DAMAGE ASSESSMENT AND SAMPLING OF THE RICE STINK BUG, *Oebalus pugnax* (FABRICIUS) (HEMIPTERA: PENTATOMIDAE), IN RICE, *Oryza sativa* L., IN TEXAS

A Dissertation

by

LUIS ESPINO VARGAS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2007

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Michael O. Way
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ABSTRACT

Damage Assessment and Sampling of the Rice Stink Bug, *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), in Rice, *Oryza sativa* L., in Texas. (August 2007)

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Co-Chairs of Advisory Committee: Dr. Michael O. Way
Dr. Jimmy K. Olson

Field and greenhouse experiments were conducted from 2003 to 2006 at the Texas A&M University Agricultural Research and Extension Center at Beaumont and commercial rice fields throughout the Texas Rice Belt with the objectives of characterizing the nature of rice stink bug, *Oebalus pugnax* (Fabricius), damage to rice, *Oryza sativa* L., and developing visual sampling methods and sequential sampling plans.

During 2005 and 2006, in greenhouse and field experiments, rice plants were caged and infested with adult or nymph *O. pugnax* during the heading, milk or soft dough stage. No differences were found in the weight of rough, brown or milled rice infested with *O. pugnax* during different stages. More peck was found in grain from plants infested during milk and soft dough than heading. Adult *O. pugnax* caused more peck than nymphs in all stages.

In field experiments conducted during 2005 and 2006, single rice panicles were caged at the onset of heading and infested with one male or female *O. pugnax* for 48 h during the heading, milk, soft or hard dough stage. No differences were found in the weight of rough rice per panicle infested with *O. pugnax.* No differences were detected

in percentage peck caused by male and female *O. pugnax*. Infestation during all stages of panicle development produced significantly more peck than no infestation.

In greenhouse experiments conducted during 2004 and 2005, rice plants at the pre-heading, heading, milk and soft dough stages were caged together and infested with male and female *O. pugnax*. Insects were observed for a period of five days and their preferences recorded. More insects were observed on milk and soft dough than on pre-heading or heading plants.

Commercial rice fields throughout the Texas Rice Belt were sampled during 2003 and 2004 and visual sampling methods were compared to the sweep net method of sampling. Analysis of covariance showed that one sweep of the "long stick" or two sweeps of the "sweep stick" compared favorably to 10 sweep net sweeps. Analyses revealed that visual sampling using the long stick is more cost-reliable than sweep net sampling for *O. pugnax* in Texas rice fields.

ACKNOWLEDGMENTS

I thank Drs. M. O. Way, J. K. Olson, L. T. Wilson and E. C. Runge for their mentoring, encouragement, advice and friendship. This research would not have been possible without the help of Glenn Wallace, Mark Nunez, Becky Pearson and other staff from the Texas A&M University Agricultural Research and Extension Center at Beaumont. Funding for this research was provided in part by the Texas Rice Research Foundation.

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CHAPTER I

INTRODUCTION

Rice is grown in at least 95 countries worldwide, on a total area estimated to be over 150 million ha (IRRI 2005). In the United States rice is grown on about 1.1 million ha and average crop value is estimated at $1.44 billion (Childs and Livezey 2006). Although the United States produces about 1.5% of the world's total annual production, it is ranked among the top five exporters of rice, together with Thailand, India, Vietnam and Pakistan (Childs 2006). Rice is a minor crop in the United States. However, it is an economically important crop regionally and locally (Childs and Livezey 2006) being produced primarily in Arkansas, California, Florida, Louisiana, Mississippi, Missouri and Texas.

A variety of insects attack rice in the United States, and each state has its own particular complex of insect pests (Way 1990). However, two are the most important: the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, and the rice stink bug, *Oebalus pugnax* (Fabricius) (Way 2003). *O. pugnax* is a pest of rice in all southern rice-producing states and is a native species found in North America east of the Rocky Mountains, as far north as New York, southern Minnesota, and southern Michigan (Sailer 1944). *O. pugnax* is a very polyphagous species that overwinters as adults in grassy areas, woodland trash and ground litter. At the beginning of spring, these insects emerge from hibernation and feed and reproduce on grassy weeds. When rice panicles become available, the insects move to rice and feed on the developing grains, causing

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This dissertation follows the style of the Journal of Economic Entomology.

reductions of rough rice yield (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1967). Also, by introducing pathogenic microorganisms while feeding, *O. pugnax* causes a discoloration of the grain known as "peck". For quality considerations, pecky kernels are considered damaged kernels. For brown rice to classify as Grade U.S. No. 1 or 2, it should have no more than 1 or 2% damaged kernels, respectively (USDA-FGIS 2002). In addition, *O. pugnax* feeding structurally weakens kernels, increasing the percentage of broken kernels after milling, which reduces head rice yield.

Numerous attempts have been made to characterize *O. pugnax* damage; however, results have been contradictory or confounded by field conditions (Douglas and Tullis 1950, Odglen and Warren 1962, Swanson and Newsom 1962, Bowling 1963, Robinson et al. 1980, Harper et al. 1993, Tindall et al. 2004, Patel et al. 2006). Currently, *O. pugnax* is considered to cause reductions in rough rice yield and grain quality (Way 2003). Economic thresholds in use consider heading and milk stages of panicle development as more susceptible to damage than soft dough stage. Nymphs are not considered damaging (Harper et al. 1994). Changes in cultivar selection and agronomic practices during the past 10 years may have influenced injury of rice by this insect, requiring updated information on insect and damage relationships.

Observations by growers and researchers suggest that *O. pugnax* is attracted to the rice crop from heading to harvest. However, information is not available regarding the relative attractiveness of different stages of panicle development. This information may help growers better monitor their fields and help researchers develop new management strategies.

In Texas, the only recommended sampling method for *O. pugnax* is the sweep net. However, sweeping is tedious and time consuming, which discourages rice producers and consultants from sampling and using treatment thresholds (Harper et al. 1990). Many times, farmers and consultants rely on subjective visual *O. pugnax* population estimates obtained as they walk in rice fields. Treatment decisions based on these observations are likely to be unreliable because farmers might overestimate or underestimate *O. pugnax* populations, resulting in unnecessary use of pesticides or crop damage. Most rice damage due to *O. pugnax* in Texas results from producers not detecting economic damaging levels or not spraying at the proper time (Harper et al. 1990). The development of a reliable visual sampling method and a sequential sampling plan for *O. pugnax* in Texas might facilitate the sampling process for farmers and consultants, and increase the adoption of treatment thresholds, eliminating unnecessary pesticide applications or substantial crop damage due to undetected high *O. pugnax* populations.

The objectives of this research were:

1. Determine the most susceptible stage of rice panicle development to *O. pugnax*.
2. Determine the effect of *O. pugnax* on rough and head rice yield and quality.
3. Compare damage potential of adult and nymph *O. pugnax*.
4. Compare damage potential of male and female *O. pugnax.*
5. Develop a visual sampling method for *O. pugnax* to be used in Texas rice fields.
6. Develop a sequential sampling plan for *O. pugnax* to be used in Texas rice fields.

CHAPTER II

LITERATURE REVIEW

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius), is one of the most important pests of rice in the southern United States (McPherson and McPherson 2000, Way 2003) attacking rice during flowering and grain development. Damage due to *O. pugnax* reduces rough and head rice yields, and grain quality. This insect feeds on developing kernels, causing partially filled grains and abortion of florets. Also, by introducing pathogenic microorganisms while feeding, *O. pugnax* causes a discoloration of the grain known as "peck", for which growers are penalized when selling the grain.

Description. Adults are straw colored, about 8 to 12 mm long, elongated and narrow in shape. They have characteristic pronotal spines that project forward. When disturbed, adults excrete a strong, disagreeable odor. Eggs are cylindrical, 0.86 mm in length and 0.65 mm in diameter. They are laid on stems, leaves or panicles, in groups of 8 to 45 arranged in two rows. Newly laid eggs are bright green, turning red as they approach eclosion. Nymphs pass through five instars. First instar nymphs have a red abdomen with two elongated dark marks that fade into a lighter color as they molt. Last instar nymphs have a tan coloration, visible wing pads, and resemble the adults (Sailer 1944, Esselbaugh 1948, Odglen and Warren 1962).

Life cycle. Nilakhe (1976a) reared *O. pugnax* under controlled conditions and found that females exiting hibernation and reared on rice have a preoviposition period of 9.9 days. They lay an average of 45 egg masses, with 17 eggs per mass, for an average

total of about 759 eggs (range 0 to 1,144). Ingram (1927) reported that the duration of the egg stage ranges from 4 to 6 days, while Naresh and Smith (1983) reported 6 days at 27°C and 5 days at 30°C.

After emerging, first instar nymphs remain aggregated near the empty eggs, and disperse after the first molt (Ingram 1927). The nymphal period lasts 22 and 18 days at 27°C and 30°C, respectively (Naresh and Smith 1983).

Naresh and Smith (1983) reported development times (egg to adult) on rice of 49, 35, 28, and 23 days at 21, 24, 27, and 30°C, respectively. Males live an average of 43 days and females 68 days (Nilakhe 1976a). Nilakhe (1976a) described the mating behavior of *O. pugnax*.

Distribution. *O. pugnax* can be found in North America, east of the Rocky Mountains, as far north as New York, southern Minnesota, and southern Michigan. It is also found in the West Indies and the northern Gulf Coast region of Mexico (Sailer 1944, McPherson 1982).

Host plants. *O. pugnax* is a polyphagous insect (McPherson and McPherson 2000). Many host plants have been identified, with most being grassy weeds found around rice fields and levees (Douglas 1939, Odglen and Warren 1962, McPherson and McPherson 2000). Vasey grass, *Paspalum urvillei* Steud., is recognized as one of the preferred wild hosts (Douglas 1939, Douglas and Ingram 1942, Naresh and Smith 1984). Other preferred hosts are broadleaf signal grass, *Brachiaria platyphylla* (Griseb.); southern crabgrass, *Digitaria ciliaris* (Retz.); jungle rice, *Echinochloa colona* (L.); and Dallis grass, *Paspalum dilatatum* Poir. (Naresh and Smith 1984). Other cultivated crops

attacked by this insect are corn, wheat, barley, rye, oats and sorghum (Odglen and Warren 1962).

Life history. *O. pugnax* overwinters as adults in grassy areas, woodland trash and ground litter (McPherson and McPherson 2000). In Louisiana, perennial bunch grasses have been found to be the primary overwintering sites, especially vasey grass, broomsedge, *Andropogon glomeratus* (Walker); and smutgrass, *Sporobolus poiretti* (R. & S.) Hitchcock. Adults enter hibernation during the first week of October and exit hibernation during the spring. Males exit hibernation 10 days before females. Hibernating females appear to be unmated (Nilakhe 1976a). After emerging, adults can be found on wild grasses on which they feed and reproduce. Ingram (1927) reports that two or three generations develop on these wild hosts; but, yearly, four to five generations are produced.

Adults move to heading rice and feed on the developing kernels. Bowling (1967) suggests that the movement to rice is due to a reduction in the suitability of wild hosts as a food source. Evidence shows that rice is preferred over grassy weeds (Naresh and Smith 1984). Nilakhe (1976a) found that first and second generation *O. pugnax* females reared on rice laid twice as many eggs as females reared on vasey grass or barnyardgrass, *Echinochloa crus-galli* Beauv. Also, Naresh and Smith (1983) determined that *O. pugnax* nymphs reared on rice and sorghum weighed more and had higher survival rates than nymphs reared on vasey grass.

Only one generation develops on the main crop because of the short time from heading to harvest (about 30 days) (Way and Bowling 1991). One or more generations

can develop on the ratoon crop, which generally matures less uniformly than the main crop. Rice panicles can be attacked any time by immigrating adults (Way and Bowling 1991). Field populations can increase dramatically due to immigration from nearby harvested sorghum fields or recently-mowed grasses (Douglas 1939).

Natural enemies. *O. pugnax* has several natural enemies. The scelionid wasps, *Ooencyrtus anasae* Ashm. and *Telenomus podisi* Ashm. have been reported to be egg parasitods (Douglas and Ingram 1942). *O. pugnax* is also attacked by the tachinid flies *Gymnoclytia immaculata* (Macquart), *Beskia aelops* (Walker), *Euthera tentatrix* Loew, and *Cylindromyia euchenor* (Walker); the nyssonid wasp *Bicyrtes fodiens* (Handlirsch); and the fungus *Sporotrichum globuliferum* Spegannini. (Ingram 1927, Douglas and Ingram 1942, Odglen and Warren 1962, McPherson 1982, Sudarsono et al. 1992). The long-horned grasshoppers *Conocephalus fasciatus fasciatus* (DeGeer), *Orchelimum laticauda* Redt., and *Neoconocephalus* sp., and the short-horned grasshopper *Melanoplus differentialis* (Thos.) have been reported to be predators of *O. pugnax* eggs. *Conocephalus fasciatus fasciatus* (DeGeer) also has been found to be predaceous on nymphs. Other important predators are ladybird beetle larvae, lacewing larvae, spiders, and the green tree frog, *Hyla cinerea* (Hylidae). Ingram (1927) reports the existence of eight bird species that feed on *O. pugnax* and considered the red-winged black bird (*Agelaius phoeniceus litoralis*) the most important one.

Parasitization by *T. podisi* has been identified as the main factor for egg mortality (Sudarsono et al. 1992). High egg parasitization rates by *T. podisi* have been reported in Texas and Arkansas (Bowling 1963, Sudarsono et al. 1992). Parasitized eggs are easily

recognized by their color. Normal nonparasitized eggs change from green to red as they develop, while parasitized eggs turn from green to dark olive green to black in 96 hours (Sudarsono et al. 1992).

Economic importance. *O. pugnax* feeds on rice grains during kernel development using its piercing-sucking mouthparts. It causes two types of damage: reduction in yield by removing the grains' contents, and reduction in the marketing quality of the grain caused by introducing pathogenic or weakly pathogenic fungi, resulting in a kernel discoloration commonly known as "peck". Pecky rice is more susceptible to breakage during the milling process, reducing the percentage of whole grains or head rice yield. Rice grains attacked during the milk stage fail to continue normal development, producing an empty glume or atrophied grain (Bowling 1967).

