MULTI-STATE SAMPLING FOR *NEOZYGITES FRESENII* IN COTTON

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Abstract

A multi-state survey of cotton aphids showed that the aphid fungus, *Neozygites fresenii*, was present in all ten states surveyed (AL, AR, GA, LA, MS, MO, NC, SC, TN, and TX). Based on an epizootic threshold level of 15% infected aphids, epizootics began in the last week of June and first week of July in most areas.

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Introduction

Since the 1980's the cotton aphid, *Aphis gossypii*, has been a serious pest of cotton in the southern and western United States (Grafton-Cardwell 1991, Smith and Hardee 1993, Hardee et al. 1993). Insecticide sprays used to control bollworms, bollweevils and plant bugs may result in aphid outbreaks (Slosser et al. 1989). High aphid populations may lower cotton yield by stunting plants and adversely affecting fruit development (Andrews and Kitten 1989; Fuson et al. 1995), by lowering boll weight and reducing square and boll retention (Fuchs and Minzenmeyer 1995), and by causing "sticky cotton" (Hardee and O'Brien 1990). Cotton aphid populations can increase rapidly and are difficult to control because of insecticide resistance and application difficulties. Annual aphid outbreaks and control difficulties have led to increased interest in the management of the cotton aphid. In the decision process of whether to spray chemical insecticides for aphid control, the impact of natural biological controls, including *Neozygites fresenii*, should be kept in mind (Harris and Furr 1994).

N. fresenii is an entomopathogenic fungus which has contributed to the reduction of aphid populations in the Mississippi Delta cotton region since 1988 (Steinkraus et al. 1991, 1995; Weathersbee and Hardee 1993). In an effort to increase our knowledge of when and where the fungus occurs and what levels of infection it causes in aphid populations in states throughout the cotton belt, we requested that cotton entomologists in the southeast, midsouth and Texas participate in a multi-state sampling survey. No multi-state survey of *N. fresenii* has been undertaken before. Our objectives were to document the occurrence of *N. fresenii* in various states, determine when it occurs, determine infection levels, and determine the feasibility of multi-state monitoring of *N. fresenii* levels. Monitoring *N. fresenii* levels may have a useful role in cotton IPM and predictions of epizootics could lead to reduced insecticide applications.

Materials and Methods

A total of 35 in 10 states took part in the program in 1995 (Table 1). Participants were supplied with a sampling kit consisting of 30 ml vials containing 70% ethanol, cardboard mailing tubes, and pre-addressed Federal Express envelopes.

Cooperators collected cotton leaves containing aphids from representative sites of fields in their areas beginning in mid-June and continuing throughout the growing season. Samples were collected from 17 counties in Arkansas and 30 counties in the other 9 states (Table 1).

All samples from outside Arkansas were sent to our laboratory in Fayetteville and processed. Fifty aphids were selected randomly from each sample. Aphids were placed in acid fuchsin-lactophenol stain (5 aphids per drop) and gently squashed under a cover slip to release bodily contents. Each aphid was examined at 200x with a phase microscope. If no sign of *N. fresenii* was present, an aphid was diagnosed as negative. If *N. fresenii* was present, the aphid was assigned to one of the following positive *N. fresenii* categories: secondary conidia attached (very early stage of infection), protoplasts/hyphal bodies present (vegetative stage of infection), conidial stage (sporulation/dissemination stage of infection), or *N. fresenii* + saprophytic fungi present (aphid was killed by *N. fresenii* at least several days prior to collection).

Previous research has indicated that epizootics are usually imminent by the time infection levels reach ≈15% (Hollingsworth et al. 1995). Therefore, we categorized infected samples into the following infection level groups: low (0.1%-3.9%), medium (4.0%-14.9%), or high (≥15%).

Arkansas samples were mailed to the Cooperative Extension Plant Diagnostic Clinic in Lonoke, Arkansas for processing. The goal of this has been to provide rapid diagnoses (within 24 hr) for growers considering insecticide sprays, therefore the number of aphids examined per Arkansas sample was lower (30).

Results and Discussion

A total of 261 samples was received in 1995; 123 from Arkansas and 138 from the other 9 participating states (Table 1). The first sample collected was on 12 June in Chicot County, Arkansas. The last sample was collected on 7 September in Tift County, Georgia.

