Field Survey of Impatiens Necrotic Spot Virus in Georgia Peanut

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ABSTRACT

A field survey to determine the incidence of Impatiens necrotic spot virus (INSV) in Georgia peanut (*Arachis hypogaea* L.) was conducted during the 1999 and 2000 growing seasons. Confirmation of INSV infection was made by enzyme-linked immunosorbent assay (ELISA). During August 1999, a total of 504 symptomatic peanut plants were sampled from 42 fields representing 15 counties. Peanut plants showing symptoms of spotted wilt from 14, 15, and 15 counties were sampled during the early (June), mid (July), and late (August/September) periods of the 2000 growing season, respectively. During 2000, a total of 1433 peanut plants were sampled over the course of the survey. The 1999 survey yielded no INSV-positive peanut plants, while 87% of the plants sampled tested positive for TSWV. During the 2000 survey, INSV was detected from 2.0% of all peanut plants sampled, while TSWV was detected in 97.8% of all plants. All plants that tested positive for INSV also tested positive for TSWV. TSWV was detected from plants in each field. INSV was only detected from seven fields in four counties. Additionally, 90 peanut plants were tested for INSV in a Coffee County field in which that virus was detected in July 2000. TSWV was detected in 94% of those plants, while INSV was detected in 20% of the plants. Double infections were detected in 17% of the plants from the field in Coffee County. The natural incidence of another *Tospovirus* in peanut could have important management implications for peanut production systems.

Key Words: *Arachis hypogaea*, *Bunyaviridae*, INSV, thrips, *Tospovirus*, TSWV.

Spotted wilt disease, caused by Tomato spotted wilt virus (TSWV), continues to be a major disease constraint to Georgia peanut (*Arachis hypogaea* L.) production. During 1998, Impatiens necrotic spot virus (INSV) was detected in Georgia and Texas peanut fields (Pappu *et al.*, 1999). INSV is an important pathogen of ornamental plants and has a major effect on the greenhouse industry (Daughtrey *et al.*, 1997). TSWV and INSV, members of genus *Tospovirus*, family *Bunyaviridae* (Moyer, 1999), are believed to exhibit similar symptoms on peanut, which include concentric ringspots, "oak leaf" patterns of chlorosis, bronzing of leaves, stunting, and/or necrosis of leaves in the terminal bud, wilting, and death. Both diseases are circulative viruses, ingested by thrips larvae and transmitted by late instars and adults during feeding (Sakimura, 1963; Ullman *et al.*, 1997). Two species of thrips, tobacco thrips (*Frankliniella fusca* Hinds) and western flower thrips (*F. occidentalis* Pergande), are considered the two main vectors of TSWV in Georgia peanut (Todd *et al.*, 1995). Currently, only *F. occidentalis* is confirmed as a vector of INSV (Peters *et al.*, 1996). Given the economic impact of spotted wilt on peanut production in Georgia, it is important to determine the prevalence of INSV in peanut. The addition of another tospovirus in peanut production systems could have important management implications for these systems, especially regarding possible differential response of cultivars with resistance to TSWV. This study was undertaken to determine the prevalence of TSWV and INSV in peanut-producing regions of Georgia during 1999 and 2000.

Materials and Methods

1999. A survey of 15 counties in Georgia was undertaken from 23 Aug.-30 Aug. 1999 (Table 1). One to four grower fields were surveyed per county. A total of 504 peanut plants were sampled. Root samples from peanut plants exhibiting symptoms of spotted wilt (chlorosis, wilting, necrotic internal taproot and crown) were taken at random from 12 plants in each field. The number of plants sampled was kept at a maximum of 12 for logistical reasons, which would not allow for large sample sizes from each field. Plants were

Table 1. Percentage of symptomatic peanut plants testing positive for tomato spotted wilt virus and Impatiens necrotic spot virus by county and field during Aug. 1999 and mean optical density values (405 nm) ± standard deviations for TSWV-positive plants; N = number of plants tested.

