2006 Beltwide Cotton Conferences, San Antonio, Texas - January 3 - 6, 2006

# FREQUENCY AND IDENTIFICATION OF COTTONSEED-ROTTING BACTERIA FROM COTTON FLEAHOPPERS

**Alois A. Bell, Juan D. Lopez and Enrique Gino Medrano**
**USDA-ARS-SPARC**
**College Station, TX**

## Abstract

Cotton fleahoppers (*Pseudatomoscelis seriatus* Reuter) were collected from two weed hosts, horsemint (*Monarda punctata* L.) and croton (*Croton capitatus* Michx.), and from cotton (*Gossypium hirsutum* L.) at periodic intervals during the 2005 cotton growing season in Texas. Insects were washed individually in sterile water which was then used to inoculate 13- to 15-day-old cotton bolls. Most male and female fleahoppers in each of seven collections yielded sufficient pathogenic bacteria to cause severe seed rot and occasionally rot of the boll wall. The causal bacteria were characterized using various microbiological tests, including API 20E test strips, and fatty acid methyl ester profile analysis. Most bacterial isolates, especially those from insects collected from croton, the host on which they overwinter, were *Pantoea* spp. Cotton fleahoppers raised on green beans in the laboratory, starting with eggs embedded in croton stems, were frequently infested with *Pantoea* and *Serratia* spp. The insects transferred these bacteria to flower buds and young bolls, when they were caged over fruiting branches. Buds or bolls that abscised consistently showed rot of the ovary wall, whereas buds or bolls that were retained on the plant had healthy ovary walls, indicating that bacterial rot of the ovary leads to abscission. Because fleahoppers feed on the pinhead squares early in the growing season, they also may be an important source of early contamination of plants and bolls with seed-rotting pathogens that could enter bolls through various wounds, including those caused by other insects.

## Introduction

Jones and Edmister (2001) described a cotton seed rot that caused extensive damage in North and South Carolina. While seeds were discolored and embryos either killed or stunted, external symptoms of boll infection were absent. Bell et al. (2004) found that symptomatic seed from South Carolina yielded a variety of fungal and bacterial pathogens that could infect bolls internally and cause seed rot and tight lock, if the pathogens were provided a puncture wound through the boll endocarp. The most frequently isolated pathogen was identified as *Pantoea agglomerans* (Medrano and Bell, 2006). Interestingly, the *P. agglomerans* type strain that is of clinical origin, also causes severe seed rot when puncture-inoculated into bolls (Medrano and Bell, 2005). None of the pathogens from rotted seed could penetrate the boll directly, indicating that a wound source is critical for infection and seed rot development.

Stink bugs and plant bugs, like seed rot, have increased in importance in recent years because of new management practices that reduce use of general insecticides that primarily target boll weevils, tobacco budworms, and bollworms. Stink bugs and plant bugs have piercing-sucking mouthparts and frequently puncture young bolls and/or flower buds. Thus, they may provide the puncture wounds needed for opportunistic infections. Bell et al. (2005) showed that stink bugs, especially the southern green stink bug, *Nezara viridula* (L.), are frequently contaminated with bacteria that can cause seed rot when a small puncture through the boll wall is provided. The pathogens most frequently isolated from stink bugs were *Pantoea* species.

Two other major insect pests, *Lygus* and cotton fleahoppers (*Pseudatomoscelis seriatus* Reuter) also pierce tissues, including young bolls and flower buds during feeding. In 2004 these insects with stink bugs were ranked among the top five insect causes of cotton yield losses: *Lygus*, 2; stink bugs, 3; and cotton fleahoppers, 5 (Williams, 2005). In 1999, the cotton fleahopper was ranked as the top cause of cotton yield losses, primarily because of severe losses in Texas in that year (Williams, 2000).

