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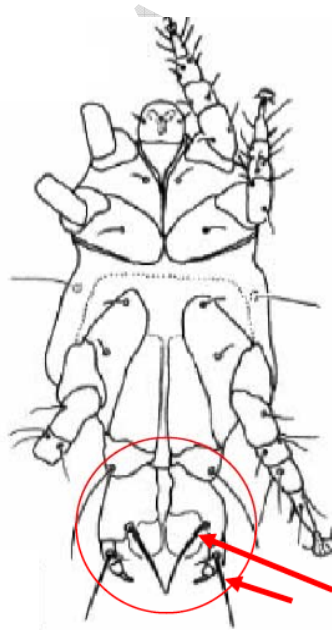
Cooperating State  
Departments of  
Agriculture

May 15, 2008

# New Pest Response Guidelines

## *Steneotarsonemus spinki* Smiley

### Panicle Rice Mite



: Cho et al., 1999.

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*Steneotarsonemus spinki* Smiley

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Version 1.3 May 15, 2008

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## INTRODUCTION

### Purpose

These New Pest Response Guidelines present available information for designing a site specific action plan to implement detection, diagnosis, containment and control or eradication of *Steneotarsonemus spinki* Smiley, the Panicle Rice Mite (PRM). Specific emergency program activity should be based on information available at that time. Any new detection may require the establishment of an Incident Command System to facilitate emergency management. This document is meant to provide the necessary information to launch a response to a detection of PRM.

The document provides background information on the mite and its hosts. The control approach is an amalgam of methods employed for PRM control in other countries and methods used to control other seedborne pests in the United States. It is intended to provide a starting point for a control/eradication program, with modifications to be made as the program and new information develops.

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) agency developed these guidelines through discussion, consultation, or agreement with other APHIS staff, the Agricultural Research Service (ARS), university advisors, States, and industry. It is to be used in conjunction with other agency regulations, guidelines, and manuals when conducting program activities. The information contained in these guidelines is based on the best scientific information available at the time of writing in consultation with States and industry. The guidelines will be updated as new information becomes available. Specific emergency program actions should be based on the best information available at the time of the incident.

### Disclaimers

***Document Comprehensiveness:*** This document is not intended to be complete and exhaustive, but to provide a basic foundation based upon available literature to assist in the development of appropriate and relevant regulatory activities. Some key publications were not available at the time of writing, and not all specialists and members of the research community were consulted in the preparation of this document.

***Commercial Suppliers or Products:*** Any references to commercial suppliers or products should not be construed as an endorsement of the company or product by the USDA.

**PRM Infestation Prevention**

Federal and state regulatory officials must conduct inspections and apply prescribed measures to ensure that this pest does not spread within or between properties. Federal and state regulatory officials conducting inspections should follow the sanitation guidelines in the beginning of the Survey procedures section before entering and upon leaving each property to prevent spreading contaminated plant material or tools to other facilities.

**Program Safety**

Safety of the public and program personnel is a priority in pre-program planning and training and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

**Support for Program Decision Making**

The USDA APHIS PPQ Center for Plant Health, Science and Technology (CPHST) provides technical support, in consultation with other scientists, including the New Pest Advisory Group, (NPAG), and a technical working group (TWG), to emergency pest response program managers concerning risk assessments, survey methods, control strategies, and other aspects of pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policy for pest response programs.



**PEST INFORMATION****Nomenclature**

<b>Phylum:</b>	Chelicerata
<b>Subclass:</b>	Acari (Acarina)
<b>Order:</b>	Acariformes
<b>Family:</b>	Tarsonemidae
<b>Subfamily:</b>	Tarsoneminae
<b>Tribe:</b>	Steneotarsonemini
<b>Genus:</b>	<i>Steneotarsonemus</i>
<b>Species:</b>	<i>spinki</i> Smiley

**Life Cycle**

Under laboratory conditions (about 24°C), this mite multiplies rapidly; the duration of the life cycle ranges from 5 to 9 days from egg to adult (Ramos and Rodriguez 2001, 2000):

Instar	Average ( $\pm$ Days)	Minimum	Maximum
Egg	2.94 ( $\pm$ 1.18)	1.75	4.77
Larva	2.22 ( $\pm$ 0.39)	2.02	2.87
Inactive larva	2.47 ( $\pm$ 1.37)	2.00	3.95
Total	7.77 ( $\pm$ 1.56)	5.75	11.59

According to Tseng (1985), the duration of the life cycle varies between 16 and 17 days at 25°C. However, in Cuba it seems that the life cycle was lessened by approximately 50%, which indicates that Cuba has favorable climatic conditions and susceptible varieties. Favorable climatic conditions and the use of susceptible varieties increase its importance as a pest (Ramos and Rodriguez 1998). At 25°C, 28°C, and 30°C, this mite required 17, 4, and 2.5 days, respectively, to complete its life cycle (Chen, Cheng, and Hsiao 1979). At 20°C and 30°C, this mite required at least 20 days and not more than 3 days, respectively, to complete its life cycle (Lo and Ho 1980).

This mite is facultatively parthenogenetic; both sexual and asexual reproduction is possible. In the Philippines, the life cycle was completed in six days. Virgin females laid only a few eggs which gave rise exclusively to males (Sogawa 1977). The descendants of virgin females were males, but the mother female could mate with its male offspring and then produce both females and males.

Under field conditions in Taiwan, populations of the mite first appeared about early May, increased in size between August and October, and declined thereafter until harvest. The mite survived the cool temperatures of winter either in stubble or ratoon rice (Lo and Ho 1980, Lo and Ho 1979a).

**Eggs:** The eggs of this mite are a translucent white, ovoid and elongated (Ramos and Rodriguez 1998). At 20°C and 30°C, females lay on average 20.0 eggs and 59.5 eggs, respectively (Lo and Ho 1980, Lo and Ho 1979a). Experiments in Cuba, using the Perla de Cuba rice variety and a temperature of 24.8°C, found that females, on average, laid 4.9 eggs per day for a total of 27.7 eggs per female (Santos et al. 2001). In the Philippines, females laid about 15 eggs per day for 5 days (Sogawa 1977).

**Larvae:** Like the eggs, the larvae are a translucent white. The larvae are elongated (Ramos and Rodriguez 1998).

**Inactive Larvae:** This phase is also a translucent white. The larvae which will become adult females are transported by the (adult) males, as is common in other species in the Family Tarsonemidae (Ramos and Rodriguez 1998).

**Adult Males:** The male of this species is characterized by (1) the presence of a pair of daggerlike setae on femur and genu IV and (2) a short, stout, blunt spurlike seta on tibia III (Smiley 1967). In Korea, males are characterized by (1) the anterior ends of apodemes II extended further than apodemes IV, (2) femur IV had a large inner median lateral flange, (3) inner anterior and outer median setae were short, about equal in length, and (4) the tarsal claw was stout and curved ventrally (Cho et al. 1999).

According to the initial description (Smiley 1967), the body of the male is elongated and broadest in the anterior region of the hysterosoma; in addition, the body is 217 microns long and 121 microns wide. In Cuba, the males were 217 microns in length and 120 microns in width (Ramos and Rodriguez 1998). In Korea, the males were 196.5 microns in length and 109.3 microns in width (Cho et al. 1999).

**Adult Females:** The body of the female is elongate and broadest in the region of the hysterosoma (Smiley 1967). In Cuba, the females were 272 microns in length and 109 microns in width (Ramos and Rodriguez 1998). In Korea, the females were 263.0 microns in length and 92.4 microns in width; the body was pale brown (Cho et al. 1999). The legs were robust except for the IV legs, which were typical tarsonemid female legs terminating in a whip-like seta two times the width of femur IV (Cho et al. 1999, Smiley et al. 1993).

**Detailed Description:** The initial description of *Steneotarsonemus spinki* was by Smiley (1967); detailed drawings were included with the description. After the detection in Cuba, Ramos and Rodriguez

(1998) again described this phytophagous mite. Identification drawings of other *Steneotarsonemus* species are in *Mites Injurious to Economic Plants* (Jeppson, Keifer, and Baker 1975) and in an illustrated key to grass-infesting species (Smiley et al. 1993).

**Ecology:** Research on ecological factors found that growth of the rice panicle mite was favored by (1) temperatures between 25.5°C and 27.5°C and (2) humidity between 83.8% and 89.5% (Miranda Cabera, Ramos, and Fernandez 2003). In India, this mite was found to infest rice plants throughout the year. The population fluctuated between a maximum during November (586.70-633.30 mites/tiller) and a minimum during February (44.30-52.70 mites/tiller). The population was greatest at the booting stage and declined thereafter as the plant matured. Correlation studies indicated that population increases were favored by low rainfall and high temperature (Ghosh, Rao, and Prakash 1997, 1999). Sterility was positively correlated with the number of mites/tiller and the percentages of mites per panicle (Lo and Ho 1979b, Lo and Ho 1977).

This mite is highly sensitive to humidity. The mortality rate increased within the range of 25°C to 32°C, as the temperature increased and the relative humidity decreased. In general, if the RH is less than 40% and if the temperature is above 30°C, all mites will die within 4 hours; if the temperature is from 25°C to 28°C, all mites will die within 6 hours. Hence, temperatures between 28°C and 30°C and a RH above 80% are optimal for this mite (Chen, Cheng, and Hsiao 1979).

**Common names:**

Panicle Rice Mite (PRM)  
Rice panicle mite  
Rice tarsonemid mite  
Spinki mite

**Pest Damage and Associated Pests**

In India, rice plants that had poorly exerted earheads and necrotic leaf sheaths were found to have rice panicle mites between the stem and the leaf sheath. Affected glumes had brownish to black lemmata and palae and shriveled ovaries (Rao and Das 1977).

In Korea, feeding by this mite caused the following symptoms: (1) deformed panicles and inflorescences, (2) lesions on the inner surfaces of leaf sheaths, and (3) browning of rice hulls (Cho et al. 1999).

**Associated Pests:** In Taiwan, the mite, in addition to its direct

damage, usually carries spores of rice sheath rot fungus (*Acrocyldrium oryzae* Sawada), which causes brownish spots on rice sheath and grains, damage termed “sterile grain syndrome.” The syndrome is manifested by (1) a loose and brownish flag leaf sheath, (2) a twisted panicle neck, and (3) impaired grain development resulting in empty or partially filled grains with diseased brown spots and the panicles standing erect (Chen, Cheng, and Hsiao 1979).

During a survey in India (Rao et al. 2000), four types of visual symptoms were observed on affected plants: (1) mite damage alone, (2) mite + saprophytic fungus, (3) mite + saprophytic fungus + sheath rot fungus, and (4) mite + white-tip nematode + other saprophytic fungal damage. After a careful examination of many samples, the researchers concluded that the mite, *Steneotarsonemus spinki*, was the dominant organism in all cases.

A disease caused by the rice tarsonemid mite virus (RTMV) is associated with a sheath browning and grain sterility syndrome of rice in Japan and the Phillipines. The virus is transmitted by *Steneotarsonemus spinki* Smiley. The virus particles are orbicular, 35 x 16 nm, and the genome consists of two single-stranded RNA segments. RTMV is not systemic and only occurs in the epidermal and mesophyll cells of the leaf sheaths and hulls of the kernels where the mites are present. RTMV is probably a mite virus that can also reproduce in plant cells (Shikata et al. 1984, Webster and Gunnell 1992).

Artificial inoculation of *Sarcocladium oryzae* onto rice was achieved in China and Japan in the 1940s and 1950s (Ou 1972) and has been confirmed by other workers and Chien and Huang (1979). Chien and Huang (1979) showed inoculation to be difficult unless the rice was first injured by the attack of mites; their *in vitro* tests indicate that several fungicides are effective (CABI-CMI No. 673 1980).

Chien (1980) found that many conidia of *Sarcocladium oryzae* were on the bodies of the rice panicle mites; pure cultures of the fungus could be obtained from the bodies, ecdysed exuviae, or eggs of the mites. Rice plants inoculated with both the mite and the fungus were more heavily infected than those inoculated with either the mite or the fungus alone (Chien 1980).