County	N	TSWV	TSWV+ O.D.$_{405\ nm}$ std. dev.	INSV
		%		%
Baker	24	92	0.65 ± 0.31	0
Berrien	36	78	0.51 ± 0.21	0
Brooks	24	71	0.61 ± 0.34	0
Burke	36	83	0.49 ± 0.24	0
Colquitt	48	88	0.73 ± 0.27	0
Dooly	48	78	0.59 ± 0.25	0
Emmanuel	24	97	0.88 ± 0.30	0
Grady	36	89	0.67 ± 0.25	0
Irwin	48	88	0.75 ± 0.27	0
Laurens	24	96	0.53 ± 0.21	0
Miller	24	100	0.66 ± 0.25	0
Mitchell	48	92	0.66 ± 0.25	0
Randolph	36	83	0.50 ± 0.28	0
Seminole	12	92	0.69 ± 0.30	0
Sumter	36	86	0.62 ± 0.26	0

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bagged separately and placed in a cooler on ice for transport back to the laboratory at the Univ. of Georgia Coastal Plain Exp. Sta., Tifton, GA. Roots were tested for presence of TSWV and INSV by enzyme-linked immunosorbent assay (ELISA) for TSWV and INSV with commercially available kits (Agdia Inc., Elkhart, IN). A possibility of false positive readings exists when assaying plant root material using horseradish peroxidase, which can react with phosphorous as a substrate from the soil. Alkaline phosphotase was used as ELISA substrate in order to avoid such positive readings. Accuracy of ELISA assays for INSV detection has been confirmed previously by immunocapture polymerase chain reaction (PCR) (Pappu *et al.*, 1999). Koch's postulates have been satisfied through experimental transmission of INSV to peanut by mechanical inoculation using inoculum isolated from field samples (B. Mandal and H.R. Pappu, unpubl. data). All plants subject to ELISA were of the Georgia Green cultivar.

2000. The study was expanded during 2000 to include surveys conducted during the early, mid, and latter parts of the peanut growing season. Sampling procedures were similar to those during 1999. Nine to 12 peanut root samples from symptomatic plants were collected from one to three fields in each county. Peanut roots were tested for TSWV and INSV by ELISA. The early season survey was conducted from 19 to 28 June over 14 counties (Table 2). Midseason surveys were undertaken from 17 to 26 July from 15 counties. The late season survey of 15 counties was conducted from 31Aug. to 12 Sept. A total of 1433 symptomatic peanut plants were sampled during 2000. Upon receiving positive results for INSV in samples from one field (Smith field) in Coffee County, an additional survey of 90 symptomatic plants from that field was conducted on 9 Aug. to gain a better understanding of the extent of INSV infection. All plants subject to ELISA were of the Georgia Green cultivar.

Results and Discussion

1999. None of the peanut plants tested were positive for INSV during the 1999 survey, while 87% of the plants sampled from all locations were positive for TSWV (Table 1). TSWV was detected in each field and in each county surveyed (Fig. 1). The highest percentages of TSWV infection were observed from plants collected from counties in the southwestern corner of Georgia. Although spotted wilt historically has been more prevalent in this region, the disease does appear to be progressing to other areas of the state.

2000. INSV was detected in 2.0% of all peanut plants sampled (n = 1433), while TSWV was detected in 97.8% of all plants. Double infections of INSV and TSWV were detected in 100% of the plants testing positive for INSV during the inital survey. TSWV was detected at all sites. INSV was only detected from seven fields in four counties (Fig. 1). During the current survey, INSV was first detected in one symptomatic peanut plant from the Smith field in Coffee County during the midseason survey in July (Table 3). This plant exhibited symptoms characteristic of TSWV infection. Samples from the same field during the late season survey yielded positive results for INSV from seven symptomatic plants (Table 4). Positive INSV results were obtained from two and four plants in two additional Coffee County peanut fields during the same period. One plant from a field in Sumter County, one plant from a field in Randolph County, and one plant from each of two fields in Colquitt County also were positive for INSV by ELISA during the late season survey (Table 4). Optical density values for INSV were lower than those for TSWV, apparently due to a lower titer of INSV in infected plants (Tables 3 and 4).

Table 2. Percentage of symptomatic peanut plants testing positive for tomato spotted wilt virus and Impatiens necrotic spot virus during June 2000 and mean optical density values (405 nm) ± standard deviations for TSWV-positive plants; N = number of plants tested.

County	N	TSWV	TSWV+ O.D.$_{405nm}$ std. dev.	INSV
		%		%
Baker	36	100	1.13 ± 1.06	0
Berrien	35	100	1.01 ± 0.32	0
Brooks	32	100	0.81 ± 0.33	0
Colquitt	23	100	0.57 ± 0.13	0
Dooly	35	100	0.55 ± 0.13	0
Grady	36	100	2.36 ± 0.95	0
Irwin	35	100	1.67 ± 0.35	0
Miller	36	100	2.48 ± 0.83	0
Mitchell	36	100	0.44 ± 0.16	0
Randolph	36	100	0.95 ± 0.29	0
Seminole	36	97	2.63 ± 0.73	0
Sumter	36	100	1.66 ± 0.37	0
Tift	24	100	0.77 ± 0.41	0
Worth	34	100	0.85 + 0.55	0

Table 3. Percentage of symptomatic peanut plants testing positive for tomato spotted wilt virus and Impatiens necrotic spot virus during July 2000 and mean optical density values (405 nm) ± standard deviations for TSWV- and INSV-positive plants; N = number of plants tested.