While cotton fleahoppers occur throughout the Cotton Belt, losses generally occur mostly in Texas followed by Oklahoma, Louisiana, Kansas, and Arizona. This is probably due to the fact that the insects generally prefer weed hosts and move to cotton only when satisfactory weed hosts are not available

(Beerwinkle and Marshall, 1999). In Central Texas, the insect overwinters primarily as eggs inserted into stems of *Croton* (*Croton capitatus* Michx.), its preferred fall host. When nymphs hatch in the spring, they move to weeds such as horsemint (*Monarda punctata* L.). Later generations move to cotton in June when the horsemint and other spring weeds begin to senesce. When cotton ceases to flower, fleahoppers move back to croton for late summer and fall generations.

Several observations indicate that fleahoppers transmit pathogenic organisms to flower and leaf buds, and that subsequent infections cause ethylene bursts resulting in abscission of buds and young bolls. Cotton fleahoppers are infested with various fungi and with bacteria putatively identified as *Xanthomonas* and *Pseudomonas* spp. (Duffey and Powell, 1979; Grisham et al., 1987; Martin et al., 1987). Criteria for the identification of the bacteria were not provided. The microorganisms were isolated from salivary glands as well as whole insects. When fleahoppers were fed on 5% sucrose containing *Xanthomonas campestris* pv. *malvacearum*, they subsequently transmitted the bacteria to cotton plants causing disease symptoms on leaves and stems (Martin et al., 1988c). Terminal bud explants of cotton planted in agar in 25-ml flasks showed a burst in ethylene production when infested with fleahoppers, or when inoculated with microorganisms associated with the insect (Duffey and Powell, 1979; Grisham et al., 1987; Martin et al., 1988a). Pectinase preparations from salivary glands also caused a burst in ethylene synthesis (Martin et al., 1988b). Ethylene bursts are symptomatic of tissue necrosis incited by microbial infections of plant tissues (Hilsop et al., 1973; Pegg, 1976).

In this study we determined the frequency of bacterial infestations of cotton fleahoppers collected in 2005, critically determined the identity of bacterial associates, and finally determined whether laboratory-reared fleahoppers could harbor and transmit bacterial pathogens to cause bud and boll rot and abscission, when the insects were caged over fruiting branches.

## Methods and Materials

### Source of Insects

Cotton fleahoppers were collected with a sweep net from areas near College Station, Texas, where the weed hosts or cotton were prevalent. After collection, the insects were immediately taken to the laboratory, sexed and individually tested for bacterial infestation. The host source, collection date and number of male and female insects examined are shown in Table 1.

Table 1. Cotton Fleahopper Collections.

| | | Sex Distribution | | |
|---|---|---|---|---|
| Host | Date | Males | Females | Total |
| Horsemint | 5/18 | 24 | 21 | 45 |
| Horsemint | 5/31 | 27 | 42 | 69 |
| Horsemint | 6/14 | 28 | 20 | 48 |
| Cotton | 6/22 | 28 | 28 | 56 |
| Cotton | 7/21 | 17 | 11 | 28 |
| Croton | 8/17 | 30 | 33 | 63 |
| Croton | 8/22 | 32 | 35 | 67 |
| | Total | 186 | 190 | 376 |

### Isolation of Bacteria from Insects and Bolls

Each insect was placed into 2 ml sterile distilled water and agitated periodically with a vortex mixer. After 1, 24, and 48 hr, a 10µl loop full of wash water was streaked on Trypticase Soy Agar (TSA) and TSA amended with 100 ppm cycloheximide to suppress fungal growth. Two or three seed from diseased locks and longitudinally sectioned buds and small bolls were soaked in sterile distilled water for 1-2 hr with intermittent agitation. Again, a 10µl sample was streaked on the media. Single bacterial colonies of different types of bacteria from separate insects or bolls/buds were used for identification or to test pathogenicity and virulence. Emphasis was given to the most prevalent bacteria from a sample.

