JUMP-STARTING EPIZOOTICS OF *NEOZYGITES FRESENII* IN COTTON APHID POPULATIONS

Don Steinkraus
University of Arkansas
Fayetteville, AR
David Wildy
Wildy Farms
Manila, AR
Dale Wells
Cotton Services, Inc.
Leachville, AR

Abstract

The objective of this work was to determine if fungal epizootics in cotton aphid populations could be initiated artificially. Greenhouse-grown cotton plants were infested with cotton aphids, *Aphis gossypii*. After aphid populations were well established they were infected with the fungus, *Neozygites fresenii*, causing "mini-epizootics" and placed in a commercial cotton field. Prevalence of the fungus was monitored adjacent to and at various distances from the release sites and compared statistically. Based on the data it appears that fungal epizootics can be initiated by early release of infected aphids on greenhouse-grown cotton plants.

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 benefited from a naturally-occurring fungus, *Neozygites fresenii*, that causes widespread epizootics in cotton aphid populations each year (Steinkraus *et al.*, 1995). For the past 8 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) (http://www.uark.edu/misc/aphid/). When fungal prevalence is high growers may avoid an insecticide application.

Each summer the earliest fungal epizootics occur in aphid populations in Louisiana or southern Mississippi 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 four potential methods of producing fungal inoculum. The first, and most obvious, is to follow the *Bacillus thuringiensis* or *Beauveria bassiana* model and 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). Recently there has been some modest success culturing related species of *Neozygites* (from thrips and mites) *in vitro* that may portend greater success with this approach in the future (Grundschober et al. 1998).

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 10 years (Steinkraus *et al.*, 1993). Aphids are infected, incubated for 3 days, then dried and frozen. In this state 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.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1141-1143 (2001)
National Cotton Council, Memphis TN

A third 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 valuable resource. Early epizootics in Louisiana, southern Mississippi, or Arkansas can be located by examining the data produced from the Arkansas Extension-Based Aphid Fungus Sampling Service (http://www.uark.edu/misc/aphid/). 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.

A fourth approach is to grow cotton plants in early spring in the greenhouse, infest them with aphids, then inoculate these aphid populations with *N. fresenii* to produce "mini-epizootics" that could be placed in fields. These plants would provide foci from which the fungus could spread throughout the fields and jump-start epizootics earlier than they would naturally occur.

The objective of this research was to determine the feasibility of initiating epizootics in northern Arkansas cotton fields by placing greenhouse-grown cotton plants with "mini-epizootics" into commerical cotton.

Methods

Inoculum

Because *N. fresenii* cannot be produced *in vitro*, infected cotton aphids were used as inoculum. Infected aphids were produced in the laboratory, dried when the fungus was in the hyphal body stage, then frozen until use (Steinkraus et al. 1993). In this paper, dried infected aphids containing hyphal bodies, are called "cadavers".

Diagnosis

All samples were diagnosed by Aphid Fungus Sampling Service technicians. Aphids were sampled in the field from release plants, adjacent plants, and at selected distances from the release areas. Portions of cotton leaves infested with aphids were placed into vials containing 70% ethanol. In the laboratory randomly-chosen subsamples of 50 aphids from each vial were squashed on microscope slides in lactophenol, and diagnosed for *N. fresenii* infections at 200x with phase microscopes. This data provides an estimate of prevalence in each sample.

"Mini-Epizootic" Release Plants

We constructed a small greenhouse at the Wildy Farm in Mississippi Co., Arkansas. Cotton was planted in 24 peat pots, allowed to grow, then infested with cotton aphids. On 15 June 2000, when aphid populations were high, the aphids were inoculated with infected aphid cadavers. On 24 June 2000 the plants were placed within the rows of a cotton field at Wildy Farm (Fig. 5). Plants were placed in an aphid-infested part of the field in groups of 4 in 6 sites. Aphid samples from the release plants, adjacent cotton plants, sites 100 m between release sites, and at selected distances from the release site were collected weekly for 3 weeks and prevalences of *N. fresenii* determined.

Statistical Analysis

Data was analyzed by ANOVA and means were separated by Tukey-Kramer HSD tests (SAS, 1995).

Results and Discussion

On June 27, one week after the greenhouse-grown "mini-epizootic" cotton plants were placed within rows of the commercial field cotton, significantly more aphids were infected with *N. fresenii* on the release plants than on adjacent plants and plants 100 meters from release sites (Fig. 1). On July 6, two weeks after the release, mean percentage prevalence of *N. fresenii* on release and adjacent plants were 84% and 74%, respectively, significantly

higher than prevalence at 100 meters from the release sites (53%), and from samples from north of the release area (Fig. 2). The pattern in Fig. 2 suggests that the releases had an effect on the spread of the fungus through the field. On July 12, three weeks post-release, a generalized epizootic was seen in the cotton field with significantly lower prevalence of *N. fresenii* only in samples from south of the release area and at 3.7 km north (Fig. 3). However, this experiment was preliminary and more intensive investigation are planned for 2001.

Conclusions

The fungus, *Neozygites fresenii*, provides important natural control of the cotton aphid in the Midsouth. In San Joaquin Valley, California, cotton production this valuable biological control agent does not appear to be present, presenting an opportunity for classical biological control. Experimental releases made in 1994 and 1995 (Steinkraus and Rosenheim 1995) indicate that this fungus can be introduced to California, infect cotton aphids there and spread somewhat. The releases made in these years, and subsequently (in cooperation with Dr. Kris Godfrey, CDFA), have been relatively small scale. We think that field collection and release of larger quantities of fungal inoculum may result in better establishment and spread of the fungus.