Fryar et al. (1986) recognized two sources of economic loss due to *O. pugnax* - a price loss and a field loss. The price loss can be direct from reducing the grain's grade, which increases the price discount due to peck. Indirect price loss is caused by an increase in proportion of broken grains after milling, reducing head yield. Their analysis shows that a 1% increase in peck produces a 1% decrease in head yield. Recent research has shown that for a 1% increase in peck, head yield decreased 0.5% (Tindall et al. 2005). Variations in cultivars and growing conditions might explain this difference. Field loss is produced by pecky grains that weigh less than normal grains. These lighter grains are not picked up by the combine during harvest. Fryar et al. (1986) determined that for every increase in percent peck in brown rice, a loss of 1.35% grain in the field was expected.

Several studies have been conducted to characterize and quantify *O. pugnax* damage to rice. Douglas and Tullis (1950) infested rice plants with *O. pugnax* from boot stage to maturity. Peck ranged from 5 to 76% and grain weight was reduced 36% in rice-infested cages. Cages of rice were also infested with various nymphal densities, causing 6 to 40% pecky rice. The percentage florets that did not develop into grains ranged from 77 in rice-infested cages to 6 in the controls.

Odglen and Warren (1962) caged rice plants and infested them at milk and dough stages of panicle development with up to 80 adult or nymph *O. pugnax*. No reduction in yield of rough, milled, head rice or grade was found among treatments, including the control. It was suggested that the micro-organisms responsible for peck were not present in the field; therefore, *O. pugnax* feeding activity failed to cause peck.

Swanson and Newsom (1962) studied the effect of adult and nymph *O. pugnax* infestations on five rice cultivars grown in cages. Severe losses in total yield were found. The percentage damaged kernels increased, while milling yield and grade decreased. The highest level of infestation (230 to 320 insects per cage) reduced yield by 50% and reduced grade to the extent that the grain was ineligible for government price support. Low (7 to 8 insects per cage) and intermediate (40 to 60 insects per cage) infestation levels had no effect on yield; however, milling yield, grade and seed viability were reduced. Cultivars were affected similarly, but percent damaged kernels and grade were more affected in medium than in long grain cultivars. The authors concluded that populations as low as 7 to 8 *O. pugnax* per 1000 panicles may cause economic damage.

Bowling (1963) conducted caged studies with varying *O. pugnax* densities and compared yield and quality of medium and long grain rice. Results showed that rough rice and milling yields decreased as *O. pugnax* caged population levels increased, but differences among *O. pugnax* population levels were not always significant. Percent pecky rice increased with increasing *O. pugnax* populations in some tests but not in others. A reduction in grade due to higher percent peck was observed in the medium but not in the long grain cultivar.

Nilakhe (1976b) screened 228 rice lines in the field for resistance to *O. pugnax* by caging nymphs on panicles. He found differences in weight loss per kernel due to *O. pugnax* damage and a positive correlation between weight loss per kernel and percent pecky grains.

Robinson et al. (1980) studied the effect of different *O. pugnax* infestation levels on the yield and quality of rice. They found a reduction in percent full grains and kernel weight as the infestation level increased. No significant differences were found in percent full undamaged grains, even though a trend of increasing damage with increasing infestation levels existed.

Harper et al. (1993) used natural populations of *O. pugnax* in rice fields to evaluate damage and found no effect on rough rice yield. They believe that this type of damage is not very important but possible.

Patel et al. (2006), using caged panicles in a greenhouse experiment, found that rice is most vulnerable to *O. pugnax* damage during the first 2 weeks after anthesis.

Proportion of filled kernels and average grain weight were lowest in panicles infested a day after anthesis while pecky rice was greater during the first 13 days after anthesis.

O. pugnax damage is least, intermediate and most prevalent in long, medium, and short grain cultivars, respectively (Swanson and Newsom 1962, Bowling 1963, Way 2003). Susceptibility of rice cultivars has been associated with differences in flowering and maturation times. Cultivars with longer flowering and kernel maturation times had higher percentages of *O. pugnax* damage .

Weeds in and around rice fields affect *O. pugnax* population levels and damage. The presence of barnyardgrass in rice fields increases the number of *O. pugnax* present in fields at heading (Odglen and Warren 1962). Tindall et al. (2004) found that *O. pugnax* populations were higher in rice grown in association with barnyardgrass than in rice grown alone. Seedheads are produced later in rice than barnyardgrass, which serves as a host for the insects while rice is still in the vegetative phase. When barnyardgrass panicles senesce, *O. pugnax* migrate to heading rice. The presence of Amazon sprangletop, *Leptochloa panicoides* (Presl.) Hitchc., broadleaf signalgrass and large crabgrass, *Digitaria sanguinalis* (L.), in rice fields also has been shown to increase *O. pugnax* populations on headed rice. As weed density increased, *O. pugnax* numbers, percent unfilled seeds and peck increased (Tindall et al. 2005).

Pecky rice. When feeding on rice grains, *O. pugnax* can penetrate the hull with its piercing-sucking mouthparts (Way and Bowling 1991), introducing microorganisms that cause peck. Pecky rice has been well described and characterized (Douglas and Tullis 1950, Lee and Tugwell 1980). Several pathogenic fungi have been associated with

pecky rice: *Curvularia lunata* (Wakker) Boedijin, *Bipolaris oryzae* (Breda de Haan) Schoem., *Cercospora oryzae* Mij, *Trichonis caudata* (Appel & Strunk) Clements, *Fusarium oxysporum* Schlect., *Alternaria alternata* (Fr.) Keissler, *Alternaria padwickii* (Ganguly) M. B. Cellis, *Nematospora coryli* Peglion, and *Phoma* spp. (Douglas and Tullis 1950, Marchetti and Petersen 1984)

Sampling. The most common sampling method for *O. pugnax* is the sweep net. Rice fields should be sampled once or twice a week from 50% heading to harvest. A 38 cm diameter net is swept from side to side with each step while walking through the field, making sure that the top of the net is flush with the top of the panicles. The number of *O. pugnax* caught in 10 consecutive sweeps represents a sample unit. Ten sample units are obtained from a field to estimate *O. pugnax* population density (Way et al. 2006). It is recommended to sample early in the morning or late in the evening when *O. pugnax* are most active and abundant on rice heads.

Rashid et al (2006) sampled different rice cultivars in Arkansas at 0900, 1330, and 1900 h and found that during hot sunny days, samples taken at 1330 h contained fewer *O. pugnax* than the earlier or later sampling times. However, other research has shown that time of day is not a significant factor in sweep net catches of *O. pugnax*. Douglas (1939) found no significant differences in the number of *O. pugnax* catches at 0800, 1330 and 1630 h. Similarly, Cherry and Deren (2000) found that sweep net samples taken at 0900, 1300 and 1700 h did not differ in the number of *O. pugnax* caught. Air temperature and wind speed were different during the sampling times, but

these differences did not affect *O. pugnax* sweep net catches during the three different sampling periods.

Visual counts and sweep net samples of grassy margins, and yellow pyramid traps have been evaluated as indicators of *O. pugnax* abundance in rice fields (Rashid et al. 2006). During rice grain development, visual and sweep net sample counts in grassy margins decreased as sweep net sample counts in rice increased. Yellow pyramid traps used male or female bugs or food as bait; however, *O. pugnax* were caught only before and after rice heading and maturation periods. These results show that *O. pugnax* disperse from weedy hosts to rice as it starts heading, and that heading rice is more attractive than yellow traps with or without bait.

Economic thresholds. Present *O. pugnax* thresholds for Texas are five or more adults per 10 sweeps with a 38 cm diameter net from 50% panicle emergence to 2 weeks later. Afterwards, the threshold is 10 or more adults per 10 sweeps (Way et al. 2006). Harper et al. (1994) developed flexible economic thresholds for *O. pugnax* management using dynamic programming. These thresholds not only consider insect counts and stage of panicle development but also the price of rice, expected yield, cost of insecticide treatment and planting date. They also determined that only adults were important in explaining increases in percentage peck and reductions in head rice yield; thus, they recommended counting only adults caught with the sweep net during the sampling process.

Control. Due to high mobility of adults and the crop's short period of susceptibility, insecticides are the main means of reducing *O. pugnax* populations (Way

1990). Current insecticides recommended for *O. pugnax* control are carbaryl, lambda-cyhalothrin, methyl parathion and zeta-cypermethrin. No resistance has been documented to these products, but control failure has been observed when large number of *O. pugnax* migrate into rice fields (Drees and Plapp 1986, Way et al. 2006). Way and Wallace (1990) found that malathion and methyl parathion provided no more than 2 days of residual activity, carbaryl provided at least 5 days of residual control, while acephate had at least 9 days of activity.

Adequate weed control in and around rice fields can help reduce sources of migrating *O. pugnax*. Selection of less preferred rice cultivars with short flowering periods and rapid maturation also can help reduce damage by this pest.

CHAPTER III

RELATIVE SUSCEPTIBILITY OF STAGES OF RICE PANICLE DEVELOPMENT TO RICE STINK BUG: GREENHOUSE AND FIELD EXPERIMENTS*

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), is a serious pest of rice, *Oryza sativa* L., in the southern United States (Way 2003). *O. pugnax* feeds on rice grains during kernel development using its piercing-sucking stylets to cause two types of damage. The first is reduction of rough rice yield (unprocessed rice that includes hull and caryopsis) by removing the grain's contents (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1967). The second is reduction in quality of the grain, which is caused by the interaction of feeding and the introduction of pathogenic microorganisms resulting in kernel discoloration commonly known as "peck" (Lee et al. 1993, Way 2003). High percentage peck has been correlated with increased percentage of broken kernels in milled rice (Way 2003). Pecky kernels may break during the milling process or appear in head rice (milled kernels at least three-fourths the length of whole kernels), giving the product a dirty appearance and reducing quality. Rice price loss due to *O. pugnax* can be direct from reducing grain grade, which increases the price

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discount due to peck, and indirect from increasing the proportion of broken grains after milling which reduces head rice yield (Fryar et al. 1986). In addition, *O. pugnax* injury significantly reduces the germination rate of rice seed (Patel et al. 2006).

Several studies have been conducted to characterize and quantify *O. pugnax* damage to rice (McPherson and McPherson 2000). In most studies, panicles were infested throughout kernel development and maturation; so, determination of susceptibility of rice to *O. pugnax* based on stage of kernel maturation was not possible. This latter information could benefit growers, plant breeders, and other researchers, allowing them to concentrate their monitoring efforts on the most susceptible stages of rice and make better informed decisions as to what control measures, if any, should be taken. Harper et al. (1993) used natural populations of *O. pugnax* in rice fields to evaluate damage and develop economic thresholds. Their results suggest that nymphal *O. pugnax* populations do not contribute significantly to damage; thus, nymphs are not considered in current economic threshold recommendations. However, previous research has shown that nymphs are capable of causing injury (Bowling 1979). As cultivars, cultural practices and management tactics change, a crop's response to insect injury also may change. Information on how *O. pugnax* affects rice needs to be updated in order to improve management of this pest.

The objective of the present study was to determine the most susceptible stage of rice panicle development to *O. pugnax* and to compare the ability of nymphs and adults to cause damage.

Materials and Methods

Experiments were conducted during 2005 and 2006 at the Texas A&M University Agricultural Research and Extension Center at Beaumont (Jefferson County), TX (Beaumont Center). Greenhouse experiments were conducted during both years, and one field experiment was conducted in 2006.

Greenhouse experiments 2005. *Experiment 1*. On 4 May, seeds of the rice cultivar Cocodrie were planted inside the greenhouse in pots (15 cm lip diameter, 10 cm base diameter, 15 cm deep) containing sifted League soil. After rice emergence, pots were moved to bins (0.9 x 0.9 m, 0.19 m deep) outside the greenhouse to provide plants with adequate light and moisture, avoid etiolation and encourage normal growth. Plants received an application of lambda-cyhalothrin (Karate Zeon FV, Syngenta Crop Protection) at 0.045 kg AI/ha using a hand held, CO_2 pressurized spray rig to eliminate attack of early season pests (Way et al. 2006). When plants reached an adequate size, bins were flooded and pots thinned to five plants per pot. Nitrogen in the form of urea was applied by hand at planting, and on 8, 20, and 27 June (64 kg N/ha each application).

On 12 July, plants reached the boot stage and pots were moved inside the greenhouse. Groups of four pots were placed in bins (0.9 x 0.9 m, 0.19 m deep) filled with water and then covered with a cylindrical cage. Cages were 45 cm in diameter and 120 cm in height, constructed with hardware cloth (3 mm x 3 mm apertures). The bottom opening of the cage was submerged in water and the top opening was covered with plastic screening (3 mm x 3 mm apertures) kept in place with an elastic band (Fig. 3.1). Cages were infested with 12 *O. pugnax* nymphs (third to fifth instars) or 12 adults (six

Fig. 3.1. Caged rice plants infested with *O. pugnax*. Greenhouse experiment 1, 2005. Beaumont, TX.

males and six females) at three stages of panicle development: heading, milk and soft dough. Insects used in these experiments were collected from untreated rice and rice field weeds at the Beaumont Center. A rice stage was considered to begin when 50% of the panicles within a cage reached the target stage. Heading was considered to begin at panicle exertion. Milk was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was milky and panicles started to bend downward due to weight of developing grains. Soft dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was soft dough (not liquid) and hulls turned from green to tan. The following treatments were included: 1) panicles at the heading stage infested with adults, 2) panicles at the heading stage infested with nymphs, 3) panicles at the milk stage infested with adults, 4) panicles at the milk stage infested with nymphs, 5) panicles at the soft dough stage infested with adults, 6) panicles at the soft dough stage infested with nymphs, 7) panicles uninfested but caged. When panicles completed the target stage, insects were killed by spraying plants with lambda-cyhalothrin at 0.228 g AI/l using a hand-held pump garden sprayer. Infestation periods were: heading, 18 to 27 July; milk, 28 July to 8 August; soft dough, 10 to 20 August. Cages were checked every 24 h to replace missing and dead insects. Nymphs that molted into adults were removed from cages and replaced with new nymphs. Bins were drained on 17 August and panicles hand cut on 29 August. Panicles were placed in paper bags and stored in a dry, cold room until processed. Grain was allowed to naturally air dry to 12% moisture. Treatments were randomly assigned to cages, with four replications per treatment.