N. fresenii was present in all 10 states and was detected in 66% of the 261 samples received. The average percentage of aphids per sample infected with *N. fresenii* for all states and all sampling dates was 33%. After 30 June the percentage of samples in which *N. fresenii* was detected increased to 89% (n=151) and the average percentage of aphids per sample infected with *N. fresenii* increased to 53% (Table 1).

The rapid rise in infection levels detected in the S. Carolina samples, which were collected from the same fields for two consecutive weeks, lends support to the hypothesis (Hollingsworth et al. 1995) that epizootics are imminent when infection levels reach ≈15% (Table 3). In S. Carolina, infection levels rose from 18% on 17 July to 80% on 24 July.

The earliest date *N. fresenii* was detected was in samples collected on 20 June in Ellis County, Texas (2%) and Natchitoches Parish, Louisiana (16%). On 22 June *N. fresenii* was detected in samples collected from Madison County, Alabama (2%) and on 23 June from Oktibbeha County, Mississippi (2%) and Dallas County, Texas (2%). The next *N. fresenii* detection was on 26 June in samples collected in Lafayette County, Arkansas (7%) and Floyd County, Georgia (30%). The fungus occurred at 4% in Madison County, Tennessee in samples collected 30 June, the only sampling date for the state. No infected aphids were detected in samples from Missouri until 28 June in New Madrid County (2%). Infection occurred even later in sample aphids from N. Carolina, where infection levels reached 16% on 15 July in one field, but only 4% on 28 July in another field. The first occurrence of *N. fresenii* in S. Carolina was in samples collected on 13 July (2%) (Tables 2 & 3). By 28 June, the infection level in Lamar County, Texas was 84%, much higher than other samples to that date.

In some locations infection levels were already moderately high (≥4.0%) by the time the first samples were collected. For example, by 20 June in Natchitoches Parish, Louisiana, 16% of the aphids were infected. Because no samples were collected in this parish prior to 20 June, we must assume that the fungus was present before 20 June. Only one sample each was received from Dunklin County, Missouri; Navarro County, Texas; and Monroe County, Arkansas (all 0% infection). This does not necessarily mean that the fungus was not present. Therefore, it is impossible from this study to provide a complete picture of the levels of the fungus in all sampling locations.

High (≥15%) *N. fresenii* infection levels occurred the last week of June in 6 states, AL, AR, GA, LA, MS, and TX (Tables 2 & 3). Based on a previous study (Hollingsworth et al. 1995) we would expect epizootics to reduce aphid populations within a week in these fields, which is substantiated by the epizootic levels of *N. fresenii* in the first week of July, except in Missouri, N. Carolina and S. Carolina. In Missouri, N. Carolina and S. Carolina, the first high infection levels were found in samples collected the 3rd week of July. The epizootic infection level (84%) in Texas on 28 June, the high infection level (16%) in Louisiana on 20 June, and the dates at which high infection levels occurred in the rest of the sampling region indicate that *N. fresenii* occurs earlier in the southwest (late June) and later in the northern areas of the cotton belt (mid to late July).

Prevailing winds may be responsible for this pattern of epizootic development. Previous research showed that aerially-borne *N. fresenii* conidia occur in vast numbers (Steinkraus et al. 1996) and are capable of causing aphid infections within a field and downwind of an epizootic field (unpublished data). Wind also disperses winged (alate) cotton aphids, many of which may be infected (Steinkraus et al. 1995). A third mechanism by which epizootics could be initiated are resting spores in the soil. The extent to which resting spores are responsible for initiation of aphid infections remains unknown. At times, often in severely

stressed cotton due to aphid damage, resting spores occur in high numbers. For example, in the field sampled in Tensas Parish, Louisiana, 6% of the aphids collected had formed resting spores. Resting spores may allow the fungus to develop independently of aerial spores or infected alatae, resulting in erratic epizootic development within certain regions. *N. fresenii* has been shown to be adversely affected by granular fungicides applied at planting (Smith and Hardee 1993), which may also affect erratic epizootic development in some fields.

We were able to gather a few post-season, word-of-mouth impressions of how the fungus performed in four of the participating states. In Texas, aphids appeared early in the season on seedlings and persisted for 3-4 weeks. The fungus controlled these aphids to some extent, but erratically. In some cases the populations resurged (A. Knutson). In Alabama the aphids were the "worst we've ever had" yet the fungus "absolutely" helped control them (B. L. Freeman). Aphid populations were also extremely high in Louisiana where the fungus controlled populations until late in the season when there was a slight resurgence (J. D. Powell).

