Progress Report to Southern Region Small Fruit Consortium

Title: *Paraphlepsius irroratus*: a potential vector of *Xylella fastidiosa*, the cause of Pierce's disease of grapes?

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Proposal type: Research

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Objectives:
The overall objective was to identify potential vectors of *Xylella fastidiosa* (*Xf*) to grapevines in the Southeast. This proposal specifically addresses the ability of the sharpshooter *Paraphlepsius irroratus (Pi)* to transmit *Xf* to grapevines. The approach we planed to take was (i) to sequence the DNA from the PCR positives collected in 2004 (19 of 40 tested) and 2005 and find if the sequences group with known grape isolates and (ii) if they group with the grape isolates, conduct transmission experiments with *Pi* to determine if it can transmit *Xf* to grapevines.

Justification:
Pierce's disease of grapevines is caused by the xylem-limited bacterium, *Xf* (2). *Xf* is transmitted by various leafhopper species (Purcell, (8)) among which *Graphocephala versuta* and *Oncometopia orbona* have been identified as vectors in North Carolina (7). Surveys of North Carolina vineyards conducted in 2002 and 2003 found two sharpshooter species, *G. versuta* and *Pi,* in consistently high numbers (5). *Pi* is known to be an important vector of X-disease of stone fruits, caused by a phloem-limited phytoplasma (3), but has not been shown to vector *Xf*. However, tests conducted in NC in 2004 and 2005 found *Xf* DNA in the mouthparts of these insects (7). These results indicate that although *Pi* is considered to feed on phloem sap, the insect can also carry species of xylem-limited bacteria. In order to investigate the implications of these findings as they relate to Pierce's disease, a first step is to determine if *Pi* is carrying strains of *Xf* associated with Pierce's disease. Secondly studies need to be conducted to determine whether *Pi* can transmit *Xf* to grapevines. We were successful in completing the first

objective but were not able to trap sufficient *Pi* to conduct the transmission studies. However we extended the trapping date in vineyards beyond previous years to learn more about the population dynamics of *Pi* and include these data.

Materials and Methods:

PCR Detection. Specimens of the species *Pi* were collected from three NC vineyards in 2004 and 2005. Samples from these collections were randomly selected for PCR assay. Insect heads were severed from their bodies and pinned through their mouthparts as described by Bextine et al (1). *Xf* DNA (if present) was extracted from insect mouthparts using the pre-extraction vacuum method (7). Extraction was completed with a Qiagen DNeasy Tissue Kit following the manufacturer's protocol. Two-step nested PCR was conducted to amplify bacterial DNA for detection. PCR products were analyzed using 1% gel electrophoresis techniques as described by Myers (7).

DNA sequencing and phylogenetic analysis. PCR products testing positive for the presence of *Xf* DNA were cleaned using a Qiagen PCR Purification Kit. Six positive PCR products, two from each vineyard sampled, were selected for sequencing and phylogenetic analysis. Samples were prepared following specifications described by the Duke University DNA Analysis Facility. Sequencing reactions were performed by Duke DNA Analysis Facility personnel using Big Dye terminator version 1.1 chemistry. Sequences obtained from Duke University were assembled with Contig Express from Vector NTI. Additional sequences that were previously obtained *in silico* from GenBank and NCBI BLAST for prior research were used for comparison. All sequences were aligned using ClustalX (9) software and viewed with BioEdit (4). Mega version 2.01 (6) was used to generate a phylogenetic tree using the Neighbor-joining method with 1000 boostraps.

Insect trapping. Populations of *Pi* were monitored in six vineyards from approximately 10 May, 2006 to 29 September, 2006 using yellow sticky traps. Thirteen traps were placed in each of two eastern-Piedmont vineyards (Cloer and Irongate), four traps in each of two Coastal Plain vineyards (Martin and Sanctuary), and thirteen and seven traps were placed in two upper-Piedmont vineyards respectively (Mize and Rockhouse). The exact start/end dates vary slightly from vineyard to vineyard. Traps were placed around the perimeter of each vineyard and were changed every 2 weeks. Sharpshooters were identified and counted with the aid of a stereomicroscope. Four sticky traps will be placed and changed biweekly in each of the two mid-Piedmont vineyards, to continue monitoring *Pi* populations throughout the winter.