*Sarcocladium oryzae* is present on seeds from sterile rice plants; no diseased seedlings resulted when infected seed was planted. The fungus survived 110 days in diseased straw piled in the field and

more than 75 days in stubble standing in the field. After being buried in wet soil, the fungus declined rapidly and was undetectable after 25 days (Hsieh, Shue, and Liang 1980). Besides *Sarcocladium oryzae* (= *Acrocylindrum oryzae*), this mite transmits a mycoplasma-like organism resembling *Spiroplasma citri* (Chow et al. 1980b).

**Dispersal:** Because clear mite symptoms were observed on leaves of young plants that were raised from infested seed material, the transmission of this tarsonemid mite from seed to plant is possible (Rao et al. 2000).

Because the initial description was from a planthopper species, *Sogata orizicola*, collected in Louisiana (Smiley 1967), long-distance dispersal on planthoppers is a probability (Ou, Fang, and Tseng 1977).

### Economic Impact

In China and Taiwan, when the mite population is large in the paddy field, the mites can transmit the fungus causing sheath rot. The production of a second rice crop in Taiwan is curtailed because of high sterility of the rice plants (Chow et al. 1980a, 1980b). In southern Taiwan, yield losses due to rice sterility induced by this mite have been severe (Lee 1980). The area of infestation in the second crop increased from 17,100 ha in 1976 to 19,146 ha (about 4.5% of the total cropping area) in 1977. The percentage of empty grains (including partially filled grains) ranged from 20% to around 60%, with a grain loss equivalent to 20,000 metric tons, valued at US \$9,200,000 (Chen, Cheng, and Hsaio 1979). In Guangdong, a province in southern China, treatment of infested rice with pesticides increased yield by 24.27% (Jiang et al. 1994). A formula has been developed for the relationship between mite density and yield production (Chen, Cheng, and Hsaio 1979).

In India, spikelet sterility or grain discoloration was observed in 24 villages that were observed in the West and East Godavari districts of Andhra Pradesh in the 1999 wet season. The pest problem had a patchy distribution in 23 villages and 1% to 21% of the rice area was affected; in the other village, 50% of the rice was affected (Rao et al. 2000).

In a Korean phytotron, a plant growth chamber, the effect of this mite on grain filling and rice quality was investigated using the varieties Suwon 441 and Ilpumbyeo. Additional information is being collected.

### Plant Hosts

The primary hosts for PRM are in the genus *Oryza*, particularly *Oryza sativa*, cultivated paddy rice and *Oryza latifolia* (weedy red

rice). . Adult PRM have been observed on numerous weeds in rice paddies, such as *Cyperus iria*, but no eggs have been observed.

**Geographic  
Distribution**

Panicle Rice Mite has been reported from Kenya, China, Taiwan, India, Japan, Korea, Philippines, Cuba, Dominican Republic, Haiti and Colombia. Recent field introductions into Puerto Rico, Texas, Louisiana, Arkansas and greenhouses in several states are currently under investigation and the impetus for this new pest response guideline.

DRAFT

## **SURVEY PROCEDURES**

- Safety** Before starting inspections, always determine if there have been recent pesticide applications that would make it unsafe to inspect the greenhouse or rice fields. Check with property owners or managers for this information. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or greenhouses.
- Introduction** Plant regulatory officials conduct detection, delimiting, and monitoring surveys. Detection surveys are performed to ascertain the presence or absence of a pest in an area where it is not known to occur. Delimiting surveys are performed to define the extent of an infestation. Monitoring surveys are performed to determine the success of control or mitigation activities conducted against a pest.
- Use this chapter as a guide to conducting a survey for Panicle Rice Mite (PRM).
- Precautions for Inspectors** Take the following precautions before starting a survey:
- Pesticide Applications**  
Before starting a survey, always determine if there have been recent pesticide applications that would make it unsafe to inspect the field or greenhouse. Check with property owners or managers for this information. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or greenhouses.
- Quarantines**  
Determine if any quarantines are in effect for other pests of rice or other crops for the area being surveyed. Comply with any and all quarantine requirements.
- Private Property**  
Obtain permission from the landowner before entering a new property. See Regulatory Procedures on page 5.1 for pertinent information.
- Sanitation**  
When visiting greenhouses or fields to conduct surveys or to take samples, everyone, including regulatory officials, must take strict measures to prevent contamination by PRM between properties during inspections.
- Before entering a new property, make certain that footwear is clean and free of soil to avoid moving soil-borne pests from one property

to another. Also ensure that clothing is carefully disinfested to avoid PRM transport from one location to the next, or utilize disposable protective outerwear, such as Tyvek suits. The possibility of movement of PRM by leafhoppers may be a greater risk in Southern States like Texas and Louisiana. Movement between greenhouses should ensure that no leafhoppers are traveling on the surveyor/inspector as it is difficult not to carry one or two when moving between greenhouses.

Wash hands with an approved antimicrobial soap. If not using a antimicrobial soap, wash hands with regular soap and warm water to remove soil and debris. Then use an alcohol-based antimicrobial lotion, with an equivalent of 63% ethyl alcohol. If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, antimicrobial lotions are less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.

Disinfest tools (when taking plant samples) with bleach (see Appendix C) to avoid spreading diseases or other pests. A brief spray or immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (common household liquid bleach) is an effective way to inactivate bacterial and other diseases and prevent their spread. (See Appendix C for more information).

### **Sampling Procedures**

**Alert:** Disinfect the tools after sampling each field or greenhouse (see Appendix C). Also be sure to disinfect any boots or clothing that comes in contact with the plant material, as these mites may hitchhike on clothing from one greenhouse or field to the next. The use of Tyvek tops or arm coverings is recommended for ease of disinfestation.

#### **Prepare Sample:**

Samples should be placed in plastic bags (place the plant parts between dry paper towels), along with their identifying numbers and the necessary collection and contact information. Samples should be stored in coolers with ice.

**Alert:** Keep the samples as cool as possible, but do not freeze.

#### **Locate source of sample:**

Mark the sampled area with flagging whenever possible and draw a map of the immediate area showing field locations so that the areas can be found in the future if necessary. Flagging or other markers in fields may help, but can become detached.



**Record Sample:**

Be sure to accurately record GPS coordinates for each field or greenhouse location so that it may be re-sampled if necessary. For greenhouses, also note the greenhouse number (or letter) designation, the bench number and approximate location on the bench. Ask the greenhouse personnel if there are specific methods used to denote location within a greenhouse, since these personnel will be the primary contacts in the event of positive samples being found.

**Ship Sample:**

Contact the laboratory by phone prior to shipping the samples via overnight delivery service (see Appendix A).

**Survey Types**

General survey strategy may be focused on finding PRM, depending on the target location.

The purpose of a detection survey is to determine that a pest is present in a defined area. This can be broad in scope, as when assessing the presence of the pest over large areas or it may be restricted to determining if a specific pest is present in a focused area.

Statistically, a detection survey is not a valid tool to claim that a pest *does not* exist in an area, even if results are negative. Negative results can be used to provide clues about mode of dispersal, temporal occurrence, or industry practices. Negative results are also important when compared with results from sites that are topographically, spatially, or geographically similar.

***Symptom surveys:*** PRM may or may not be severe enough to cause symptoms. For PRM, symptom surveys will likely not be utilized. Some symptoms from pathogens associated with PRM may be present, however and may present opportunities to target sampling to potentially infested panicles. Symptomatic panicles should therefore not be ignored when observed.

***General detection surveys:*** A general detection survey for PRM may consist of visual observation for pest damage or mites or collection of plant tissue for dissection, depending on the target location.

***Targeted surveys:*** Sometimes referred to as “Hot Zone” or “demographic” surveys. In this case, greenhouses where rice is produced and rice fields associated with high risk pathways will be used for targeted surveys.

### **Trace-back and Trace-forward Investigations**

Trace-back and trace-forward investigations help determine priorities for delimiting survey activities after an initial US detection. Trace-back investigations attempt to determine the source of infection. Trace-forward investigations attempt to define further potential dissemination through means of natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

For greenhouse hosts, a list of facilities associated with infected greenhouse stock from those testing positive for PRM will be compiled. These lists will be distributed by the state to the field offices, and are not to be shared with individuals outside USDA APHIS PPQ regulatory cooperators.

**Grower names and greenhouse and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited. Each state is only authorized to see locations within their state and sharing of confidential business information may be restricted between state and federal entities. Check the privacy laws with the State Plant Health Director for the state.**

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received PRM infested material. Speak to the growers or farm managers and obtain proper permission before entering private property.

Several actions need to occur immediately upon confirmation that a greenhouse or field sample is positive for PRM. Check greenhouse records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from infested greenhouse) during the previous six months. (See the Regulatory and Control Sections for more information).

### **Delimiting Survey after Initial US Detection**

After a new detection in the US, or if a new area is confirmed, host surveys in commercial and research field and rice greenhouse properties in the area will be conducted.

If available, data collection can be simplified by the use of pre-programmed hand-held units that allow ease of data recording with GPS capability. The data collected during surveys should include:

- 1) date of collection,

- 2) sample number from predetermined numbering system,
- 3) collector's name and affiliation,
- 4) Full name of Business/Institution/Agency,
- 5) full address, including county and State,
- 6) type of property (i.e., commercial field, research field, greenhouse),
- 7) grower's field or greenhouse ID numbers if appropriate,
- 8) GPS coordinates of the host plant and property,
- 9) host species, and cultivar,
- 10) general conditions or any other relevant information,
- 11) positive or negative results from testing (recorded later).

Recording negative results in surveys is just as important as positive detections since it helps define an area of infestation. A system of data collection should include an efficient tracking system for suspect samples such that their status is known at various stages and laboratories in the confirmation process.

**PRECAUTIONS:** Before starting inspections, always determine if there have been recent pesticide applications that would make it unsafe to inspect the rice plants. Check with property owners or greenhouse/field managers for this information. Look for posted signs indicating recent pesticide applications, particularly in commercial and research fields. Miticides and insecticides can be highly toxic to humans. Do not disregard posted warnings.

Before entering a new property, ensure you have permission and make certain that footwear is clean and free of soil to avoid moving soil-borne pests from one property to another. Disinfest tools with bleach (see Appendix C) to avoid spreading other pathogens or pests.

Surveys should be most intensive around the known positive detection(s) and any discovered through trace-back and trace-forward investigations. These surveys should include greenhouse and commercial fields with the results mapped to develop potential regulatory boundaries.

Survey task forces should consist of an experienced survey specialist, entomologist or acarologist familiar with the mite and symptoms caused by the mite and personnel responsible for sample collection and properly recording the data and GPS coordinates.

**Guidelines for  
Commercial or  
Research Field  
Sampling****Field Survey***Location:*

Rice-producing states.

A. Delimiting survey for fields found positive for PRM in 2007.

Survey the 2007 positive field and a minimum of three associated fields (use adjacent or nearby fields). The purpose of this survey is twofold: First, to determine if the mites survived the winter and second to determine the extent of the infestation. The delimiting survey will use the same sampling protocol that is outlined in this document.

B. Detection survey in rice-producing states not found positive for PRM in 2007.

Survey 5% of fields within rice-producing states. Use the sampling protocol that is outlined in this document.

*Timing of the Sampling:*

Samples should be collected when the majority of rice plants within the field are at the heading to milk stage, when mite populations are expected to be at their highest. At this time, the panicle is fully exerted beyond the boot and symptoms of panicle blight and other panicle disorders are observable. Identify when rice will be at this physiological stage in your state and conduct sampling during those weeks. Sampling at these stages will ensure the highest likelihood of collecting the mite if an infestation is present. In ratoon crops, sampling at earlier physiological stages of rice is an option.

In positive fields from 2007 and fields in close proximity to positive 2007 fields, sampling can begin at the seedling stage. For these fields, if the mite is present, populations would be expected to be higher than in newly infested fields. If the mites were able to survive the winter, then the mites will likely be present on the seedlings.

