Genetic Characterization of the Panicle Rice Mite, *Steneotarsonemus spinki* (Acari: Tarsonemidae)

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**ABSTRACT**

The panicle rice mite, *Steneotarsonemus spinki* Smiley, has been a serious pest of rice (*Oryza sativa*) across tropical Asia and was recently introduced to the Americas in the late 1990s. The mite was recently found in the United States, primarily in greenhouse and research facilities in Texas, Puerto Rico, Louisiana, Arkansas, New York, and California. The panicle rice mite has the potential to cause major damage to the United States rice industry if it becomes established in commercial fields. We have begun to obtain population genetic data from the panicle rice mite in order to aid in identification, and detection so as to hinder the spread of the mite in the United States and minimize crop losses. This project will provide the basic scientific information necessary to lay the foundation for an effective biological control program before the mite becomes an uncontrollable pest. We sequenced the mitochondrial cytochrome oxidase subunit I (COI) for several U.S. populations of *S. spinki* and found that all COI sequences are identical, supporting the hypothesis that these infestations are very recent invasions into these research greenhouses, likely occurring around the same time period since they haven’t had a chance to diverge yet.

**INTRODUCTION**

The panicle rice mite, *Steneotarsonemus spinki* Smiley, has been a serious pest of rice (*Oryza sativa*) across tropical Asia (Tseng, 1984) and was recently introduced to the Americas in the late 1990s. Since introduction, *S. spinki* has been responsible
for crop losses ranging from 30% to 90% per year since introduction to the Caribbean and Central America (Almaguel et al., 2000).

The United States produces approximately 9 million metric tons of rice, worth roughly $2.5 billion, yearly and is one of the top five rice exporters worldwide. When in association with sheath rot fungus (*Sarocladium oryzae*), which it is thought to commonly vector among rice plants, losses and plant sterility are often greater than 70% (Chen et al., 1979). Damage caused by mite infestations and the vectored sheath rot fungus leads to deformation, yield loss, and sterility in rice plants. Discovery of the mite in 2008 in Puerto Rico, which provides seed to rice researchers and certain commercial seed companies, and in Louisiana, Arkansas, Texas, and recently California, sets up the United States rice industry for increased problems. Successful establishment of the mite in U.S. commercial rice fields could result in severe economic losses. Once established, the mite is very difficult to treat with conventional pesticides due to governmental regulations and where it resides in the plant. Additionally, many pest mites have shown abilities to quickly form resistance to pesticides, increasing the yearly expense for control and the hazards for producers and consumers. The ideal approach would be to effectively maintain a quarantine of this mite and prevent any of the greenhouse outbreaks from spreading to the production areas. Also, the intensive use of natural predators to control mite populations has potential to limit the problem if rice mites become established in rice growing areas.

Traditionally with invasive species, U.S. policy has dictated that a project is not worth funding until the invasive organism has reached pest status (e.g., Emerald Ash Borer, Red Fire Ants, Zebra Mussels). Unfortunately, once an invasive species becomes established and widespread it is often very difficult to control or eradicate. Additionally, most research on control programs take numerous years before any applicable procedures or treatments are ready for wide-scale field testing. Because of the early detection of this mite, we have the opportunity to get ahead in the game and develop control methods before the mite becomes established and causes major damage. The objective of this study was to determine the genetic diversity of U.S. rice panicle mite populations and compare them to foreign mite specimens to understand where U.S. populations came from. A subsequent objective of this study is to develop molecular detection tools for rapid and conclusive identification of the pest and to assist quarantine efforts in preventing infested seed lots (a suspected source of entry) from being imported to the U.S.

**PROCEDURES**

The identity of ethanol preserved rice panicle mite samples received from APHIS was confirmed by visual examination using a dissecting microscope. Representative mites from each sample were slide mounted and several individual mites from each sample were selected for DNA extraction. Mite DNA was extracted using the QIAamp DNA Micro Kit from (Qiagen Inc. Valencia, Calif.). The COI gene and ITS region was amplified using Platinum TAQ DNA polymerase (InvitrogenTM1, Carlsbad, Calif.)
RESULTS AND DISCUSSION

We began work on this project in April 2008 to determine the genetic diversity, occurrence, and mode of dispersal of the panicle rice mite (Steneotarsonemus spinki) in the rice-growing area of Arkansas. Our initial objective was to determine the genetic diversity of the panicle rice mite, as other objectives are dependent on this.