Panicles per cage were counted and threshed by hand. Filled grains were manually separated from empty grains, counted and weighed. Empty grains were weighed and their number estimated by comparing total weight to the weights of three, 100 grain samples in each replication. Filled grains were hulled using an Automatic Rice Husker (TR200, Kett Electric Laboratory, Japan) to obtain brown rice. Brown rice was weighed and manually inspected for peck, and kernels with peck were weighed. The United Sates Department of Agriculture (USDA) Federal Grain Inspection Service defines pecky kernels as "whole and broken kernels of rice that have one or more black, brown, red or other discolored spots or areas on them caused by fungus growth or insects" and consider them as a type of "damaged kernels" (USDA-FGIS 1994). For purposes of this investigation, only pecky rice caused by *O. pugnax* was considered, which is characterized by circular lesions that range from small to fairly large, covering most of the grain in some cases (Lee et al. 1993, Tindall et al. 2005). Brown rice was milled using a grain polisher (Pearlest, Kett Electric Laboratory, Japan). Milled kernels were weighed, manually separated into whole and broken kernels and whole kernels weighed. Milled kernels at least three fourths the length of unbroken kernels were considered whole kernels. Percentage peck was calculated as (weight of pecky kernels/weight of brown rice) x 100. Percentage whole kernels was calculated as (weight of milled whole kernels/weight of filled grains) x 100.

Experiment 2. Materials and methods were the same as in experiment 1 with the following differences. Planting date was 3 June. Urea was applied at planting and on 1 July, 19 and 1 August (64 kg N/ha each application). Plants were moved inside the

greenhouse and covered with cages on 9 August. On 15 August, plants were sprayed with a mixture of azoxystrobin and propiconazole (Quilt, Syngenta Crop Protection) at 0.19 kg AI /ha + 0.32 kg AI/ha, respectively, using a hand held, CO_2 pressurized spray rig, to control panicle and foliage diseases. Infestation dates were: heading, 10 to 18 August; milk, 19 to 29 August; and soft dough, 31 August to 12 September. Bins were drained on 14 September and panicles hand cut on 28 September. For this experiment, the percentage of pecky kernels was calculated as (number of pecky kernels/number of filled grains) x 100.

Greenhouse experiment 2006. Materials and methods were the same as in 2005, with the following differences. Planting was 17 April with seeds treated with fipronil (Icon 6.2 FS, Bayer CropScience) at 0.042 kg AI/ha. Pots were thinned to three plants per pot. Urea was applied at planting, 22 May and 5 June (64 kg N/ha each application). On 20 June, at early boot stage, plants were sprayed with a mixture of azoxystrobin and propiconazole at 0.19 kg AI /ha + 0.32 kg AI/ha, respectively. Plants were moved inside the greenhouse and covered with cages on 5 July. Infestation dates were: heading, 6 to 16 July; milk, 17 to 27 July; and soft dough, 28 July to 7 August. At the end of the infestation period, insects were killed by spraying plants with zeta-cypermethrin (Mustang Max, FMC) at 0.192 g AI/l with a hand held pump garden sprayer. On 10 July, all plants received an application of spinosad (Spin Tor 2SC, Dow AgroSciences) at 0.11 kg AI/ha due to a fall armyworm (*Spodoptera frugiperda* [J. E. Smith]) infestation; spinosad has not been found to have a major effect on hemipterans (Bret et al. 1997).

Bins were drained on 7 August and panicles hand cut on 18 August. During grain

processing, the total number of filled grains was estimated by weighing 10, 100 grain samples. Before hulling, total filled grains per cage was divided in two samples of approximately the same weight and then hulled and milled. Percentage peck and whole kernels were determined for each of these samples.

Field experiment 2006. On 6 April, seeds of the rice cultivar Cocodrie were planted in circular areas of 0.16 m^2 in a research block at the Beaumont Center. Twenty eight planted areas were arranged in four rows, seven planted areas per row, in approximately a 3 x 2 m grid. Seeds were treated with fipronil at 0.042 kg AI/ha. Water management, weed control, and other cultural practices were performed following the Texas Rice Production Guidelines (McCauley 2006, McCauley and Chandler 2006). Permanent flood was applied on 5 May and planted areas were thinned to 26 plants, a density equivalent to 162 plants/m^2. Nitrogen in the form of urea was applied at planting, 5, 22 May (64 kg N/ha each application), and 12 June (45 kg N/ha). On 20 June, at early boot stage, plants were sprayed with a mixture of azoxystrobin and propiconazole at 0.19 kg AI /ha + 0.32 kg AI/ha, respectively, using a hand held, CO_2 pressurized spray rig to control panicle and foliage diseases.

On 26 June, when plants were in late boot stage, cages identical to the ones used for the greenhouse studies were placed over each of the circular areas, covering all plants (Fig. 3.2). Cages were secured to the soil using wooden stakes. Infestation density and treatments were the same as in the greenhouse studies. Infestation dates were: heading, 29 June to 9 July; milk, 10 to 20 July; and soft dough, 21 to 31 July. At the end of each infestation period, insects were killed by spraying plants with zeta-cypermethrin at 0.192

Fig. 3.2. Caged rice plants infested with *O. pugnax*. Field experiment 2006. Beaumont, TX.

g AI/l with a hand held pump garden sprayer. The field was drained on 28 July and panicles hand-harvested on 14 August. Treatments were randomly assigned to cages, with four replications per treatment. During the experiment, one of the uninfested control cages was contaminated with adult *O. pugnax.* Panicles and grain from this cage were not considered in the analysis, and other control cages were continuously inspected to negate insect contamination.

Panicles per cage were counted, cut and threshed by hand. Filled grains were separated from empty grains using a seed aspirator (Seedburo Equipment Company, Chicago, IL) and then weighed. Total number of filled grains was estimated by weighing 10, 100 grain samples. Empty grains were weighed and their number estimated by weighing three, 100 grain samples. Six 25 g samples of filled grain per cage were hulled and milled in the same manner as in the greenhouse experiments. Whole kernels were separated from broken kernels using a # 6 plate (USDA-FGIS 1994). Percentage peck and whole kernels were determined as in the greenhouse experiments.

Data analysis. Statistical analyses were performed using the SPSS package (SPSS Inc. 2005). For each experiment, number of panicles, number of filled and empty grains, weight of filled and empty grains, percentage peck, and percentage whole kernels per cage were analyzed using a two-way analysis of variance (ANOVA), with factors panicle and insect stages. Tukey's honestly significant difference (HSD) test (Tukey 1953) was used for mean separation of significant effects. When the assumptions of normality of residuals and constant variances were not met, the data were transformed before applying ANOVA. The Box-Cox procedure was used to determine the best

transformation (Kutner et al. 2005). To determine the amount of peck produced by nymphs relative to adults, data from all experiments were pooled and percentage peck caused by adults (independent variable) regressed against percentage peck caused by nymphs (dependent variable) for each panicle stage, with the intercept forced through the origin. To examine the relationship between percentage peck and whole kernels, linear regression analysis was performed between percentage peck (independent variable) and percentage whole kernels (dependent variable). The level of alpha used in all tests was 0.05.

Results

Number of panicles per cage was not significantly affected by *O. pugnax* infestation in any of the experiments ($P > 0.05$). Average number of panicles per cage was 56.3 ± 1 for greenhouse experiment 1, 2005; 41.2 ± 1 for greenhouse experiment 2, 2005; 42.4 ± 0.6 for greenhouse experiment 2006; and 81.8 ± 1.4 for field experiment 2006.

Infestation with *O. pugnax* adults or nymphs during different stages of panicle development did not significantly affect the number or weight of filled grains produced per cage in any of the experiments (Table 3.1). Average number and weight of filled grains per cage were 1917.5 ± 77.7 and 33.6 ± 1.7 g for greenhouse experiment 1, 2005; 1882.8 ± 61.8 and 38.0 ± 1.4 g for greenhouse experiment 2, 2005; 2535.7 ± 51.6 and 53.6 ± 1.2 g for greenhouse experiment 2006; and 8759 ± 218.6 and 202.9 ± 4.9 g for field experiment 2006.

Table 3.1. Statistical analyses of yield components of cages infested with adult or nymph *O. pugnax* during three stages of panicle development. Beaumont, TX, 2005 and 2006

Experiment	Factors	Number of empty grains			Weight of empty grains (g)			Number of filled grains		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
GH[a] 1, 2005	Panicle stage	9.536	2, 21	0.001	11.014	2, 21	0.001	0.158	2, 21	0.855
	Insect stage	0.005	1, 21	0.945	0.005	1, 21	0.946	0.463	1, 21	0.504
	Panicle stage x Insect stage	0.760	2, 21	0.480	0.236	2, 21	0.792	1.084	2, 21	0.356
GH 2, 2005	Panicle stage	0.132	2, 21	0.877	1.398	2, 21	0.269	0.446	2, 21	0.646
	Insect stage	0.036	1, 21	0.852	0.666	1, 21	0.424	0.245	1, 21	0.626
	Panicle stage x Insect stage	0.003	2, 21	0.997	0.440	2, 21	0.650	0.751	2, 21	0.484
GH 2006	Panicle stage	4.791	2, 21	0.019	6.353	2, 21	0.007	1.734	2, 21	0.201
	Insect stage	1.479	1, 21	0.238	3.081	1, 21	0.094	2.346	1, 21	0.141
	Panicle stage x Insect stage	0.594	2, 21	0.561	0.846	2, 21	0.443	0.432	2, 21	0.655
Field 2006	Panicle stage	0.800	2, 20	0.463	1.450	2, 20	0.258	0.131	2, 20	0.878
	Insect stage	1.015	1, 20	0.326	0.624	1, 20	0.439	0.061	1, 20	0.807
	Panicle stage x Insect stage	1.818	2, 20	0.188	1.084	2, 20	0.357	2.042	2, 20	0.156

Table 3.1. Continued

Experiment	Factors	Weight of filled grains (g)			% peck			% whole kernels		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
GH 1, 2005	Panicle stage	0.073	2, 21	0.93	7.645	2, 18	0.004	1.785	2, 21	0.192
	Insect stage	0.015	1, 21	9.04	17.055	1, 18	0.001	3.13	1, 21	0.091
	Panicle stage x Insect stage	0.979	2, 21	0.392	0.318	2, 18	0.732	0.468	2, 21	0.632
GH 2, 2005	Panicle stage	0.625	2, 21	0.545	22.449	2, 21	< 0.001	0.511	2, 21	0.607
	Insect stage	0.042	1, 21	0.840	11.238	1 21	0.003	1.142	1, 21	0.297
	Panicle stage x Insect stage	1.042	2, 21	0.370	1.612	2, 21	0.223	0.903	2, 21	0.420
GH 2006	Panicle stage	0.922	2, 21	0.413	53.012	2, 49	< 0.001	2.790	2, 49	0.071
	Insect stage	2.295	1, 21	0.145	147.801	1, 49	< 0.001	0.593	1, 49	0.445
	Panicle stage x Insect stage	0.248	2, 21	0.783	2.956	2, 49	0.061	2.422	2, 49	0.099
Field 2006	Panicle stage	0.182	2, 20	0.835	33.972	2, 155	< 0.001	0.959	2, 153	0.385
	Insect stage	0.148	1, 20	0.705	0.059	1, 155	0.809	0.327	1, 153	0.568
	Panicle stage x Insect stage	1.897	2, 20	0.176	2.729	2, 155	0.068	0.004	2, 153	0.996

[a]GH, greenhouse experiment.

Number and weight of empty grains per cage were significantly affected by *O. pugnax* infestation in greenhouse experiment 1, 2005, and greenhouse experiment 2006 (Table 3.1). The interaction panicle by insect stages and the insect stage main effect were not significant ($P > 0.05$), while the panicle stage main effect was significant. In greenhouse experiment 1, 2005, cages of rice infested with *O. pugnax* during heading had significantly more empty grains and higher empty grain weight than cages of rice infested during soft dough; however, none of the treatments were significantly different from the uninfested control (Tables 3.2A and 3.3A). In greenhouse experiment 2006, the number of empty grains from cages infested with *O. pugnax* during heading, milk and soft dough were not significantly different, and cages infested during heading and soft dough had significantly more empty grains than the uninfested control. In this same experiment, empty grain weight from cages infested with *O. pugnax* during heading was significantly higher than empty grain weight from infested cages during milk and uninfested control cages (Table 3.2B and 3.3B). *O. pugnax* had no effect on the mean number or weight of empty grains per cage in greenhouse experiment 2, 2005, and in field experiment 2006 (Table 3.1). Average number and weight of empty grains were 1719 ± 50.25 and 5.49 ± 0.16 g for greenhouse experiment 2, 2005, and 1591.26 ± 81.55 and 7.04 ± 0.38 g for field experiment 2006.

