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PEST MANAGEMENT

Population Growth and Characterization of Plant Injuries of *Steneotarsonemus spinki* Smiley (Acari: Tarsonemidae) on Rice

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Keywords

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Abstract

Rice is attacked by *Steneotarsonemus spinki* Smiley, a mite that has dispersed throughout many countries causing important loss on rice production. Rice plants of the variety Morelos A-92 were infested with *S. spinki*, and its population growth was estimated along plant development. Further, the morphological and histological injuries associated to the mite attack were characterized. The highest infestation level was obtained 13 weeks after plant infestation, with an average of 58.5 mites per plant, predominantly females. Morphological injuries were categorized from level 0 (no injuries from uninfested plants) to level 3, characterized by the highest injuries represented by blotches on the adaxial epidermis of the leaf sheath and on panicles and grains. Plants ranked within levels 0, 1, and 2 for morphological injury did not exhibit clear histological injuries, while those at level 3 exhibited histological injury characterized by destruction of cells of the adaxial epidermis, disorder, color change, and hypertrophy in the mesophyll cells, as well as color change in the abaxial epidermis. Thus, it presented a significant correlation between morphological injuries and mite density level, which can be further adopted to help the control decision-making process for this mite on rice.

Introduction

Rice is one of the most important tropical crops. It is a source of energy and protein for human diet worldwide. Mexico has a total rice-growing area of 34,018 ha and an estimated yield of 179,776 t (SIAP 2014) ranking as the fourth produced cereal in Mexico, after maize, wheat, and barley (SIAP 2013). Rice is cultivated in 13 states, mostly in coastal lowlands using the variety Milagro Filipino, followed by the variety Morelos, subvarieties A-92 and A-98 (SIAP 2013, Orona Castro 2008, CONAPAMEX 2013).

The panicle rice mite, *Steneotarsonemus spinki* Smiley (Acari: Tarsonemidae), is a very destructive pest species widespread throughout rice-producing countries (Rodríguez Morell & Quirós-McIntire 2009). Its life cycle and behavior are characterized by a high reproductive rate, short life cycle, and hidden habitat, as this mite is protected by leaf sheaths (Almaguel Rojas & Botta Ferret 2005). *Steneotarsonemus spinki* causes direct injuries by sucking the cell contents of leaf sheaths (Comité Técnico Nacional del Complejo Ácaro-Hongo-Bacteria 2005, Orona Castro 2008) and indirect injuries due its association with plant pathogens, the fungus *Sarocladium oryzae* and the bacteria *Burkholderia glumae* (Correa-Victoria 2006). As a result of the injuries produced by mite feeding and plant infection by these microorganisms, the plant produces empty or partially filled panicles, undulated peduncles, and light brown to black-stained grains, and necrotic leaf sheaths will appear (Santos Herrera *et al* 2002, Almaguel Rojas & Botta Ferret 2005, Hummel *et al* 2009). The combination of these injuries and hence the reduction in the plant photosynthetic activity result in lower yield and production of deformed grains, and vain grains, compared to healthy plants (Comité Técnico Nacional del Complejo Ácaro-Hongo-Bacteria 2005).

The rice panicle mite was first reported in Mexico in 2006 (NAPPO 2007), and both the cultivated area with rice and

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yield were reduced by 50% in the following years (SIAP 2014). Therefore, it is relevant to know how the injuries proceed in order to help in the decision-making process for selection of control strategies. This work aimed to study the injuries caused by *S. spinki* on rice var. Morelos A-92, expressed as morphological and histological injuries, in response to mite population density.

Material and Methods

Collection of mites and plants

Mite colonies were established by collecting rice plants exhibiting injuries caused by *S. spinki* in the states of Veracruz (Piedras Negras, 18°45′57N, 96°11′41″W), Campeche (Palizada, 18°02′49″N, 91°52′53″W), and Tabasco (Cárdenas 18°09′23″N, 93°29′42″W), Mexico. The plants were potted and kept at 29°C and under photoperiod of 14:10 (L/D) to allow mite reproduction. Mites from these colonies were later used in the experiments.

Rice plants variety Morelos subvariety A-92 (nearly 45 days old) were collected in Mazatepec, Morelos, Mexico (18°43′26″N, 99°22′27″W). Despite this is a currently *S. spinki*-free area, the plants were fully inspected to confirm the absence of infestation. Then, plants were transplanted into trays containing argillaceous soil, also collected in Mazatepec, which was previously sterilized (3 h at 121°C).