Extracting a sufficient quantity of DNA from an individual microscopic panicle rice mite for PCR amplification is technically challenging. Previously to this project, we used the DNeasy kit from Qiagen for other mite species, but switched to the DNA Micro Kit for the especially small panicle rice mite and found it to be superior. We then tested the effect of freezing the panicle mite in the kits suspension buffer, as other researchers reported that this improved DNA yield. No improvement in PCR amplification was observed in our hands. We also found that the addition of trehalose to the PCR reaction mixture improved PCR amplification over the standard PCR conditions using Invitrogen’s Platinum TAQ. This protocol was developed by the Canadian Centre for DNA Barcoding and is published on their website (http://www.dnabarcoding.ca/pa/ge/research/protocols/amplification).

We have extracted and sequenced the mitochondrial cytochrome oxidase subunit I (COI) for numerous U.S. populations of S. spinki collected from 2007 to 2009. Specimens from 2007 came from a Cornell greenhouse (N.Y.); Rice Tec, Inc. (Houston, Texas); a Texas research greenhouse, and LSU research fields (La.). Specimens from
2008 were obtained via USDA, APHIS Inland Inspection (Austin, Texas) from locations in New York (Cornell University Greenhouse), Arkansas (Dale Bumpers National Rice Research Center), and Texas (Rice Tec, Inc.). Specimens from 2009 were again from Texas (Rice Tec, Inc.). All samples obtained to date have been extracted and all successful extractions have been amplified and sequenced. Cytochrome oxidase I (COI) was the gene of choice for this study because of its high copy number, fast mutation rate, and a comparatively small variance within species making it an excellent candidate for molecular diagnostics as well as for the analysis of genetic diversity. Multiple COI sequences have been obtained for *S. spinki* from all locations previously mentioned and results have indicated that all possess identical COI sequences.

We have also sequenced COI for *Tarsonemus bilobatus* found in rice from a research field at LSU collected in 2007. *T. bilobatus* is another mite commonly found in rice plants and we need these sequences to distinguish them from the panicle rice mite sequences. This is especially useful for the purpose of molecular diagnostics. We will continue to receive and analyze other rice associated specimens in order to distinguish all possible species from PRM.

Attempts to sequence ITS, a quickly evolving nuclear gene, mostly failed. Direct amplification techniques through PCR failed to produce enough useable sequences even after all variables and parameters were exhausted. This gene has been known to be difficult to sequence in some animals, but is also more variable than COI and worth the effort. Attempts to clone the ITS region from the panicle mite resulted in positive colony growth. Unfortunately, all sequences were from fungi rather than mites. We are currently trying to figure out the problem with ITS and may employ one more tactic to obtain the data.

To date, confirmed panicle rice mites have been collected from Arkansas, Louisiana, Texas, and New York. We have also received mites from China and have been in contact with researchers in numerous Latin American countries; however, none of these contacts have resulted in actual specimens. We are continuing to pursue panicle rice mite DNA from Latin America and other rice-growing regions in the world.

In order to further characterize the genetic diversity of rice panicle mites we will require sequences from many international regions and must be able to amplify and sequence another gene region such as ITS. Until we can obtain these additional mite samples, the project cannot progress.

**SIGNIFICANCE OF FINDINGS**

These findings lead to a few preliminary conclusions. First, this likely indicates that these are very recent invasions into these research greenhouses, likely occurring around the same time period since they haven’t had a chance to diverge yet. This contradicts the belief that this mite is a common pest of no importance around since at least the 1960s. If this were the case we would expect to see significant divergence between these populations. Additionally, we would expect to find specimens outside of research greenhouses and plots. Also, panicle rice mites often reproduce parthenogenetically, so
it is possible that using a maternally inherited gene like COI may have confounded the results, but many asexual mite groups still show COI divergence across populations, so we do not feel that this is necessarily the case. Either way, sequencing of a nuclear gene will shed more light on the situation. We cannot make any claims as to where this mite came from at this point because we have not yet obtained specimens from any localities outside the United States. We have contacted numerous international researchers in order to obtain PRM, but to this point have been unsuccessful. A colleague at the USDA is also contacting international colleagues on our behalf.

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LITERATURE CITED