Percentage pecky kernels was significantly affected by *O. pugnax* infestation during different stages of panicle development in all experiments (Table 3.1). Percentage peck varied considerably between years, averaging 7.4 for 2005 and 1.7 for 2006. In all experiments, peck was observed in infested and uninfested control cages; however,

Table 3.2A. Mean number of filled and empty rice grains per cage ± SEM infested with *O. pugnax* during three stages of panicle development. Beaumont, TX, 2005

Panicle stage	Experiment			
	Greenhouse			
	1 – 2005		2 – 2005	
	Filled	Empty	Filled	Empty
Heading	1852.0 ± 160.4	3260.6 ± 115.3a	1959.1 ± 102.5	1798.0 ± 76.6
Milk	1942.4 ± 118.4	2867.9 ± 78.3ab	1817.5 ± 81.2	1727.6 ± 100.5
Soft dough	1968.9 ± 177.0	2132.2 ± 203.0b	1817.5 ± 146.4	1753.9 ± 95.4
Uninfested control	1895.8 ± 207.2	2628.2 ± 483.4ab	1991.1 ± 217.9	1475.9 ± 132.0

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

Table 3.2B. Mean number of filled and empty rice grains per cage ± SEM infested with *O. pugnax* during three stages of panicle development. Beaumont, TX, 2006

Panicle stage	Experiment			
	Greenhouse		Field	
	2006		2006	
	Filled	Empty	Filled	Empty
Heading	2419.4 ± 87.7	1393.3 ± 79.8a	8888.6 ± 367.5	1635.6 ± 125.4
Milk	2619.5 ± 53.6	1094.5 ± 75.4ab	8705.0 ± 496.5	1460.6 ± 175.2
Soft dough	2427.4 ± 87.7	1368.9 ± 73.8a	8592.8 ± 351.2	1720.9 ± 157.6
Uninfested control	2817.3 ± 185.2	1025.3 ± 95.0b	9000.7 ± 887.2	1475.7 ± 241.9

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

Table 3.3A. Mean weight (g) of filled and empty rice grains per cage ± SEM infested with *O. pugnax* during three stages of panicle development. Beaumont, TX, 2005

Panicle stage	Experiment			
	Greenhouse			
	1 - 2005		2 - 2005	
	Filled	Empty	Filled	Empty
Heading	32.9 ± 3.1	9.2 ± 0.3a	39.6 ± 2.3	6.0 ± 0.2
Milk	33.2 ± 2.6	7.9 ± 0.2ab	36.2 ± 1.8	5.3 ± 0.3
Soft dough	34.6 ± 3.9	6.2 ± 0.5b	35.9 ± 3.0	5.5 ± 0.3
Uninfested control	33.7 ± 4.4	7.2 ± 1.1ab	42.6 ± 5.0	4.9 ± 0.4

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

Table 3.3B. Mean weight (g) of filled and empty rice grains per cage ± SEM infested with *O. pugnax* during three stages of panicle development. Beaumont, TX, 2006

Panicle stage	Experiment			
	Greenhouse		Field	
	2006		2006	
	Filled	Empty	Filled	Empty
Heading	50.8 ± 2.1	5.6 ± 0.3a	205.9 ± 8.6	7.2 ± 0.6
Milk	54.6 ± 1.3	4.3 ± 0.3b	198.9 ± 10.4	6.3 ± 0.8
Soft dough	52.2 ± 1.8	5.5 ± 0.2abc	199.6 ± 7.8	8.0 ± 0.6
Uninfested control	60.0 ± 4.2	3.9 ± 0.4c	214.2 ± 20.1	6.1 ± 1.5

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

percentage peck in uninfested control cages was always lower than in infested cages. The interaction panicle by insect stages was not significant in all experiments (Table 3.1); therefore, only main effects will be discussed.

In greenhouse experiment 1, 2005, main effects of panicle and insect stages were significant (Table 3.1). *O. pugnax* infestation during heading, milk and soft dough caused significantly higher percentage peck than the uninfested control (Table 3.4, Fig. 3.3A). No significant differences were found in peck produced during milk and soft dough, or milk and heading. Peck produced during soft dough was significantly higher than during heading. Across all panicle stages, adult *O. pugnax* caused significantly more peck than nymphs, and both insect stages caused significantly more peck than the uninfested control (Table 3.5, Fig. 3.4A). In greenhouse experiment 2, 2005, panicle and insect stages main effects were significant (Table 3.1). *O. pugnax* infestation during milk and soft dough caused higher percentage peck than infestation during heading or in the uninfested control. No significant differences were observed in percent peck from panicles infested during heading and the uninfested control (Table 3.4; Fig. 3.3B). Comparison of adult and nymph *O. pugnax* injury across all panicle stages showed that percentage peck was significantly higher for adults than nymphs, and both insect stages caused significantly more peck than the uninfested control (Table 3.5, Fig. 3.4B).

In greenhouse experiment 2006, panicle and insect stages main effects were significant (Table 3.1). *O. pugnax* infestation during milk caused the highest percentage peck, followed by soft dough and heading. The uninfested control exhibited significantly

Table 3.4. Mean percent peck and percent whole kernels ± SEM of cages infested with *O. pugnax* during three stages of panicle development. Beaumont, TX, 2005 and 2006

Panicle stage	Experiment			
	Greenhouse			
	1 - 2005		2 - 2005	
	% peck	% whole kernels	% peck[a]	% whole kernels
Heading	4.8 ± 1.0b	51.1 ± 0.6	3.7 ± 0.6b	23.3 ± 1.7
Milk	9.7 ± 1.8ab	46.1 ± 1.9	8.5 ± 0.5a	23.1 ± 0.7
Soft dough	12.6 ± 4.8a	47.5 ± 3.5	12.1 ± 2.1a	24.7 ± 1.3
Uninfested control	0.4 ± 0.1c	50.7 ± 2.7	2.3 ± 0.8b	25.4 ± 0.5

Table 3.4. Continued

Panicle stage	Experiment			
	Greenhouse		Field	
	2006		2006	
	% peck	% whole kernels	% peck	% whole kernels
Heading	1.8 ± 0.2c	56.6 ± 0.8	0.7 ± 0.05b	45.9 ± 0.4
Milk	3.8 ± 0.4a	52.9 ± 1.3	1.6 ± 0.1a	46.3 ± 0.4
Soft dough	2.6 ± 0.2b	54.4 ± 1.2	1.3 ± 0.1a	46.5 ± 0.3
Uninfested control	0.2 ± 0.1d	56.8 ± 1.5	0.4 ± 0.05c	44.3 ± 0.5

[a] Percentage peck calculated as (number of pecky kernels/number of filled grains) x 100

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

Table 3.5. Mean percent peck ± SEM of cages infested with adult or nymph *O. pugnax*. Beaumont, TX, 2005 and 2006

Insect Stage	Experiment			
	Greenhouse			Field
	1 - 2005	2 – 2005[a]	2006	2006
Adults	12.4 ± 0.3a	10.0 ± 1.7a	3.7 ± 0.3a	1.2 ± 0.1a
Nymphs	5.3 ± 3.2b	6.2 ± 0.9b	1.7 ± 0.1b	1.2 ± 0.1a
Control	0.4 ± 0.1c	2.3 ± 0.8c	0.2 ± 0.1c	0.4 ± 0.1b

[a] Percentage peck calculated as (number of pecky kernels/number of filled grains) x 100

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

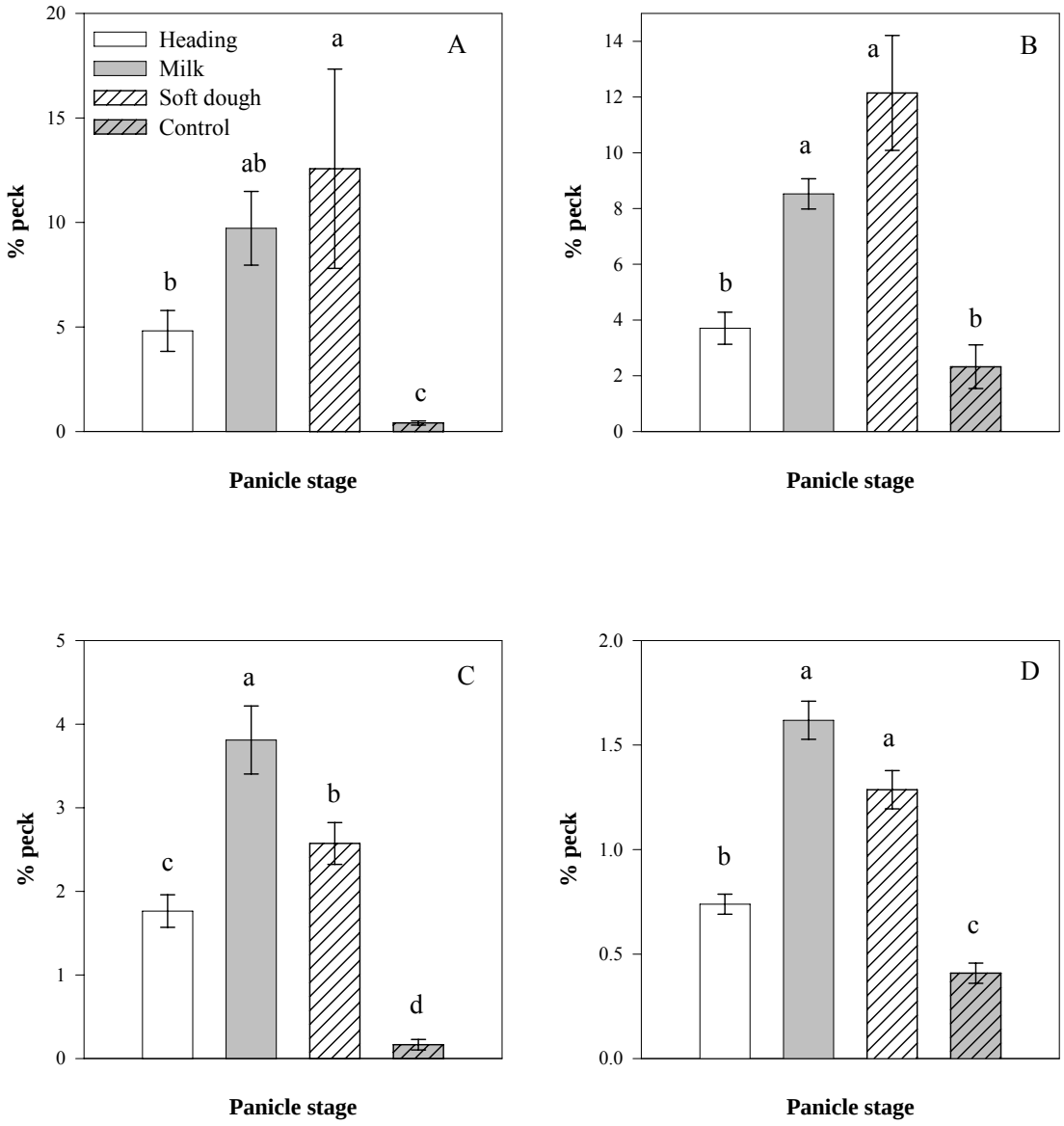

Fig. 3.3. Mean percentage peck (± SEM) of rice infested with *O. pugnax* during three stages of panicle development. (A) Greenhouse experiment 1, 2005; (B) greenhouse experiment 2, 2005; (C) greenhouse experiment 2006 and (D) field experiment 2006; Beaumont, TX. Bars with the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

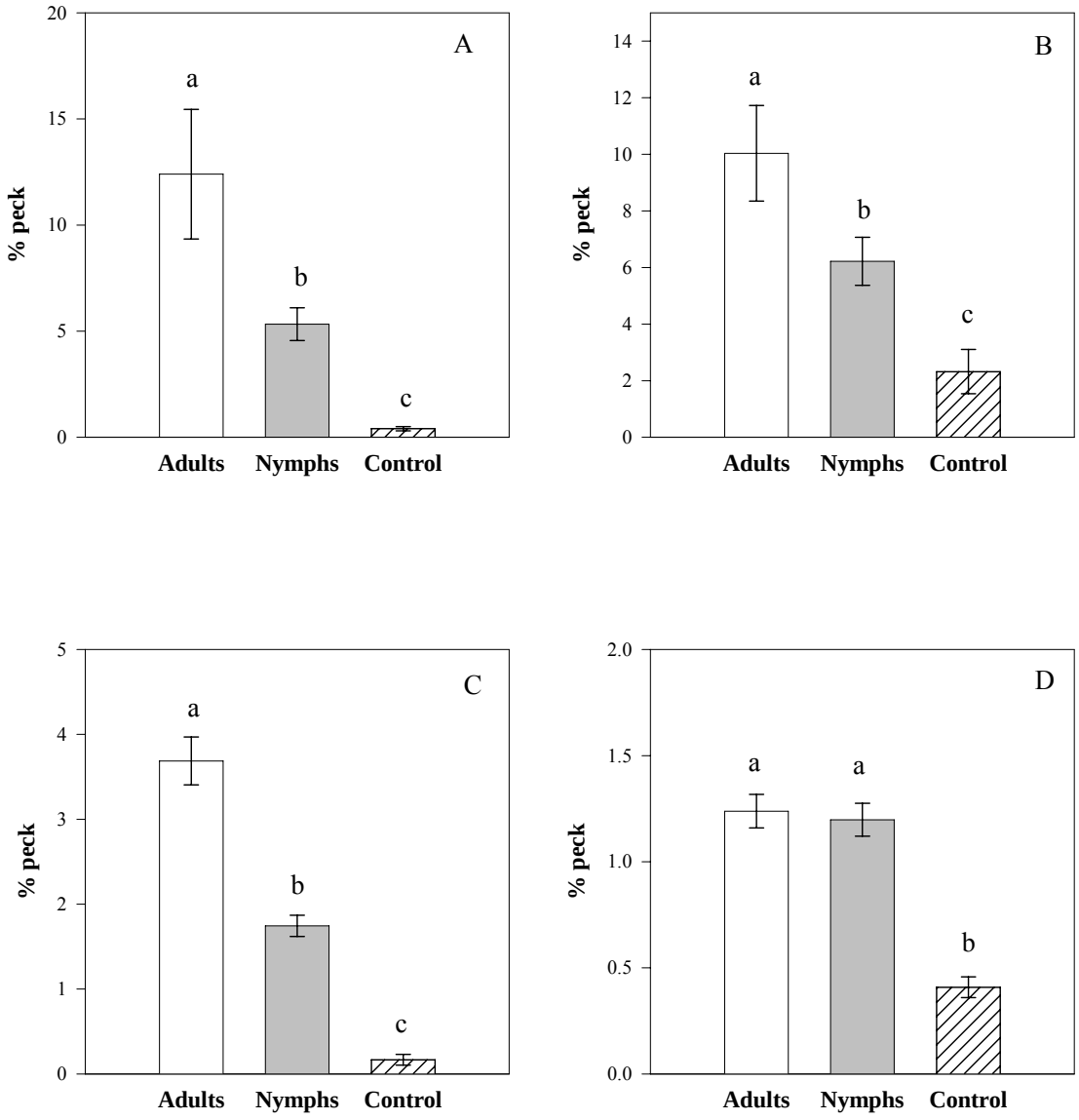

Fig. 3.4. Mean percentage peck (± SEM) of rice infested with adult or nymph *O. pugnax*. (A) Greenhouse experiment 1, 2005; (B) greenhouse experiment 2, 2005; (C) greenhouse experiment 2006 and (D) field experiment 2006; Beaumont, TX. Bars with the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

less peck than any of the other treatments (Table 3.4; Fig. 3.3C). Comparison of adult and nymph *O. pugnax* injury across all panicle stages showed that percentage peck was significantly higher for adults than nymphs, and both insect stages caused significantly more peck than the uninfested control (Table 3.5, Fig. 3.4C).