County	N	TSWV	TSWV+ O.D.$_{405nm}$ std. dev.	INSV	INSV+ O.D.$_{405nm}$ std. dev.	Double infection
		%		%		%
Baker	36	100	2.94 ± 0.95	0	□	0
Berrien	36	100	3.02 ± 0.21	0	□	0
Brooks	34	100	2.99 ± 0.25	0	□	0
Colquitt	24	100	2.43 ± 0.41	0	□	0
Coffee	9	100	2.66 ± 1.50	11	0.15+0.0	11
Dooly	36	97	2.89 ± 0.87	0	□	0
Grady	36	97	2.68 ± 0.35	0	□	0
Irwin	35	100	1.63 ± 0.43	0	□	0
Miller	36	95	3.33 ± 0.52	0	□	0
Mitchell	36	94	2.54 ± 0.57	0	□	0
Randolph	36	100	2.43 ± 0.59	0	□	0
Seminole	36	100	3.14 ± 0.69	0	□	0
Sumter	36	80	1.29 ± 0.45	0	□	0
Tift	24	96	2.14 ± 0.54	0	□	0
Worth	35	100	2.09 ± 0.41	0	□	0

A more extensive survey of 90 symptomatic plants in the Smith field showed 94 and 20% positive infections for TSWV and INSV, respectively. Mean optical density values and standard deviations for TSWV and INSV ELISA-positive plants were 3.44 ± 0.67 and 0.057 ± 0.11, respectively. Double infections of both tospoviruses were observed in 17% of the plants tested. These results, along with those of the regular mid and late season surveys, show that INSV is not as common in peanut production systems as TSWV; however, when INSV does

