EXPANSION OF EXTENSION-BASED APHID FUNGUS SAMPLING SERVICE TO LOUISIANA AND MISSISSIPPI D. C. Steinkraus and G. O Boys **University of Arkansas** Fayetteville, AR **R. D. Bagwell** Louisiana Cooperative Extension Service Winnsboro, LA D. R. Johnson, G. M Lorenz and H. Meyers **Arkansas Cooperative Extension Service** Little Rock, AR M. B. Lavton **Mississippi Cooperative Extension Service** Mississippi State, MS P. F. O'Leary **Cotton Incorporated** Raleigh, NC

Abstract

An extension-based sampling service for cotton aphid fungus was expanded from Arkansas to include Louisiana and Mississippi. This service provides timely information on the progress of fungal epizootics in cotton fields. This information is used in making IPM decisions for aphid control.

Introduction

The cotton aphid, *Aphis gossypii*, has become an increasingly important cotton pest during the past decade. This is due to a variety of factors, including insecticide resistance (Grafton-Cardwell 1991, Kerns and Gaylor 1992, O'Brien et al. 1992, Harris and Furr 1993) and changes in aphid phenology. Prior to 1988 *A. gossypii* occurred primarily in early and late season cotton (Isely 1946). In recent years, aphid populations have been heavy during mid-season as well (Hardee and Herzog 1992, Godfrey et al. 1997).

Cotton aphid damage to cotton is problematic. Some studies have shown severe yield losses due to *A. gossypii* (Andrews and Kitten 1989, Fuchs and Minzenmayer 1995, Fuson et al. 1995, McNally and Mullins 1996). Other studies indicate that the cotton plant can compensate for some aphid damage (Rosenheim et al. 1997, Hardee unpublished data). Control of the aphid is difficult due to insecticide resistance, the unpredictability of the resistance level of an aphid population to a particular chemical, difficulties associated with insecticide application and coverage, insecticide destruction of natural enemies, and the rapid resurgence of aphid populations. Yield losses due to the cotton aphid are complicated by drought and heat stress,

nematode populations, and other factors. There is an acute need for additional research to determine yield losses due to aphid populations and determine economic injury levels under different circumstances.

Since 1993 we have utilized the cotton aphid fungus, Neozygites fresenii, in an IPM program in Arkansas (Steinkraus and Boys 1997). Our objective has been to sample aphid populations in Arkansas throughout the season, determine the percentage of infected aphids from fields, and make predictions of the natural control provided by the fungus. This information can then be used by consultants, growers, and extension personnel to help them make IPM decisions. Research in Arkansas showed that the declines in aphid populations due to this fungus are widespread, somewhat predictable, and can result in rapid reductions of aphid populations (Steinkraus et al. 1995). Generally once 15% of the aphid population is infected a decline will occur within a week or so (Hollingsworth et al. 1995). When this fungus level is reached, it may be more economical and advantageous for the grower to let the fungus reduce the aphid population than to apply an insecticide.

In 1997, with support from Cotton Incorporated, we expanded the Extension-Based Aphid Fungus Sampling Service to include Louisiana and Mississippi. We also developed an Internet site that provides daily results from this service. The objective of this project is to provide cotton growers, consultants, and extension personnel with timely information on the status of this valuable natural enemy that can be used to improve IPM decisions.

Materials and Methods

Participants

Participants were selected by the Cooperative Extension coordinators in their respective states; Drs. Gus Lorenz (Arkansas), Ralph Bagwell (Louisiana), and Blake Layton (Mississippi). Each state coordinator provided a list of participants' names, addresses, phone/FAX numbers and email addresses. Most participants were county extension agents, private consultants, growers, or researchers.

Sampling Kits and Instructions

Each participant was supplied with a sampling kit containing instructions, vials containing 70% ethanol, data sheets, and pre-addressed Federal Express return envelopes. Participants were asked to collect cotton leaves containing aphids from representative sites in their fields as soon as aphids were seen and especially when aphid populations were building. The aphid samples, along with data sheets specifying the degree of urgency of diagnostic results, were sent via 2-day delivery to our laboratory in Fayetteville for processing.

