POTENTIAL FOR SPEEDING UP APHID FUNGUS IN NORTHEASTERN ARKANSAS

D. Steinkraus[1], D. Wildy[2], D. Wells[3], M. Vingaard[1,] and G. Daniels[4]
[1]Department of Entomology, Univ. of Arkansas, Fayetteville, AR,
[2]Wildy Farms, Manila, AR, [3]Cotton Services, Inc., Leachville, AR,
[4]Louisiana Cooperative Extension Service, Vidalia, LA

Abstract

Early-season epizootics of the aphid pathogenic fungus, *Neozygites fresenii*, were identified in Concordia Parish, Louisiana. Aphid/fungus-infested plants from a commercial cotton field were collected and transported to commercial cotton fields in Mississippi Co., Arkansas in order to inoculate the fields with fungus. Significantly more aphids were infected near release sites than 60 meters distant at both 1 and 2 weeks after the releases were made. The results support the idea that fungus can be introduced to a field and spread in the aphid population.

Introduction

The cotton aphid, *Aphis gossypii*, is a persistent, common problem throughout U.S. cotton and can be difficult and expensive to control with insecticides. Cotton growers in the Midsouth and Southeastern U.S. have benefitted from a naturally-occurring fungus, *Neozygites fresenii*, that causes widespread epizootics in cotton aphid populations each year (Steinkraus *et al.*, 1995). For the past 7 years Cotton Incorporated has helped fund an Extension-Based Aphid Fungus Sampling Service for detection and prediction of the aphid fungus epizootics in cotton fields in AL, AR, GA, LA, and MS (Steinkraus and Boys, 1997). When fungal prevalence is high growers may avoid an insecticide application.

Each summer the earliest fungal epizootics occur in aphid populations in Louisiana and several weeks later similar epizootics occur in northern Arkansas. This difference in the timing of epizootics suggests that it may be possible to introduce fungal inoculum to northern cotton fields early in the season and speed up the occurrence of epizootics.

There are several potential methods of producing fungal inoculum. The first, and most obvious, is to culture *N. fresenii in vitro*, produce large quantities, and develop methods of storing and applying the fungus. While *in vitro* production is desirable, repeated attempts by many mycologists to culture *N. fresenii in vitro* have failed, or had negligible results (Steinkraus *et al.*, 1991).

A second method involves culture of *N. fresenii in vivo* in its natural host, *A. gossypii*. This method has been successful and our laboratory has maintained an *in vivo* culture for 9 years (Steinkraus *et al.*, 1993). Aphids are infected, incubated for 3 days, then dried and frozen. In this state the aphid mummies can be stored for years. When fungus is needed the aphid mummies are rehydrated at room temperature and sporulate within two hours. Unfortunately, labor costs for maintaining the necessary aphid culture and handling infected aphids results in a cost of approximately $1 per aphid mummy, precluding mass production of infected aphids. Fungal inoculum produced using this *in vivo* method has been successfully used in experiments to introduce *N. fresenii* into cotton aphid populations in the San Joaquin Valley and Arkansas (Steinkraus and Rosenheim, 1995).

A third potential source of fungal inoculum is the tremendous quantities produced during natural epizootics in cotton fields. Research has demonstrated that during the middle of an epizootic millions of aphids are infected in a cotton field (Steinkraus *et al.*, 1999). These infected aphids represent a potential resource. Early epizootics in Louisiana or southern Mississippi can be identified by examining the data produced from the Arkansas Extension-Based Aphid Fungus Sampling Service. These fields could be used as a source for large quantities of aphids infected with *N. fresenii* for use in speeding up epizootics in northern Arkansas counties. The objective of this study was to determine if it is feasible to transport aphid-infested cotton plants from early epizootic fields in Louisiana to northern Arkansas to speed up epizootics.

Materials and Methods

The Extension-Based Aphid Fungus Sampling Service at the University of Arkansas was used to identify the earliest epizootics. Aphid samples sent from Glen Daniels of the Louisiana Cooperative Extension Service were among the earliest epizootics reported. His cooperation was essential in this endeavor.