Population growth

Ninety-six plants were separated into two groups: 48 plants were subjected to infestation with *S. spinki*, and the 48 remaining plants were used as an uninfested control treatment. Plants were infested by transferring a piece of a leaf sheath carrying 10 *S. spinki* individuals of undefined age to each one; this was done to prevent excessive handling of mites. A second infestation was carried out 30 days later, again using 10 individuals per plant. The experiment was arranged in a completely randomized design with four replications considering 12 plants each. The plants were kept at 29°C and 14:10 h (L/D) photoperiod under laboratory conditions.

After infestation, one plant per replication was evaluated to record the number of mites present as well as to determine the life stages and gender of the mites at approximately every 3 weeks. Leaves were trimmed; their sheaths were opened and observed under a stereomicroscope at 40× magnification. The evaluation consisted of recording all immature developmental stages, males and females separately. At the end of the study, the plants in the control treatment were fully inspected to confirm they remained uninfested. During the destructive plant inspection, data were taken to describe the morphological injuries and the effects on plant development.

Morphological injuries

During the destructive plant inspection, samples of plant tissue were taken to histological injury studies. To associate mite densities with morphological injuries, color changes and presence of blotches on leaf sheaths were classified into an injury rank level from 0 to 3. Level 0 represents a healthy plant, while level 3 represents maximal injury (Fig 1): *injury 0* stands for undamaged and mite-free plants, without a pattern of blotches on leaf sheaths, without injuries or blotches on spikes, succulent tissues, and light green in color; *injury 1* stands for plants with injuries scarcely visible to the naked eye, average of mite number per plant lower than 10 individuals, with small, isolated, light brown feeding blotches on leaf sheaths, occupying at most 30% of leaf sheaths, spikes without apparent blotches or, if present, they are tenuous and light brown; *injury 2* stands for plants with injuries visible with the naked eye, more than 50 mites per plant, isolated dark brown blotches on leaf sheaths occupying more than 40% of their surface, and spikes with blotches partially covering the grain; and *injury 3* stands for plants with injuries evident to the naked eye, lower than 10 live mites but many dead ones, with dark brown blotches on leaf sheaths occupying more than 80% of their surface, tissues are mostly necrotic, and spikes with blotches, grain absent, or fragmented.

Histological injuries

Small pieces of healthy and mite-injured plant tissues were processed for observation under a light field microscope (Carl Zeiss). They were fixed in formaldehyde–acetic acid–ethanol (FAA) during at least 24 h, washed with tap water, dehydrated in a series of increasing concentrations of ethanol (from 10 to absolute ethanol), 4 h each, cleared with xylene (4 h), and then included in Paraplast (Sigma®). Transversal cuts were made using a rotatory microtome (American Optical) and stained with safranine–fast green (López Curto *et al* 2005). Healthy plants were set as number 0, while plants exhibiting any kind of injury caused by the mite were set as 1 for statistical analysis. In addition, plant tissues with injuries were dissected and observed under a scanning electron microscope (JEOL Model JSM 6390). The process included fixation in formaldehyde, dehydration in a graded series of ethanol (1 h each), critical point drying, and coating with ionized gold (Bozzola & Russell 1992, with slight modifications).

