpus physocarpus* E. Meyer. As-TSV isolates studied previously (8) appeared to be serologically identical in gel diffusion tests but gave less leaf margin distortion in tobacco than isolates from Beerburrum.

DISCUSSION

The unusually high incidence of TSV in tobacco at Beerburrum is probably attributable to infection derived from weed hosts outside the crop. Incidence in tobacco was highest on both sides of the weed-infested grassy roadway in the middle of the crop and presumably originated from either *A. houstonianum*, *B. pilosa*, *C. bonariensis*, or *H. radicata*, which were shown to be infected by TSV. Of these weeds, *A. houstonianum* could be the main source of infection, because it was by far the most prevalent weed and had a 50% incidence of TSV. Also, the pollen was shown to blow readily in a light breeze over a distance adequate to contaminate the leaves of the tobacco, and *M. abdominalis*, which infested flowers of *A. houstonianum*, was shown to transmit pollen-associated TSV to tobacco and other plants under laboratory conditions. The presence of leaves with TSV symptoms low on most affected plants indicates the epidemic was largely complete by the time our observations commenced, and therefore, we were not able to observe this part of the epidemic. However, the weeds were present on the roadway throughout the crop life, and the thrips populations and *A. houstonianum* flowers infected with TSV persisted throughout the 4 mo of our observation of the area.

Thrips taken directly from *A. houstonianum* flowers with a small amount of pollen adhering to them were less efficient in causing infection than when they were provided with additional pollen on the test plant surface. Thus, wind-blown pollen and species of thrips other than *M. abdominalis*, arriving independently, may well contribute to an epidemic. Thrips free of pollen and pollen free of thrips failed to cause infection. These and previous data (8) indicate that both pollen and thrips are required for TSV infection, although with another pollen-borne virus (raspberry bushy dwarf virus), transmission by thrips was not shown (1).

Because *A. houstonianum* associated with the tobacco crop could have played a key role in providing both virus and thrips vector, destruction of this weed near the crop would presumably have reduced the incidence of TSV. Even regular, close mowing, which would have controlled flowering and, hence, production of pollen carrying TSV, might have prevented any TSV epidemic. Because tobacco plants flower only at the end of the crop cycle, further spread of TSV within the crop by means of thrips and pollen from infected tobacco plants should not cause any great loss in yield.

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