After Louisiana fields with early epizootics were located Arkansas growers (D. Wildy and C. Hawkins) chartered a 6-passenger plane which left the airport in Manila, Mississippi Co., AR, at 1100 h on 1 July 1999. At 1230 h we arrived in Vidalia, Concordia Parish, LA, and were met by Glen Daniels. We drove to a cotton field of grower Bill Campbell in Ferriday, Concordia Parish, LA. His Stoneville 474 cotton was at the 14-15 node stage and had a heavy aphid population that appeared to be in the middle of an *N. fresenii* epizootic. The grower had no plans to apply insecticides for the aphid population due to the fact that the fungal epizootic was in

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1165-1167 (2000)
National Cotton Council, Memphis TN

progress. The aphid population in this cotton field was sampled and examined immediately using standard diagnostic procedures (Steinkraus *et al.*, 1999). We squashed aphids on slides in lactophenol fixative and examined them under a phase microscope at 200x. Both naked-eye and microscopic examination showed that between 50 and 75% of the aphids in the field were infected with *N. fresenii*. The field was ideal because it had large numbers of live aphids still present and was in the middle of a *N. fresenii* epizootic. We collected aphid-infested leaf strips from 20 plants into vials of 70% ethanol for laboratory determination of *N. fresenii* prevalence.

We dug up approximately 100 entire plants and placed them in plastic 5 gallon buckets, about 10 to a bucket. Soil was used to cover their roots and the plants were watered. The buckets were loaded into the back of the truck then we returned to the airport in Vidalia and loaded the plane. We flew back to Manila, AR, airport, again loaded the buckets of plants into trucks and drove to 2 aphid-infested fields in Leachville, AR. Field 1 had been planted 8 May 1999 with BXN-47 cotton and Field 2 had been planted 12 May 1999 with BXN-47 cotton.

The cotton plants from Louisiana were divided into two equal groups and transplanted into the two fields. They were placed adjacent to cotton plants within the rows in the field and were supported by wrapping plastic flagging tape around their stems and the stems of adjacent plants. Twenty release plants in each field were given a number. The plants were then watered. Each plant from LA was at least several meters from its nearest neighbor.

Aphid samples were collected from the release sites on 29 June (before the release) and on 8 July and 15 July (one week and two weeks after the release). Samples were not taken after this time because the aphid populations crashed due to the fungus.

At each sampling date we collected aphid-infested leaves from mainstem leaves 5-6 nodes below the terminal and placed them in vials containing 70% ethanol. In each field we collected leaves from a plant immediately adjacent to 20 of the Louisiana release plants (0 meters). We also collected aphid-infested leaves from 4 plants 1 meter in the 4 cardinal directions away from each release plant and pooled the sample. In order to determine if the fungus was spreading from release sites we took 4 additional samples 30 meters from the release area in the 4 cardinal directions and 8 samples 60 meters away in the 4 cardinal directions and SE, SW, NE, and NW. Therefore, a total of 52 samples were collected in each field per date.

From each sample a random subsample of 50 aphids was removed, squashed on a microscope slide in lactophenol, and diagnosed for *N. fresenii* stages at 200 x under a phase microscope. The fungal stages recorded were: secondary spores, hyphal bodies or protoplasts, conidial stage, or infected with saprophytes.

The data were analyzed by ANOVA and means were separated by the Tukey-Kramer HSD test (SAS, 1995).

Results and Discussion

The Extension-Based Aphid Fungus Sampling Service proved useful for identifying Louisiana fields with early epizootics and northeastern Arkansas fields with aphids but little or no fungal prevalence. The cooperation we received from the Louisiana Cooperative Extension Service and growers in both AR and LA was remarkable.

Examination of the data generated by the Service enabled us to identify fields in Louisiana with epizootics before any fungus was reported from Arkansas (Table 1). The data from the Service is precise enough to indicate fields in which the fungal epizootic is still in the early stages such as secondary spores (infective spores) attached to the hosts, protoplasts (vegetative stages), or sporulating. The field we chose to use (Campbell) had 65% *N. fresenii*-infected aphids on 1 July 1999, of which, 10%, 14% and 36% were in the secondary spore, protoplast, and conidial stages, respectively.

There is little doubt that the aphid-infested plants transplanted from the epizootic field in Louisiana to northeastern Arkansas resulted in a rapid spread of the fungus from the release sites (Table 2). More than 10,000 aphids were individually diagnosed from the two fields. Significantly more aphids were infected adjacent to release plants (0 meters) in both fields one week after the plants were put in the field, than at 1, 30, or 60 meters. In Field 1 the difference was striking with 70.3% infected aphids adjacent to release plants, 38.3% at 1 meter, and only 7 and 3.8% at 30 and 60 meters distant, respectively. The data from Field 2 was less clear. Naturally-occurring fungus may have been present earlier in this field, but this is not certain. In both fields on 15 July there still existed a statistically significant increase in the fungal prevalence adjacent to and at 1 meter compared to 30 and 60 meters.