These recommendations are based on the biology of the mite and promote sampling during plant growth stages

when mite populations are expected to be highest. However, states may adjust the timing of sample collection to accommodate the safety of inspectors, differences in rice production techniques within their states, and other factors as necessary.

Sampling at the seedling stage may find an infestation early enough to provide eradication or management options to the grower and possibly save the crop. This would provide an incentive for grower cooperation in the survey.

If no mites are found at the seedling stage, then the survey should be repeated during the heading to milk stage.

*Sampling Unit:*

Three tillers, preferably with panicles, from each sampling location within the field.

By collecting tillers instead of whole plants, the amount of collected plant material will not vary as greatly between hybrid and conventional plants and between varieties.

*Sampling Universe:*

Individual field.

For this survey, an individual field is defined as an area planted to rice (regardless of the number of varieties), with a physical separation of more than 5 feet from other rice fields by canals, berms, roads, or other physical attributes.

*Number of Sample Locations per Field:*

In fields that may have been recently infested by PRM, the PRM population may still be small and difficult to detect.

In order to detect a new, possibly small infestation of PRM, from a statistical and scientific standpoint, it is advisable to take as many samples as possible. Ideally, 300 samples would be collected per field which would provide a 95% confidence level of detecting a 1% infestation level, assuming a 100% extraction efficiency of the sample. However, feasibility, logistics, fiscal and

survey has determined a target detectable infestation level of 5%. Therefore, 59 samples would be collected per field to provide a 95% confidence level at this percentage level.

It should be noted that the detectable infestation level will increase as the number of samples collected decreases; therefore, smaller infestations may be less likely to be detected (See Table 1). This table assumes an unknown distribution of PRM within the field and a 100% extraction efficiency of the sample.

**Table 1. Relationship between detectable infestation level of PRM and number of samples collected per field.**

Detectable Infestation Level of PRM	Number of samples per field to achieve a 95% CL
1%	300
2%	149
3%	99
4%	74
5%	59

*Sampling Method:*

Samples should be taken along the field perimeter. The four corners of the field and the water inlet/ outlet should be included in the sampling.

If it is prohibitive to sample from all sides of a field, due to the presence of canals, etc., samples should be distributed evenly between the sides of the perimeter that are accessible.

Little is known about PRM distribution within newly infested fields. Therefore, when only sampling the perimeter of the field, we can only be confident about detecting a PRM infestation in the perimeter.

If the surveyor is able to identify bacteria panicle blight (*Burkholderia glumae*) and sheath rot (*Sarocladium oryzae*), the surveyor should collect approximately 10-20% of the total number of samples from plants showing symptoms of either of these two diseases. If the surveyor is not familiar with rice diseases and their symptoms, sample collection should not be biased

towards plants with damage. Multiple diseases and other causes of damage that are not related to PRM infestations may be present in the field and this could impact sampling results.

*Selection of Fields to Sample:*

Fields with the following characteristics are more likely to have been exposed to mites and may be easier to access:

- Fields near roads (especially dirt roads) that are traveled by equipment. Mite populations tend to increase in dry, dusty conditions.
- Fields near paved roads. These fields are likely to have been chosen for scouting purposes. Within these fields, choose logical points of entry that may have been used for scouting in the past.
- Fields that are bordered by levees.

*Re-sampling Positive Fields:*

If only one mite is detected in a field, inspectors should return to the field and re-sample. One mite could be the result of contamination and therefore may be an isolated incident. Inspectors should re-sample using the same sampling method described above and collect the same number of samples.

*Sample Processing:*

**All** sampled material collected from the field should be processed in the lab. See the Panicle Rice Mite New Pest Response Guidelines for further detail on sample processing.

**Guidelines for  
Greenhouse  
Sampling**

**Greenhouse Survey**

*Location:*

Greenhouses with permits to import rice and greenhouses that were found positive for PRM in 2007 will be surveyed. In addition, surveys should be performed on any trace-forward/ trace-back greenhouses that received material from the positive greenhouse and any that are associated. Surveys will be conducted at all other facilities as deemed necessary.

*Timing of the Sampling:*

Sampling will begin as soon as funding has been made available.

Inspectors should sample greenhouses once per month. In lieu of monthly inspections, the inspector may place the greenhouses under a Compliance Agreement.

*Sampling Unit:*

Visual Inspection: one tiller per plant.

Sample Collection: one tiller per plant (a tiller that is not needed for research/breeding as identified by the researcher).

*Sampling Universe:*

Individual research block.

An individual research block should be sampled instead of the entire greenhouse because of the likely differences in plant varieties and maturity.

*Sampling Method:*

Inspectors should perform both visual inspection and sample collection during each monthly survey in each research block in the greenhouse.

*Visual Inspection:*

Inspectors should familiarize themselves with the size and visual characteristics of PRM before performing surveys (See New Pest Response Guidelines for images and resources).

Inspectors should visually inspect 50% of plants on the perimeter of each research block and focus their selection on plants with an unhealthy appearance. Inspectors should inspect one tiller per plant. Inspectors should unroll the sheath and inspect the plant down to the node (if possible) with a 20X hand lens.

Inspectors should collect any suspicious-looking mites by placing a section of the infested leaf sheath in 95% ethanol for identification at the lab.

*Sample Collection:*

Researchers at each greenhouse should instruct inspectors which tillers can be collected (tillers not



needed for research). Researchers should focus on plants with an unhealthy appearance.

Inspectors should collect one tiller per plant from 10% of plants in a research block, as identified by the researcher (if any suspicious samples were taken during the visual survey, the inspector can count those as part of the 10% collected total).

If a greenhouse is confirmed positive during the 2008 PRM survey or from the prior year, the inspector may use sentinel plants for sampling. If sentinel plants are used, they should be sampled prior to miticide treatments. Sampling prior to spraying is necessary to determine if mites are present prior to application.

**Monitoring Surveys**

After any control or eradication procedures are conducted, it is necessary to do follow-up monitoring surveys to assess the success of the program. The duration of monitoring should be determined in consultation with the PRM TWG.

**Survey Timing**

Research in China and other countries suggests that field sampling should be conducted in late summer, when the tillers have filled and harvest is about to begin. This is usually when the populations of PRM are at a peak and detection is most likely.

For those fields where ratoon cropping occurs, this cycle may not be as important, with more green material available at other times of the year (such as during the second ratoon crop).

In fields and around fields that had high infestation rates in late Summer of 2007, sampling fields soon after second leaf.

## PEST DIAGNOSTICS AND IDENTIFICATION

<b>Importance</b>	Accurate identification of this quarantine pest is pivotal to assessing its potential risk, developing a survey strategy, and deciding the level and manner of control.
<b>Authorities</b>	<p>A USDA-recognized national authority for the regulatory taxon must positively identify the suspected pest before initiation of any program regulatory activities.</p> <p>Final confirmatory identification of first detections within a State must be done at the Systemic Entomology Laboratory by Dr. Ron Ochoa (these will be forwarded by Eric McDonald).</p> <p>See below for more information on identifiers doing confirmation.</p> <p>In the future, other laboratories may obtain approval to make suspect positive determinations but must abide by guidelines set under various permits and authorizations maintained by APHIS Plant Protection and Quarantine.</p> <p>PPQ permit requirements for plant pests and laboratories fall under the authority of the Plant Protection Act (7 CFR Part 330). Diagnostic laboratories receiving plant samples from other states are required to have PPQ permits and proper containment.</p> <p>The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status. If any material is shipped interstate, it is a requirement that the receiving laboratory has a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at:  <a href="http://www.aphis.usda.gov/ppq/permits/">http://www.aphis.usda.gov/ppq/permits/</a> or contact PPQ Permit Services at (301) 734-8758.</p>
<b>Risk Factors For Detection</b>	<p><b>Factors that increase the chance of detecting PRM in a greenhouse.</b></p> <ul style="list-style-type: none"> <li>• Site history- continual and successive cropping of rice, lack of a host-free period;</li> <li>• Host age - young foliage, new plantings, in the same location as older rice plants;</li> <li>• Host susceptibility - age, genotypic characteristics (variety);</li> <li>• Proximity to other positive fields or greenhouses.</li> </ul>
<b>Sample Storage and Forwarding</b>	Tillers with seed samples are preferred for diagnostic testing since the mite is many times found under the seed panicle. Seed that are

attached to stems are desirable. The entire plant above the water line should be sampled.

Place leaf and stem samples with paper towels in zip-lock bags and remove as much of the air out of the bag as possible prior to sealing the bag. Include dry paper towels in the bag with the sample so they can absorb moisture that will cause plant material to degrade. Keep samples cool, but not frozen, preferably in an ice chest, while transferring them and waiting for preparation for mailing to the screening laboratory. Write the sample ID number legibly using a permanent marker on the Ziploc bag.

**Sample Extraction and Preparation**

Samples from the field or greenhouse must be extracted at a diagnostic laboratory according to procedures provided in Appendix A. Check with the regional office if a diagnostic laboratory to extract the samples is not available.

**Sample Packaging and Documentation**

Extracted samples must be sent by overnight delivery. Ice packs are not needed or recommended. Packaging of all extracted samples should be in a larger zip-lock bag made leak proof, then must be placed in a sturdy cardboard outer box with insulation to prevent movement within the box during shipping. Include the completed PPQ form 391, and any relevant tags or barcodes that came with the sample.

Only send samples by overnight delivery Monday through Thursday. Saturday delivery may not be accepted unless special arrangements with the receiving laboratory are made prior to shipping.

**Sample Labeling, Numbering, and Record Keeping**

It is recommended that both the Integrated Survey Information System (ISIS) and the National Agricultural Pest Information System (NAPIS) databases be utilized to input PRM survey data. The use of ISIS is important to gather timely and accurate data in order to assist in the regulatory decision process. Those States surveying for PRM as part of the Cooperative Agricultural Pest Survey (CAPS) funding must enter data into NAPIS. An ISIS worksheet (Appendix H) and NAPIS worksheet (Appendix I) have been developed to assist in collecting survey information. The Incident Commander or Program officials should provide the appropriate equipment for recording the sample collection information. Complete a PPQ form 391 (*Specimens for Determination*) for each sample.

Composite samples should have one PPQ form 391 but document the subsample numbers on the form for what it represents.

The submitter should complete and include a hard copy of the PPQ form 391 inside the outside bag of double-bagged samples. Assign and record for each sample a unique ID sample number with a predetermined format. Assure that the sample is linked to any survey data collected for that sample by including the Survey ID number on the form. This will enable the linkage of the sample to all the field collection information.

In block 1 of the PPQ form 391, enter and label the assigned sample ID number first with the first two letters designating the state two letter code. If sample is being processed at an identifier location, enter the information from the PPQ form 391 in the Pest ID program that will generate an interception number. Also enter the survey ID in parenthesis. The state's own lab sample accession number can also be added for record keeping. If the laboratory does not have a numbering convention for samples, use the following format:

Sample ID # XX-00000 (Survey ID # \_\_\_\_\_)

XX is the two letter state abbreviation. In the remarks section (block 22), give the name of the office or diagnostic laboratory forwarding the sample, plus a contact name, e-mail address, and phone number of the contact.

In block 23, enter the preliminary diagnosis (e.g., "Suspect PRM").

Inspectors must provide all relevant collection information with samples. This information should be communicated within a State and with the regional office program contact. If a sample tracking database is available at the time of the detection, please enter collection information in the system as soon as possible.

### **State Extraction and Identification Procedures**

While each region will be providing a list of PPQ-approved laboratories for centralized area for plant washing, extraction, and identification, States may perform their own washing and extraction of mites in accordance with the procedures in Appendix A. Plant samples sent to these locations will need to contact the laboratory and obtain a copy of the permit for inclusion with shipment. They will then send any mites obtained to the identifier with a completed a PPQ form 391 for each sample by over night delivery properly mounted on slides or all mites extracted in tightly sealed in a vial of 70% ethyl alcohol, with no plant material.