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Processing Samples

Fifty aphids were randomly selected from each sample. Aphids were placed in a drop of acid fuchsin-lactophenol stain (5 aphids per drop) and gently squashed under a cover glass to release bodily contents. Each aphid was examined at 200x with a phase microscope for signs of fungus infection. Aphids were diagnosed as negative if no sign of *N. fresenii* was present. If *N. fresenii* was present, the aphid was assigned to one of the following positive 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 2-3 days before collection).

Reporting Diagnostic Results

The diagnostic results of each sample were reported according to the degree of urgency specified on the accompanying data sheet. Participants requiring results as soon as possible were sent results within 24 hours of receipt of their samples. All other results were reported within 48 hours of receipt of the sample. In addition, a summary of diagnostic results was faxed to state coordinators once per week so that they could disseminate this information within their respective states. In Arkansas, Dr. Don Johnson of the Cooperative Extension Service and his assistant, Hal Meyers, were instrumental in developing an Internet website containing all Arkansas results.

Follow-up Survey of Participants

A follow-up survey of all participants who sent samples was conducted at the end of the sampling season. The objective of the survey was to evaluate the overall usefulness of the sampling service and determine to what extent the information it provided was used in making aphid management decisions.

Results and Discussion

Participants

A total of 97 participants were sent sampling kits. We received samples from 64% of these participants. The total number of participants in each state who sent samples was 23 from Arkansas, 15 from Louisiana, and 23 from Mississippi. The percentages of participants in each state who sent samples were 59% (n=39), 54% (n=28), and 77% (n=30) from Arkansas, Louisiana, and Mississippi, respectively.

Processing Samples

We received samples from a total of 54 counties or parishes; 13 counties in AR, 11 parishes in LA, and 30 counties in MS. A total of 469 samples were received; 162 from AR, 109 from LA, and 198 from MS. The total number of acres sampled was 34,271; 9,470 in AR, 7,698 in LA, and 17,103 in MS (Tables 1 and 2). Each sample took approximately 2 hours to process. We estimate the final cost of each sample was approximately \$25.00 including supplies, shipping, and diagnosis.

General Results

Aphid samples were collected from the first week of June through the third week of August. There was no fungus present in any samples collected the first week of June. The first sample containing infected aphids (4%) was collected in Franklin parish, LA, on 12 June (Table 3). The first MS sample containing infected aphids (2%) was collected in Attala county on 25 June. The first AR sample containing infected aphids (2%) was collected in Chicot county on 3 July. The first samples containing 15% or more infected aphids were collected in Franklin parish, LA on 23 June, in Leflore and Sunflower counties MS on 3 July, and in Ashley county, AR, on 7 July. We consider infection levels of 15% or higher significant because they are usually followed within a few days by aphid population declines, sometimes making aphid sprays unnecessary.

For the most part a temporal relationship was observed between states with regard to infection levels and sampling intensity. Infection levels and sampling intensity in AR consistently lagged behind those of MS, which in turn consistently lagged behind those of LA. The first samples with infected aphids in MS and AR lagged behind those of LA by ca. 2 weeks and 3 weeks, respectively. For the first samples with 15% or greater infection, the lag time decreased to 10 days in MS and 2 weeks in AR. This lag time in infection levels between states seems indicative of the general south-to-north progression of the fungus which we have observed in previous years of the sampling service (Table 3, Figs. 1-4).

The data in Table 1 provide a general view of infection levels and sampling in each state for the entire sampling period. However, Figure 4 illustrates the range of infection levels and numbers of samples per day in just one county in Even within one county infection levels vary AR. enormously on a given date. This indicates that individual fields need to be scouted and sampled in order to accurately predict the occurrence of epizootics. These daily variations in infection levels from field to field within the same county may be due in part to the effect of wind on spore dispersal. Prevailing winds tend to result in a general south-to-north progression of the fungus. However, since we did not track field locations within counties, we do not know to what extent the dynamics of prevailing winds were responsible for these particular variations in infection levels.