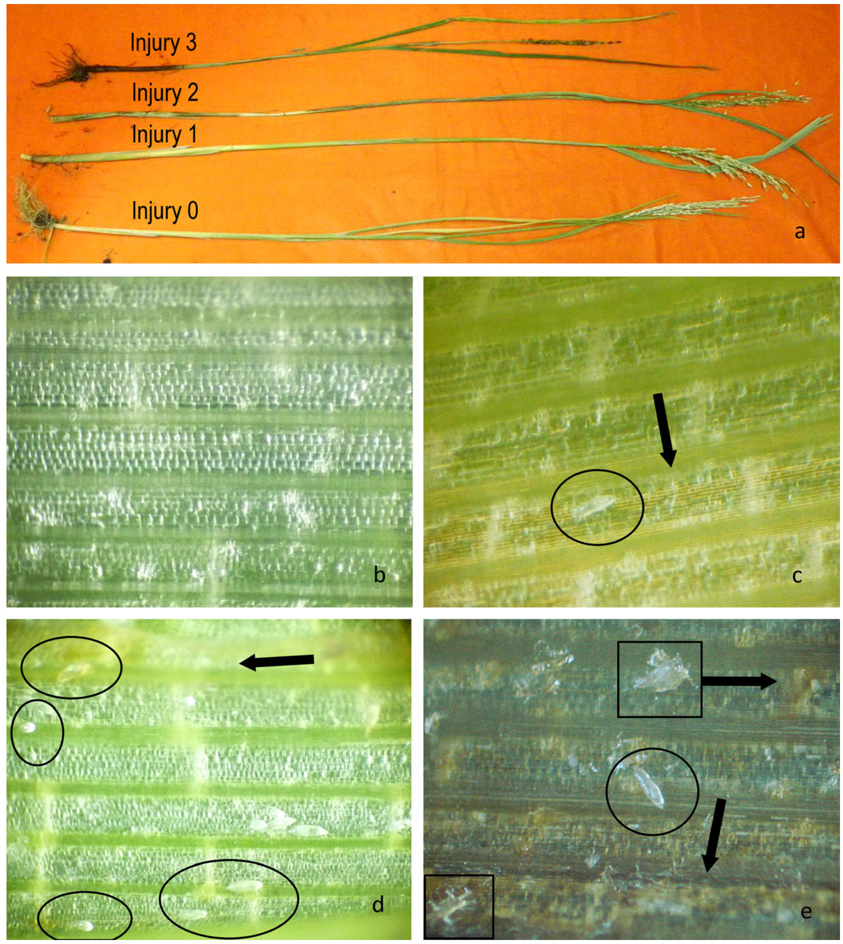

Fig 1 Morphological injury levels on rice plants. **a** Comparative view among levels. **b** Injury level 0 inside of leaf sheath (no injury, no blotches). **c** Injury level 1 inside of leaf sheath (some scattered blotches). **d** Injury level 2 inside of leaf sheath (*dark brown* blotches). **e** Injury level 3 inside of leaf sheath (necrotic areas). The *arrow* shows blotches. Encircled there are mites. Inside the *square*, there are dead mites.

Effects on plant growth

Plant growth response was measured considering the height of plants from the lowest end of the stem to the tip of the flag leaf, as well as the total number of tillers produced in uninfested and mite-infested plants. These measurements were subjected to analysis of variance (ANOVA) between treatments (infested and uninfested treatments) to determine possible effects on plant growth. Spearman's correlations were estimated among morphological and histological injuries, plant height, and number of tillers. Morphological injuries were expressed as levels of the scale 0–3; histological injuries were expressed as 0, absence and 1, presence. The SAS® System for Windows 9.0 was used for statistical analysis.

Results

Population growth

The average of mite infestation 2 weeks after the onset of the experiment was 38.3 mites per plant (Fig 2). Four weeks after infestation, no mites were found on observed plants; at that date, the plants were reinfested with 10 mites of mixed developmental stages. Once plants were reinfested, an increase in mite density was observed, with a maximum of 58.5 mites per plant. Mite density decreased afterwards to

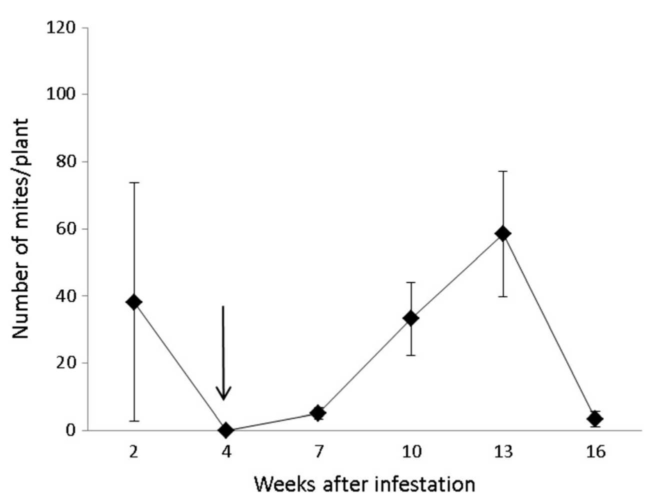

Fig 2 Mean total *Steneotarsonemus spinki* specimens per plant. *Bars* represent standard errors, and the *arrowhead* indicates the day when plants were reinfested with 10 specimens of the same species.

reach an average of 3.3 mites per plant 16 weeks after the first infestation.