**Inoculation of Bolls With Wash Water or Bacteria**

Wash water from each plant tissue or insect was used to test for the presence of pathogens. Coker 312 and Deltapine 458B/RR plants were grown in the greenhouse under a rigid insect control regime. Newly opened flowers were tagged daily so that bolls of a specific age could be inoculated. A 40µl drop of wash water from an insect was placed over the suture of a 13- to 15-day-old boll midway between the apex and base. A 28-gauge needle was placed through the drop and then 2-3 mm into the boll passing through the suture. Bolls were sectioned longitudinally through the suture at 7 or more days after inoculation to observe disease progress, or bolls were allowed to mature, and then final symptoms were observed. Suspensions of bacteria in sterile water were inoculated into bolls to confirm pathogenicity.

**Characterization of Bacteria**

Remote colonies of bacteria that were isolated, inoculated, and then recovered from diseased bolls were used for characterization and/or identification. Colony morphology was observed on TSA, King's B-pectin agar (KBP), and potato dextrose agar containing .8 g/L of fine $CaCO_3$ (PDAC). Anaerobic growth was determined on a medium containing peptone, 2.0 g; NaCl, 5.0 g; $KH_2PO_4$, 0.3 g; agar, 3.0 g; bromothymol blue (1% aqueous solution), 3.0 ml; glucose 1.0 g; and water, 1 liter. Ingredients were dissolved with minimal heat, and 5 ml of solution was dispensed into 13-ml tubes before sterilizing at 121˚C for 15 min. The tubes were stabbed with a bacterial suspension using a plastic probe, and the medium was covered immediately with sterile mineral oil. Anaerobes acidified the medium turning it yellow within 4 to 8 hr at 30˚C; tubes were scored for anaerobic growth after 24 hr. Other phenotypic tests were performed using protocols described by Schaad et al. (2001). Representative isolates of different groups of bacteria determined from the above criteria were submitted to the Texas Plant Disease Diagnostic Laboratory for fatty acid methyl ester (FAME) profile analysis. Possible species identification was determined by best fit (SIM index) to the database for bacteria, Sherlock Version 4.5 (0209B); TSBA 40 4.10. Isolates that grew anaerobically also were tested with the API 20E strip (Biomérieux, Hazlewood, MO) to determine possible species identification.

**Bacterial Transmission and Damage from Laboratory-Reared Insects**

Fleahoppers were reared in the laboratory using methods similar to those described by Beerwinkle and Marshall (1999). Newly-emerged adults were caged over fruiting branches using a styrofoam cylinder enclosed with a section of nylon mesh hose fitted over the cylinder and fruiting branch stem and tied at both ends after 3 fleahoppers were placed in the cage. Thirty-four cages were used. After 9 days the insects were removed from the cages. Buds were sectioned longitudinally with a razor blade and examined for tissue necrosis in the anthers, stigma, and ovary. Both insects and sectioned tissues from individual cages were tested separately for the presence of seed-rotting bacteria.

## Results and Discussion

Wash waters from the 376 cotton fleahoppers (Table 1) were examined directly for bacteria and were used as inoculum for young bolls. Over 60% of the insects yielded sufficient pathogenic bacteria in the wash water to cause moderate to severe seed rot (Table 2). There were no apparent differences between insect sexes or among fleahoppers collected from different crops. The lower values on 5/31 may have been due to the large number of *Penicillium* spores in the wash water from those insects; these fungi showed antibiotic activity against bacteria.

Table 2. Percentages of Insects from Different Sources and Collection Dates Yielding Infectious Bacteria.*

| Host Source | Date Collected | Percentage of Infested Insects | | |
|---|---|---|---|---|
| | | Males | Females | Total |
| Horsemint | 5/18 | 88 | 71 | 80 |
| Horsemint | 5/31 | 37† | 41† | 39† |
| Horsemint | 6/14 | 86 | 70 | 75 |
| Cotton | 6/22 | 50 | 75 | 59 |
| Cotton | 7/21 | 77 | 73 | 75 |

| | | | | |
|---|---|---|---|---|
| Croton | 8/17 | 43 | 49 | 44 |
| Croton | 8/22 | 66 | 69 | 67 |
| MEAN | | 64 | 64 | 63 |

* Inoculation of 13- to 15-day-old bolls with wash water from insect caused rot of 40-100% of seed in the inoculated lock.
† High numbers of *Penicillium* propagules in the wash water may have suppressed bacteria.