Mean percentages of infection provide a somewhat inaccurate indication of peak infection levels when sample numbers were low (Table 1). For example, the highest mean percentage infection in LA occurred the week of 20 July, but this figure is based upon only two samples. It is, therefore, not an entirely accurate indication of peaks of infection. Figures 1-4 show actual percentages of infection for each sample in each state by date and, therefore, more accurately indicate peak infection levels. These data show that in LA peak sampling occurred the week of 22 June followed by peak infection levels the weeks of 28 June and 5 July. A second peak infection level occurred the week of 17 August, but was based on only four samples. Peak sampling in MS occurred the week of 6 July followed by peak infection levels the week of 12 July. Sampling and infection levels in AR both peaked the week of 20 July. These findings and those of other researchers support the idea that *N. fresenii* is most prevalent in hot weather, unlike many aphid fungal pathogens which occur in cooler weather during the spring and fall.

There was little relationship between sampling intensity and infection levels. During times of intensive sampling, mean percentages of infection were sometimes lower than in times of less intensive sampling, and vice versa (Table 1). Actual percentages of infection (Figs. 1-4) show that sampling intensity is not necessarily indicative of infection levels. Several factors may be responsible for these findings. Sampling may intensify as soon as aphid populations begin to build, but the fungus may take several days beyond this time to establish and spread. On the other hand, sampling may slow down as aphid populations decline, but infection levels in those few samples may be high.

Reporting Diagnostic Results

Because of the lag time involved in collecting, shipping, and processing aphid samples, it is crucial that samples be shipped in a timely manner in order for diagnostic results to be useful in management decisions, especially if an aphicide is being considered. If there is no fungus present in a field being considered for an aphid insecticide, then a treatment may in order, though at the risk of reducing beneficial arthropods. On the other hand, if the infection level in the field is 15% or greater, a treatment may be unnecessary. Infection levels should increase in the few days between the time of collection and the receipt of diagnostic results.

Diagnostic results were sent, usually by FAX, to participants within 24-48 hours of receipt of their sample. These results were often shared with other agents, consultants, and growers in the area from which the sample was collected. State coordinators were also sent weekly results and circulated these within their states. Dr. Blake Layton in MS published results in his weekly newsletter. Dr. Don Johnson in AR and his assistant, Hal Meyers, developed Internet a n website (http://ipm.uaex.edu/Insects/Aphid/StartHre.htm) for results, which proved very popular. We are planning to upgrade our computer system so that we can more rapidly enter data onto this site for the 1998 sampling service.

Follow-up Survey of Participants

The results of the follow-up survey of participants were excellent, both in the number of responses and participant opinions concerning the usefulness of the sampling service. Seventy-one percent (n=62) of the participants who sent

samples responded to the follow-up survey. Eighty-six percent (n=44) said that the service saved them or their growers money by not using insecticide treatments for aphids when the fungus was present. Ninety-eight percent (n=44) said the service was helpful, that they used the information it provided in making aphid management decisions, and that the service should be continued in 1998. We received many positive comments regarding the sampling service, the following are ten examples:

"This program was very helpful and saved thousands of dollars in insecticide costs."

"Able to piggy back aphid control with other sprays."

"We had 5 growers prepared to spray ca. 2500 acres until the survey revealed that the fungus was present. At \$7.50/A, this was a significant savings."

"Without it I would have made a follow-up aphid spray. It saved the farmer money."

"Knowing the fungus was in the county encouraged some growers to delay treatments."

"Detection of fungal infestations before they are observed visually is extremely helpful."

"Speed with which you identified % aphids having fungus was excellent."

"Information gained from the service made decisions easier on many fields not sampled."

"Gave the grower and myself and other consultants a way to define what was happening in the field instead of just wondering."

"Participating helped my understanding of how/when the fungal disease works & how we can best fit reliance of this disease into our pest management program."

Late-Season Aphids

In late August and early September we received reports (but few aphid samples!) of heavy late-season aphid populations on cotton and queries regarding the value of the aphid fungus, *N. fresenii*, in controlling these late populations. From Alabama we received samples of aphids that a consultant had observed co-existing with an aphid fungus, but not controlling the aphid population. We examined these aphids and found that a second species of fungus, *Erynia neoaphidis*, was present in 33% of the aphids and no *N. fresenii* was present. Research in many areas of the world has shown that *N. fresenii* is most effective in very hot, humid weather, such as found in July in the Midsouth, whereas, *E. neoaphidis* is more abundant in cool, rainy weather. Our preliminary conclusions are that late-season aphids are unlikely to be controlled to any significant degree by *N. fresenii. Erynia neoaphidis*, provides some natural mortality of late aphids, but is less likely produce rapid epizootics like those caused by *N. fresenii* in July. Therefore, if late season aphids are causing significant honeydew problems, a good insecticide should be used.