In Arkansas the first sample containing infected aphids was collected on 26 June in Lafayette County (7%) (Table 2). The first high level of infection occurred in Miller County on 29 June (30%). These were the two southwestern counties sampled in Arkansas. In general, high infection occurred approximately one week later (the first week of July) in southeastern and east central Arkansas . High levels of infection in northeastern Arkansas occurred approximately one week after this (the second week of July).

In August 1995 surveys, Arkansas cooperators responded that insecticide treatments for aphids were postponed in 47 of 49 sample fields due to the expectation that the fungus would soon control the aphids. Information considered in their decisions to postpone spraying insecticides were, 1) results from the diagnostic service in Lonoke, 2) word-of-mouth information that fungus epizootics were occurring in the area, and 3) detection of fungus-killed aphids in sample fields. Cooperators indicated that the fungus provided good control of aphids in 42 of the fields in which insecticide applications were postponed.

Conclusions

N. fresenii was detected in all 10 participating states. The fungus first appeared in the most southern and western sampling areas (Texas and Louisiana) and somewhat later in the northern and eastern sampling areas. High infection levels ($\geq$15%) followed the same temporal and spatial pattern. There was a large increase in the percentage of samples and aphids per sample infected with *N. fresenii* after 30 June. Most epizootic levels occurred in late June and early July with the exception of Missouri, North Carolina and South Carolina where the fungus did not reach epizootic levels until the third week of July.

Although we detected *N. fresenii* in all participating states and the fungus appeared to provide satisfactory control of many aphid populations, careful, systematic sampling and microscopic diagnoses are essential before insecticide treatment is postponed in expectation of a fungal epizootic. Importantly, if aphid levels are above economic thresholds in a field and *N. fresenii* is not present, the most prudent course for growers would be to apply an effective insecticide and not rely on the fungus.

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References Cited

Andrews, G. L. and W. F. Kitten. 1989. How cotton yields are affected by aphid populations which occur during boll set, pp. 291-293. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Fuchs, T. W. and R. Minzenmayer. 1995. Effect of aphids on cotton development and yield in West Texas, pp. 890-892. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Fuson, K. J., L. D. Godfrey, and P. F. Wynholds. 1995. Agronomic and environmental factors influencing cotton aphid (*Aphis gossypii* Glover) insecticide efficacy, pp. 995-997. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Grafton-Cardwell, E. E. 1991. Geographical and temporal variation in response to insecticides in various life stages of *Aphis gossypii* (Homoptera: Aphididae) infesting cotton in California. J. Econ. Entomol. 84: 741-749.

Hardee, D. D. and P. J. O'Brien. 1990. Cotton aphids: current status and future trends in management, pp. 169-171. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Hardee, D. D., M. T. Smith, and A. A. Weathersbee III. 1993. Sampling of the cotton aphid, *Aphis gossypii* Glover, in cotton, pp. 738-740. *In* Proceedings, Beltwide Cotton Production and Research Conference, National Cotton Council of America, Memphis, TN.

Harris, F. A. and R. E. Furr, Jr. 1994. Strategies for chemical control of cotton aphid, pp. 134-136. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Hollingsworth, R. G., D. C. Steinkraus and R. W. McNew. 1995. Sampling to predict fungal epizootics in cotton aphids (Homoptera: Aphididae). Environ. Entomol. 24: 1414-1421.

Smith, M. T. and D. D. Hardee. 1993. Influence of granular fungicides applied at planting on seasonal development of the entomopathogenic fungus, *Neozygites fresenii* in the cotton aphid. pp. 744-746. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Slosser, J. E., W. E. Pinchak, and D. R. Rummel. 1989. A review of known and potential factors affecting the population dynamics of the cotton aphid. Southwest. Entomol. 14: 302-313.

Smith, M. T. and D. D. Hardee. 1993. Influence of granular fungicides applied at planting on seasonal development of the entomopathogenic fungus, *Neozygites fresenii* in the cotton aphid. pp. 744-746. *In* Proceedings, Beltwide Cotton Production and Research Conference. National Cotton Council of America, Memphis, TN.

Steinkraus, D. C., T. J. Kring, and N. P. Tugwell. 1991. *Neozygites fresenii* in *Aphis gossypii* on cotton. Southwest. Entomol. 16: 118-122.