*Penicillium* species and several other fungi have been reported previously from fleahoppers collected with sweep nets (Duffey and Powell, 1979; Grisham et al., 1987; Martin et al., 1987). The *Penicillium* species produced ethylene when grown on culture media and enhanced ethylene production of excised cotton plant terminals planted into water agar and puncture-inoculated with the fungus in sealed flasks (Martin et al., 1988b). We rarely recovered *Penicillium* species from any boll inoculated with wash water from insects even when the fungus was in the inoculum. Thus, the *Penicillium* spp. were not pathogenic to young bolls. Also fleahoppers raised in the laboratory usually were free of *Penicillium* spores yet still caused marked abscission of squares and bolls. *Penicillium* spores probably contaminate the insects mostly during their collection from senescing plants. Accordingly, collections of fleahoppers from cotton which had fewer senescent tissues also yielded much less *Penicillium* contamination. These same arguments are applicable to reports of other fungi and their induction of ethylene synthesis in explants (Duffey and Powell, 1979; Grisham et al., 1987).

The pathogens found in wash water and responsible for seed rot were mostly bacteria (Table 3). Only two infections from a fungus, *Fusarium semitectum* (Berk. & Ravenel), were observed and these might have originated from surface contamination of the boll by airborne fungal spores. Most of the bacterial pathogens were facultative anaerobes, including *Pantoea*, *Serratia*, *Enterobacter*, and *Klebsiella* species (Tables 3 and 4). *Pantoea* species were by far the most frequent pathogen found and were especially dominant in fleahoppers collected from croton, the host on which the insect overwinters. The first collection from horsemint (5/18), which may have originated from eggs in croton, also contained mostly *Pantoea*. Subsequent collections from horsemint showed progressively greater numbers of other bacteria, especially strict aerobes. The greatest numbers of the latter occurred on cotton and included mostly *Pseudomonas* spp. (Table 4).

Table 3. Distribution of Bacterial Isolates Causing Seed Rot Among Taxonomic Groups.

| | | Percentage of Total Pathogens | | |
|---|---|---|---|---|
| Host Source | Date Collected | Facultative Anaerobes | *Pantoea* spp. | Strict Aerobes |
| Horsemint | 5/18 | 97 | 78 | 3 |
| | 5/31 | 93 | 93 | 7 |
| | 6/14 | 68 | 61 | 39 |
| Cotton | 6/22 | 47 | 30 | 53 |
| | 7/21 | 86 | 54 | 14 |
| Croton | 8/17 | 100 | 95 | 0 |
| | 8/22 | 97 | 97 | 0 |

Table 4. Seed-Rotting Bacteria Other than *Pantoea* Isolated from Cotton Fleahopper.

| Facultative Anaerobes (11)* | Strict Aerobes (19)* |
|---|---|
| *Serratia* spp. | *Pseudomonas pudita* |
| *Klebsiella* spp. | *Flavimonas oryzihabitans* |
| *Enterobacter* spp. | *Pseudomonas chlorophis* |
| | *Pseudomonas syringe* |
| | *Pseudomonas flectens* |

* Total number for all collections

*Xanthomonas* was thought to be the predominant bacteria infesting fleahoppers in previous studies (Duffey and Powell, 1979; Grisham et al., 1987; Martin et al., 1987). However, no data were presented to support this conclusion. We did not find a single colony of *Xanthomonas* among the hundreds of yellow bacteria that were tested. *Pantoea* can be confused with *Xanthomonas*, because both form yellow mucoid colonies on PDA and King's B medium, which were used in the previous studies. The two species, however, are readily distinguished in anaerobic culture where *Pantoea* grows readily but *Xanthomonas* does not grow because it is an obligate aerobe.