### **Centralized**

After the initial US detection of Panicle Rice Mite (PRM), a

**Authorized  
Laboratories for  
Survey Sample  
Extraction and  
Identification**

national survey conducted in rice growing states requires consistency in diagnostic procedures. For states not performing their own extractions and mite screening, a centralized USDA, APHIS, PPQ laboratory has been designated to perform extractions from other states and screen suspect mites from the samples. This laboratory has the permits and proper containment to handle potentially infested plant samples from other states.

The laboratory is the USDA, APHIS, PPQ, CPHST lab - Mission, Texas.

Properly packaged plant samples and PPQ form 391 for each shipment will be sent by overnight carrier to the following address:

Attn: Josie Salinas  
USDA, APHIS, PPQ, CPHST lab  
22675 N. Moore Field Rd.  
Moore Airbase, Bldg. S-6414  
Edinburg, TX 78541-5033

Phone: 956-580-7301 (Main)  
956-580-7278 (Lab)

E-mail: [Elma.J.Salinas@aphis.usda.gov](mailto:Elma.J.Salinas@aphis.usda.gov)

Please send by overnight carrier according to instructions and label the box “plant samples for PRM analysis”.

Please notify the regional program manager in your region and the laboratory by email that plant material is being sent with the overnight service name and tracking number.

**Approved  
Identification of *S.  
spinki***

Once the plant material washed and extractions made, screening at the centralized laboratory will isolate the mites on slides and/or in vials of 70% ethyl alcohol. This facility will forward suspect PRM's with the PPQ form 391 to identifier, Eric McDonald in Humble, TX. Please notify the regional program manager in your region and the laboratory by email that plant material is being sent with the overnight service name and tracking number.

In the case of PRM, the first suspect positives from a new state or county, are considered Potentially Actionable Suspect Samples (PASS), until confirmed by the USDA Systematic Entomology Laboratory in Beltsville, MD. An inconclusive result, any suspect positive from a new host, or other unexpected or unusual find should also be treated as PASS samples.

***This laboratory does not have all the necessary permits or containment protocols to handle the sample extractions. Please only send extracted samples in vials with 70% ethanol or mites mounted on slides to this Facility.***

To: Eric M. McDonald  
USDA, APHIS, PPQ  
Plant Inspection Station  
19581 Lee Road  
Humble, TX 77338

Tel: 281-230-7204  
Fax: 281-230-7203

**States with No  
Previous Positive  
Confirmations for  
PRM**

The authorized identifier, Eric McDonald, has identification authority for *S. spinki*. If a positive ID is made from a state that has no previous determinations of *S. spinki*, he will have the information from the PPQ form 391 entered in the Pest ID database and send specimens to the USDA Systematic Entomology Laboratory (SEL) in Beltsville, MD. (These will be routed to Dr. Ronald Ochoa, the national mite identification specialist for final confirmation).

The address for sending suspect *S. spinki* for SEL confirmation is:

Location Leader  
Systematic Entomology Laboratory  
Attn: Communication and Taxonomic  
Services Unit  
Building 005, Room 137, BARC-West  
10300 Baltimore Avenue  
Beltsville, MD 20705

Phone: (301) 504-7041 (for Fedex ONLY, do not call SEL for status of samples. Call NIS at 301-734-5312.

Also, send an e-mail a notification with the following text in the subject line: SUSPECT FWD: *S. spinki* (PRM) in XX (state two letter abbreviation) to the following e-mail address

[ppq.nis.urgents@aphis.usda.gov](mailto:ppq.nis.urgents@aphis.usda.gov)

The PPQ National Identification Staff (NIS) in Riverdale, MD will notify PPQ Emergency and Domestic Programs (EDP) who will forward it to program managers, SPHD's and SPRO's from the state of origin.

Once a final determination is received from SEL, the same notification procedure as above will be followed to communicate the results.

**States with New  
Positive  
Confirmations for  
PRM**

Any positive sample from a new State processing their own samples should send suspect positives to Eric McDonald, who will also forward them for verification by the SEL in Beltsville, MD.

The authorized identifiers at the above locations have identification authority for *S. spinki*, and if a positive ID is made from a state that is already positive for *S. spinki*, they will e-mail a PDF file of the final determination record from PestID to the following e-mail address:

[ppq.nis.urgents@aphis.usda.gov](mailto:ppq.nis.urgents@aphis.usda.gov)

The PPQ National Identification Staff (NIS) in Riverdale, MD will forward it to PPQ Emergency and Domestic Programs (EDP) who will forward it to program managers, SPHD's and SPRO's from the state of origin.

Negative determinations can be communicated directly back to the State of origin by the authorized identifier without notifying NIS.

**REGULATORY PROCEDURES****Instructions  
To Officials**

Agricultural officials must follow instructions for regulatory control measures, treatments or other procedures when authorizing the movement of regulated articles. A full understanding of the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by quarantine and regulations. Only authorized treatments may be used in accordance with labeling restrictions. During all field visits, please ensure that proper sanitation procedures are followed as outlined in the Survey section.

**Regulatory  
Actions and  
Authorities**

After an initial suspect positive detection, an Emergency Action Notification (PPQ form 523, Appendix D) may be issued to hold articles or facilities, pending positive identification by a USDA APHIS PPQ recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register*.

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides for authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under state authority. State departments of agriculture normally work in conjunction with federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the state's measures are inadequate, USDA can take intrastate regulatory action provided that the governor of the state has been consulted and a notice has been published in the *Federal Register*. If intrastate action cannot or will not be taken by a state, PPQ may find it necessary to quarantine an entire state.

PPQ works in conjunction with state departments of agriculture to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property in the absence of owner permission. PPQ prefers to work with the state to facilitate access when permission is denied; however, each state government has varying authorities regarding entering private property. A General



Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas where PPQ and the state department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected state.

### **Tribal Governments**

PPQ also works with Federally Recognized Indian tribes to conduct surveys, enforce regulations and take control actions. Each tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to state governments. Permission is required to enter and access tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Indian tribal governments about actions that may have substantial direct effects on tribes. Whether an action is substantial and direct is determined by the tribes. Effects are not limited to current tribal land boundaries (reservations) and may include effects on off-reservation land or resources which tribes customarily use or even effects on historic or sacred sites in states where tribes no longer exist.

Consultation is a specialized form of communication and coordination between the federal government and tribal government. Consultation must be conducted early in the development of a regulatory action to ensure that tribes have opportunity to identify resources which may be affected by the action and to recommend the best ways to take actions on tribal lands or affecting tribal resources. Communication with tribal leadership follows special communication protocols.

For additional information, contact PPQ's Tribal Liaison.

Christina Jewett  
National Program Manager for Native American  
Program Delivery and Tribal Liaison  
USDA\_APHIS\_PPQ  
14082 S. Poston Place  
Tucson, AZ 85736

Phone: 520-822-5440  
Fax: 520-822-5440 call first

To determine if there are Federally Recognized Tribes in a state, contact the State Plant Health Director (SPHD). To determine if there are sacred or historic sites in an area, contact the State

Historic Preservation Officer (SHPO).

For clarification, check with your SPHD or State Plant Regulatory Official (SPRO) in the affected state.

**Overview of  
Regulatory  
Program for PRM  
after a US  
Detection**

Once an initial US detection is confirmed, holds will be placed on the property by the issuance of an EAN.

Trace-back and trace-forward investigations from the property will determine the need for subsequent holds for testing and/or further regulatory actions.

**Record Keeping**

Record keeping and documentation is important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by the owners, researchers or manager for information on destination of shipped plant material, movement of plant material within the facility, and any management (cultural or sanitation) practices employed.

Keep a detailed account of the numbers and types of plants held, destroyed, and/or requiring treatments in control actions. Consult a master list of properties, distributed with the lists of suspect nurseries based on trace-back and trace-forward investigations, or nurseries within a quarantine area. Draw maps of the facility layout to located suspect plants, and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout, and document plant propagation methods, labeling, and any other information that may be useful for further investigations and analysis.

Keep all written records filed with Emergency Action Notification (EAN, PPQ form 523) copies, including copies of sample submission forms, documentation of control activities, and related State issued documents if available.

**Issuing an  
Emergency Action  
Notification**

An EAN is issued to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested, or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision to destroy plants is made, or in the case of submitted samples, once positive confirmation is received, the same EAN which placed plants on hold also is used to document any actions

taken, such as destruction and disinfection. Additional action may be warranted in the case of other fields or greenhouses testing positive for PRM.

If plant lots or shipments are held as separate units, it is advisable to issue separate EAN's for each unit of suspected plant material and associated material held. EAN's are issued under the authority of the Plant Protection Act of 2000 (statute 7 USC 7701-7758). **States are advised to issue their own hold orders parallel to the EAN to ensure that plant material cannot move intrastate.**

When using EAN's to hold articles, it is most important that the EAN language clearly specify actions to be taken. An EAN issued for positive testing and positive associated plant material must clearly state that the material must be disposed of, or destroyed, and areas disinfected. Include language that these actions will take place at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, then document on the same EAN, any disposal, destruction, and disinfection orders resulting from investigations or testing.

For Block 1, enter the name and location of the nearest PPQ office. Under "Name of Article" in block 3, enter the host scientific name and cultivar. In Block 4, enter the property address, greenhouse, or field number or name or other information indicating the location of the plant material held. In the Shipper Block 6, enter the plant material source if known. Blocks 7 and 8 can be left blank unless that information is known.

To place plant material on a property on "Hold", in Block 12 of the EAN, enter for the Pest: "suspect Panicle Rice Mite, *Steneotarsonemus spinki*". The authority under which actions are taken is The Plant Protection Act of 2000, Statute 7 USC 7701-7758. In block 15, the Action Required with suggested text as follows:

**"All host plants of the Panicle Rice Mite, *Steneotarsonemus spinki*, (PRM) are prohibited from movement from the property pending further notification by USDA APHIS PPQ and/or the State department of agriculture. No other host plant material, including harvested rice and blow out material, may leave the property until further evaluations can be made. After further investigations are conducted on the listed plants and other host material, if a positive detection is confirmed on the property, [plant material] will be treated/destroyed under**

**supervision, with approved methods in accordance with USDA and state policies. Any additional hosts of PRM on the property are subject to Federal and State quarantine requirements prior to movement from the property.”**

**Regulated Articles**

Once initial detections are confirmed in an area (positive research or commercial rice field or greenhouse with predetermined buffer area around positive finds after a thorough delimiting survey), regulated articles include all live host plant material in that area.

The PRM is spread by seed and fresh propagative plant material harboring the bacteria within the phloem of the plant. Whole live plants, stems, leaves, and seed are regulated. Other potential pathways of PRM include personnel and equipment and possibly leafhoppers coming in contact with infested rice plants. Steps should be taken to ensure that proper sanitation around exists in infested greenhouses to include sanitation of all personnel and equipment that comes into contact with plant material.

**Panicle Rice Mite Hosts**

The main hosts of PRM are *Oryza* spp. and are restricted and subject to regulatory treatments in areas where the Panicle Rice Mite has been detected. *Oryza sativa*, *Oryza latifolia* (red rice or wild red rice) and *Cyperus iria* are known hosts of PRM.

**Shipment of Host Plant Material**

Shipment of host plant material from a greenhouse or field that is under EAN for PRM is prohibited unless the rice seed is treated with an APHIS-approved treatment. These treatments may be found in Appendix E. See also Control Section.

**Regulatory Treatments for Mites in Infested Greenhouses and Fields**

See Appendix E for information on labeling and products available. Also see Control Section.

**Regulated Area**

The regulated area will consist of the area under the EAN and a specified buffer around that area. If a greenhouse is found to be infested with PRM and placed under EAN, then the head house of the greenhouse and areas surrounding the greenhouse (especially outside of any air handling equipment) will be placed under restrictions. These restrictions may include a designated host-free area and entry and exit sanitation requirements for personnel and equipment.