Steinkraus, D. C., R. G. Hollingsworth, and P. H. Slaymaker. 1995. Prevalence of *Neozygites fresenii* (Entomophthorales: Neozygitaceae) on cotton aphids (Homoptera: Aphididae) in Arkansas cotton. Environ. Entomol. 24: 465-474.

Steinkraus, D. C., R. G. Hollingsworth, and G. O. Boys. 1996. Aerial spores of *Neozygites fresenii* (Entomophthorales: Neozygitaceae): density, periodicity, and potential role in cotton aphid (Homoptera: Aphididae) epizootics. Environ. Entomol. 25: (in press).

Weathersbee, A. A. III, and D. D. Hardee. 1993. Indices of relative abundance for the cotton aphid, *Aphis gossypii* Glover, and associated parasitoids, pathogens, and predators on six cotton cultivars, pp. 733-737. *In* Proceedings, Beltwide Cotton Conferences, National Cotton Council of America, Memphis, TN.

Table 1. Average percentages of samples and aphids per sample in which *N. fresenii* was detected.

			Percentage *N. fresenii* Positive			
			% of samples		Mean % of aphids	
States and Counties	**Sample no.**	**Cooperator**	**All Samples**	**Samples After 30 June**	**All Samples**	**Samples After 30 June**
Alabama	**12**		**75**	**100**	**28**	**62**
Autauga	1	Gaylor	100	100	96	96
Colbert	2	Freeman	50	--	2	--
Lee	3	Gaylor	100	100	60	60
Limestone	5	Freeman	60	100	11	32
Madison	1	"	100	--	2	--
Arkansas	**123**		**62**	**91**	**32**	**51**
Ashley	3	Williams	100	100	53	53
Chicot	8	Denver	25	100	8	30
Clay	6	Vangilder	67	100	19	50
Craighead	18	Burns, Culp	62	79	41	52
Crettendon	16	Rodery, Thompson	56	100	30	55
Drew	6	Wall, Wiley	33	100	25	75
Jefferson	3	Harmon	67	100	23	35
Lafayette	11	Vestal	82	88	43	58
Lee	10	Kennedy	60	100	37	81
Lincoln	2	Sites	100	100	84	84
Lonoke	14	Klein,	50	100	16	19
Miller	2	Welch	100	--	19	--
Mississippi	11	Culliphen Smith,	55	78	29	37
Monroe	1	Studebaker	0	--	0	--
Phillips	1	Crow	100	100	87	87
Poinsett	8	Payne	75	100	40	53
Woodruff	2	McNeely Crow	100	100	52	97
Georgia	**9**		**78**	**100**	**38**	**52**
Floyd	6	Ruberson	67	100	32	54
Tift	3	"	100	100	49	49
Louisiana	**14**		**93**	**100**	**47**	**69**
Bossier	2	Leonard	50	100	27	54
Caddo	2	Powell	100	100	33	40
Natchitoches	2	"	100	--	31	--
Tensas	8	Leonard	100	100	57	77
Mississippi	**48**	**Luttrell**	**71**	**100**	**50**	**80**
Coahoma	1	Harris	100	100	92	92
Holmes	1	"	100	100	96	96
Oktibbeha	36	Parker	67	100	54	92
Washington	10	Harris	80	100	24	35
Missouri	**6**		**67**	**75**	**32**	**47**
Dunklin	1	Sorenson	0	0	0	0
New Madrid	5	"	80	100	38	63
North Carolina	**2**		**100**	**100**	**10**	**10**
Gates	2	Bacheler				
South Carolina	**23**		**61**	**70**	**27**	**31**
Barnwell	10	Turnipseed	40	50	14	21
Darlington	13	Durant	77	83	34	37
Tennessee						
Madison	3	Lentz	33	--	1	--

Table 1. Average percentages of samples and aphids per sample in which *N. fresenii* was detected. Continued.

States and Counties	Sample no.	Cooperator	Percentage *N. fresenii* Positive: % of samples: All Samples	% of samples: Samples After 30 June	Mean % of aphids: All Samples	Mean % of aphids: Samples After 30 June
Texas	**21**		**52**	**67**	**16**	**19**
Bell	1	Knutson	100	100	47	47
Collin	4	"	0	--	0	--
Dallas	3	"	33	0	1	0
Delta	1	"	100	100	4	4
Ellis	5	"	40	--	1	--
Hill	2	"	100	100	6	6
Lamar	2	"	100	--	72	--
Navarro	1	"	0	0	0	0
Williamson	2	"	100	100	48	48

Table 2. Infection levels of *Neozygites fresenii* in Arkansas aphid samples.