In field experiment 2006, panicle stage main effect was significant and insect stage main effect was not (Table 3.1). No significant differences were observed in percentage peck caused by *O. pugnax* infestation during milk and soft dough, and peck during these two stages was significantly higher than heading. The uninfested control showed significantly less peck than any of the other treatments (Table 3.4, Fig. 3.3D). Across all panicles stages, no significant differences were detected between percentage peck caused by adults and nymphs. Panicles from cages infested with adults or nymphs showed significantly more peck than panicles from uninfested control cages (Table 3.5, Fig. 3.4D).

Infestation of cages with *O. pugnax* did not significantly affect percentage whole kernels (Table 3.1 and 3.4). Mean percentage whole kernels per cage was 50.1 ± 0.7 for greenhouse experiment 1, 2005; 23.9 ± 0.6 for greenhouse experiment 2, 2005; 54.9 ± 0.6 for greenhouse experiment 2006; and 46 ± 0.2 for field experiment 2006.

Regression of percentage peck caused by nymphal feeding vs. percentage peck caused by adult feeding revealed that on average, nymphs (3^{rd} instars and older) caused 0.52 ± 0.03 as much peck as adults. Regression analysis between percentage peck and percentage whole kernels yielded a significant linear relationship for greenhouse experiment 1, 2005 (Table 3.6). The R-square value for this experiment shows that

Table 3.6. Linear regression analyses of whole kernels (dependet variable) against percentage peck (independent variable) of rice infested with adult or nymph *O. pugnax* at different stages of panicle development. Beaumont, TX, 2005 and 2006

Experiment	*n*	*F*	*P*	r^2
Greenhouse experiment 1, 2005	28	59.673	< 0.001	0.722
Greenhouse experiment 1, 2005[a]	26	4.047	0.057	0.162
Greenhouse experiment 2, 2005	28	0.525	0.475	0.020
Greenhouse experiment, 2006	56	1.412	0.24	0.025
Field experiment, 2006	162	1.722	0.191	0.011

[a]Linear regression excluding two suspected outliers.

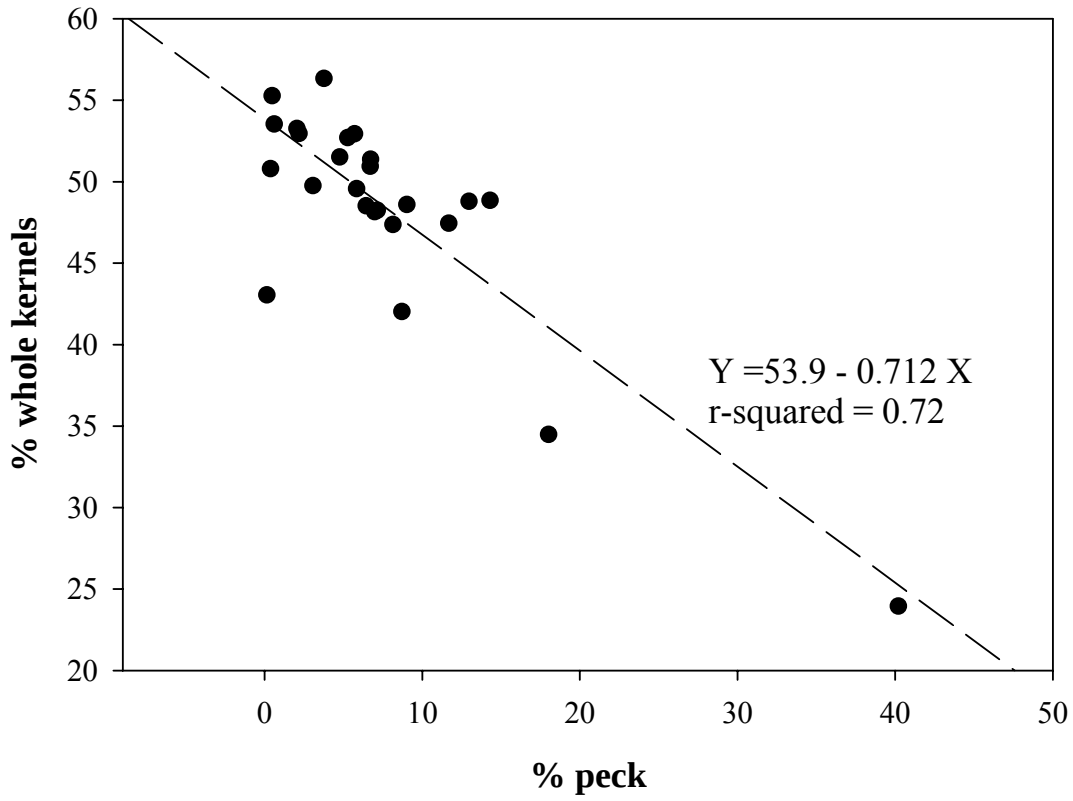

Fig. 3.5. Linear regression between percentage peck and percentage whole kernels of rice infested with *O. pugnax* during three stages of panicle development. Greenhouse experiment 1, 2005, Beaumont, TX.

percentage peck explains 72% of the variation in percentage whole kernels (Fig. 3.5). However, two data points fall outside the grouping of 26 data points. If these two points are removed from the analysis, the linear regression is nonsignificant (Table 3.6).

Discussion

Past research concerning *O. pugnax* damage suggests that infestations during the heading of rice reduce rough rice yield by increasing the number of empty grains, while infestations during milk and soft dough increase peck and reduce milling quality (Bowling 1967, Way et al. 2006). Based on the results of the current study, *O. pugnax* feeding did not affect rough rice yield (filled grain weight). Previous research has found no effect of *O. pugnax* feeding on rough rice yield. Odglen and Warren (1962) presented data from a single experiment using cages, while Harper et al. (1993) used data from naturally occurring infestations of *O. pugnax* collected during three years and from two locations in Texas. Tindall et al. (2005) also used natural infestations and found a reduction in the number of filled grains in unprotected rice plots; however, rough rice yield losses due to *O. pugnax* were not detected.

Other experiments using cages have found an effect of *O. pugnax* on rough rice yield (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963). However, in these experiments, infestation population levels used were much higher than are found normally in Texas rice fields (Harper et al. 1993). Pantoja et al. (2000), working with *O. ornatus* (Sailer), an important pentatomid pest of rice in Colombia, found a significant reduction in rough rice yield due to adult infestation during heading and milk. However,

action thresholds developed showed that, at population levels found in Colombian rice fields, rough rice yield reductions will rarely occur. Reductions in kernel weight and number of filled kernels due to *O. pugnax* feeding have been found in more recent experiments (Nilakhe 1976b, Patel et al. 2006), but these studies restricted insect feeding to a single panicle which overlooked the response of the whole plant.

Although the number and weight of empty grains in the current experiments tended to be higher in treatments infested during heading, no significant differences were found in the number and weight of filled grains. Panda and Khush (1995) consider compensatory responses of plants as a mechanism of tolerance to insects, and propose that sink-limited plants usually do not suffer yield reduction when injured, while source-limited plants undergo yield reduction due to insect injury. *O. pugnax* feeds on developing grains, which constitute a 'sink' for the rice plant, without affecting the 'source' (roots and foliage). Insect feeding may have caused an increase in the number of empty grains, but plants may have compensated for this injury by filling more grains or increasing the movement of photosynthates to grains not fed on by *O. pugnax*.

Differences in filled grain weight among experiments were due mainly to plant density. Plant density in greenhouse experiment 2006 (three plants per pot) was optimal, while plant density during the 2005 greenhouse experiments was higher (five plants per pot), causing a reduction in filled grain weight per cage. Also, in greenhouse experiment 1, 2005, plants were infected with kernel smut (*Tilletia horrida* Takah.), stem rot (*Sclerotium oryzae* Cattaneo) and black sheath rot (*Gaeumannomyces graminis* [Sacc.] Arx and D. Olivier var. *graminis*), causing some panicles to 'blank out', reducing filled

grain weight per cage. This explains the higher number of empty grains in this experiment (Table 3.2). During field experiment 2006, growing conditions were ideal and optimal plant density was used in a larger area, resulting in higher filled grain weight per cage than in any of the greenhouse experiments.

O. pugnax adults and nymphs can cause peck (Table 3.5, Fig. 3.4). Past experiments included nymphs in infestations (Douglas and Tullis 1950, Odglen and Warren 1962); however, nymphs were left to feed on panicles until they molted to adults, making difficult the assessment of the contribution of nymphs to damage. Bowling (1979) infested panicles with second to fifth instar nymphs for 48 hours and found that nymphs produced as much peck as adults. Harper et al. (1993, 1994) found that nymph populations were not a good predictor of peck. In the present study, it was found that adults cause higher percentage peck than nymphs. Across all experiments, adults caused twice as much peck as nymphs. Only in the field experiment was percentage peck caused by adults and nymphs similar. During the greenhouse experiments, newly-molted adults were relatively easy to locate and remove from selected cages. Cages were lifted off the plants which were examined daily. However, during the field experiment, newly-molted adults were difficult to locate. Cages could not be lifted because they were secured to the ground; and after heavy rains, driving to the field to check them was impossible. Also, the higher number of plants per cage allowed insects to hide in the foliage and panicles. These factors could have contributed to an adult 'contamination' of cages infested with nymphs, increasing percentage peck in these cages.

Milk and soft dough were the most susceptible stages of panicle development to *O. pugnax* feeding. When comparing injury from *O. pugnax* infestations during different stages, heading was the stage with the least percentage peck in three experiments. Milk showed significantly higher percentage peck than soft dough in one experiment, and milk and soft dough showed no significant differences in the other three experiments. Patel et al. (2006) found that the highest percentage peck was observed when panicles were infested with *O. pugnax* during the soft dough stage. Lee et al. (1993) determined that symptoms of insect damage were more prevalent when *O. pugnax* fed 15 d after anthesis. Anthesis occurs after panicle exertion and usually lasts 5 d (Moldenhauer and Gibson 2003), which could be considered the mid point of the heading stage. Thus, the results described herein are in agreement with these experiments. It is interesting to note that, in all experiments, a low percentage of peck was detected in uninfested control cages. This has been reported by other workers (Patel et al. 2006). As mentioned earlier, peck can be caused by several pathogenic microorganisms and we can not rule out the possibility of small insects infesting the cages and feeding on developing grains. However, clearly *O. pugnax* was the major cause of pecky grains.

In the current experiments, *O. pugnax* feeding did not affect percentage whole kernels. Due to lack of electricity caused by hurricane Rita during two days in the greenhouse, panicles in greenhouse experiment 2, 2005, were exposed to temperatures approaching 50^{o} C during the final stages of grain maturation. High temperatures prior to harvest have been found to reduce grain moisture (Wang and Luh 1991). Rice kernels are more likely to fissure on the panicle if left to dry below a certain minimum moisture

content, causing a reduction in head rice yield (Cnossen et al. 2003). The low percentage of whole kernels found in experiment 2, 2005 (23.9 ± 0.6%), may be explained by high temperatures in the greenhouse before harvest.

Previous work has found an effect of *O. pugnax* feeding on milling quality of rice (Swanson and Newsom 1962, Bowling 1963, Robinson et al. 1980, Harper et al. 1993, Tindall et al. 2005). When feeding on rice grains, *O. pugnax* can penetrate the hull with its piercing-sucking mouthparts, introducing microorganisms that cause peck (Way and Bowling 1991). Several pathogenic fungi have been associated with peck (Marchetti and Petersen 1984, Lee et al. 1993). In the current experiments, percentage peck was found at low levels in three experiments. Fungicide use may have played a role in reducing the incidence of peck in these experiments. Odglen and Warren (1962) failed to detect differences in percentage peck among treatments infested with varying densities of *O. pugnax*, and attributed this to the absence of fungal inoculum in the field. Greenhouse experiment 2, 2005, and the 2006 experiments were sprayed with fungicides at the boot stage, possibly causing a reduction in percentage peck.

In greenhouse experiment 1, 2005, peck reached values of 18 and 40% in two cages. These values are considerably higher than other peck values from similar infested cages. Considering these extreme values, a significant negative slope was found between percentage peck and percentage whole kernels (Table 3.6; Fig. 3.5). Regression analysis showed that for an increment of 1% peck, percentage whole kernels was reduced by approximately 0.8%. Similarly, Fryar et al. (1986) determined that a 1% increase in peck produced a 1% decrease in head yield, and Tindall et al. (2005) found a reduction of

0.5% in whole kernels for every percentage increase in pecky rice. However, if the two extreme values are not considered in the analysis, the regression becomes not significant. A significant relationship was not detected in the other experiments, which could have been due to the low percentage peck observed in these experiments. Also, the manner in which rice was dried and milled may have influenced the percentage of whole kernels obtained. Percentage whole kernels in milled rice decreases as drying air temperature increases. In order to maximize head yield, the lowest air temperature possible should be used to dry rice (Wang and Luh 1991). In the current experiments, rice samples were air dried at a lower temperature than commercial rice drying. In addition, milling in the current study was probably not as intense as commercial milling. Lower quality and lower price is associated with a less than optimal degree of milling (Wadsworth 1991); however, as degree of milling increases, the proportion of broken kernels also increases. These two factors, optimal air drying and less intense milling, possibly prevented some pecky grains from breaking during the milling process in the current experiments.

