Winter and Early-Spring Survey of Thrips Vectors and Host Plants of Tomato Spotted Wilt *Tospovirus* in and near a Flue-Cured Tobacco Farmscape[1]

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Abstract The tobacco thrips, *Frankliniella fusca* (Hinds), is an economic pest of flue-cured tobacco because it vectors tomato spotted wilt tospovirus. Other species of thrips are also vectors of spotted wilt in tobacco, including the western flower thrips, *F. occidentalis* (Pergande). This study examined the presence of thrips species on alternate plant hosts associated with the tobacco farmscape and surrounding area. Weed hosts were sampled from December through April from 1998 through 2001 to assess which plants provide suitable refuge and nutrients for thrips survival, reproduction, and spotted wilt infection. Thrips were identified to species and confirmed as potential vectors of spotted wilt by using ELISA to test for the presence of a non-structural tomato spotted wilt virus protein. Wild radish (*Raphanus raphanistrum* L.), broomsedge (*Andropogon virginicus* L.), and narrowleaf vetch (*Vicia sativa* L. subsp. *nigra* (L.) Ehrh.) are common late-winter weeds in the farmscape that harbor spotted wilt vectors. Cutleaf evening primrose, *Oenethera laciniata* Hill, and volunteer soybean, *Glycine max* (L.) Merrill, also were hosts of spotted wilt vectors in the tobacco farmscape. Numerous other weed hosts were present in the tobacco farmscape but either had no thrips collected from them or thrips were not confirmed as potential spotted wilt vectors from these host plants. Several other plants near the tobacco farmscape also were infected with spotted wilt, and three of these host plants, common chickweed (*Stellaria media* (L.) Cyrillo), carrot (*Daucus carota* L.), and flowering dogwood (*Cornus florida* L.) had confirmed vectors (ELISA) collected from them. Henbit (*Lamium amplexicaule* L.), wild radish, cutleaf evening primrose, narrowleaf vetch, carrot, curly dock (*Rumex crispus* L.), red sorrel (*Rumex acetosella* L.), and common chickweed were confirmed as positive plant hosts in this study for spotted wilt using ELISA. *Frankliniella fusca* appears to be the most abundant thrips vector on these alternate plant hosts and is the predominate thrips species collected on the flue-cured tobacco, *Nicotiana tabacum* L. However, *F. occidentalis*, *Haplothrips graminis* Hood, and *Chirothrips* spp. also were confirmed in this study to be potential vectors in the tobacco farmscape. Weed hosts in the farmscape appear to be influential as refuge and nutrients for vectors and an innoculant source of tomato spotted wilt virus in the flue-cured tobacco farmscape.

Key Words *Frankliniella fusca, F. occidentalis, Haplothrips graminis, Chirothrips* spp., wild radish, broomsedge, narrowleaf vetch, thrips ecology, thrips vectors

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Thrips continue to increase in importance as economic pests of flue-cured tobacco, *nicotiana tabacum* L., in Georgia (McPherson et al. 2002). Direct thrips feeding on tobacco is considered to be of no economic consequence (Jones and McPherson 1997); however, the ability of thrips to vector tomato spotted wilt virus causes losses to the crop in excess of $28 million in certain years (Bertrand 2000). Spotted wilt is also a serious disease of peanut, vegetable crops, and ornamentals (Johnson et al. 1996). Several thrips species are reported as vectors of tomato spotted wilt virus (Ullman et al. 1997), and two of these species, *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande), commonly occur on tobacco (McPherson et al. 1999), peanut (Chamberlin et al. 1992), and tomato (Riley and Pappu 2001) in Georgia.

Thrips can vector spotted wilt after acquiring the virus as larvae feeding on infected host plants. Tomato spotted wilt has a wide host range, including over 900 plant species (Groves et al. 2002) representing 50 botanical families (Johnson et al. 1996). The ingested virus in the larval thrips crosses the midgut and enters the salivary glands. Transmission occurs when saliva of primarily the adult thrips vector enters the plant tissue during feeding (Ullman et al. 1997). An infected thrips remains a potential vector throughout life (Pappu et al. 1998).