Results:

PCR detection. Thirty three percent of the *Pi* trapped in 2004 and 2005 tested positive for *Xf* (7).
DNA sequencing and phylogenetic analysis. Of the six samples submitted, four quality sequences were obtained from the Duke University DNA Analysis Facility. NCBI BLAST searches indicate that the sequences are all similar to known strains of *Xf*. Two sequences from Cloer's vineyard and one sequence each from Silk Hope and Iron Gate vineyards were compared with sequences of known *Xf* strains obtained for previous research. This comparison yielded one phylogenetic tree produced using a 1000 bootstrap majority rule (50%) with the neighbor-joining method (Fig 1). All four sequences obtained grouped with known Pierce's disease strains of *Xf*, including strains isolated from *G. versuta* and *O. orbona* collected from the same vineyards. The

tree displayed three well-defined groups. Two samples both isolated from *Pi* but from different locations and trapping dates, formed a subgroup within the Pierce's disease group.
Trap catches. *Pi* populations tended to be much higher in the vineyards in the Mountains and Piedmont than the two vineyards in the Coastal Plain (Figs 2, 3, 4, 5). They peaked in mid to late-May, declined rapidly in late May and remained low during the summer months. They also peaked in the two vineyards in the northeastern Coastal Plain in mid to late-May but there were smaller peaks during the summer. Counts are still underway for traps at Mize and Irongate vineyards.

Discussion:
Sequences of the four PCR products obtained from *Pi* all aligned with the Pierce's disease group. Two strains formed a subgroup within the Pierce's disease group. The significance of this subgroup is not known. As shown previously by Myers et al. (7) *Xf* strains seemed to group according to host. The three observable groups were isolated from grape/NC insects, almond/oleander, and citrus/coffee. There was an exception of one individual occurring from an oleander host. A subgroup was found within the grape/NC insects group, with all members being isolated from *Pi*. Additional isolates need to be sequenced to determine the significance of the sub-grouping.

Pi was one of the most common leafhopper species found in surveys of insects in NC vineyards in 2002 and 2003 (5). *Pi* has at least two generations per year although we found the first (overwintering) to be the largest. . Populations have been reported to peak from mid-May to mid-June in other studies (7). Because systemic infections are most likely to develop from early season inoculations, control of the overwintering population of *Pi* would be very important.

Conclusions:
Large numbers of *Pi* were found in vineyards in the Piedmont and Mountains during May. The population remained low during the remainder of the season. *Pi* was shown to carry grape strains of *Xf* indicating that it is a potential vector of the pathogen. However, transmission studies are needed to confirm this.

Impact statement:
The results of this study provide further evidence that *Pi* is a potential vector of *Xf*. If transmission studies confirm this, then we will need to include it in an overall management plan for the disease in the Southeast.

Publications:
None

Literature Cited:

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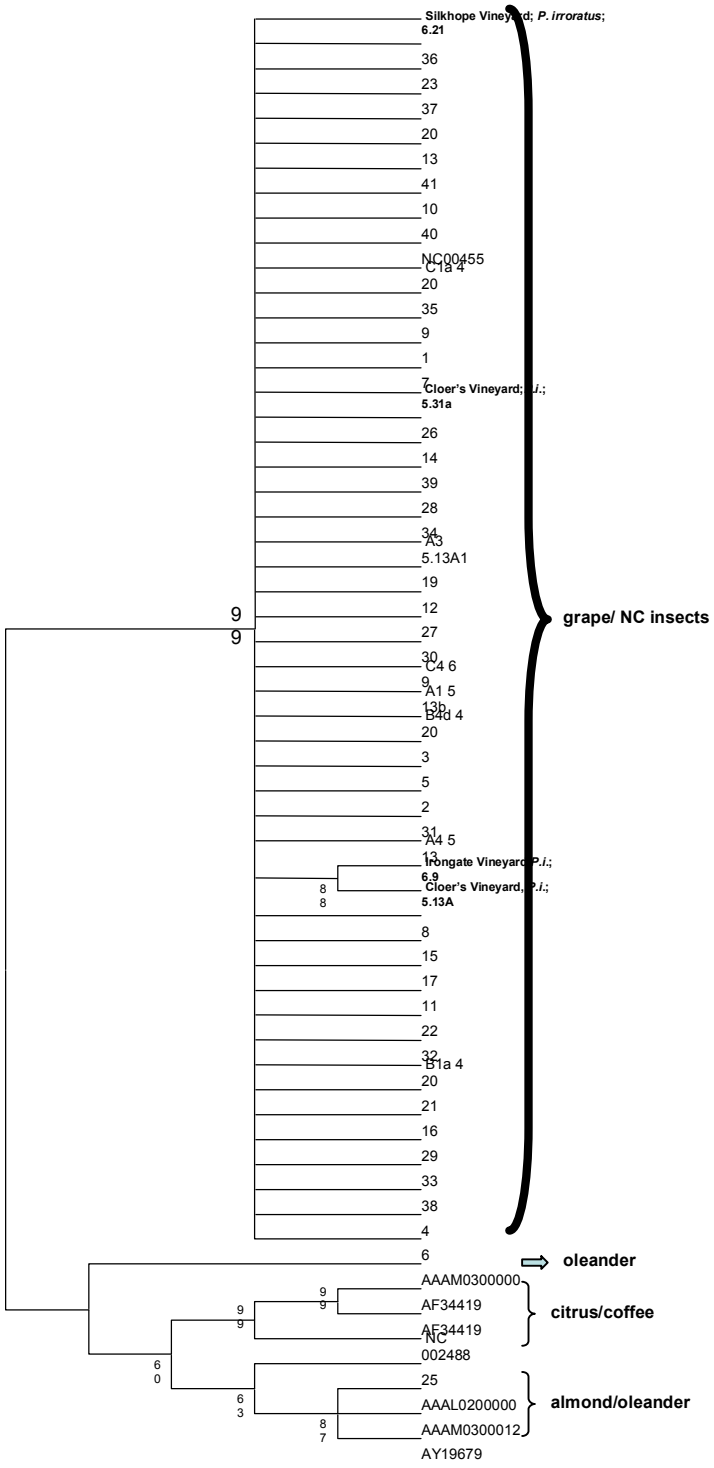

Figure 1. Tree showing phylogenetic relationships and grouping of *Xf* strains. The tree was constructed using Mega version 2.01.