The current studies have shown that *O. pugnax* adults and nymphs feeding on rice reduce the quality of grain by causing peck but do not affect rough rice yield. Milk and soft dough are the most susceptible stages of panicle development to *O. pugnax* attack. Peck also was observed when panicles were infested during the heading stage; however, percentage peck during this stage was lower. Injury caused by nymphs was about half the injury caused by adults. Currently, nymphs are not considered when sampling and determining the need for controlling *O. pugnax* (Way et al. 2006). Thus, economic thresholds for *O. pugnax* may need revision, especially when late instar

nymph populations are high. For example, in California, Gutierrez et al. (1977) determined that males and nymphs of *Lygus hesperus* (Knight) cause less damage than females in cotton, and modified the economic threshold to reflect the real contribution to damage of each stage. *O. pugnax* economic thresholds may be modified in a similar fashion to incorporate nymphal damage potential. A strong relationship between percentage peck and percentage whole kernels was not detected. However, past research suggests a strong relationship; consequently, more research is needed to better quantify this association.

CHAPTER IV

RELATIVE SUSCEPTIBILITY OF STAGES OF RICE PANICLE DEVELOPMENT TO ADULT FEMALE AND MALE RICE STINK BUG FEEDING

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius), is one of the most important pests of rice in the southern United States (McPherson and McPherson 2000, Way 2003) attacking rice during flowering and grain development. Damage to rice due to *O. pugnax* reduces rough and head rice yields, and grain quality (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Harper et al. 1993, Tindall et al. 2005, Patel et al. 2006). This insect feeds on developing kernels, causing partially-filled grains and abortion of florets. Also, by introducing pathogenic microorganisms while feeding, *O. pugnax* causes a discoloration of the grain known as "peck" (Lee et al. 1993).

Several studies have been conducted to characterize and quantify *O. pugnax* damage to rice. In recent years, research has been performed with the objective of determining the susceptibility of rice to *O. pugnax* attack during different stages of panicle development (Patel et al. 2006). Current economic thresholds for *O. pugnax* vary as the grain matures. However, as a result of changes in cultural practices and cultivar selection in recent years, economic thresholds need to be revised. The objective of the present study is to determine the effect of *O. pugnax* on rice grain production during different stages of panicle development and to compare the damage potential of male and female adults.

Materials and Methods

Field experiments were conducted in 2005 and 2006 at the Texas A&M University Agricultural Research and Extension Center at Beaumont in Jefferson Co., TX (Beaumont Center). On 10 June 2005 and 4 April 2006, plots 5.5 m long, seven rows wide (0.18 m row spacing) were planted with the rice cultivar Cocodrie at a seeding rate of 102 kg/ha. Seeds were treated with fipronil at 0.042 kg AI/ha to suppress rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, population density. Water management, weed control, and other cultural practices were performed following the Texas Rice Production Guidelines (McCauley 2006, McCauley and Chandler 2006). Nitrogen in the form of urea was applied at planting, 11 July and 02 August 2005, and 5, 22 May 2006 (64 kg N/ha each application), and 12 June 2005 (45 kg N/ha). On 16 August 2005 and 12 June 2006, at early boot stage, plants were sprayed with a mixture of azoxystrobin and propiconazole at 0.19 kg AI /ha + 0.32 kg AI/ha, respectively, using a hand-held, CO_2 pressurized spray rig, to control panicle and foliage diseases.

At panicle exertion, 30 August 2005 and 27 June 2006, 10 plants per plot were selected and one panicle per plant in nine of the 10 selected plants caged. In 2005, five plants per row on two border rows were selected; while in 2006, five plants per row on two inside rows were selected. Plants on the same row were approximately equidistant from one another. Cages were constructed with white No See-Um netting (Outdoor Wilderness Fabrics Inc., Nampa, Idaho) and were 33 cm long by 13 cm wide. One end of the cage was closed using Velcro glued and stapled to the netting. Inside the cage, two stereo foam rings (2.5 cm wide, 9 cm diameter) were inserted to give the cage cylindrical

form. The selected panicle was guided through the Velcro end of the cage, and then the Velcro secured so that the panicle was upright and insects were unable to enter or exit the cage. The other end of the cage was closed using a twist-tie. The twist-tie was attached to a wire (10 cm long), which was secured perpendicularly to a bamboo stake (1.2 m long) inserted in the ground next to the selected plant (Fig. 4.1).

Selected panicles within a plot were randomly assigned to the following treatments: (1) panicle at heading infested with a male *O. pugnax*, (2) panicle at heading infested with a female *O. pugnax*, (3) panicle at milk infested with a male *O. pugnax*, (4) panicle at milk infested with a female *O. pugnax*, (5) panicle at soft dough infested with a male *O. pugnax*, (6) panicle at soft dough infested with a female *O. pugnax*, (7) panicle at hard dough infested with a male *O. pugnax*, (8) panicle at hard dough infested with a female *O. pugnax* (9) uninfested caged panicle, and (10) uninfested non-caged panicle. Panicles remained caged until harvest. Insects used in the experiments were collected from untreated rice and rice field weeds at the Beaumont Center. Treatments were arranged in a completely randomized design with six replications, each replication consisting of a different rice plot. Caged panicles were infested with one adult male or female *O. pugnax* for 48 h at four stages of panicle development: heading, milk, soft dough or hard dough. Since the feeding rate of *O. pugnax* has not been determined, a 48 h time interval was selected to allow insects to acclimate to the cage environment and feed normally. A shorter time interval may not allow detection of differences in susceptibility to *O. pugnax* injury across panicle stages. A longer time interval may result in abnormal injury and/or feeding behavior due to limited number of grains

Fig. 4.1. Caged rice panicle infested with *O. pugnax*. Beaumont, TX.

available to the caged insects. Cages were infested when all grains in the panicle reached the target stage. Heading was considered to begin at panicle exertion. Milk was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was milky and panicles started to bend downward due to weight of developing grains. Soft dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was soft dough (not liquid) and hulls turned from green to tan. Hard dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was hard and hulls were tan. To determine if cages had an effect on grain production, one panicle was not caged but protected from natural *O. pugnax* infestation by spraying it every 3 or 4 days with lambda-cyhalothrin at 0.228 g AI/l using a hand-held pump garden sprayer.

Cages were checked at least twice daily throughout the entire grain maturation period to ensure they were not damaged and to replace any dead or missing insects during the 48 h infestation period. Infestation dates were: heading, 1 September 2005 and 28 June 2006; milk, 7 September 2005 and 6 July 2006; soft dough, 12 September 2005 and 13 July 2006; hard dough, 20 September 2005 and 28 July 2006. Plots were drained on 14 September 2005 and 28 July 2006 and panicles hand harvested on 22 September 2005 and 8 August 2006 and stored in paper bags in a dry, cold room until processed. Grain was allowed to naturally air dry to 12% moisture.

Panicles were threshed by hand and all grains counted. Filled grains per panicle were manually separated from empty grains, counted and weighed. Filled grains were hulled using an Automatic Rice Husker (TR200, Kett Electric Laboratory, Japan) to

obtain brown rice. Brown rice was weighed and manually inspected for peck, and kernels with peck were weighed.

The United Sates Department of Agriculture (USDA) Federal Grain Inspection Service defines pecky kernels as "whole and broken kernels of rice that have one or more black, brown, red or other discolored spots or areas caused by fungus growth or insects" and consider them as a type of "damaged kernels" (USDA-FGIS 1994). For the purposes of the current study, only pecky rice caused by *O. pugnax* was considered, which is characterized by circular lesions 1-2 mm in diameter to covering most of the grain (Lee et al. 1993, Tindall et al. 2005). Brown rice was milled using a grain polisher (Pearlest, Kett Electric Laboratory, Japan). Milled kernels were weighed, manually separated into whole and broken kernels and whole kernels weighed. Milled kernels at least three fourths the length of unbroken kernels were considered whole kernels (USDA-FGIS 1994). Percentage peck per panicle was calculated as (weight of pecky kernels/weight of brown rice) x 100. Percentage whole kernels per panicle was calculated as (weight of milled whole kernels/weight of filled grains) x 100.

Data analysis. Statistical analyses were performed using the SPSS package (SPSS Inc. 2005). Total number of grains, number of filled grains, weight of filled grains, percentage peck and percentage whole kernels per panicle were analyzed for each year using a two-way analysis of variance (ANOVA) with factors panicle stage of infestation and insect gender. Tukey's honestly significant difference (HSD) test (Tukey 1953) was used to compare means of significant effects. To determine if cages affected the variables analyzed, linear contrasts were used to compare caged and non-caged

uninfested control panicles. If no significant differences between the two controls were detected, data were pooled and compared to other treatments as a single control. When the assumptions of normality of residuals and constant variances were not met, the data were transformed before applying ANOVA. The Box-Cox procedure was used to determine the best transformation (Kutner et al. 2005). To examine the relationship between percentage peck and whole kernels, linear regression analysis was performed between percentage peck (independent variable) and percentage whole kernels (dependent variable).

Results

During the 2006 experiment, two panicles infested during the hard dough stage were damaged due to storms. These panicles were not harvested. Three cages infested with females during the heading stage were contaminated with nymphs. Female *O. pugnax* in these cages laid eggs that were not detected and destroyed before causing injury. Yield component data from these panicles were not included in the analysis.

Linear contrasts revealed no significant differences ($P > 0.05$) in total number of grains, number of filled grains, weight of filled grains, percentage peck and percentage whole kernels between the uninfested caged and non-caged control panicles. Therefore, data from these two treatments were pooled and considered as a single uninfested control treatment for the remainder of the analysis.

Total number of grains, number of filled grains, weight of filled grains and percentage of whole kernels per panicle were not significantly affected by the treatments in 2005 or 2006 (Table 4.1). In 2005, panicles had an average of 150.42 ± 3.16 grains,

Table 4.1. Statistical analyses of yield components of panicles infested with male or female *O. pugnax* at different stages of panicle development. Beaumont, TX, 2005 and 2006

Variable/panicle	Factors	Year			
		2005		2006	
		F[a]	P	F[b]	P
Total number of grains	Panicle stage	0.259	0.854	0.049	0.985
	Insect gender	0.139	0.711	1.299	0.260
	Panicle stage x Insect gender	0.649	0.587	1.394	0.257
Number of filled grains	Panicle stage	0.108	0.955	0.844	0.477
	Insect gender	1.014	0.319	2.314	0.135
	Panicle stage x Insect gender	0.524	0.668	1.127	0.348
Weight of filled grains	Panicle stage	0.315	0.815	0.685	0.566
	Insect gender	0.414	0.523	2.875	0.097
	Panicle stage x Insect gender	0.122	0.947	0.805	0.497
% peck	Panicle stage	5.832	0.002	0.681	0.568
	Insect gender	0.083	0.774	1.935	0.171
	Panicle stage x Insect gender	1.001	0.400	2.776	0.052
% whole kernels	Panicle stage	0.102	0.958	1.785	0.163
	Insect gender	0.094	0.760	0.955	0.334
	Panicle stage x Insect gender	1.946	0.134	0.842	0.478

[a]Panicle stage df: 3, 51; Insect gender df: 1, 51; Panicle stage x Insect gender df: 3, 51.
[b]Panicle stage df: 3, 46; Insect gender df, 1, 46: Panicle stage x Insect gender df: 3, 46.

124.02 ± 2.68 were filled and weighed 2.87 ± 0.06 g. In 2006, panicles had an average of 105.82 ± 1.75 grains, 91.89 ± 1.67 were filled and weighed 2.28 ± 0.05 g. Percentage of whole kernels was 47.31 ± 0.64% and 36.64 ± 0.97% in 2005 and 2006, respectively.

In 2005, percentage peck was significantly affected by *O. pugnax* infestation (Table 4.1). Panicle stage by insect gender interaction and insect gender main effect were not significant, while panicle stage main effect was significant. Peck was significantly higher in panicles infested during heading, milk and soft dough, but no significant differences were found among these stages (Fig. 4.2A). No significant differences were found between uninfested control panicles and panicles infested during hard dough. Peck in panicles infested during heading, milk and soft dough was on average 5.4 times higher than in the uninfested control. Across all panicle stages, no significant differences were detected between percentage peck produced by male and female *O. pugnax*, but both sexes produced significantly more peck than observed in the uninfested control (Fig. 4.3A).