Many of the identified host plants of tomato spotted wilt virus are closely related to the endemic weed species in the southeastern USA (Johnson et al. 1996, 2000). Susceptible crops, such as peanut, tobacco, and vegetable crops, often emerge as volunteers in the fall and early spring and would be considered as weeds during this time of the year. The weed hosts and volunteer crops serve as refuge and nutrient source for thrips survival and are attractive to potential thrips vectors (Groves et al. 2001, 2002, Cho et al. 1986, Chamberlin et al. 1992, 1993, Stewart et al. 1989, Yudin et al. 1998, Stobbs et al. 1992, Latham and Jones 1997). The impact that these alternative host plants have on thrips abundance and spotted wilt epidemiology in the flue-cured tobacco farmscape is unknown. Thus, this study was initiated to examine the thrips species present in the tobacco farmscape and to assess which plant hosts provide suitable refuge and nutrients for thrips survival, reproduction, and tomato spotted wilt virus infection.

Materials and Methods

From December through April of 1998 through 2001, the commonly observed weeds and volunteer crop plants were collected every 2 to 3 wks from the flue-cured tobacco farmscape at the Coastal Plain Experiment Station Entomology Farm in Tifton, GA. The plant material was separated by species and placed into separate brown paper bags and returned to the laboratory. Plant material was further separated as being either "in" the tobacco farmscape if it was collected in the field or immediate surrounding area where tobacco was planted or "near" the farmscape if plants were collected more than 50 m from the tobacco field borders. Up to ten plants were placed into each bag (if that many plants were available) and up to three bags (30 plants) were collected for each plant species on each sampling date. In the laboratory, individual plant material (by species) was either visually examined for the presence of thrips or placed into aluminum Berlese extraction funnels (Beshear 1973). The direct visual sampling technique included both direct observation of the plant foliage, stems and blooms plus shaking the plant material over white posterboard that was covering the laboratory bench. All the thrips observed for each plant host through the direct observation were collected and placed into 1-dram glass vials

containing either 70% ethyl alcohol (to be mounted for ID only) or a phosphate-buffered saline containing 2% polyvinyl pyrrolidone and 0.02% sodium azide (for tomato spotted wilt assay). The Berlese samples were kept separated by plant species, with three funnels on each wooden holding rack containing the same plant host. Additional holding racks, with three Berlese funnels each, were isolated near each wall of the laboratory and contained other plant species to be sampled for thrips. Up to four racks (12 Berlese funnels) were maintained simultaneously. Each Berlese funnel was covered with an overlapping lid and contained a single 60-watt light bulb; the base of the funnel was submerged in a 473-mL plastic cup containing either 70% alcohol or the buffered solution. Once tobacco was transplanted in late-March, tobacco plants were sampled for thrips weekly from early April until mid-June, when the plants were topped (blooms removed from the plants). All thrips observed on 10 to 20 random plants were collected with a fine artist brush and placed into a 1-dram vial containing either 70% alcohol (to be mounted for ID) or buffer (for spotted wilt assay). The majority of all the thrips collected in this study were placed in a buffered solution, but a subsample from each collection was placed in alcohol so they could be positively identified.

Thrips collected for identification were mounted on microscope slides for detailed study under a dissecting microscope using procedures described by Beshear (1973). Thrips collected for confirmation as potential vectors of spotted wilt were assayed by enzyme-linked immunosorbent assay (ELISA) to determine the presence of a nonstructural (NSs) tomato spotted wilt virus protein (Bandla et al. 1994). The protein is only produced when the virus is replicating in the host and, therefore, serves as a reliable indicator of vector competence. One thrips was ground and placed in each well of the micro titer plate. Thrips reared without access to spotted wilt virus served as negative controls. To confirm the presence of tomato spotted wilt virus in the host plants, representative plants from each species were tested by ELISA using a commercial kit (Agdia, Eklhard, IN).