Several potential problems still need to be solved. First, the cotton plants from Louisiana began to wilt and dry due to heat and wind during trips in the trucks and the plane. This may be solved by collecting plants in the evening or by using a closed, refrigerated truck. Second, because the development of an epizootic is a natural process and takes several weeks, in order to significantly speedup epizootics in the north, it will be essential to identify epizootic fields as soon as possible and collect and move plants north without delay. Third, the usefulness of this method as shown by reductions in insecticide application for aphids needs to be

verified in larger trials. Fourth, the optimum number of plants/field to be released and their spacing need to be determined.

Acknowledgments

We gratefully acknowledge C. Hawkins and Cotton Incorporated for partial funding of this project. We thank B. Campbell and Jackson Farms for access to their cotton. We thank G. Boys, J. Zawislok, and J. Laslovich for laboratory assistance.

Summary

It seems clear that it is possible to 1) identify early epizootic fields using the Extension-Based Aphid Fungus Sampling Service, 2) collect aphid/fungus-infested plant material from early fields and use it to inoculate northern cotton fields, and 3) this inoculum can successfully start foci of fungus in fields. The potential for this procedure to start early epizootics on an areawide or statewide basis is evident.

References

SAS Institute. 1995. JMP Statistical Software. SAS Institute, Cary, NC.

Steinkraus, D. C., G. O. Boys, and P. H. Slaymaker. 1993. Culture, storage, and incubation period of *Neozygites fresenii* (Entomophthorales: Neozygitaceae) a pathogen of the cotton aphid. Southwest. Entomol. 18: 197-202.

Steinkraus, D. C. and G. O. Boys. 1997. Update on prediction of epizootics with extension-based sampling service. Proc. Beltwide Cotton Conf., pp. 1047-1048.

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., M. N. Howard, R. G. Hollingsworth, and G. O. Boys. 1999. Infection of sentinel cotton aphids (Homoptera: Aphididae) by aerial conidia of *Neozygites fresenii* (Entomophthorales: Neozygitaceae). Biol. Control 14: 181-185.

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. and J. Rosenheim. 1995. Biological factors influencing the epizootiology of cotton aphid fungus. Proc. Beltwide Cotton Conf., pp. 887-889.

Table 1. Prevalence of *N. fresenii* infections in cotton aphids in fields in Concordia Parish, Louisiana, as determined by the Extension-Based Aphid Fungus Service on 24 June 1999.

	% *N. fresenii*		% Fungal Stage	
Field Name	infections	2° Spore	Protoplasts	Conidial
Barn	40	20	4	12
T&W Farms	44	12	0	32
House Field	22	20	0	2
Denning Field	0	0	0	0
Hwy 565	26	22	4	0
Vangilder	2	2	0	0
Miller	0	0	0	0

Table 2. Prevalence of *N. fresenii* infections 7 and 14 days after fungus release in *A. gossypii* at 0, 1, 30, and 60 meters distance from release plants in 1999.

		% *N. fresenii*-Infection	
Date	Distance (m)[1]	Mean[2]	(SE)
Field 1			
July 8	0	70.3 a	(4.0)
	1	38.3 b	(3.0)
	30	7.0 c	(4.4)
	60	3.8 c	(3.8)
July 15	0	74.6 a	(3.4)
	1	70.8 a	(3.9)
	30	28.0 b	(8.1)
	60	20.6 b	(2.2)
Field 2			
July 8	0	45.2 a	(3.9)
	1	24.4 b	(2.3)
	30	21.5 b	(5.9)
	60	24.4 b	(9.0)
July 15	0	82.5 a	(3.4)
	1	75.8 ab	(2.6)
	30	60.5 bc	(2.2)
	60	47.9 c	(5.6)

[1] Aphids were sampled from plants 0 (adjacent to release plants), 1, 30, and 60 meters distant from the release area.
[2] Means were derived from random subsamples of 50 aphids diagnosed from each of 20 samples at 0 and 1 meters, and 4 plant samples 30 meters from the release area, and 8 plant samples at 60 meters.
Means within columns within a field and date not sharing a common lower case letter are significantly different, Tukey-Kramer HSD.