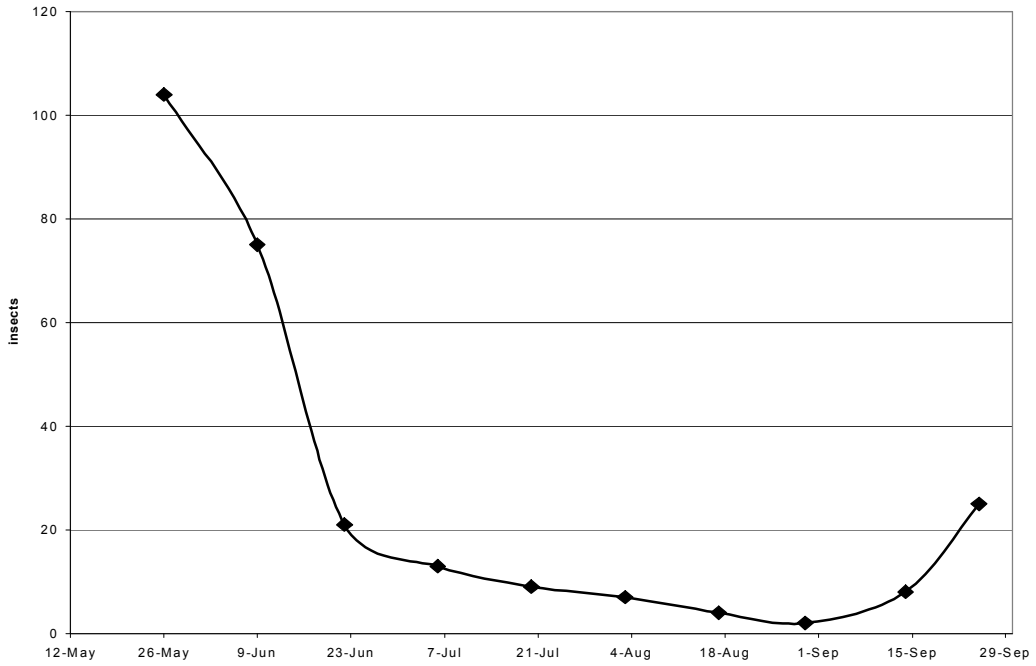

Fig.2. *Paraphlepsius irroratus* trapped on sticky traps placed at Rock House Vineyards (Polk Co. NC) from May 26, 2006 to September 25, 2006.

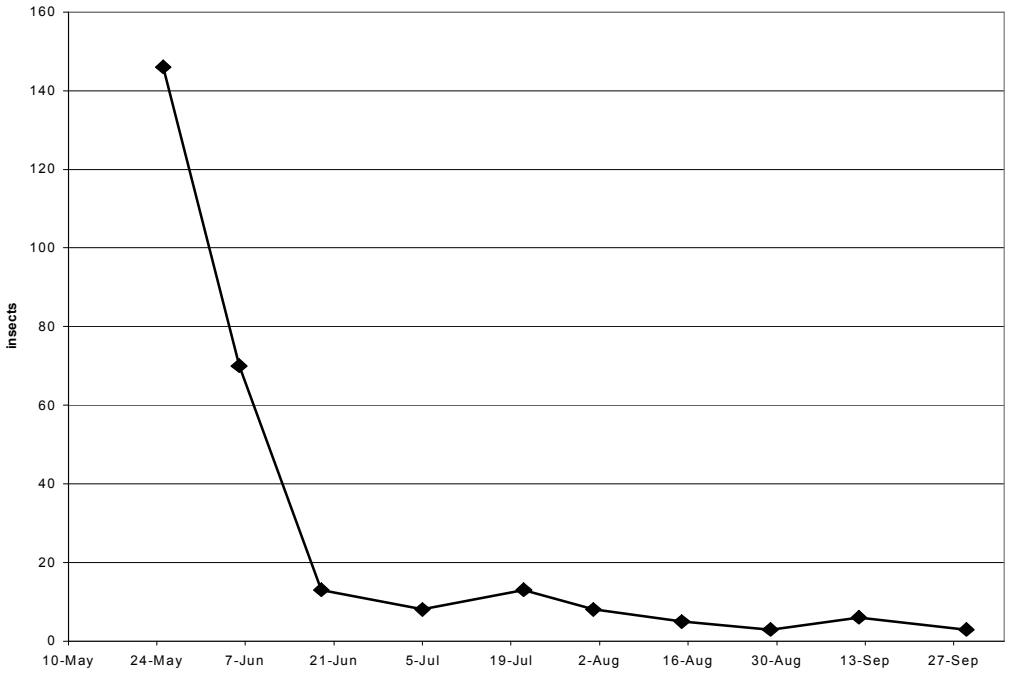

Fig. 3. *Paraphlepsius irroratus* trapped on sticky traps at Cloer's vineyard (Wake Co. NC) from May 25, 2006 to September 29, 2006.