Results and Discussion

During this 3-yr study, 2619 thrips were collected in alcohol, mounted and identified to species from the weed hosts and tobacco grown in and near the farmscape in Tifton, GA. Another 10,140 thrips were collected in the buffered solution and tested with ELISA to confirm the presence of the NSs protein of tomato spotted wilt virus. The number of thrips collected on plant hosts and the number of confirmed tomato spotted wilt vectors are recorded in the farmscape (Table 1) and for plant hosts near the farmscape (Table 2). Wild radish, broomsedge, cutleaf evening primrose, volunteer soybeans, and tobacco foliage/blooms were infested with thrips that are capable of transmitting tomato spotted wilt on each of the years that these plants were sampled in the farmscape (Table 1). Narrowleaf vetch in the farmscape (Table 1) and carrot, common chickweed, and flowering dogwood collected near the farmscape (Table 2) also harbored low numbers of thrips vectors. All of these host plants previously mentioned with confirmed thrips vectors also tested positive for tomato spotted wilt virus except for broomsedge and soybean. Henbit (*Lamium amplexicaule* L.), curly dock (*Rumex crispus* L.), and red sorrell (*Rumex acetosella* L.) also tested positive for spotted wilt, but the thrips collected from them were not confirmed as vectors in this study. Most of the host plants in the farmscape and near the farmscape had immature thrips collected from them, indicating that these plants are suitable for

Table 1. Number of thrips collected from different plant species in a Georgia flue-cured tobacco farmscape and numbers of confirmed vectors of tomato spotted wilt virus using ELISA (% vectors), 1999-2001

Plant host	1999 N	1999 Vectors (%)	2000 N	2000 Vectors (%)	2001 N	2001 Vectors (%)
Small hop clover* *Trifolium dubium* Sibth.	24	0	70	0	45	0
Henbit*++ *Lamium amplexicaule* L.	24	0	24	0	30	0
Wild radish *++ *Raphanus raphanistrum* L.	71	4 (5.6)	221	6 (2.7)	42	2 (4.8)
Narrowleaf vetch*++ *Vicia sativa* L. subsp. *nigra* (L.) Ehrh.	2	0	256	4 (1.6)	75	2 (2.7)
Rye *Secale cereale* L.	8	0	22	0	—	—
Cutleaf evening primrose*++ *Oenothera laciniata* Hill	13	1 (7.7)	—	—	35	1 (2.9)
Volunteer soybean* *Glycine max* (L.) Merrill	121	5 (4.1)	25	1 (4.0)	—	—
Broomsedge* *Andropogon virginicus* L.	—	—	3880	63 (1.6)	445	29 (6.5)
Pampasgrass *Cortaderia selloana* (Schult.) Asch. & Graebn.	—	—	13	0	17	0
Tobacco foliage*++ *Nicotiana tabacum* L.	1300	60 (4.6)	863	9 (1.1)	874	23 (2.6)
Tobacco blooms*++ *N. tabacum*	—	—	448	4 (0.9)	670	33 (4.9)

* Immature stages of thrips observed on this host plant, ++ indicates positive ELISA on at least one of the plant samples tested for this plant host.

thrips survival and reproduction (Tables 1,2). Volunteer peanuts in the farmscape also can be an effective reservoir for tomato spotted wilt virus and thrips vectors in the southeastern U.S. (McPherson et al. 1997, Johnson et al. 1996, 2000); however, no volunteer peanuts were observed in the tobacco farmscape at our test site. Peanuts had not been produced on this land for 2 yrs prior to this study. Volunteer peanuts are abundant in most fields in the fall and early-spring following their harvest (Johnson et al. 1996, 2000). Small hop clover (*Trifolium dubium* Sibth), rye (*Secale cereale* L.), and pampasgrass (*Cortaderia selloana* (Schult.) Asch. & Graebn.) were collected in the tobacco farmscape, but these host plants neither tested positive for spotted wilt

Table 2. Number of thrips collected from different plant species near a Georgia flue-cured tobacco farmscape and number of confirmed vectors of tomato spotted wilt virus using ELISA (% vectors), 1999-2001