We thank Cotton Incorporated for providing the funding to continue the sampling service in Arkansas in 1998. We hope to make it as useful and successful as the service in 1997.

Acknowledgments

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Table 1. Number of samples, number of acres sampled, and mean percentage infection for each week of sampling in Arkansas, Louisiana and Mississippi.*

		No. Samples			No. Acres		Mean %		
		Col	lected		Sam	pled		Infect	ion
Week	AR	LA	MS	AR	LA	MS	AR	LA	MS
Jun 1-7	1	2	1	112	120	-	0	0	0
Jun 8-14	0	8	1	0	547	97	-	0.5	0
Jun 15-21	2	13	8	40	850	664	0	0.2	0
Jun 22-28	5	37	32	220	2168	3100	0	8	0.4
Jun 29-Jul 5	9	24	54	313	1458	5570	0.9	44	4
Jul 6-12	31	13	70	1600	1115	4937	20	44	16
Jul 13-19	48	2	29	2898	540	2415	14	40	57
Jul 20-26	61	2	2	4091	100	270	38	66	97
Jul 27-Aug 2	3	0	0	126	0	0	28	-	-
Aug 3-9	2	0	0	70	0	0	11	-	-
Aug 10-16	0	4	1	0	400	50	-	58	14
Aug 17-23	0	4	0	0	400	0	-	91	-

*Does not include data of 6 samples received from Alabama.

Table 2. Total numbers of counties sampled, samples received and acres sampled.*

State	# Counties	# Samples	# Acres**	
Arkansas	13	162	9,470	
Louisiana	11	109	7,698	
Mississippi	30	198	17,103	
Totals	54	469	34,271	

*Does not include 3 counties, 6 samples and 300 acres sampled in Alabama.

**Includes repeated sampling of some fields.

Table 3. Dates (counties/parishes) of first samples collected and first samples containing infected aphids.

	Arkansas	Louisiana	Mississippi
First Aphids Collected	Jun 5 (Chicot)	Jun 4 (Tensas)	Jun 5 (George)
First Aphids with Fungus Present	Jul 3 (Chicot)	Jun 12 (Franklin)	Jun 25 (Attala)
First Sample with 4%-10% Infection	Jul 3 (Ashley)	Jun 12 (Franklin)	Jun 27 (Sunflower)
First Sample with 15% or > Infection	Jul 7 (Ashley)	Jun 23 (Franklin)	Jul 3 (Leflore & Sunflower)

*Does not include data of 6 samples received from Alabama.

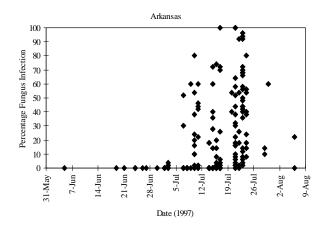
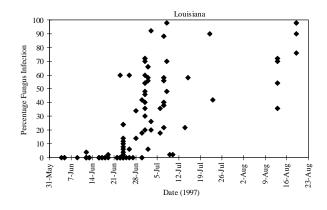


Figure 1. Percentage of aphids infected with *N. fresenii* from Arkansas cotton aphid samples.



igure 2. Percentage of aphids infected with *N. fresenii* from Louisiana cotton aphid samples.

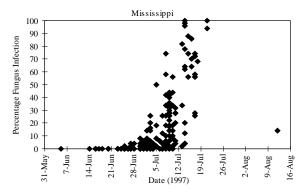


Figure 3. Percentage of aphids infected with *N. fresenii* from Mississippi cotton aphid samples.

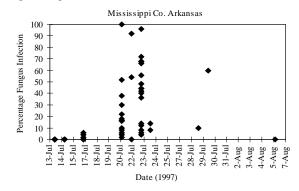


Figure 4. Percentage of aphids infected with *N. fresenii* from one county in Arkansas. This data indicate that even within a county there is great variation in the percentage of aphids infected within individual cotton fields on any given date. For instance, on 20 July the fungus levels ranged from 0 to 100%. This shows the necessity of scouting and sampling individual fields and not totally relying on reports of the fungus from a county.