Representative isolates of *Pantoea* from the seven fleahopper collections were examined for variability and virulence specialization. Isolates of all four *Pantoea* species proposed in the API 20E test software were found. However, most isolates did not fit cleanly into one of these species but rather showed similarity to two or three species. The species assignments in Table 5 are based on greatest similarity in the API 20E tests. Likewise, fatty acid methyl ester (FAME) analyses showed profiles from fleahopper isolates with similarity to those of *Pantoea agglomerans*, *Pantoea annanas*, and *Enterobacter intermedius* (Table 6). Isolates assigned to *Pantoea* species 2 in the API 20E tests were divided among three different species in the FAME test. We found no indication of virulence specialization among groups based on the API 20E tests (Table 7). Our results indicate that the *Pantoea* isolates do not show sufficient variability to designate them as anything other than *Pantoea agglomerans*.

Table 5. Frequency of *Pantoea* Isolates from Cotton Fleahoppers Assigned to Four Species by API 20E Tests.

| Host | Collection | No. Isolates in *Pantoea* spp. | | | |
|---|---|---|---|---|---|
| Source | Date | 1 | 2 | 3* | 4 |
| Horsemint | 5/18 | 0 | 6 | 0 | 1 |
| | 5/31 | 1 | 5 | 0 | 0 |
| | 6/14 | 1 | 2 | 4 | 1 |
| Cotton | 6/22 | 0 | 4 | 2 | 0 |
| | 7/21 | 0 | 2 | 1 | 0 |
| Croton | 8/17 | 0 | 7 | 0 | 0 |
| | 8/22 | 0 | 6 | 0 | 0 |
| Green Beans | (Lab Reared) | 0 | 1 | 2 | 1 |
| | TOTAL | 2 | 33 | 9 | 3 |

*The profile for this species is identical to that of the *P. agglomerans* type strain.

Table 6. Identification of Six Isolates Based on Fatty Acid Methyl Ester (FAME) Profiles.

| Isolate No. | API 20E ID | FAME ID | SIM Index |
|---|---|---|---|
| 1 | *Pantoea* sp. 3 | *Pantoea agglomerans* | 0.89 |
| 2 | *Pantoea* sp. 3 | *Pantoea agglomerans* | 0.64 |
| 3 | *Pantoea* sp. 2 | *Pantoea agglomerans* | 0.1 |
| 4 | *Pantoea* sp. 1 | *Pantoea ananas* | 0.57 |
| 5 | *Pantoea* sp. 2 | *Pantoea ananas* | 0.45 |
| 6 | *Pantoea* sp. 2 | *Enterobacter intermedius* | 0.22 |

Table 7. Pathogenicity of *Pantoea* Species Designated by API 20E Tests.

| *Pantoea* Species | No. of Isolates | Seed Rot Index* | | Boll Wall Rot (No. of Isolates) |
|---|---|---|---|---|
| | | Range | MEAN | |
| 1 | 2 | 4-5 | 4.5 | 0 |
| 2 | 26 | 3-5 | 4.6 | 4 |
| 3 | 7 | None | 5 | 0 |
| 4 | 1 | None | 5 | 1 |

* Based on a scale of 0 = no symptoms to 5 = rot of all seed.

Cotton fleahopper nymphs that hatched from overwintering eggs embedded in croton stems were reared on beans in the laboratory. When these insects were caged over fruiting branches (three insects in each of 34 cages), they caused severe damage to cotton fruiting forms. All 48 pinhead squares were blighted and killed, and most abscised. Twenty-four of 29 larger flower buds (3-7 mm wide) died and abscised. Eight of 14 young bolls (7-9 mm wide) became blackened and abscised. Also, protuberances from insect feeding or ovipositing appeared on fruiting branches, leaves and flower petals.

Abscission was consistently associated with necrosis and damage of the ovary wall (Table 8). This symptom in abscised squares also was reported by Mauney and Henneberry (1979, 1984) but was found only occasionally. Both abscised and retained buds showed necrotic spots among the anthers or on the stygma and style. This symptom is considered diagnostic for square abscissions caused by fleahoppers (Mauney and Henneberry, 1979; Williams et al., 1987). Damage to the ovary, however, appears to be most critical for inciting abscission.