Plant host	1999 N	1999 Vectors (%)	2000 N	2000 Vectors (%)	2001 N	2001 Vectors (%)
Broccoli* *Brassica oleracea* L.	—	—	42	0	—	—
Carrot*++ *Daucus carota* L.	40	1 (2.5)	—	—	—	—
Wild iris *Iris* spp.	—	—	64	0	—	—
Japanese honeysuckle* *Lonicera japonica* Thunberg	—	—	25	0	6	0
Azalea blooms* *Rhododendron* spp.	—	—	70	0	150	0
Curly dock*++ *Rumex crispus* L.	—	—	20	0	—	—
Red sorrel*++ *Rumex acetosella* L.	—	—	15	0	—	—
Common chickweed*++ *Stellaria media* (L.) Cyrillo	—	—	—	—	50	1 (2.0)
Eastern braccerus	—	—	—	—	10	0
Privet* *Ligustrum sinense* Lour.	—	—	—	—	3	0
Flowering dogwood blooms* *Cornus florida* L.	—	—	—	—	27	1 (3.7)

* Immature stages of thrips observed on this host plant, ++ indicates positive ELISA on at least one of the plant samples tested for this plant host.

nor had confirmed thrips vectors present on them (Table 1). Similarly, in the area near the tobacco farmscape, several host plants were neither positive for spotted wilt nor had confirmed thrips vectors. These host plants near the farmscape included broccoli, wild iris, Japanese honeysuckle, azalea blooms, eastern braccerus and privet (Table 2).

Frankliniella fusca was the most common vector species both in number collected and in the number of host plants collected from (5 of the 10 plant hosts) (Table 3). *Frankliniella fusca* also was reported as the most abundant spotted wilt vector among the 28 common weed hosts monitored in North Carolina (Groves et al. 2002). Three flower thrips (*F. occidentalis, F. tritici* and *F. bispinosa* Morgan) in the flower thrips complex also were confirmed as potential vectors in this study. Not all of the *F. occidentalis, F. bispinosa,* and *F. tritici* could be taxonimically separated in the buffer

Table 3. Thrips species that were confirmed vectors [ELISA] of tomato spotted wilt virus and the host plants on which the thrips were collected in or near a Georgia tobacco farmscape, 1999-2001

Plant host	Thrips species confirmed as vector (year)
Cutleaf evening primrose *Oenothera laciniata* Hill	*Frankliniella fusca* ('99, '01)
Wild radish *Raphanus raphanistrum* L.	*Flower thrips complex* ('99, '00, '01)*
Carrot *Daucus carota* L.	*F. fusca* ('99)
Common chickweed *Stellaria media* (L.) Cyrillo	*F. fusca* ('01)
Flowering dogwood *Cornus florida* L.	*F. occidentalis* ('01)
Narrowleaf vetch *Vicia sativa* L. Subsp. *Nigra* (L.) Ehrh.	*F. fusca* ('00, '01)
Broomsedge *Andropogon virginicus* L.	*Haplothrips graminis* & *Chirothrips* spp. ('00, '01)
Soybean *Glycine max* (L.) Merrill	*F. occidentalis* ('99, '00)
Tobacco foliage *Nicotiana tabacum* L.	*F. fusca* ('99, '00, '01)
Tobacco blooms *Nicotiana tabacum* L.	*F. occidentalis* & *Flower thrips complex* ('00, '01)*

* Flower thrips complex consisted of *F. occidentalis, F. bispinosa,* and *F. tritci* combined (unable to separate to species in the buffer solution).

solution because these specimens could not be mounted on slides when used in the ELISA tests (they could be separated to species if they were preserved in alcohol and mounted on slides). When a positive vector was confirmed in the flower thrips complex, the vector was most probably either *F. occidentalis* or *F. bispinosa* because these species are reported vectors of spotted wilt (Sakimura 1962, Webb et al. 1997) and *F. tritici* is not a reported vector. Some thrips collections from host plant blooms contained only *F. occidentalis* in the alcohol subsamples and, thus, could be assumed to be the vector species confirmed by ELISA. It is interesting to note that *Haplothrips graminis* Hood and *Chirothrips* spp. (*C. crassus* Hinds and *C. mexicanus* Crawford, combined) were confirmed as potential vectors in the farmscape and were collected on broomsedge, which was present along fence rows and ditch banks. This host plant, which often had high numbers of thrips present (several hundred per plant), was never confirmed as a positive host of tomato spotted wilt virus in over 20 ELISA tests run over 2 yrs. Immature stages of thrips were observed on broomsedge, but because this plant was never confirmed as a positive host of tomato spotted wilt, the