**Grower Requirements**

Depending upon decisions made by Federal and State regulatory officials in consultation with the PRM Technical Working Group,

- Under Regulatory Control** quarantine areas may have certain other requirements for commercial or research rice fields in that area, such as plant removal and destruction, mite cultural control measures, or plant waste material disposal.
- Any regulatory treatments used to control PRM or herbicides used to treat plants will be labeled for that use or exemptions will be in place to allow the use of other materials.
- Establishing a Federal Regulatory Area or Action** Regulatory actions undertaken using EAN's continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfestation orders or longer term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers, and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.
- Results analyzed from investigations, testing, and risk assessment will determine the area to be designated for a federal and parallel state regulatory action. Risk factors will take into account positive testing, positive associated, and potentially infested exposed plants. Boundaries drawn may include a buffer area determined based on risk factors and epidemiology.
- Removing Areas from Regulatory Control** If investigations determine the regulatory restrictions on fields are adhered to over the prescribed time periods, actions are documented and fields can be released from regulatory restrictions. Notify growers that their fields may be subject to additional monitoring by State or Federal officials for the presence of PRM. Furthermore, permit requirements for rice greenhouses may be changed to include additional seed treatments to prevent accidental introduction of PRM to the field.
- Regulatory Records** Maintain standardized regulatory records and database(s) in sufficient detail to carry out an effective, efficient, and responsible regulatory program.
- Use of Chemicals** The PPQ Treatment Manual and this Guideline identify the authorized chemicals, and describe the methods and rates of application, and any special application instructions. See the Control section for more information. Concurrence by PPQ is necessary before using any other chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for Panicle Rice Mite.

## CONTROL PROCEDURES

<b>Overview</b>	Plant Protection and Quarantine develops and makes control measures available to involved states. Environmental Protection Agency (EPA) approved and labeled treatments will be recommended when available. If additional treatments selected are not labeled for use against the organism or in a particular environment, an emergency exemption can be requested and obtained under Section 18, or 24(c), special local need (SLN), of FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act), as amended.
<b>Control Decisions and Oversight</b>	<p>All regulatory actions related to destruction are to be witnessed, supervised, and documented by a federal and/or state plant regulatory official whenever possible. Proper supervision and documentation of destruction of infected plant material is critical. If a PPQ representative is not available, a State cooperating inspector can witness and document the disposal.</p> <p>The control measures are currently divided into two different segments. Control of PRM in the field and Greenhouse treatment and eradication of PRM.</p>
<b>Control in Research and Commercial Rice Fields</b>	<p><b><i>Grain (rice for consumption):</i></b></p> <ol style="list-style-type: none"> <li>1. Harvest grain from EAN fields. Implement safeguards for personnel working in the field and at the processor to minimize dispersal of mites. Sanitize harvesting equipment after EAN field harvest is completed or at the end of the work day, whichever is first.</li> <li>2. Safeguard all aspects of transportation of grain.</li> <li>3. Sanitize equipment used to transport, unload, and process the rice from infested fields. Sanitization should be by either high pressure washing or steam treatment (preferred).</li> <li>4. Process the grain, including the hot air process, whereby the rough rice enters a concrete tumbler dryer which has hot air forced through it. Ideally, seed storage conditions should allow for seed to be maintained at a moisture content of 14% moisture or less. The temperature of seed is maintained at or near 100° F with humidity in the tumbler below 40% relative humidity (i.e. temperatures and relative humidity appropriate for commercial grain drying).</li> <li>5. Hulls should not be introduced (e.g., spread as a mulch) back into the field. Dispose of rice hulls by deep burial at a minimum depth of six feet. Seed processing by-products must be safeguarded prior to and during transport for disposal.</li> </ol>

Exposure to this level of heat for this time period is expected to kill any mites that are associated with the grain, and no further treatment of the processed grain or grain by-products is necessary. Processed grain and grain by-products will be sampled to confirm that hot air processing killed *S. spinki* from infested fields.

6. Sampling of processed grain and grain by-products harvested from each field:

1. For grain:
  - a. Randomly take 10- 50 g samples of processed grain to make up one approximately 500 g composite per field.
  - b. Three sub-samples are drawn from these composites to confirm effectiveness of processing to kill *S. spinki* on harvested grain.
2. For grain by-products (hulls, bran, and defective grains):
  - a. Randomly take 10- 50 g samples of each by-product to create one 500 g composite of hulls, one 500 g composite of bran, and one 500 g composite of defective grains per field.
  - b. Three sub-samples are drawn from these composites to confirm effectiveness of processing to kill *S. spinki* on grain by-products.

***Seed (rice for planting, for either production or research use):***

1. Harvest seed from the EAN fields. Implement safeguards for personnel working in the field and at the processor to minimize dispersal of mites.
2. Safeguard all aspects of transportation of grain. Sanitize equipment used to transport, unload, and process the rice (if this equipment will be used again for non-infested grain or for seed). Sanitization should be by either high pressure washing or steam treatment (preferred)
3. Process the rice according to standard processing practices.
4. Treat seed (in individual, gas-permeable bags) by **ONE** of the following methods (A through D):

**A. Phosphine treatment:**

In recent efficacy trials, rice stems infested with live *S. spinki* were exposed to phosphine\* at an initial dosage of 30-90g/1000 ft<sup>3</sup>, at NAP (normal atmospheric pressure) in a chamber at > 83°F for 72 hrs. To be effective, phosphine should be applied at a rate in the range of 750 to 2250 ppm/1000 ft<sup>3</sup> at the discretion of the fumigator dependent on the leakage of the fumigation structure. Treatment

concentration readings should not fall below the minimum 350 ppm/1000 ft<sup>3</sup> over the 72 hours (readings should be taken at 24 48 and 72 hrs to document treatment).

No live mites were retrieved after phosphine treatment, nor were live mites detected after 6 days of incubation after treatment, indicating phosphine's effectiveness on adults, nymphs and eggs of *S. spinki*. Live mites were detected in untreated infested control stems up to 6 days after collection and initiation of experiments. The treatment would likely be similarly effective if used to treat infested rice seed, although experimental evidence for seed is not yet available. During fumigation, sacks of seed should be elevated off of the floor level and placed on pallets in a single layer to facilitate even application of the fumigant.

\* The intent is to allow flexibility in the form of phosphine used for rice fumigation. Fumigators may use either the Aluminum or Magnesium forms of phosphine applied in gas, liquid or tablet form, as long as the guidelines for treatment outlined are met.

#### **B. Methyl bromide treatment:**

Rice stems infested with live *S. spinki* were treated with methyl bromide at 1.25 lbs /1000 ft<sup>3</sup>, at NAP (normal atmospheric pressure) in a chamber, for 12 hours at > 80 °F. Non-infested rice seed were exposed to each methyl bromide treatment to assess impact on germination. Results of germination tests are still pending. No live mites were retrieved after methyl bromide treatment or after 6 days of incubation after treatment, demonstrating methyl bromide's effectiveness on adults, nymphs and eggs of *S. spinki*. Live mites were detected in untreated infested control stems up to 6 days after collection and initiation of experiments. The treatment would likely be similarly effective if used to treat infested rice seed, although experimental evidence for seed is not yet available.

Methyl bromide treatment should be applied when the seed's moisture content is between 14.2% and 8.9% ensuring a germination rate of between 93% and 92%, respectively (see Table 6-1 for higher temperature recommendations and rates). During fumigation, sacks of seed should be elevated off of the floor level and placed on pallets in a single layer to facilitate even application of the fumigant.



**Table 6-1: Methyl Bromide Fumigation of Rice Seed: Recommendations for Control of Insect Pests\***

Temperature	Dosage rate (lb/ 1,000 ft <sup>3</sup> )	Duration	Minimum concentration readings (ounces) at:	
			Seed moisture %	Germination %
50°F	5 lbs	12 hrs	17.0	9
50°F	5 lbs	12 hrs	14.2	93
50°F	5 lbs	12 hrs	8.9	92
51-65°F	4 lbs	12 hrs	17.0	27
51-65°F	4 lbs	12 hrs	14.2	95
51-65°F	4 lbs	12 hrs	8.9	94
≥80°F	1.25 lbs	12 hrs	---	80

\* In the interest of seed quality, the Treatment Quality Assurance Unit (TQAU) recommends all rice varieties be tested using the recommended Methyl Bromide treatments. These treatment recommendations are not for bulk seed. Since these treatments have not been tested for *Steneotarsonemus spinki* Smiley, the TQAU does not accept legal responsibility for damage to rice seed or control failure resulting from the above recommended treatments.

**C. Cold treatment:** Rice stems infested with live *S. spinki* were treated at -8 °C for 72 hours. No live mites were retrieved after cold treatment or after 6 days of incubation after treatment, demonstrating the cold treatment effectiveness on adults, nymphs and eggs of *S. spinki*. Live mites were detected in untreated infested control stems up to 6 days after collection and initiation of experiments. The treatment would likely be similarly effective if used to treat infested rice seed, although experimental evidence for seed is not yet available. This treatment would likely be most feasible for small scale seed treatment.

**D. Storage at low relative humidity:** Hold seed at the storage facility in proximity to where it has been processed for a minimum of three months. Ideally, seed storage conditions should allow for seed to be maintained at a moisture content at no more than 14% moisture. If seed is to be moved to a storage facility at a different location from where it has been processed, then the seed must be moved in a covered vehicle with the vehicle being cleaned after delivery.

1. Processed seed will be sampled to confirm that the chosen treatment killed *S. spinki* from each of the infested fields.
  - a. From each field, randomly take 10- 50 g samples of processed seed to make up one approximately 500 g composite per field.
  - b. Three sub-samples are drawn from these composites to confirm effectiveness of processing to kill *S. spinki* on harvested grain and grain by-products.
2. Implement safeguarding measures to prevent re-infestation of treated seed.
3. Ship seed as needed.
4. Dispose of seed processing by-products, including, but not limited to rice hulls, sweeps, broken and heavy grain by deep burial. Deep burial will be at a minimum depth of six feet. Safeguard by-products of seed processing prior to and during transport for disposal.
5. Use appropriate sanitation for equipment used to harvest, till, etc. to prevent re-infestation of the crop. Sanitization should be by either high pressure washing or steam treatment (preferred). Also, personnel working in the field and at the processor should change their outer clothes (or utilize disposable outerwear, such as Tyvek suits) or spray themselves with 70% ethanol when working in areas/materials that were previously exposed to mites to minimize the potential of dispersal. Furthermore, workers should avoid entering “clean” fields (fields not known to contain *S. spinki*) once they have entered a greenhouse or field previously found positive for *S. spinki* without taking the appropriate safeguards.

#### ***Fields (positive grain and seed fields)***

1. Burn if possible then disk the stubble soon after harvest where the soil can be worked. Repeat disking at two-week intervals as needed to further break down stubble and kill volunteer plants and weeds.
2. Establish a host free area (buffer) at a minimum of 25 feet around the perimeter of each positive field, a greater distance is preferred when possible.
3. Control volunteer rice plants and alternate hosts (*Oryza sativa*, *Oryza latifolia* (red rice or wild red rice) and *Cyperus iria*) (if present), by applying an appropriate herbicide to the field and buffer area or by other mechanical means where chemicals are not permitted.
4. Ideally, do not plant rice after rice or use ratoon cropping.
5. Fields should be fallow and free of rice or alternate hosts of *S. spinki* for a minimum of 3 months. Rotate rice with a

non-host crop, (*i.e.*, soybean, grain sorghum, etc.) or leave the field fallow for three months or longer. Scout fields at regular intervals during the fallow period to assure that no *S. spinki* hosts are growing.\*

6. Use appropriate sanitation for equipment used to harvest, till, etc. to prevent re-infestation of the crop. Sanitization should be by either high pressure washing or steam treatment (preferred). Also, personnel working in the field and at the processor should change their outer clothes\*\* or spray themselves with 70% ethanol when working in areas/materials that were previously exposed to mites to minimize the potential of dispersal. Furthermore, workers should avoid entering “clean” fields (fields not known to contain *S. spinki*) once they have entered a greenhouse or field previously found positive for *S. spinki* without taking the appropriate safeguards.