In the Midsouth, natural epizootics are extremely important in limiting high cotton aphid populations in July. However, some growers may make an aphicide application prior to the occurrence of natural epizootics. Early epizootics can be located from the data from the Extension-Based Cotton Aphid Fungus Sampling Service. This suggests that it may be possible to speed up the progression of epizootics in northern fields by transfer of epizootic material from early southern epizootics (Steinkraus et al. 2000) or by starting epizootics in aphid populations on greenhouse-grown cotton plants and placing them in cotton fields. Both these methods appeared to have potential based on our results.

The methods developed in this research may also have application for the other aphid situations, such as the newly-discovered soybean aphid, *Aphis glycines*, in U.S. soybean production.

Acknowledgments

Aphid slides and diagnoses were made by G. Boys, J. Zawislak, J. Fiser, and R. Strausberg. We appreciate the input and support of Dr. Pat O'Leary of CI. This research has been supported by Cotton Incorporated, USDA-NRI grants, the CDFA, and the Arkansas Experiment Station.

References

Grundschober, A., Tuor, U. and Aebi, M. 1998 *In vitro* cultivation and sporulation of *Neozygites parvispora* (Zygomycetes: Entomophthorales). Systematic and Applied Microbiology 21: 461-469

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

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

Steinkraus, D. C., R. G. Hollingsworth & 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. & J. Rosenheim. 1995. Biological factors influencing the epizootiology of cotton aphid fungus, pp. 887-889. Proceedings Beltwide Cotton Conference, 1995, Jan. 4-7, San Antonio, TX.

Steinkraus, D. C. and G. O. Boys. 1997. Update on prediction of epizootics with extension-based sampling service, pp. 1047-1048. Proc. Beltwide Cotton Conferences, Jan 6-10, 1997, New Orleans. National Cotton Council of America, Memphis, TN.

Steinkraus, D. C., M. N. Howard, R. G. Hollingsworth & G. O. Boys. 1999. Infection of Sentinel Cotton Aphids (Homoptera: Aphididae) by Aerial Conidia of *Neozygites fresenii* (Entomophthorales: Neozygitaceae). Biological Control 14: 181-185.

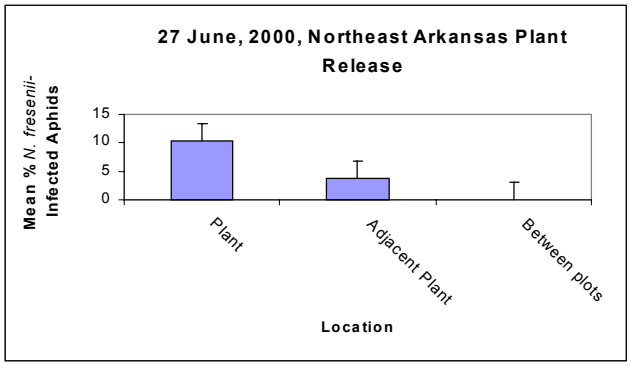

Figure 1. Mean percentage prevalence of *N. fresenii*-infected aphids on "mini-epizootic release plants", adjacent to release plants, and at various distances from release plants on 27 June 2000.

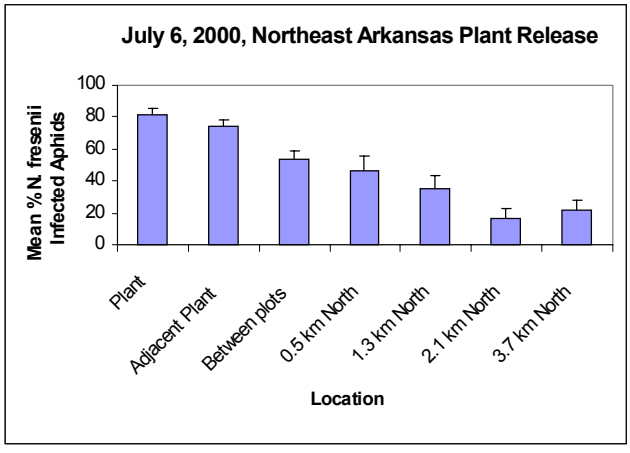

Figure 2. Mean percentage prevalence of *N. fresenii*-infected aphids on "mini-epizootic release plants", adjacent to release plants, and at various distances from release plants on 12 July 2000.

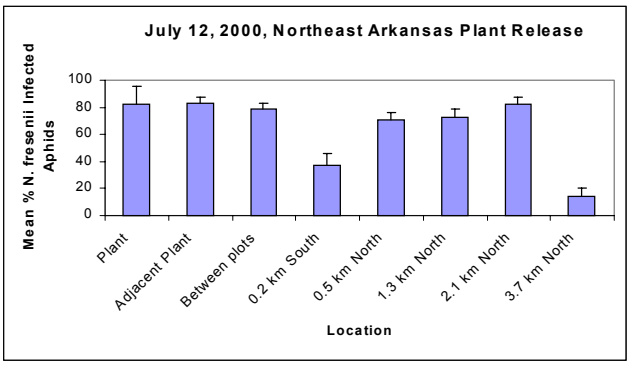

Figure 3. Mean percentage prevalence of *N. fresenii*-infected aphids on "mini-epizootic release plants", adjacent to release plants, and at various distances from release plants on 21 July 2000.