Differences in the relative abundance of each developmental stage and gender of adults were observed (Fig 3). Two weeks after infestation, the predominant stages were eggs and nymphochrysalis, with means of 13.5 and 11.8 mites per plant, respectively. Later, adult females were predominant, with 29 females per plant 13 weeks after infestation. This abundance of adults and corresponding scarcity of immature stages (34.5 mites/plant, given by 29 females and 5.5 males) suggested that population would soon collapse and, as expected, it happened on week 16.

Morphological injuries

Two weeks after infestation, all levels of the injury scale were found. However, injuries representing level 3 consistently corresponded to high numbers of *S. spinki* or cumulated feeding, while injuries at the other levels corresponded to fewer mites. Seven weeks after infestation, the most common injury level was 1 (Fig 1c); 13 weeks after infestation, with the highest numbers of mites, the most common level was 2 (Fig 1d). Finally, the 16th week after infestation was characterized by maximal injury level and even several dead plants. At that time, the number of mites was reduced, but it was evident that an extensive colonization had taken place because many dead mites were observed (Fig 1e).

Histological injuries

Plants with injury ranked as levels 0 to 2 did not show histological injuries (Fig 4a) in epidermis or in the parenchymatous tissues of the mesophyll. Similarly, vascular tissues did not

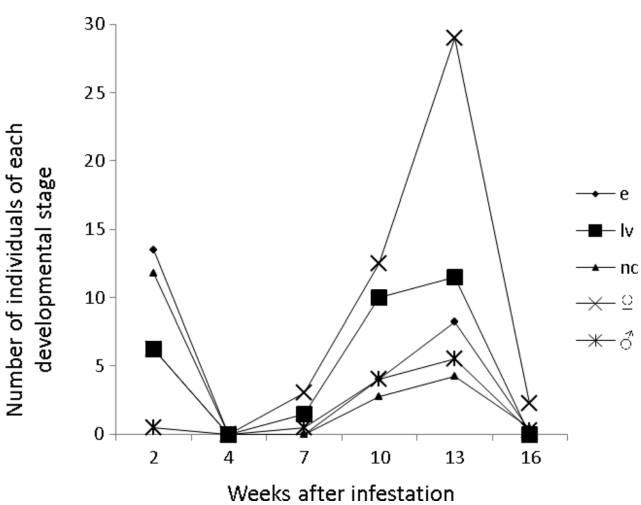

Fig 3 Number of individuals of each developmental stage (*e* egg, *lv* larva, *nc* nymphochrysalis, ♀ female, ♂ male) of *Steneotarsonemus spinki* per plant along periodical observations.

show alterations. When morphological injury was ranked as level 3 (Fig 4b), the adaxial epidermal cells were necrosed, and cells in mesophyll appeared disordered, with some cells displaying hypertrophy. Hyphae of unidentified fungi were present, and the accumulation of brown or red material, possibly of phenolic nature, was also observed.

The adaxial surface of leaf sheaths was observed with the aid of a scanning electron microscope to determine possible effects of *S. spinki*. In plants with injury at levels 0 to 1, the epidermis was naked (Fig 5a, b) but, as injury increased to levels 2 to 3, the surface of leaf sheaths became covered by a layer, apparently formed by bacteria and fungi. Mites wandered over and below that layer (Fig 5c, d).

Effect on plant growth

No significant differences were observed in plant height ($F_{df=1}$= 2.49, p=0.15) or number of tillers ($F_{df=1}$=0.11, p=0.75), when comparing infested and uninfested plants at 16 weeks after infestation. Spearman's correlation coefficient indicated a highly significant and positive association (r=0.96, p<0.0001) between the number of mites and the morphological injury levels. Likewise, significant and positive correlations were found between number of mites and histological injuries (r=0.30, p<0.0001) and between histological and morphological injuries (r=0.48, p<0.0001). Surprisingly, low but significant and positive correlations were found for histological (r=0.20, p=0.01) and morphological (r=0.16, p=0.04) injury levels and plant height.