Counties	First Low Infection Date	% Infection	First Med. Infection Date	% Infection	First High Infection Date	% Infection	Highest Infection Date	% Infection
Ashley	--	--	--	--	5 Jul	30	5 Jul	83
Chicot	--	--	7 Jul	7	8 Jul	53	8 Jul	53
Clay	--	--	10 Jul	14	10 Jul	23	10 Jul	50
Craighead	--	--	7 Jul	10	7 Jul	27	13 Jul	97
Crittendon	7 Jul	3	--	--	7 Jul	20	12 Jul	90
Drew	--	--	29 Jun	13	12 Jul	70	12 Jul	80
Jefferson	--	--	7 Jul	13	7 Jul	57	7 Jul	57
Lafayette	--	--	26 Jun	7	10 Jul	43	17 Jul	100
Lee	--	--	29 Jun	7	29 Jun	43	13 Jul	90
Lincoln	--	--	--	--	10 Jul	77	10 Jul	90
Lonoke	--	--	7 Jul	7	27 Jun	17	30 Jun	87
Miller	--	--	29 Jun	7	29 Jun	30	29 Jun	30
Mississippi	--	--	6 Jun	6	3 Jul	17	11 Jul	80
Monroe	--	--	--	--	--	--	--	--
Phillips	--	--	--	--	11 Jul	87	11 Jul	87
Poinsett	5 Jul	3	5 Jul	7	10 Jul	53	17 Jul	100
Woodruff	--	--	28 Jun	7	11 Jul	97	11 Jul	97

Table 3. Infection levels of *N. fresenii* in multi-state samples outside Arkansas.

Counties	First Low Infection Date	% Infection	First Med. Infection Date	% Infection	First High Infection Date	% Infection	Highest Infection Date	% Infection
Alabama								
Autauga	--	--	--	--	21 Jul	96	21 Jul	96
Colbert	--	--	29 Jun	4			29 Jun	4
Lee	--	--			10 Jul	60	11 Jul	84
Limestone	--	--	30 Jun	4	27 Jun	20	7 Jul	32
Madison	22 Jun	2	--	--	--	--	22 Jun	2
Georgia								
Floyd	--	--	--	--	26 Jun	30	14 Jul	62
Tift	--	--	--	--	1 Jul	22	7 Sep	88
Louisiana								
Bossier	--	--	--	--	6 Jul	54	6 Jul	54
Caddo	--	--	--	--	27 Jun	26	6 Jul	40
Natchitoches	--	--	--	--	20 Jun	16	28 Jun	46
Tensas	--	--	28 Jun	9	29 Jun	24	5 Jul	85
Mississippi								
Coahoma	--	--	--	--	3 Jul	92	3 Jul	92
Holmes	--	--	--	--	2 Jul	96	2 Jul	96
Oktibbeha	23 Jun	2	24 Jun	4	24 Jun	26	4 Jul	100
Washington	23 Jun	2	--	--	30 Jun	30	7 Jul	64
Missouri								
Dunklin	--	--	--	--	--	--	--	--
New Madrid	28 Jun	2	6 Jul	4	15 Jul	90	15 Jul	94
N. Carolina								
Gates	--	--	28 Jul	4	15 Jul	16	15 Jul	16
S. Carolina								
Barnwell	--	--	--	--	17 Jul	18	24 Jul	80
Darlington	13 Jul	2	13 Jul	6	20 Jul	82	4 Aug	96
Tennessee								
Madison	--	--	30 Jun	4	--	--	30 Jun	4
Texas								
Bell	--	--	--	--	3 Jul	47	3 Jul	47
Collin	--	--	--	--	--	--	--	--
Dallas	23 Jun	2	--	--	--	--	23 Jun	2
Delta	--	--	4 Aug	4	--	--	4 Aug	4
Ellis	20 Jun	2	--	--	--	--	20 Jun	2
Hill	--	--	3 Jul	4	--	--	3 Jul	8
Lamar	--	--	--	--	28 Jun	60	28 Jun	84
Navarro	--	--	--	--	--	--	--	--
Williamson	--	--	--	--	3 Jul	48	3 Jul	64