*\*Prevention and Scouting:* Personnel working in fields and greenhouses should be made aware of the mite and the symptoms it can cause on rice. Training materials should be designed for *S. spinki* early detection to be used.

*\*\*Clothes should be washed (hot water, long cycle >10 min.) after exposure to fields previously found positive for *S. spinki* before re-using them.*

### Control in Greenhouses

1. Safeguard infested facilities by posting notices at entrances about the infestation. Restrict movement of personnel, plant material, and equipment into or out of the greenhouse. Access of personnel to infested greenhouses should be restricted, except if precautions are taken to prevent moving *S. spinki* outside of the infested greenhouse.
2. Seed may be harvested but must be treated according to instructions below for ***Seed (rice for planting, for either production or research use)***.
3. After harvest, remove and dispose of harvested plant material, other than seed, by bagging and autoclaving, double bagging and deep burial, or by incineration.
4. Items in the infested greenhouse such as pots, tools, labcoats, etc. that may harbor *S. spinki* should be cleaned and disinfected to eliminate the mite.
5. Disinfest the entire facility by completing steps 1-4 under this section, followed by one of the treatment options listed below (A through C).

#### **A. Plant free period:**

1. Destroy, remove, and dispose of all plant material

and potting medium in the infested greenhouse and within a 5 ft host free buffer zone outside of the infested greenhouse.

2. Treat greenhouse with appropriate, labeled disinfectant.
3. Wait one month before planting hosts of *S. spinki* in these greenhouses to interrupt the life cycle of the mite and prevent re-infestation. During the waiting period, planting of non-infested dicotyledonous plants is permitted.
4. Continue safeguarding measures (see above: Infested Greenhouse steps 1-4) to prevent re-infestation.
5. Only treated seed or seed that did not originate from an infested greenhouse can be used in the greenhouse to prevent re-infestation related to seed source.
6. Continued monitoring for the pest is recommended at the discretion of the greenhouse facility management.

**B. Steam heat treatment (T408-f):**

1. Destroy, remove, and dispose of all plant material and potting medium in the infested greenhouse and within a 5 ft host free buffer zone outside of the infested greenhouse.
2. Continue safeguarding to prevent re-infestation. Steam treatment can be used to treat infested surfaces and equipment
3. Planting of hosts of *S. spinki* may resume after the cumulative exposure time at the minimum temperature has been reached.
4. Only treated seed or seed that did not originate from an infested greenhouse can be used in the greenhouse to prevent re-infestation related to seed source
5. Continued monitoring for the pest is also recommended, at the discretion of the greenhouse facility management.

**C. Methyl bromide treatment:**

Fumigate the greenhouse with methyl bromide to eliminate remaining mites in the structure. The methyl bromide treatment recommended to provide quarantine security is T403-e-1-1 (Table 6-2). The agency does not have any mortality data to reduce the exposure times listed on this treatment. Treatment rate is dependent upon temperatures during exposure. Treatment duration is 12 hours.

**Table 6-2: USDA APHIS PPQ Treatment T403-e-1-1: Methyl bromide (“Q” label only) at NAP (Normal atmospheric pressure) under tarpaulin**

<i>Temperature</i>	<i>Dosage rate (lb/ 1,000 ft<sup>3</sup>)</i>	<i>Minimum concentration readings (ounces) at:</i>		
		<i>0.5 hrs</i>	<i>2.0 hrs</i>	<i>12 hrs</i>
90°F or above	2.5 lbs	30	20	15
80-89 °F	3.5 lbs	42	30	20
70-79 °F	4.5 lbs	54	40	25
60-69 °F	6.0 lbs	72	50	30
50-59 °F	7.5 lbs	90	60	35
40-49 °F	9.0 lbs	108	70	40

### Control Records

Also attach any documentation, receipts, etc. that document these actions.

Program personnel must maintain records and maps noting the locations of all detections, the number and type plants subjected to control actions, and the materials and formulations used in each treated area. Attach all documentation to the office EAN copy.

**ENVIRONMENTAL COMPLIANCE**

- Overview** A key element in designing a program or an emergency response is consultation with Environmental Services (ES), a unit of APHIS' Policy and Program Development Staff (PPD). ES prepares environmental documentation such as environmental impact statements (EIS) and environmental assessments (EA) to aid in program operational decisions, as well as Endangered Species consultation. ES also coordinates pesticide registration and approvals for APHIS pest control and eradication programs, ensuring that registrations and approvals meet program needs and conform to pesticide use requirements. Refer to the Resources Section of this document for additional information.
- Disclaimer** All uses of pesticides must be registered or approved by appropriate Federal, State, and/or Tribal agencies before they can be applied. The information provided on pesticide labels may not reflect all of the actual information, including precautions and instructions for use, which you are required to follow in your specific State or locality. It is the responsibility of persons intending to use a pesticide to read and abide by the label, including labeling approved for the particular State or locality in which the chemical is to be used, and to comply with all Federal, State, Tribal, and local laws and regulations relating to the use of the pesticide. APHIS program staffs are responsible for their compliance with applicable environmental regulations.
- National Environmental Policy Act** Agencies should prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) concurrently and integrated with environmental impact analyses, surveys, and studies required by the Fish and Wildlife Coordination Act, National Historic Preservation Act of 1966, Endangered Species Act, and other laws and executive orders. Environmental documents prepared to comply with other acts also may be incorporated into National Environmental Policy Act (NEPA) documents as part of the NEPA process.
- Categorical Exclusion** Categorical exclusions (CE) are categories of actions that do not have a significant effect on the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is generally required.
- APHIS managers are encouraged to use categorical exclusions where appropriate to reduce paperwork and speed up decision making. Proposed actions are subject to sufficient environmental

review to determine whether they fall within the broadly defined categories. Each time a specific categorical exclusion is used, the required review must be done. An EA may be prepared for proposed actions otherwise excluded when the manager determines that the action may have potential to significantly affect the environment or an EA would be helpful in planning or decision making.

**Environmental Impact Statement**

An environmental impact statement (EIS) is a detailed statement that must be included in every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment. The primary purpose of an EIS is to serve as an action-forcing device to insure that the policies and goals defined in the National Environmental Policy Act (NEPA) are infused into the ongoing programs and actions of the Federal government. Generally, EIS's are prepared when Federal agencies recognize that their actions have the potential for significant environmental effects (adverse or beneficial), or when an environmental assessment leads to a finding of potentially significant impact.

APHIS prepares EIS's for administrative proceedings that establish broad scale significant impact-generating strategies, methods, or techniques such as large-scale aerial pesticide applications. This can include contingency or emergency strategies that are comprehensive in scope or long-range plans with potential for significant environmental impact. APHIS also prepares programmatic EIS's to examine strategies and options for dealing with issues with important implications for the maintenance and enhancement of environmental quality.

**Environmental Assessment**

An environmental assessment (EA) is a concise public document that briefly provides sufficient evidence and analysis for determining whether to prepare an environmental impact statement (EIS) or a finding of no significant impact (FONSI). An EA aids an agency's compliance with the National Environmental Policy Act (NEPA) when no EIS is necessary and facilitates the preparation of an EIS when necessary. Generally, an EA leads to a FONSI or an EIS, but it could also lead to abandonment of a proposed action.

The content of an EA must include brief discussions of the need, alternatives, and potential environmental impacts of the proposal, and a list of agencies and persons consulted.

**Environmental Monitoring**

PPQ requests assistance from ES before PPQ personnel or funding are used for control operations. Additionally, program staff should

consult with PPQ EDP Environmental Monitoring staff (Appendix G) to determine if an environmental monitoring plan is required for the operation. State, regional, and national program managers determine counties where treatments may be needed.

Program personnel should evaluate the need for and success of biological control agents and herbicide treatments used in eradication or suppression of the target Foreign Noxious Weed or host weeds and avoid damage to non-target plants.

**Biological  
Assessment**

A biological assessment (BA) is an analysis of the effects that a Federal agency action may have on listed or proposed endangered or threatened species and designated critical habitat. The Endangered Species Act (ESA) requires this analysis if the proposed action may affect a listed species. In such a case, consultation with the U.S. Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS) is required. Federal agencies are required to insure that any action authorized, funded, or carried out is not likely to jeopardize listed species or result in adverse modification of designated critical habitat.



## SCIENTIFIC RESEARCH

### Overview

Another very important part of the New Pest Response Guidelines is determining what is not known about the pest. Once these research areas are identified, additional funding may be secured to evaluate the potential effectiveness of these different areas on control of PRM.

Panicle Rice Mite is a regulated pest. If research is to be conducted on this pest, a permit is required. See Appendix G for contact information.

### Research Needs

- Definitive information regarding lethal temperature
- Effects of temperature on all life stages
- Effective chemical treatments for all PRM life stages
- Genetic resistance/Tolerance to PRM
- Role of “Transient” hosts - other plants that some life stages of *S. spinki* can live on. Are they bridging hosts while fields are free of *Oryza* spp?

**DEFINITIONS**

<b>Delimiting Survey</b>	After the initial first detection in an area, this type of survey is conducted to define the geographic range of the infection/infestation.
<b>General Detection Survey</b>	A survey conducted over a large area to discover new potential infestations/infections in areas where the pest/disease is not known to occur.
<b>Host Plant</b>	A plant which is invaded by a parasite or pathogen and from which it obtains its nutrients.
<b>Identification Authority</b>	Authority to confirm the presence of a particular pest organism issued by the APHIS National Identification Services to diagnosticians that have demonstrated proficiency in identifying.
<b>Incident Command System</b>	An expandable and contractible system to manage emergencies, based on the Forest Service's Forest Fire Management System.
<b>Monitoring or Evaluation Survey</b>	A survey conducted at a site where a disease was found and where an eradication program is being performed.
<b>Parthenogenesis</b>	Development of an unfertilized egg into an adult female. This type of asexual reproduction occurs in many different types of invertebrate animals, including the panicle rice mite.
<b>Pathogen</b>	Any organism that can incite a disease.
<b>Potentially Actionable Suspect Sample</b>	Also know as PASS, a suspect positive sample diagnosed or identified by provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation.
<b>Suspect Positive</b>	Such a result may require confirmatory testing if the sample is a PASS sample.
<b>Symptom</b>	The external and internal reactions or alterations of a plant as the result of a pest, pathogen, environmental effect or injury.
<b>Targeted Survey</b>	Choosing an area, usually residential, to concentrate surveys based on known pathway information with zipcode-based demographic information or other scientifically-based information, also known as a "hot zone" survey.
<b>Trace-back</b>	To investigate the origin of infested plants from initial detection

location back through intermediate steps in commercial distribution channels to the origin.

**Trace-forward**

To investigate where infected plants may have been distributed from a known infestation through steps in commercial distribution channels or wholesale or retail procurement.

DRAFT

**APPENDIX A. SAMPLE PROCESSING AND SHIPPING****Collection and Preparation of PRM Specimens**

Collect as many specimens, adults and nymphs, as possible for identification, by the local designated identifier and/or subsequent analysis for presence of the mite. Do not mix samples. Be sure to separate mites into vials by greenhouse or field location. Prepare the PPQ form 391 (Specimens for Determination) and be sure to include information as noted:

- date of collection,
- sample number from predetermined numbering system,
- collector's name and affiliation,
- Full name of Business/Institution/Agency,
- full address, including county and State,
- type of property (i.e., commercial field, research field, greenhouse),
- grower's field or greenhouse ID numbers if appropriate,
- GPS coordinates of the host plant and property,
- host species, and cultivar,
- general conditions or any other relevant information,
- positive or negative results from testing (recorded later).

