Development of host plant resistance to Lygus feeding damage in alfalfa, beans and cotton.

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Justification and Problem Statement Lygus bugs (Order Hemiptera, suborder Heteroptera, family Miridae) are a serious pest of many agricultural crops, including alfalfa, beans, and cotton. Lygus bugs feed on various plant tissues using piercing and sucking mouthparts. During penetration and feeding, saliva (containing many enzymes and amino acids) from the bug is injected into the target tissue in a "lacerate-and-flush" action. Damage is manifested in tissue necrosis, distortion and abscission of fruits, growth retardation, and discoloration. This damage is due to maceration by the salivary enzymes, principally polygalacturonase (PG). Producers of these crops currently treat extensively with insecticides to control these pests. In the absence of either chemical or effective biological control (including genetic resistance), Lygus bug feeding will result in nearly total destruction of a crop. Lygus bugs readily develop resistance to agricultural chemicals, particularly organophosphates. Furthermore, many of the effective insecticides are organophosphates and their long-term labeling is uncertain. Therefore, development of host plant resistance to the Lygus bug is a priority. However, attempts to develop genetic resistance have not resulted in effective economic control of Lygus. We have verified that damage to bug feeding is associated with the PG in the bug's saliva, and we have determined that PG inhibitor proteins (PGIP) capable of inhibiting crude preparations of polygalacturonase from *Lygus hesperus* (PG_L) are present in cotton (also alfalfa and dry beans). We have developed methods of "harvesting" Lygus saliva. Using this material we have determined that more than one PG_L and amylase are increased in cotton plant tissue following Lygus feeding.

A whole plant screening protocol for PGIP in cotton has been developed and the technique is being improved to include screening for Lygus bug amylase inhibitors. We propose to continue to advance our knowledge of the mechanism of Lygus bug damage (for the purpose of improving the screening protocol) and to apply the screening protocol to identify and select, using conventional plant breeding, lines with superior levels of PGIP activity and amylase inhibition, and develop plant breeding and genetic information focused on reduction (elimination) of damage, caused during and after Lygus feeding, by salivary enzymes. Ultimately we expect to develop, test, and make available, cotton genetic stocks that resist Lygus bug PG_L and amylase (A_L) induced feeding damage.

The project needs to be conducted at Shafter Research and Extension Center (SREC) because it is the most suitable location to grow diverse lines of cotton.

1. **Objectives**

   Conduct a survey of cotton germplasm sources to identify most useful sources of polygalacturonase and amylase inhibitor proteins (PGIP and AIP, respectively) in cotton.

2. **Procedure**

   The experimental design will be a randomized complete block design in a 1-acre field. "Treatments" will be different plant introductions. These are being grown for the sole purpose of screening for potential source of PGIP and AIP . Standard crop management will be needed. When the cotton plants are beginning to bloom, leaf tissue will be collected from representative plants of each cotton accession. The samples will be frozen on dry-ice, transported to UC Davis and analyzed in the laboratory. It will be desirable to maintain the field even after tissue collection so that potentially desirable genotypes can be resampled for purposes of verification. There is no plan to harvest lint or seed from these plantings.

3. Previous Work and Present Outlook

In a preliminary study using microinjection with a pressure probe (11), Shackel and Teuber (unpublished) injected three compounds (PG [not from Lygus], sodium azide, and 1-aminocyclopropane-1-carboxylic acid [ACC, the metabolic precursor to the gaseous plant hormone ethylene]) into the peduncle of alfalfa inflorescences. These compounds were selected based on the hypothesis that PG may induce bud necrosis and/or that ethylene production may be involved in the necrosis that is associated with feeding (ACC synthesis can be induced by PG). When observed 25 days after treatment, buds injected with PG showed aberrant development. They were straw-colored and incompletely developed - typical of Lygus damage. Buds developing at the same time, but on untreated stems or on untreated plants showed no symptoms. During the past two years we have refined the injection process and have clearly demonstrated the ability to cause "Lygus like" symptoms on cotton plants injected with crude preparations of Lygus saliva. We have also made substantial progress in characterizing the Lygus salivary content, We have collected, concentrated, and fractionated the salivary enzymes. Studies are in progress to determine the exact contribution that each component is making to the development of Lygus feeding damage. A screening protocol has been adapted from the protocol initially developed for alfalfa. That protocol was successfully used in 2001and 2002 to evaluate ~1554 cotton accessions representing a broad range of materials. Importantly significant variation was identified for PGL inhibition and we have developed preliminary information to indicate that inhibitors of amylase are also present in cotton. Germplasm sources with the highest levels of PGIP activity have been identified and are being incorporated into the development of breeding populations that exhibit inhibitory activity against Lygus salivary enzymes. We are hopeful that these lines will lead to the development of commercially desirable germplasm that is resistant to the Lygus bug.

There are over 7,000 accessions representing 40 species in the world collection of cotton. New world species *Gossypium barbadense* (1,065 accessions) *Gossypium hirsutum* (4,360 accessions) represent a very large proportion of this collection. We are systematically working through these materials and expect to continue to identify sources of high inhibition. The project might expand over the next three years as desirable materials are identified.