Table 8. Association of Infection and Rot of Ovary Walls with Bud and Boll Abscission.

| | Frequency of Rot of Ovary Walls in | |
|---|---|---|
| Bud/Boll Fate | Flower Buds | Young Bolls |
| Abscised | 21/24 | 7/8 |
| Retained | 0/5* | 0/6 |

*Necrosis of anthers and/or stigma, but not the ovary, occurred in these buds.

The laboratory-reared fleahoppers were frequently infested with seed-rotting bacteria, especially *Pantoea* and *Serratia* spp. (Table 9). In 16 of 34 cages the same major bacterium was obtained from the insects and the damaged buds and bolls. The array of pathogenic bacteria transmitted by the laboratory insects (Table 9) was very similar to that found infesting the insects from the field (Tables 3 and 4). The somewhat higher frequency of *Serratia* might have been due to the rearing conditions or bean diet. Most *Serratia* isolates from fleahoppers showed API 20E test reactions typical of the type species isolate of *S. marcescens*. Thus, isolates from fleahopper are different from the *S. marcescens* strain that causes cucurbit yellow vine disease (Rascoe et al., 2003). The *P. agglomerans* isolates from fleahoppers were similar to those previously found associated with stink bugs (Bell et al., 2005; Medrano and Bell, 2006).

Table 9. Frequency of Seed-Rotting Bacteria from Caged Cotton Fleahoppers and Bud/Bolls.

| | No. of Cages Yielding Pathogens from | |
|---|---|---|
| Bacterial Pathogen | Insects | Buds/Bolls |
| One or more species | 28* | 32* |
| Facultative anaerobes | | |
| *Pantoea* spp. | 5 | 14 |
| *Serratia* spp. | 17 | 13 |
| *Klebsiella* spp. | 1 | 10 |
| Aerobes | | |

| | | |
|---|---|---|
| *Flavimonas oryzihabitans* | 1 | 3 |
| *Pseudomonas* spp. | 5 | 6 |
| *Bacillus* spp. | 0 | 1 |

*****16 of 34 total cages yielded the same pathogenic bacteria from both the insects and the buds/bolls.

## Conclusions

Cotton fleahoppers are frequently infested with *P. agglomerans* strains that cause severe necrosis and rot of flowering buds, young bolls and seed within older bolls. Other pathogenic bacteria occasionally are carried by fleahoppers and also can contribute to damage caused by the insect. Cotton fleahoppers apparently introduce these bacteria into ovaries of buds and into young bolls where they cause rot and subsequent abscission. The small size of the insect (2.5-3 mm long) might seem to preclude this possibility. However the proboscis and stylet of the insect is usually about 1.5 mm long, while the distance to the ovary from the outside of even a large bud is usually no more than 1mm. The bacterial infections, especially of the ovary, are likely the primary direct cause of ethylene bursts that occur in squares injured by fleahoppers and lead to abscission.

## Acknowledgements

The authors are grateful to Jose Quintana, Chris Parker, Kathryn Wittie and Renata Luff for technical assistance. We also recognize Dr. Larry Barnes for performing the FAME analyses.

## References

Beerwinkle, K.R. and H.F. Marshall. 1999. Cotton fleahopper (Heteroptera: Miridae) responses to volatiles from selected host plants. J. Cotton Sci. 3: 153-159.

Bell, A.A., J. Lopez, J. Esquivel, and E.G. Medrano. 2005. Isolation of cottonseed-rotting *Pantoea* spp. from stink bugs and plant bugs. Pages 186-193 in Proceedings of the Beltwide Cotton Conferences. National Cotton Council of America.

Bell, A.A., E.G. Medrano, and M.A. Jones. 2004. Frequency and pathogenicity of microorganisms associated with cotton seed rot in South Carolina. Pages 378-382 in Proceedings of the Beltwide Cotton Conferences. National Cotton Council of America.

Duffey, J.E. and R.D. Powell. 1979. Microbial induced ethylene synthesis as a possible factor of square abscission and stunting in cotton infested by cotton fleahopper. Ann. Entomol. Soc. Am. 72: 599-601.