Prepare specimens according to the following protocols:

- ***Only send extracted samples. No plant material. The PIS does not have the required permits nor containment required for processing plant samples for PRM. Check with the regional office to determine appropriate laboratories to conduct necessary sample extractions.***
- gather nymphs/adults from the host plant, place in the same vial;
- label the vial with a sample number, date, locale, etc.;
- preserve the mites in 70% ethyl alcohol (95% for PCR analysis);
- Fed-Ex vials in well-padded box, with absorbent materials in case of vial breakage or leaks, and place in a Ziploc bag;
- include a completed PPQ form 523 (with the submitter's e-mail address on the form).

Submit specimens to your state or cooperating university entomologist for screening. When the suspect specimen represents a potentially new detection for a state, please forward to the appropriate specialist for confirmation (Table B.1). Include PPQ Form 391 (Specimens for Determination) marked "Urgent" (see PPQ General Operational Procedures Manual M390.500) with all specimens.

**Panicle Rice Mite Extraction**

The objective of this method is to remove mites from rice plants collected from the field or greenhouse for later identification by

**Protocol**

qualified identifiers. To accomplish this, the following materials will be needed:

- Standard sieves
  - #10 to #24 for debris
  - #60 for middle
  - #400 for bottom
- Sink with spray attachment
- 70% ethanol squirt bottle
- Watch glass or Petri dishes
- 5 Gallon bucket
- Tray for cutting plant material
- Tub
- Latex gloves
- Exacto knife or razor
- Quarantine/autoclave garbage receptacle
- Bleach disinfectant
- Spray bottles
- Pipette, hog-hair bristle brush, probe
- Vials for storage and shipment

Samples should be stored in a cooler at 4-10 degrees C, labeled with appropriate field and sample information, and logged in appropriately. Samples will need to be logged out and taken to the lab.

The rice panicle and leaf tissue will need to be cut off the stem and separated longitudinally into 3-4 inch segments. Place all cut tissue in a disinfected 5 gallon bucket. The bucket can be loosely filled to approximately halfway. Fill bucket approximately ½ full of water. Carefully mix the leaf and stem material with your hands for 1-2 minutes. Stack sieves in the sink, with the 400 on bottom, the 60 in the middle and the largest sieve on top to catch the large debris pieces. Pour the mixture, including the large pieces, into the top sieve. Allow the plant/water mixture to drain through the sieves and then spray hot water into the top sieve at medium pressure for 2 minutes.

After the water has gone through the sieves, collect the debris from the bottom sieve using 70% ethanol squirt bottle. Rinse the debris to the bottom of one side then backwash the sieve to dislodge any mites that might be attached to the # 400 sieve. Rinse all debris into a Petri dish or watch glass with the 70% ethanol. Approximately half of the mites will float in the surface film of the 70% alcohol. Mites should be transferred to a vial for later identification.

**Shipment and Screening of**

If no mite screening capability exists in a state (PPQ identifiers or state cooperators):

**Suspect mite  
Samples**

1. Perform the extraction following the extraction instructions outlined above;
2. Complete a PPQ form 391 for each sample;
3. Send with form by over night delivery all mites extracted in tightly sealed in a vial of 75% ethyl alcohol with no plant material.

To: Eric M. McDonald  
 USDA, APHIS, PPQ  
 Plant Inspection Station  
 19581 Lee Road.  
 Humble, TX 77338

Tel: 281-230-7204

Fax: 281-230-7203

If screening and identifier capability *does* exist in a state:

1. Perform the extraction following the extraction instructions outlined above;
2. Screen the extracted samples to remove suspect mites;
3. Only send *Steneotarsonemus* spp., with forms, preferably mounted on slides or in tightly sealed in a vial of 70% ethyl alcohol with no plant material;
4. To identifiers at the same address as above with PPQ form 391's.

**Communication**For states which already have confirmed detections of *Steneotarsonemus spinki*:

1. Eric McDonald will make determinations and communicate them to,
2. National Identification Service (NIS) who will notify:
3. Emergency and Domestic Programs (EDP) to forward the determinations to the national and region program managers, SPHD and SPRO.
4. The SPHD or SPRO should be responsible for communicating the determination back to the originating laboratory or identifier.

For new state suspect detections:

1. Eric McDonald will forward the specimens to SEL specialist for final determination.
2. SEL will identify and inform NIS
3. NIS to EDP and to the national/regional program manager, SPHD, and SPRO.

The SPHD or SPRO should be responsible for communicating the determination back to the originating laboratory or identifier.

**APPENDIX B. IDENTIFICATION AID****Identification Guide**

Only those individuals with specific training to identify Tarsonemid mites should attempt identification of these mites to species. Any extracted sample with Tarsonemid mites should be sent to the identifiers as outlined in the Appendix A. Ethan Kane of the USDA APHIS PPQ NIS has produced a guide to identification that is available on the USDA PRM website at:

[www.aphis.usda.gov/plant\\_health/Plant\\_pest\\_info/rice\\_mite/index.shtml](http://www.aphis.usda.gov/plant_health/Plant_pest_info/rice_mite/index.shtml)

**Identifying characteristics**

*S. spinki* are small mites (~200-275  $\mu\text{m}$ ) with an overall morphology typical of the Tarsonemidae. The color of the mites range from pale white to a darker yellow depending on life stage or feeding conditions. Males are distinguished by their smaller size than the females and a highly modified pair of hind legs.

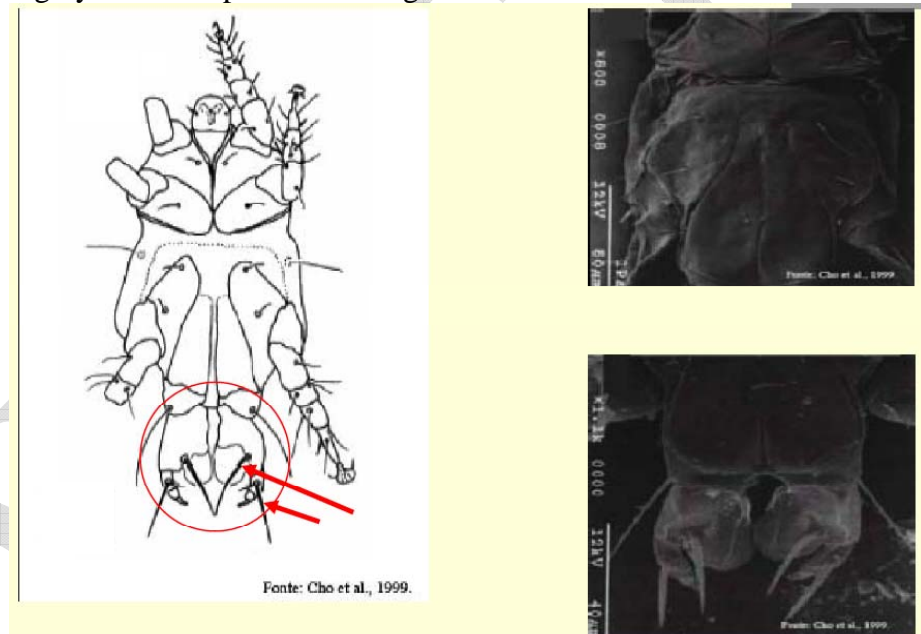


Figure B-1. Line drawing and electromicrographs of male *Steneotarsonemus spinki*, with emphasis on the Leg IV.

## APPENDIX C. DISINFECTION PROCEDURES

**Overview** Any tools used to cut samples should be disinfested prior to use on a new field or greenhouse to avoid spreading the mites from one location to another.

A spray, or brief immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (common household liquid bleach) or 70% ethanol are effective ways to inactivate mites and prevent their spread.

**CAUTION:** Household liquid bleach is caustic. Avoid spilling or splashing it onto skin, eyes, or clothing. Note all precautions on the bleach container label.

**Usage Instructions** Use a fresh bottle of household bleach, since the efficacy of sodium hypochlorite solutions is reduced over time. The bleach label should indicate the concentration of sodium hypochlorite in the bleach. Use a brand of liquid bleach which contains at least a 5.25% sodium hypochlorite solution (this is a common strength). If stronger, the bleach will need to be diluted with water to achieve a final working concentration of 5% sodium hypochlorite.

Use a thick-walled non-breakable plastic container for the bleach solution, with a top opening large enough to easily dip the cutting surfaces of the tools into the bleach. Alternatively, the bleach can be kept in a spray bottle. If a dipping method is used, pour sufficient bleach into the container to allow easy dipping of the cutting surfaces of the tools. The bleach should be replaced every 2-3 hours, as it “breaks down” when exposed to sunlight and organic matter.

When sampling is completed for the day, disinfect the tools by dipping in the bleach and then rinse thoroughly with water to remove all bleach solution. To minimize corrosive effects of the bleach on the cutting tool, dry the equipment after the water rinse and coat the cutting surfaces with a thin film of lubricating oil.



# Appendix D. Forms

# Panicle Rice Mite

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make and accurate record of plant pest conditions.

**FORM APPROVED**  
**OMB NO. 0579-0010**

<b>U.S. DEPARTMENT OF AGRICULTURE</b> <b>ANIMAL AND PLANT HEALTH INSPECTION SERVICE</b>  <b>SPECIMENS FOR DETERMINATION</b>		Instructions: Type or print information requested. Pres hard and print legibly when handwritten. Item 1 assign number for each collection beginning with the year, followed by collector's initials and collector's number. Example (collector, John J. Dingle); 83-JJD-001. <u>Pest Data Section</u> – Complete Items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete Items 17 and 18 if a trap was used.					<b>FOR IIBIII USE</b> Lot No. _____  PRIORITY _____				
1. COLLECTION NUMBER		2. DATE MO   DA   YR			3. SUBMITTING AGENCY						
SENDER AND ORIGIN	4. NAME OF SENDER				INTERCEPTION SITE	5. TYPE OF PROPERTY (Farm, Feedmill, Nursery, etc.)					
	6. ADDRESS OF SENDER					7. NAME AND ADDRESS OF PROPERTY OR OWNER					
	CITY, STATE					COUNTRY/COUNTY					
	ZIP										
PURPOSE	8. REASON FOR IDENTIFICATION ("X" ALL Applicable Items)										
	A. <input type="checkbox"/> Biological Control (Target Pest Name )				E. <input type="checkbox"/> Livestock, Domestic Animal Pest						
	B. <input type="checkbox"/> Damaging Crops/Plants				F. <input type="checkbox"/> Possible Immigrant (Explain in Remarks)						
	C. <input type="checkbox"/> Suspected Pest of Regulatory Concern (Explain in Remarks)				G. <input type="checkbox"/> Survey (Explain in Remarks)						
D. <input type="checkbox"/> Stored Product Pest				H. <input type="checkbox"/> Other (Explain in Remarks)							
9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS"											
HOST DATA	10. HOST INFORMATION				11. QUANTITY OF HOST						
	NAME OF HOST (Scientific name when possible)				NUMBER OF ACRES/PLANTS		PLANTS AFFECTED (Insert figure & indicate number or percent)				
							<input type="checkbox"/> Number <input type="checkbox"/> Percent				
	12. PLANT DISTRIBUTION				13. PLANT PARTS AFFECTED						
<input type="checkbox"/> LIMITED <input type="checkbox"/> SCATTERED <input type="checkbox"/> WIDESPREAD				<input type="checkbox"/> Leaves, Upper Surface <input type="checkbox"/> Trunk/Bark <input type="checkbox"/> Bulbs, Tubers, Corns <input type="checkbox"/> Seeds <input type="checkbox"/> Leaves, Lower Surface <input type="checkbox"/> Branches <input type="checkbox"/> Buds <input type="checkbox"/> Petiole <input type="checkbox"/> Growing Tips <input type="checkbox"/> Flowers <input type="checkbox"/> Stem <input type="checkbox"/> Roots <input type="checkbox"/> Fruits or Nuts							
PEST DATA	14. PEST DISTRIBUTION										
	15. <input type="checkbox"/> INSECTS <input type="checkbox"/> NEMATODES <input type="checkbox"/> MOLLUSKS										
	<input type="checkbox"/> FEW <input type="checkbox"/> COMMON <input type="checkbox"/> ABUNDANT <input type="checkbox"/> EXTREME		NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYPHPS	JUVS.	CYSTS
			ALIVE								
			DEAD								
16. SAMPLING METHOD			17. TYPE OF TRAP AND LURE			18. TRAP NUMBER					
19. PLANT PATHOLOGY – PLANT SYMPTOMS ("X" one and describe symptoms)											
<input type="checkbox"/> ISOLATED <input type="checkbox"/> GENERAL											
20. WEED DENSITY				21. WEED GROWTH STAGE							
<input type="checkbox"/> FEW <input type="checkbox"/> SPOTTY <input type="checkbox"/> GENERAL				<input type="checkbox"/> SEEDLING <input type="checkbox"/> VEGETATIVE <input type="checkbox"/> FLOWERING/FRUITING <input type="checkbox"/> MATURE							
22. REMARKS											
23. TENTATIVE DETERMINATION											
24. DETERMINATION AND NOTES (Not for Field Use)							FOR IIBII USE				
							DATE RECEIVED				
							NO.				
							LABEL				
							SORTED				
							PREPARED				
							DATE ACCEPTED				
							RR				
SIGNATURE _____					DATE _____						