infected thrips probably acquired the virus on some other host plant as immatures then migrated as adults to broomsedge. Both *Haplothrips* spp. and *Chirothrips* spp. are found on flue-cured tobacco in Georgia (McPherson et al. 1992) along with 43 different thrips species and now must be considered as potential vectors of tomato spotted wilt virus on tobacco.

Frankliniella fusca, a known vector of tomato spotted wilt (Sakimura 1963), was the predominate thrips species on flue-cured tobacco foliage during this study (Table 4). *Frankliniella occidentalis,* another reported vector of spotted wilt (Sakimura 1962), also was present in low numbers in flue-cured tobacco blooms on all 3 yrs of this study and was sometimes the only thrips of the flower thrips complex present on the blooms. Several other thrips species were collected on tobacco during this investigation and included *Caliothrips* spp., *Plesiothrips perplexus* (Beach), *Microcephalothrips abdominalis* (Crawford, D. L.), *Limothrips cerealium* (Haliday), *Scirtothrips* spp., *Haplothrips graminis* Hood, and *Chirothrips mexicanus* Crawford. On tobacco foliage, *F. fusca* comprised 94.9, 82.7, and 95.8% of the thrips complex in 1999-2001, respectively.

Weeds have been reported to have a significant impact on the development of spotted wilt epidemics in some cropping systems (Bautista and Mau 1994, Cho et al. 1987, Johnson et al. 1996, 2000), and in the overwintering of *F. fusca* and tomato wilt virus (Groves et al. 2001). The results reported herein suggest that certain weeds in the tobacco farmscape are also important in providing thrips refuge and nutrients for survival and a virulent innoculant source for tomato spotted wilt virus during the pre-transplanting and early post-transplanting periods of flue-cured tobacco production. Four different thrips vector species were confirmed in the tobacco farmscape during this period, including *F. fusca, F. occidentalis, H. graminis,* and *Chirothrips* spp. One or more of these species was present in the farmscape on every date that thrips were collected from December through April, thus it appears that the weed hosts in the farmscape are influential as refuge and nutrients for vectors and innoculant source of tomato spotted wilt virus in tobacco. Although this study concentrated

Table 4. Incidence of thrips species on flue-cured tobacco foliage and blooms and the percentage of each species in the thrips complex, Tift County Georgia, 1999-2001

Thrips species collected	1999* N (%)	2000 N (%)	2001 N (%)
Frankliniella fusca	1234 (94.9)	843 (64.3)	838 (54.3)
Frankliniella occidentalis	9 (0.7)	124 (9.5)	15 (0.9)
Flower thrips complex++	39 (3.0)	324 (24.7)	655 (42.4)
Other species†	18 (1.4)	20 (1.5)	36 (2.3)
Total collected	1300	1311	1544

* Only tobacco foliage sampled in 1999. From foliage samples: 94.9, 82.7, and 95.8% were *F. fusca* in 1999–01.

++ Flower thrips complex comprised of *F. occidentalis, F. bispinosa,* and *F. tritici* combined.

† Other species include *Caliothrips* spp., *Plesiothrips perplexus, Microcephalopthrips abdominalis, Limothrips cerealium, Scirtothrips* spp., *Haplothrips graminis,* and *Chirothrips mexicanus.*

on the weeds and potential thrips vectors of tomato spotted wilt virus in and near a flue-cured tobacco farmscape, the results could be equally important to the vegetable and peanut farmscapes as well. Many of the same weed hosts and thrips species are present in south Georgia fields where tomato, bell pepper, and peanut are being produced. These three crops are being planted/transplanted about the same time as tobacco and are infected annually with tomato spotted wilt virus.

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