In 2006 no differences were found in percentage peck of panicles infested during heading, milk, soft dough or hard dough. No significant differences were found in percentage peck caused by *O. pugnax* infestation during soft dough, hard dough and the uninfested control (Fig. 4.2B). On average, percentage peck in infested panicles was 3.7 times higher than in uninfested control panicles (Fig. 4.2B). Across all panicle stages, no

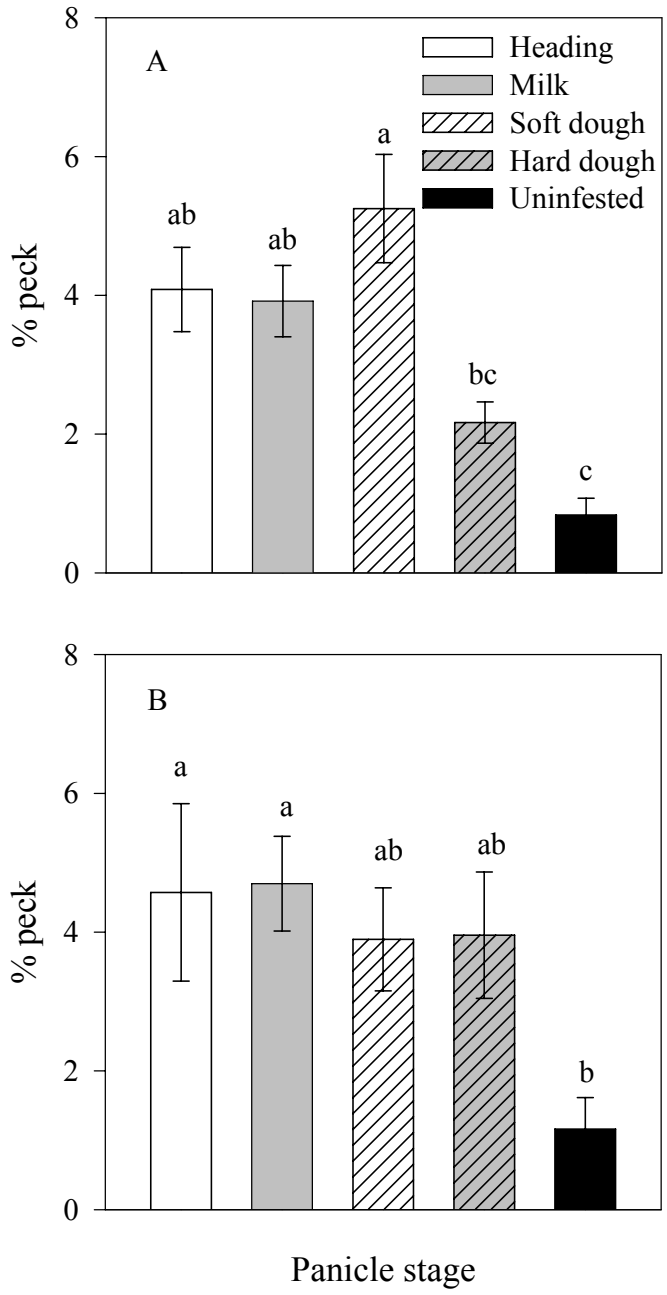

Fig. 4.2. Mean percentage peck ± SEM in panicles infested with *O. pugnax* during four stages of panicle development in (A) 2005 and (B) 2006. Bars followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD).

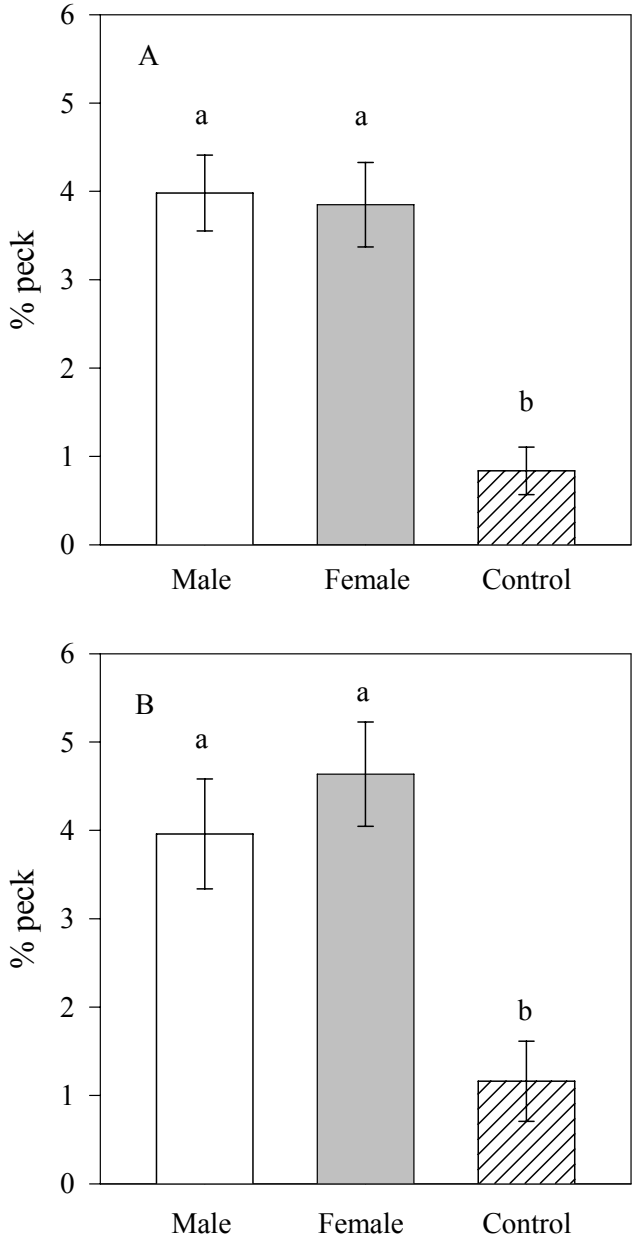

Fig. 4.3. Mean percentage peck ± SEM in panicles infested with male or female *O. pugnax* in (A) 2005 and (B) 2006. Bars followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD).

significant differences were detected between percentage peck produced by male and female *O. pugnax*, but both sexes produced significantly more peck than observed in the uninfested control (Fig. 4.3B).

Regression analyses between percentage peck and percentage whole kernels were not significant for both years ($F = 0.259$; df = 1, 58; $P = 0.613$ for 2005; $F = 0.105$; df = 1, 53; $P = 0.747$ for 2006).

Discussion

In the current experiments, infestation with *O. pugnax* for 48 h during different stages of panicle development did not affect filled grain weight or number of filled grains per panicle. Past research has found reductions in kernel weight and percentage of filled grains when individual panicles were infested with *O. pugnax.* Nilakhe (1976b) screened 228 rice lines for resistance to *O. pugnax* by caging the insects for four weeks on field grown plants. He found differences in weight loss per kernel due to *O. pugnax* feeding and a positive correlation between weight loss per kernel and percent pecky grains. Robinson et al. (1980) found a reduction in percentage of filled grains and kernel weight per panicle when panicles were infested for seven or eight days with *O. pugnax*. Patel et al. (2006) infested individual rice panicles with one or two *O. pugnax* for four days. They found higher percentage of empty grains when panicles were infested during the heading stage and a significant reduction in grain weight when panicles were infested during heading, milk and soft dough stages.

Results from other studies in which whole plants were caged and infested with *O. pugnax* or in which natural infestations were used have been contradictory (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Harper et al. 1993, Tindall et al. 2005). Currently, *O. pugnax* is considered to cause reductions in number of filled grains and grain weight (Way 2003, Way et al. 2006). However, as shown in Chapter III, the capacity of rice plants to compensate for these types of damage in field situations may reduce the effect of *O. pugnax* on rice yield. In the current experiments, the short period of infestation may explain the lack of differences among treatments in filled grain number and weight per panicle.

Differences were not found in percentage peck caused by male or female *O. pugnax* across all stages of panicle development (Table 4.1, Fig. 4.3). Bowling (1979) used stylet sheaths as an indicator of *O. pugnax* feeding. He determined that females produced twice the number of stylet sheaths compared to males when feeding on rice; however, he did not associate feeding with damage. Rashid et al. (2005) exposed male and female *O. pugnax* to different host plants and artificial diet in the laboratory and found that females fed more than males over a 24 h period. The results described herein do not agree with these observations. Changing weather conditions during panicle infestation may have caused a reduction in insect feeding rates. For example, frequent temperature and wind speed fluctuations in the field may affect insect mobility and feeding behavior. Under laboratory conditions, these weather effects are minimized.

Also, age and feeding status differences among field collected insects used in the experiments may have influenced feeding rates. Finally, the infestation period used (48 h) may have been too short to permit differentiation between female and male feeding.

Percentage peck per stage in infested cages for both years were similar (Fig. 4.2) and ranged from 2.19 to 5.20 in 2005 and 3.90 to 4.57 in 2006. Previous research reported that milk and soft dough stages of panicle development are the most susceptible to *O. pugnax* damage (Lee et al. 1993, Patel et al. 2006). Results of the 2005 experiment suggest that heading, milk and soft dough are equally susceptible (Fig. 4.2A). Percentage peck caused by *O. pugnax* infestation during the hard dough stage was not significantly different from percentage peck detected in the uninfested control. This was expected because, as grains harden, they become less suitable for *O. pugnax* feeding (Patel et al. 2006). In 2006, no differences were found in percentage peck caused by *O. pugnax* infestation during heading, milk, soft or hard dough (Fig. 4.2B). Also, percentage peck during soft and hard dough was not significantly different from the uninfested control. Plots in the 2006 experiments were drained the same day hard dough panicles were infested, while in 2005 hard dough panicles were infested 6 days after plots were drained. Possibly in 2006, due to late drainage and presence of water in the plots, not all grains on the panicles at the moment of infestation were in the hard dough stage. Grains at the top of panicles mature faster than those at the bottom (Counce et al. 2000). Grains at the bottom were in late milk stage probably were still susceptible to *O. pugnax* feeding. In both experiments, low percentage of peck was detected in the control panicles. This has been previously reported by others (Patel et al. 2006). Peck is associated with several

pathogenic fungi that are introduced into the kernel by *O. pugnax* feeding (Douglas and Tullis 1950, Lee and Tugwell 1980), but development of these pathogens in rice kernels without *O. pugnax* assistance is possible (Lee et al. 1993).

O. pugnax feeding did not affect whole kernel percentages in 2005 or 2006 (Table 4.1). Past research determined an effect of *O. pugnax* feeding on milling quality of rice (Swanson and Newsom 1962, Bowling 1963, Robinson et al. 1980, Harper et al. 1993, Tindall et al. 2005). Pecky grains are structurally damaged and tend to break during milling, reducing the percentage of whole kernels and the quality of the grain (Odglen and Warren 1962). In the present study, percentage whole kernels values obtained were considerably lower than typical percentage whole kernels values for Cocodrie (McClung et al. 2006). A possible explanation is that milling in the present experiment was more intense than commercial milling. Extended milling has been found to decrease percentage of whole kernels due to increase breakage caused by mechanical stress (Wadsworth 1991). In the present study, grains from a single panicle were milled for 20 s. This time might have been excessive for such a reduced mass of kernels, causing an increase in the fraction of broken kernels in all treatments. The high proportion of broken kernels due to intense milling may have rendered the relationship between percentage peck and percentage whole kernels nonsignificant.

Economic thresholds for *O. pugnax* management are currently available (Harper et al. 1994, Way et al. 2006). The thresholds increase as grain matures, conveying that susceptibility of rice to *O. pugnax* damage decreases as panicles age from heading to milk to soft dough. The results described herein indicate that *O. pugnax* can cause peck

during all stages of panicle development and that heading, milk and soft dough stages seem to be most susceptible. Unlike previous research, no effect of *O. pugnax* was found on the number or weight of filled grains. However, infestation duration in the present studies was shorter than in previous experiments. These results indicate that *O. pugnax* economic thresholds in rice may need to be revised. The use of more accurate thresholds could benefit growers by reducing *O. pugnax* damage to the crop by avoiding unnecessary control actions.

CHAPTER V

ATTRACTIVENESS OF STAGES OF RICE PANICLE DEVELOPMENT TO RICE STINK BUG

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), is a serious pest of rice, *Oryza sativa* L., in the southern United States (Way 2003) attacking the crop from flowering to grain maturity. This insect is responsible for reductions of rough and head rice yields, and grain quality by feeding on developing kernels, introducing pathogenic fungi and causing a discoloration of the grain known as "peck" for which growers are penalized (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Harper et al. 1993, Tindall et al. 2005, Patel et al. 2006).

O. pugnax is a polyphagous insect (McPherson and McPherson 2000) with many host plants, most of which are grassy weeds found around rice fields and levees (Douglas 1939, Odglen and Warren 1962, McPherson and McPherson 2000). Vasey grass, *Paspalum urvillei* Steud., has been recognized as one of the preferred wild hosts (Douglas 1939, Douglas and Ingram 1942, Naresh and Smith 1984) as well as barnyardgrass, *Echinochloa crusgalli* (L.), broadleaf signal grass, *Brachiaria platyphylla* (Griseb.); southern crabgrass, *Digitaria ciliaris* (Retz.); jungle rice, *Echinochloa colona* (L.); and Dallis grass, *Paspalum dilatatum* Poir. (Naresh and Smith 1984, Tindall et al. 2004, Rashid et al. 2005, Tindall et al. 2005). Rice is a preferred cultivated host (Naresh and Smith 1984). Other cultivated crops attacked by this insect are corn, wheat, barley, rye, oats and sorghum (Odglen and Warren 1962).

Adult *O. pugnax* typically move to rice fields from weeds or sorghum fields when the rice crop starts to head (Way 2003). However, rice panicles can become infested at any moment of maturity (Way and Bowling 1991) and field populations can increase dramatically in a very short time (Douglas 1939). Numerous field studies have determined that *O. pugnax* move to heading fields regardless of calendar date of heading (Ingram 1927, Douglas 1939, Douglas and Tullis 1950, Odglen and Warren 1962, Jones and Cherry 1986, Rashid et al. 2006). Bowling (1967) suggested a reduction in the suitability of wild hosts as food source due to aging may trigger *O. pugnax* movement to rice, while Rashid et al. (2006) conjectured stronger attractiveness of rice panicles over weed hosts. However, the relative attractiveness of different stages of panicle development has not been reported. The objective of the present study is to address this knowledge gap.

Materials and Methods

Greenhouse experiments were conducted during 2004 and 2005 at the Texas A&M University System, Agricultural Research and Extension Center at Beaumont (Jefferson County), TX. Seeds of the rice cultivar Cocodrie were planted on 15, 21 and 30 June and 6 July 2004, and 4 and 24 May, and 3 and 13 June 2005 in pots (15 cm lip diameter, 10 cm base diameter, 15 cm deep) containing sifted League soil. Pots were placed in bins (0.9 x 0.9 m, 0.19 m deep) and when plants reached the tillering stage, bins were flooded. In 2005, after rice emergence, pots were moved to bins outside the greenhouse to provide plants with adequate light to avoid etiolation and encourage

normal growth. On 8 August 2005, plants were returned to the greenhouse. One week after emergence, plants received an application of lambda-cyhalothrin (Karate Zeon FV, Syngenta Crop Protection) at 0.045 kg AI/ha using a hand-held, CO_2 pressurized spray rig to suppress rice water weevil *Lissorhoptrus oryzophilus* Kuschel attack (Way et al. 2006). Nitrogen in the form of urea was applied by hand at planting and at the beginning of tillering in 2004, and at planting, beginning of tillering, and 2 weeks later in 2005 (64 kg N/ha each application). Before the tillering nitrogen application, pots were thinned to four plants/pot.