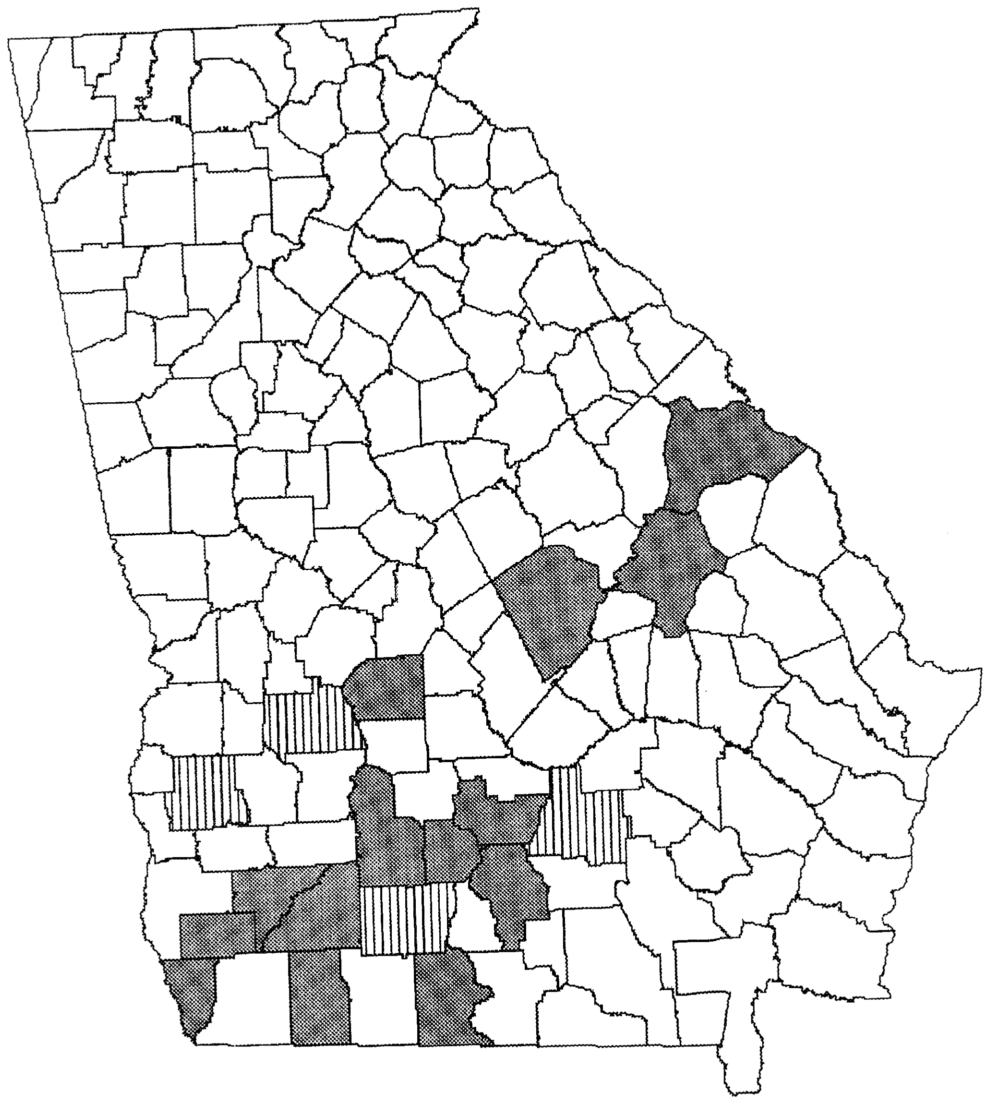

Fig. 1. Map of Georgia counties surveyed during 1999 and 2000 where shaded counties had TSWV only and counties overlayed with vertical lines had both INSV and TSWV.

Table 4. Percentage of symptomatic peanut plants testing positive for tomato spotted wilt virus and Impatiens necrotic spot virus during Aug./Sept. 2000 and mean optical density values (405 nm) ± standard deviations for TSWV- and INSV-positive plants; N = number of plants tested.

County	N	TSWV	TSWV+ O.D.$_{405nm}$ std. dev.	INSV	INSV+ O.D.$_{405nm}$ std. dev.	Double infection
		%		%		%
Baker	36	83	0.91 ± 0.19	0	□	0
Berrien	36	97	0.75 ± 0.19	0	□	0
Brooks	36	100	0.94 ± 0.24	0	□	0
Colquitt	24	100	3.52 ± 0.26	8	0.06+0.01	8
Coffee	36	97	3.06 ± 0.92	36	0.16+0.07	36
Dooly	36	97	2.85 ± 0.74	0	□	0
Grady	36	89	3.43 ± 0.62	0	□	0
Irwin	36	100	0.85 ± 0.26	0	□	0
Miller	35	97	1.19 ± 0.87	0	□	0
Mitchell	36	95	3.10 ± 0.62	0	□	0
Randolph	36	92	1.74 ± 0.80	3	0.12+0.00	3
Seminole	24	100	2.35 ± 0.75	0	□	0
Sumter	36	97	2.28 ± 0.73	3	0.17+0.00	3
Tift	24	100	3.53 ± 0.30	0	□	0
Worth	35	94	0.85 ± 0.27	0	□	0

occur, it is usually found in a plant infected with TSWV. INSV infection of peanut also occurred most often in double infection with TSWV in Texas, where incidence of INSV ranged from 13-56% in commercial peanut fields (Black *et al.*, 2000).

Of the two tospoviruses, INSV is typically considered more of a threat to greenhouse systems, while TSWV is a common threat to field crops such as peanut, tomato (*Lycopersicon esculentum* Miller), pepper (*Capsicum frutescens* L.), and tobacco (*Nicotiana tabacum* L.). Tomato and pepper have been shown previously to be susceptible to INSV; however, symptoms of INSV often are less severe on these crops than those caused by TSWV (Daughtrey *et al.*, 1997). Where INSV has been shown to occur in field crops, the source usually has been traced back to greenhouse-grown transplants (Daughtrey *et al.*, 1997), which is a scenario that would not occur in peanut production. INSV does not infect burley tobacco (Daughtrey *et al.*, 1997) and has not been detected in flue-cured tobacco. INSV was first detected from Georgia peanut fields at one site in Mitchell County and at one site in Tift County during the 1998 peanut growing season (Pappu *et al.*, 1999). These same fields were surveyed for detection of INSV during the 1999 and 2000 growing seasons; however, INSV was not detected from either location.

Detection of INSV in peanut remains a difficult task due to the apparent similarity of symptoms between INSV and TSWV. As a result, INSV may be overlooked or dismissed as TSWV. However, it may be more common in Georgia peanut agroecosystems than is currently realized. The abundance of potential plant hosts and thrips vectors shared between the two tospoviruses favor continued spread of INSV in peanut production areas. The current effect of INSV, alone or in combination with TSWV, and its potential effect on peanut production in Georgia remains unknown at this time. Production strategies included in the TSWV risk assessment index (Brown et al., 2000) may help manage INSV as well; however, the response of INSV to production practices geared toward management of TSWV remains unknown, particularly the response of moderately resistant cultivars such as Georgia Green with moderate levels of field resistance to TSWV. Continued monitoring for INSV is important in order to develop appropriate management strategies for spotted wilt disease in peanut.

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