PPQ FORM 391 Previous editions are obsolete. (JUL 86)

PPQ Form 523, Emergency Action Notification  
<http://www.aphis.usda.gov/library/forms/pdf/ppq523.pdf>

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. **FORM APPROVED - OMB NO. 0579-0102**

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE PLANT PROTECTION AND QUARANTINE  <b>EMERGENCY ACTION NOTIFICATION</b>	SERIAL NO. 1. PPQ LOCATION 2. DATE ISSUED 4. LOCATION OF ARTICLES 5. DESTINATION OF ARTICLES 7. NAME OF CARRIER 8. SHIPMENT ID NO.(S) 10. PORT OF LADING 11. DATE OF ARRIVAL 12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S) 12a. PEST ID NO. 12b. DATE INTERCEPTED 13. COUNTRY OF ORIGIN 14. GROWER NO. 15. FOREIGN CERTIFICATE NO. 15a. PLACE ISSUED 15b. DATE
3. NAME AND QUANTITY OF ARTICLE(S)	
6. SHIPPER	
9. OWNER/CONSIGNEE OF ARTICLES  Name: _____  Address: _____  _____  PHONE NO. _____ FAX NO. _____  SS NO. _____ TAX ID NO. _____	

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and/or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

**AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:**

16. ACTION REQUIRED

TREATMENT: \_\_\_\_\_

RE-EXPORTATION: \_\_\_\_\_

DESTRUCTION: \_\_\_\_\_

OTHER: \_\_\_\_\_

**Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.**

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):	18. SIGNATURE OF OFFICER:
--	---------------------------

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION  
*I hereby acknowledge receipt of the foregoing notification.*

SIGNATURE AND TITLE: _____	DATE AND TIME: _____
----------------------------	----------------------

19. REVOCATION OF NOTIFICATION

ACTION TAKEN: \_\_\_\_\_

SIGNATURE OF OFFICER: _____	DATE: _____
-----------------------------	-------------

**APPENDIX E. TREATMENTS FOR CONTROL**

Methyl bromide treatment should be applied when the seed's moisture content is between 14.2% and 8.9% ensuring a germination rate of between 93% and 92%, respectively (see Table E-1 for higher temperature recommendations and rates). During fumigation, sacks of seed should be elevated off of the floor level and placed on pallets in a single layer to facilitate even application of the fumigant.

**Table E-1: Methyl Bromide Fumigation of Rice Seed: Recommendations for Control of Insect Pests**

Temperature	Dosage rate (lb/ 1,000 ft <sup>3</sup> )	Minimum concentration readings (ounces) at:		
		Duration	Seed moisture %	Germination %
50°F	5 lbs	12 hrs	17.0	9
50°F	5 lbs	12 hrs	14.2	93
50°F	5 lbs	12 hrs	8.9	92
51-65°F	4 lbs	12 hrs	17.0	27
51-65°F	4 lbs	12 hrs	14.2	95
51-65°F	4 lbs	12 hrs	8.9	94
≥80°F	1.25 lbs	12 hrs	---	80

The Treatment Quality Assurance Unit is concerned with the quality of rice seed which is treated with Methyl Bromide when all varieties have not been tested with the recommended treatment. Phosphine is currently being evaluated by TQAU for efficacy on PRM.

Table E-2. The following chemicals have been labeled for use in **greenhouses to control mites**. *Please note that there is currently nothing labeled by the EPA for use on panicle rice mite in the United States.* Also, check with state registration agencies to

# of Products	Active Ingredient(s)	Chemical Code	Comments
1	Abamectin	122804	
2	Azadirachtin	121701	
2	Beauveria bassiana GHA	128924	Not a quarantine treatment
1	Clarified hydrophobic neem oil	25007	Not a quarantine treatment
2	Dihydroazadirachtin	121702	
5	Piperonyl butoxide and Pyrethrins	67501 & 69001	

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**APPENDIX G. RESOURCES****Diagnostic Tools and Equipment**

Sieves and other equipment

Ben Meadows            <http://www.benmeadows.com/store/item/134572/>

Fisher Scientific        <http://www.fishersci.com>

VWR                        [http://vwrlabshop.com/product.asp\\_Q\\_pn\\_E\\_0012572](http://vwrlabshop.com/product.asp_Q_pn_E_0012572)

**Environmental Compliance**

Susan J. O'Toole  
USDA, APHIS, PPQ  
Environmental Services  
Pesticide Labeling Issues  
4700 River Road  
Riverdale, MD 20737  
Telephone: (301) 734-5861

Robert Baca  
USDA, APHIS, PPQ  
Environmental Monitoring  
4700 River Road  
Riverdale, MD 20737  
Telephone: (301) 734-7175

**PPQ Permits**

[http://www.aphis.usda.gov/permits/ppq\\_epermits.shtml](http://www.aphis.usda.gov/permits/ppq_epermits.shtml)

To use ePermits, you must have a user id and password provided by the USDA eAuthentication system. USDA agencies use USDA eAuthentication to enable customers to obtain accounts that will allow them to access USDA Web applications and services via the Internet.

The USDA eAuthentication system supports different levels of authentication. ePermits currently requires all users to register for Level 2 eAuthentication.

**APPENDIX H. GENERAL WORKSHEET FOR ISIS DATA ENTRY**

April 4, 2008

The Integrated Survey Information System (ISIS) as a field data collection tool is not required. However, operationally specific data is of great importance and the ISIS application should be utilized as a centralized database for information.

The ISIS database is housed inside the APHIS network and is accessible to employees who have direct access to the APHIS network and to co-operators with APHIS Virtual Private Network (VPN) accounts. After receiving a username and password for ISIS, users can log into the system and utilize any of the three data entry tools. These tools include; a web interface, a web upload tool, and a Personal Digital Assistant (PDA) software application.

Users are encouraged to access the PDA portion of ISIS. Organizations utilizing methods other than a PDA (paper, spread sheets, or third party software platforms) can enter data directly into the web interface or “bulk” upload data from flat file spread sheets using the web upload tool.

The ISIS team is always available to discuss end user needs and/or other solutions available regarding data collection and data management issues. Assistance and support is available from the ISIS help desk.

**National Support**

[ISIS.Support@aphis.usda.gov](mailto:ISIS.Support@aphis.usda.gov)

1-866-910-9091

**ER ISIS Support**

LaWan A. Foster

[lawan.a.foster@aphis.usda.gov](mailto:lawan.a.foster@aphis.usda.gov)

919-855-7754

**WR ISIS Support**

Ryan J. Reynolds

[ryan.j.reynolds@aphis.usda.gov](mailto:ryan.j.reynolds@aphis.usda.gov)

970-494-7557

**ISIS**  
Integrated Survey Information System VERSION

Users | Designs | Data | Report John | Logout

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**PRM\_Test102**

**Main**

SurveyDate: 4/4/2008  
 TargetPest: ILATAIA: RICE PANICLE MITE, STENEOTARSONEMUS SPINKI  
 Comments: Comments  
 TemplateVersion: 2

**Site**

Location/Site\_Name: San Francisco  
 Location\_ID: 102  
 Contact\_Name: Slate  
 EPASite: 28072: RICE, ORYZA SATIVA  
 CropSituation: 61015: GREENHOUSE  
 AddressLine1: 123 Shade Turn  
 AddressLine2:  
 City: San Francisco  
 State:  
 FIPS:  
 Zip:  
 Latitude: 38.12345  
 Longitude: -83.12345

Location/Site_Name	Location_ID	Contact_Name	EPASite	CropSituation	AddressLine1	AddressLine2	City	State
San Francisco	102	Slate	28072: RICE, ORYZA SATIVA	61015: GREENHOUSE	123 Shade Turn		San Francisco	

**Service**

ServiceDate: 4/4/2008  
 Primary\_Surveyor: Sam  
 Service\_Comments: Look in tree

ServiceDate	Primary_Surveyor	Service_Comments
4/4/2008	Sam	Look in tree

**Pest**

Sample\_Taken (Y/N)   
 SurveyMethod: 58: GENERAL PEST OBSERVATION; LAB CONFIRMED  
 EPAPest: ILATAIA: RICE PANICLE MITE, STENEOTARSONEMUS SPINKI

: NEGATIVE (NOT PRESENT IN SAMPLE)  
 3: NEW OR REINTRODUCED IN THE COUNTY  
 4: POSITIVE (PRESENT)

**PestStatus**  
 A: KNOWN TO BE ESTABLISHED  
 1: NEW OR REINTRODUCED IN THE U.S.  
 2: NEW OR REINTRODUCED IN THE STATE  
 B: NOT KNOWN TO BE ESTABLISHED

SampleID: 120  
 Count: 6  
 DescriptorUnits: 376: NURSERY/GREENHOUSE

Sample Taken (Y/N)	SurveyMethod	EPAPest	PestStatus	SampleID	Count	Descriptor
Yes	58: GENERAL PEST OBSERVATION; LAB CONFIRMED	ILATAIA: RICE PANICLE MITE, STENEOTARSONEMUS SPINKI	: NEGATIVE (NOT PRESENT IN SAMPLE);	120	6	376: NURSERY/GREENHOUSE

Assign Record (If using bi-directional synchronization, clicking this box will assign this record to the current user for use on the PDA.)

**APPENDIX I. GENERAL WORKSHEET FOR NAPIS DATA ENTRY**

April 4, 2008

Observation Number	Observation Date [YYYYMMDD]	Data Source
create your own record identifier	(Year)(month)(day) (when found)	11-Federal 13-State 16-Joint

State-County	EPA Site Code	Crop Life Stage	Crop Situation
State & county FIPS codes from reference file	5 digit code from REF-Crop file or 99999 - none specified		61015-greenhouse 28037-field/agronomic

Latitude	Longitude	EPA Pest Code
I L A T A I A ,		
often left blank, but not always		

Pest Life Stage	Pest Status	Survey Method	Quantification
		0 0 0 5 8 ,	
often optional	minimum entry: + present - not found	general pest obs w/lab confirmation	0-negative 1-positive
	1 New or re-introduced USA record 2 New or re-introduced state record 3 New or re-introduced county record		

plus either :  
A - established or  
B - not known to be established

Descriptor Units	Total Units Checked	Positive Units	Observation Duration	Diagnostic Lab
337-field 376-nursery/ greenhouse	1 ,	leave blank	leave blank	code from Ref-Diagnostic-Lab

Confirmation Method	Diagnostic Lab Date (YYYYMMDD)	Biocontrol Target
-- -- -- -- --	-- -- -- -- --	-- -- -- -- --
'	'	'
-- -- -- -- --	-- -- -- -- --	-- -- -- -- --
code from Ref- Survey-Method > 89999	Date of Diagnosis by lab	leave blank

Notes
-- -- -- -- --
-- -- -- -- --
Comments in English or prearranged code about optional information 40 character comment length