Discussion

The first infestation of mites on rice plants did not succeed because mites did not settle down in all plants, seemingly because tissues of the host were too young; mostly eggs and larvae were found, showing that not a single life cycle was completed. Then, a second infestation was made at that time in which a profuse proliferation of tillers had taken place, so plant tissues seemed more appropriate as mite habitat and food source, allowing mite population to grow. This agrees with observations made by Ramos & Rodríguez (2001) and Miranda Cabrera *et al* (2003), who found that plants in the early stages of development are mite-free and that their infestation occurs after the formation of tillers.

Seven weeks after infestation and later on, the numbers of all developmental stages increased, mostly composed by adults. Sixteen weeks after infestation, mite numbers decreased due to depletion of plants as food source, and all plants exhibited severe morphological injuries. Few live mites but mostly dead ones were observed at this time and probably other mites absconded, indicating the end of the population.

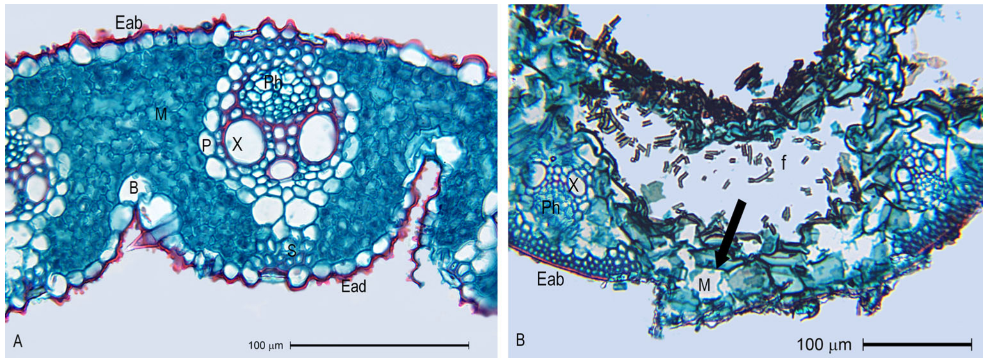

Fig 4 Health (**a**) and injured tissues (**b**) after mite infestation. *Ead* adaxial epidermis, *M* mesophyll, *X* xylema, *Ph* phloema, *P* parenchyma, *S* schlerenchyma, *Eab* abaxial epidermis, *B* bulliform cells, *f* injury associated to presence of fungi. *Arrow* shows the hypertrophy in the mesophyll cells.

In previous studies, other rice varieties exhibited either lower or higher numbers of *S. spinki* individuals. For example, short-cycle varieties evaluated by Almaguel *et al* (2000) presented less than 10 mites per plant, while Leyva Fernández *et al* (2003) observed 141 mites/plant in the variety J-104 and 58 mites/plant in the variety IACuba 28. On the other hand, Quirós-McIntire & Camargo Buitrago (2011) counted up to 532 mites/plant in the variety Idiap-22. Since maximal infestation of *S. spinki* in the variety A-92 was 58.5 mites/plant, its level of susceptibility seems to be similar to IACuba 28, considered a moderately tolerant variety.

Injuries were mainly observed on leaf sheaths and panicles and were characterized by blotches on adaxial and abaxial surfaces, varying in intensity depending on the severity. Previous studies indicated that *S. spinki* causes other injuries, as reduced panicle weight, increased rate of sterility, curvature of panicles (Thuy *et al* 2012), reduced quantity of entire grains, and also fragmented, stained, and empty grains (Ramos & Rodríguez Dueñas 2003).

The most severe injuries were observed on leaf sheaths with level 3. However, such injuries cannot be unequivocally attributed to *S. spinki* since fungi were also present and could result in hypertrophy and accumulation of phenolic compounds (Smith *et al* 2007). Ramos & Rodríguez (2000) observed hypertrophy and disorder in epidermal cells; however, in our work, hypertrophy was observed in mesophyll cells.