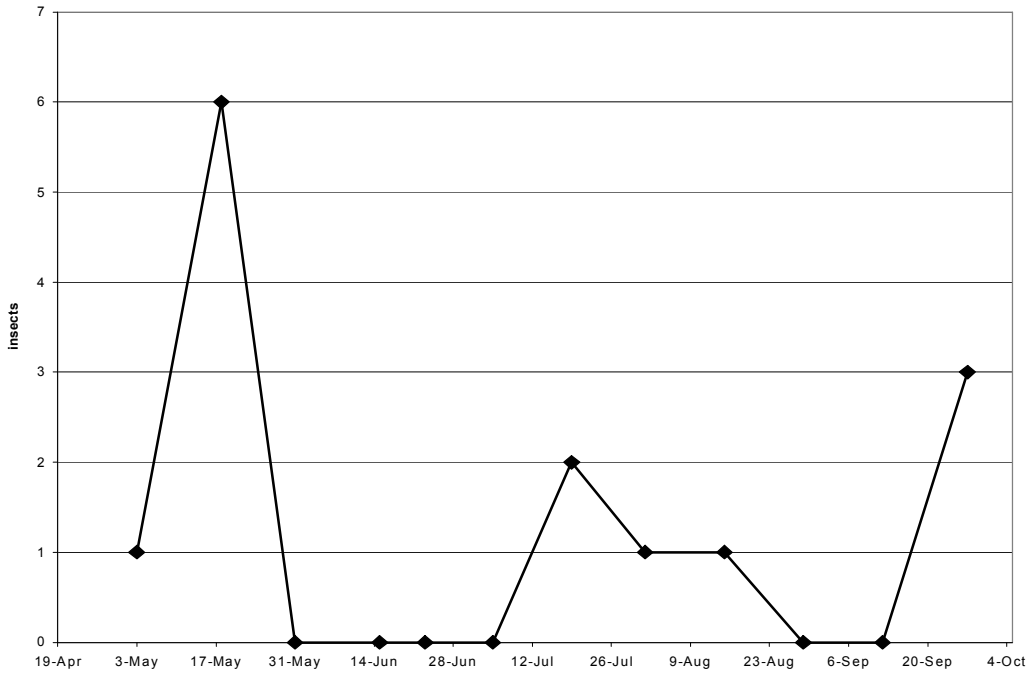

Fig 4. *Paraphlepsius irroratus* trapped on sticky traps at Martin Vineyards (Currituck Co. NC) from May 3, 2006 to September 27, 2006.

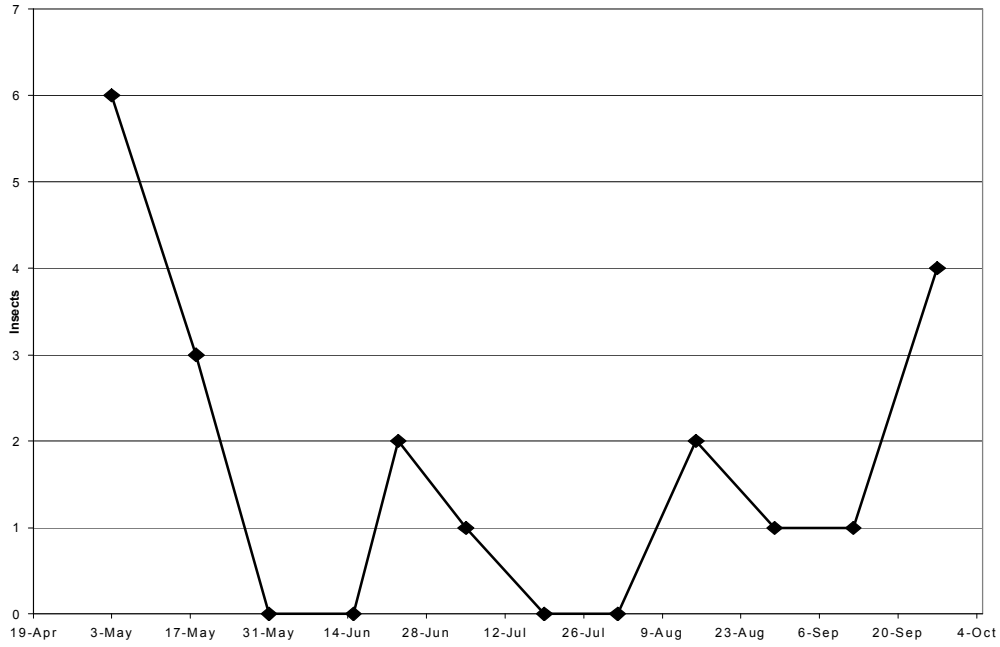

Fig 5. *Paraphlepsius irroratus* trapped on sticky traps at Sanctuary Vineyards (Currituck Co. NC) from May 3, 2006 to September 27, 2006.