Grisham, M.P, W.L. Sterling, R.D. Powell and P.W. Morgan. 1987. Characterization of fleahopper (*Pseudatomoscelis seriatus* [Reuter]) (Hemiiptera: Miridae)-microorganism induction of stress ethylene synthesis in cotton. Ann. Entomol. Soc. Am. 80: 411-416.

Hilsop, E.C., G.V. Hoad and S.A. Archer. 1973. The involvement of ethylene in plant disease. Pages 87-113 in R.J. Byrde and C.V. Cutting [eds.], Fungal Pathogenicity and the Plants Response. Academic Press, New York.

Jones, M.E. and K. Edmister. 2001. Incidence and description of seed rot. Pages 116-117 in Proceedings of the Beltwide Cotton Conferences. National Cotton Council of America.

Martin, W.R., Jr., M.P. Grisham, C.M. Kenerley, W.L. Sterling, and P.W. Morgan. 1987. Microorganisms associated with cotton fleahopper, *Pseudatomoscelis seriatus* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 80:251-255.

Martin, W.R., Jr., P.W. Morgan, W.L. Sterling, and C.M. Kenerley. 1988a. Cotton fleahopper and associated microorganisms as components in the production of stress ethylene by cotton. Plant Physiol. 87:280-285.

Martin, W.R., Jr., P.W. Morgan, W.L. Sterling, and R.W. Meola. 1988b. Stimulation of ethylene production in cotton by salivary enzymes of the cotton fleahopper (Heteroptera: Miridae). Environ. Entomol. 17:930-935.

Martin, W.R., Jr., W.L. Sterling, C.M. Kenerley, and P.W. Morgan. 1988c. Transmission of bacterial blight of cotton, *Xanthomonas campestris* pv. *malvacearum*, by feeding of the cotton fleahopper: Implications for stress ethylene-induced square loss in cotton. J, Entomol. Sci. 23:161-168.

Mauney, J.R. and T.J. Henneberry. 1979. Identification of damage symptoms and patterns of feeding of plant bugs in cotton. J. Econ. Entomol. 72:496-501.

Mauney, J.R. and T.J. Henneberry. 1984. Causes of square abscission in cotton in Arizona. Crop Sci. 24:1027-1030.

Medrano, E.G. and A.A. Bell. 2005. Infection of cotton bolls by a *Pantoea agglomerans* strain originally isolated from a human wound. Joint Meeting of the 3 Divisions of the International Union of Microbiology Societies 2005, Abstracts of the International Congress of Bacteriology and Applied Microbiology, p. 26.

Medrano, E.G. and A.A. Bell. 2006. Role of *Pantoea agglomerans* in opportunistic bacterial seed and boll rot of cotton grown in the field. J. Appl. Microbiol. (in press).

Rascoe, J., M. Berg, U. Melcher, F.L. Mitchell, B.D. Bruton, S.D. Pair and J. Fletcher. 2003. Identification, phylogenetic analysis, and biological characterization of *Serratia marcescens* strains causing cucurbit yellow vine disease. Phytopathology 93:1233-1239.

Pegg, C.F. 1976. The involvement of ethylene in plant pathogenesis. Pages 582-591 in R. Heitfuss, and P.H. Williams [eds.], Encyclopedia of Plant Physiology, New Series, Vol 4. Springer-Verlag, Heidlberg.

Schaad, N.W., J.G. Jones and W. Chen, eds. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria, Third Edition. APS Press, The American Phytopathological Society, St. Paul, Minnesota.

Williams, L., III, J.R. Phillips and N.P. Tugwell. 1987. Field technique for identifying causes of pinhead square shed in cotton. J. Econom. Entomol. 80:527-531.

Williams, M.R. 2000. Cotton insect loss estimates - 1999, pp. 884-887. Proc. 2000 Beltwide Cotton Conferences. National Cotton Council.

Williams, M.R. 2005. Cotton insect losses – 2004, pp. 1828-1843. Proc. 2005 Beltwide Cotton Conferences. National Cotton Council.