Scanning electron microscope images show that the adaxial surface of healthy leaf sheaths appeared smooth, naked, and with succulent aspect. As injury level increased, an irregular layer covered the epidermis, formed by what seemed to be bacteria and fungi. Correa-Victoria (2006) reported dark blotches on rice as a result of infections with the

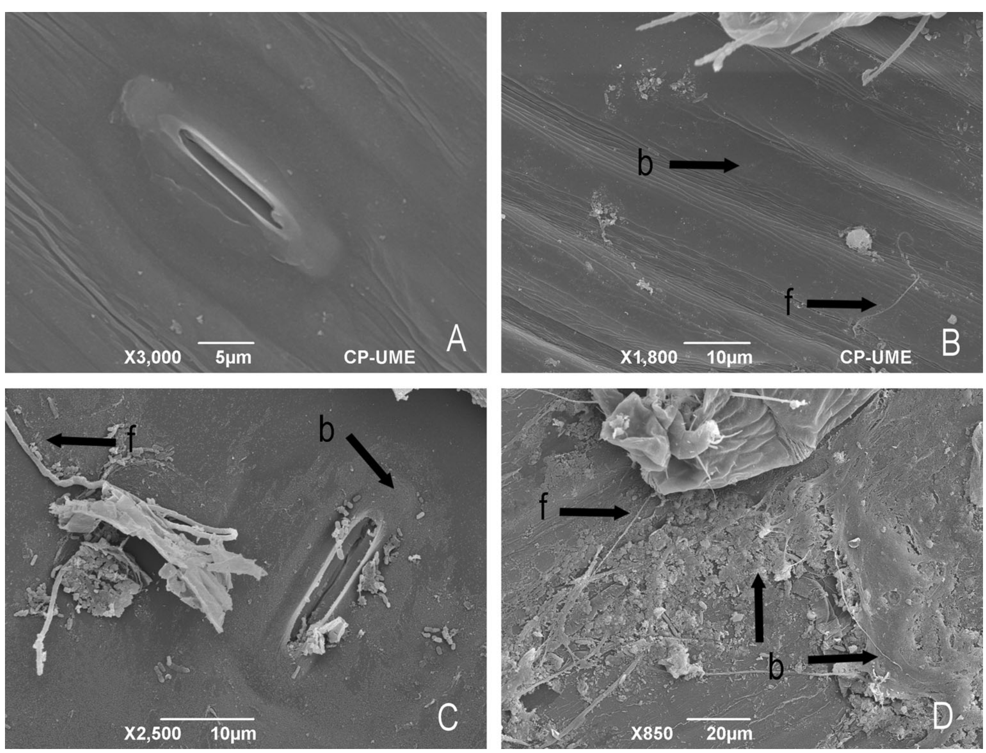

Fig 5 Surface of adaxial epidermis of leaf sheaths observed with scanning electron microscope. **a** Injury level 0. **b** Injury level 1. **c** Injury level 2. **d** Injury level 3. *b*: presence of bacteria, *f*: fungal hyphae.

pathogenic fungus *S. oryzae*, which caused grain rot in association with *S. spinki*. Ramos & Rodríguez Dueñas (2003) indicated that the associated mite–fungus attacking rice resulted in grain sterility.

Mite infestation did not significantly affect plant height, but a correlation was observed of both morphological and histological injuries with plant height. Karmakar (2008) did not find a significant correlation of mite population with the length of flag leaves or panicles (both associated to plant height). By contrast, Thuy *et al* (2012) reported that infestations of *S. spinki* cause certain degree of dwarfness, opposite to our results in which infested plants were slightly but significantly taller than uninfested plants. It is possible that such differences in results are associated with the degree of susceptibility among rice varieties.

Patterns of spots or blotches are a good indicator of injury level, as shown by a positive significant correlation. The abundance of mites would determine the degree of risk the crop is facing. Then, blotches on leaf sheaths are a good indicator for decision-making for mite control. Similarly, Nahrung & Waugh (2012) observed a positive correlation between the number of mites (Eriophyidae) and the severity of injuries to its host plant (Myrtaceae). In such cases, mites are so small that observation of visible injuries becomes a good strategy to estimate mite population.

Since a relatively low population of *S. spinki* was observed in the rice variety A-92 under laboratory conditions, this variety could be moderately tolerant to *S. spinki*. However, field tests would be required to confirm the moderate tolerance of this variety to this mite species.

The small size and hidden habitat of *S. spinki* make evaluation of its infestation a difficult task. The use of the numerical scale of injury we used is proposed here as a practical method to determine the presence and cumulative injury of *S. spinki* on rice based on the characteristic color changes, as these morphological characteristics are closely associated with mite population.

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