

Creamer Lab Research

The Creamer lab research programs focus on plant-associated microorganisms of importance to New Mexico agriculture. We have sought to develop better methods for the detection of the organisms, determine how the microorganisms cause disease, and develop means to manage the organisms. The research has focused primarily on two pests that are economically important problems throughout the western US: curtovirus disease in chile pepper and fungal endophytes of locoweed.

Curly top viruses (curtoviruses) are leafhopper-transmitted viruses that cause disease on crops such as peppers, tomatoes, beans, sugarbeets, and spinach, as well as infecting many dicot weeds. Our research focuses on the ecology, epidemiology and predictive management of the disease caused by curtoviruses in chile. We study the molecular genetics, as well as the ecology of curtoviruses in New Mexico. We surveyed curtoviruses in chile and the weeds surrounding chile fields over a five-year period. We identified and compared the virus strains found in chile and weeds, assessed their genetic variability, and analyzed their spatial and temporal distribution. We determined which weeds were most likely to be infected by curtoviruses and the time of the year they were infected. We have been able to identify and characterize two new curtoviruses from chile in New Mexico, pepper yellow dwarf and pepper curly top virus. These viruses, which are both recombinant viruses, have each been found for multiple years. We were able to show that pepper yellow dwarf was prevalent in weed hosts as well as peppers, and proportionally increased compared to other curtoviruses from 2001 through 2005. Our work has demonstrated the very high level of variability and recombination found in curtoviruses compared to other plant viruses and showed that the variability does not appear to be associated with a single plant host.

Curtoviruses are transmitted by the beet leafhopper, so to understand the movement of the virus we have conducted research on the insect vector. We monitored the leafhopper populations throughout southern New Mexico every two weeks for eight years. In doing so we were able to determine when the leafhoppers arrive in chile fields, when they left the fields, and the population trends. Collaborative work with Dr. Jill Schroeder, Dr. Leigh Murray, and Dr. Scott Bundy, has revealed the role of London rocket, an annual weed, as a primary winter host for both the vector insect and the virus. Through a cooperative project with Dr. David Richman, we have determined that leafhopper feeding behavior differs on different plants and differs in leafhoppers from New Mexico and California.

Since management of curly top virus is an important concern for chile producers, we have used data gathered on the leafhopper and London rocket host to develop a disease prediction model in chile based on the effects of precipitation and temperature on overwintering weed hosts of the leafhopper. The model, developed with Drs. Jill Schroeder and Leigh Murray, has resulted in accurate prediction of the level of disease for the last seven years. Although the model is further refined somewhat almost every year with new information and was developed for southern New Mexico chile producers, growers of several crops and extension personnel in Arizona, Colorado, and Oklahoma, in addition to New Mexico routinely use this information to make cropping decisions and recommendations. Disease management is optimally accomplished through plant resistance and thus we have screened tomatoes and chile peppers for resistance to curtoviruses. We identified two pepper types, Tabasco and NuMex Las Cruces Cayenne that are field resistant. In cooperation with Dr. Steve Hanson, we developed a vascular puncture inoculation method to screen plants for R gene resistance. We also adapted a stylet sheath

staining procedure to determine how much the leafhoppers are actually feeding on a plant. These methods are now in use by other groups to study resistance to curtoviruses in other crops. Using these methods, we have found that leafhoppers do not prefer eating these 'resistant' peppers. Similarly, we have found several tomato varieties that are field resistant, but are not preferred by the leafhoppers.

The other research focus of the laboratory is on fungal endophytes of locoweed. Locoweeds are leguminous plants that are toxic to grazing animals. Our work showed that the toxin, swainsonine, is produced by fungi that live endophytically inside the locoweed plants. By better understanding the fungi and their interaction with their plant hosts, we hope to be able to develop options to help rangeland managers avoid or control the animal disease. In a cooperative project with Dr. Barry Pryor (University of Arizona), using morphological and molecular methods, we showed that the fungi were novel and developed a new genus for them, *Undifilum*. Through genetic and morphological studies, we will soon establish several new species of the genus that infect different locoweed genera and species. We developed a PCR method for specific rapid detection of the fungi and a quantitative PCR method to determine the amount of fungus in infected plants. We have localized the fungi in the plant seed coat, showing that these fungi are maternally transmitted and move through the seed. We developed an *in vitro* culture system to study the locoweed plants with or without the endophytic fungus. We have cooperated with researchers at the USDA Poisonous Plant Laboratory to characterize field samples of locoweeds from throughout the western US and China. We are currently cooperating with several laboratories in China to compare the locoweed endophytes in different areas of China to those found in the western US. We have shown that the concentrations of the toxin swainsonine are only correlated with the amount of fungi in very young *in vitro* cultured plants, and not in mature field plants. We have shown that while levels of swainsonine vary seasonally in field plants, the levels of fungi remain the same.

We have a focused effort to investigate the swainsonine biosynthetic pathway. We have genetically characterized one of the intermediates in the pathway, saccharopine reductase. We developed a transformation system for the fungus, which allows us to genetically manipulate the fungus. We successfully knocked out saccharopine reductase, which verified that it is part of the swainsonine degradation pathway, resulting in increased swainsonine production. We are comparing the proteomes of the mutant and wild type *U. oxytropis* with other species of *Undifilum*. At the same time, we are characterizing another swainsonine-producing fungus (*Rhizoctonia leguminicola*) to determine if the pathways are similar in different fungi.