On 30 August 2004 and 8 August 2005, groups of four pots were placed in bins filled with water and then covered with a cylindrical cage. Cages were 45 cm in diameter and 90 cm in height, constructed with hardware cloth (3 mm x 3 mm apertures). The bottom opening of the cage was submerged in water and the top opening was covered with plastic screening (3 mm x 3 mm apertures) kept in place with an elastic band. Each of the pots in a bin represented a different planting date; therefore, each pot contained plants in one of four different stages of development: pre-heading, heading, milk, and soft dough. Plants were considered to be in the pre-heading stage before panicle exertion or boot split. Heading was considered to begin at panicle exertion. Milk was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was milky and panicles started to bend downward due to weight of developing grains. Soft dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was soft dough (not liquid) and hulls turned from green to tan. The arrangement of pots within cages was changed so that each plant stage occupied a

unique cardinal direction position in each cage, yielding four arrangements. These arrangements were repeated four times for a total of 16 cages.

Cages were infested with five male and five female *O. pugnax* from 3 to 8 September 2004 and 9 to 14 August 2005. Male *O. pugnax* were marked with a black permanent marker on the scutellum to easily differentiate them from females. Insects were collected from untreated rice and rice field weeds at the Beaumont Center using an insect sweep net. One day after infestation, cages were visually inspected twice daily for five days. Sex and number of *O. pugnax* observed on pre-heading plants or plants with panicles in heading, milk or soft dough stages and on the inside cage surfaces were recorded. These sites where *O. pugnax* were observed will be referred to as location. Observations were made during the morning, between 0900 and 1100 h CDT, and afternoon, between 1500 and 1700 h CDT. Dead or missing insects were replaced after each inspection. Distance between bins allowed for free movement around cages to facilitate observation and data recording.

Data analysis. All analyses were performed using SPSS (SPSS Inc. 2005). Number of *O. pugnax* was analyzed in a repeated measures analysis of variance (ANOVA) with cages as subjects, location (pre-heading vs. heading vs. milk vs. soft dough plants vs. cage surface), sex (male vs. female), day of observation (first through fifth), and time (morning vs. afternoon) as within-subjects factors, and arrangement of pots within a cage as between-subjects factor. To stabilize variances, number of *O. pugnax* was transformed to ln (x + 1) before ANOVA. To determine if the covariance of the dependent variable met the sphericity assumption, Mauchly's test was performed. If

the assumption was not met, the Huynh-Feldt correction was applied when necessary. Comparisons between levels of significant factors were made using Fisher's least significant difference (LSD) test. The level of alpha used in all analyses was 0.05.

Results

For both years, the interactions of location by day by time, and location by sex by time were significant, and the main effect arrangement was not significant (Table 5.1, Fig. 5.1 and 5.2). Fig. 5.1 and table 5.2 show changes in the mean number of *O. pugnax* per location across days for morning and afternoon inspections in 2004. During morning inspections, number of *O. pugnax* across days increased significantly on pre-heading plants, remained constant on heading plants, significantly decreased during day 3 on milk and soft dough plants, and significantly increased during day 3 on the cage surface. During afternoon inspections, number of *O. pugnax* across days remained constant on pre-heading, heading and milk plants; significantly increased on day 4 on soft dough plants; and significantly increased on day 1 on the cage surface.

Figs. 5.3 and 5.4 show the mean number of *O. pugnax* observed per location during morning and afternoon inspections on different days for 2004. On days 1, 3, and 5, significantly more *O. pugnax* were observed on heading, milk and soft dough than pre-heading plants during morning and afternoon inspections. On day 2, during the morning inspection, significantly more insects were observed on soft dough than pre-heading or heading plants. Number of insects observed on milk plants was not

Table 5.1. Analysis of variance (ANOVA) table for number of *O. pugnax* per location during morning and afternoon inspections during 5 days. Beaumont, TX. 2004 and 2005

Effects	2004			2005		
Within-subjects effects	df	*F*	*P*	df	*F*	*P*
Location	3.6, 43.6[b]	33.055	< 0.001	3.6, 43.4[b]	78.154	< 0.001
Location x Arr[a]	12, 48	1.280	0.261	12, 48	1.138	0.353
Sex	1, 12	5.340	0.039	1, 12	4.795	0.049
Sex x Arr	3, 12	0.018	0.997	3,12	1.069	0.399
Day	4, 48	2.982	0.028	3.6, 42.7[b]	3.476	0.018
Day x Arr	12, 48	1.211	0.304	12, 48	1.259	0.273
Time	1, 12	0.345	0.568	1, 12	0.844	0.376
Time x Arr	3, 12	0.426	0.738	3, 12	0.814	0.514
Location x Sex	4, 48	14.687	< 0.001	4, 48	18.780	< 0.001
Location x Sex x Arr	12, 48	1.252	0.278	12, 48	0.831	0.618
Location x Day	16, 192	1.650	0.060	16, 192	4.727	< 0.001
Location x Day x Arr	48, 192	0.954	0.563	48, 192	0.839	0.760
Sex x Day	4, 48	0.994	0.420	4, 48	0.789	0.538
Sex x Day x Arr	12, 48	0.458	0.929	12, 48	1.357	0.220
Location x Sex x Day	16, 192	0.252	0.175	16, 192	1.398	0.146
Location x Sex x Day x Arr	48, 192	0.903	0.653	48, 192	0.940	0.588
Location x Time	4, 48	4.146	0.006	3.4, 40.8[b]	47.517	< 0.001

Table 5.1. Continued

Effects	2004			2005		
Within-subjects effects	df	*F*	*P*	df	*F*	*P*
Location x Time x Arr	12, 48	1.853	0.066	12, 48	1.358	0.219
Sex x Time	1, 12	0.367	0.556	1, 12	5.341	0.039
Sex x Time x Arr	3,12	0.064	0.978	3, 12	2.641	0.097
Location x Sex x Time	4, 48	4.169	0.006	4, 48	11.858	< 0.001
Location x Sex x Time x Arr	12, 48	1.317	0.240	12, 48	0.642	0.796
Day x Time	4, 48	2.470	0.057	4,48	2.855	0.033
Day x Time x Arr	12, 48	0.733	0.713	12, 48	1.703	0.096
Location x Day x Time	16, 192	1.934	0.020	16, 192	2.116	0.009
Location x Day x Time x Arr	48, 192	0.608	0.978	48, 192	0.764	0.864
Sex x Day x Time	4, 48	0.425	0.790	4, 48	1.903	0.125
Sex x Day x Time x Arr	12, 48	1.860	0.065	12, 48	1.032	0.436
Location x Sex x Day x Time	16, 192	0.907	0.563	16, 192	0.306	0.996
Location x Sex x Day x Time x Arr	48, 192	1.041	0.413	48, 192	1.357	0.078
Between-subjects effect						
Arr	3, 12	2.522	0.107	3, 12	1.156	0.367

[a] Arrangement of pots within a cage.

[b]df adjusted using the Huynh-Feldt correction.

Table 5.2. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections for 5 days. Beaumont, TX, 2004

Day	Morning				
	Pre-heading	Heading	Milk	Soft dough	Cage
1	0.063 ± 0.063bc	1.500 ± 0.357a	2.125 ± 0.222a	1.938 ± 0.403ab	3.063 ± 0.499ab
2	0 ± 0c	1.250 ± 0.302a	2.375 ± 0.418a	2.875 ± 0.459a	2.750 ± 0.492b
3	0.188 ± 0.095abc	1.563 ± 0.242a	1.250 ± 0.342b	1.250 ± 0.255b	4.250 ± 0.566a
4	0.250 ± 0.114ab	1.438 ± 0.380a	2.438 ± 0.425a	2.063 ± 0.567ab	3.188 ± 0.291ab
5	0.438 ± 0.149a	1.563 ± 0.194a	2.563 ± 0.452a	1.500 ± 0.454ab	3.500 ± 0.487ab
Day	Afternoon				
	Pre-heading	Heading	Milk	Soft dough	Cage
1	0.125 ± 0.072a	1.438 ± 0.384a	1.563 ± 0.387a	1.500 ± 0.245b	5.125 ± 0.350a
2	0.188 ± 0.140a	0.875 ± 0.335a	1.563 ± 0.397a	1.563 ± 0.237b	3.688 ± 0.504b
3	0.313 ± 0.140a	1.313 ± 0.317a	1.813 ± 0.277a	1.438 ± 0.407b	4.063 ± 0.422ab
4	0.375 ± 0.210a	1.563 ± 0.258a	1.625 ± 0.375a	2.813 ± 0.422a	3.125 ± 0.375b
5	0.250 ± 0.177a	1.125 ± 0.255a	2.125 ± 0.378a	2.063 ± 0.536b	3.688 ± 0.512b

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; LSD).

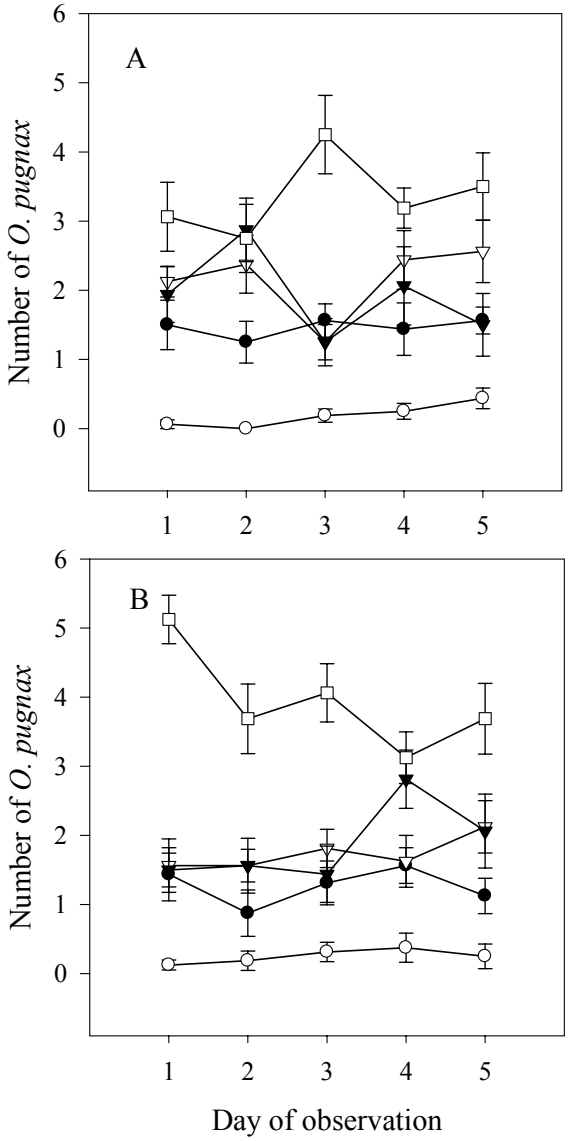

Fig. 5.1. Mean number of *O. pugnax* ± SEM (n = 16) per location during morning (A) and afternoon (B) inspections for 5 days. Locations are pre-heading plants (–○–), heading plants (–●–), milk plants (–Δ–), soft dough plants (–▼–) and cage surface (–□–). Beaumont, TX, 2004.

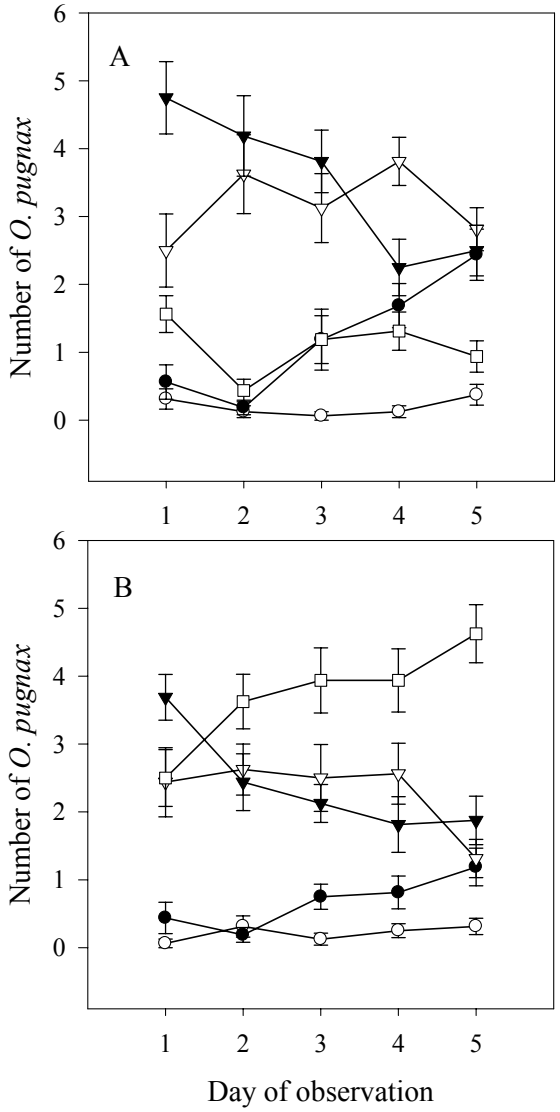

Fig. 5.2. Mean number of *O. pugnax* ± SEM (*n* = 16) per location during morning (A) and afternoon (B) inspections for 5 days. Locations are pre-heading plants (—○—), heading plants (—●—), milk plants (—Δ—), soft dough plants (—▼—) and cage surface (—□—). Beaumont, TX, 2005.

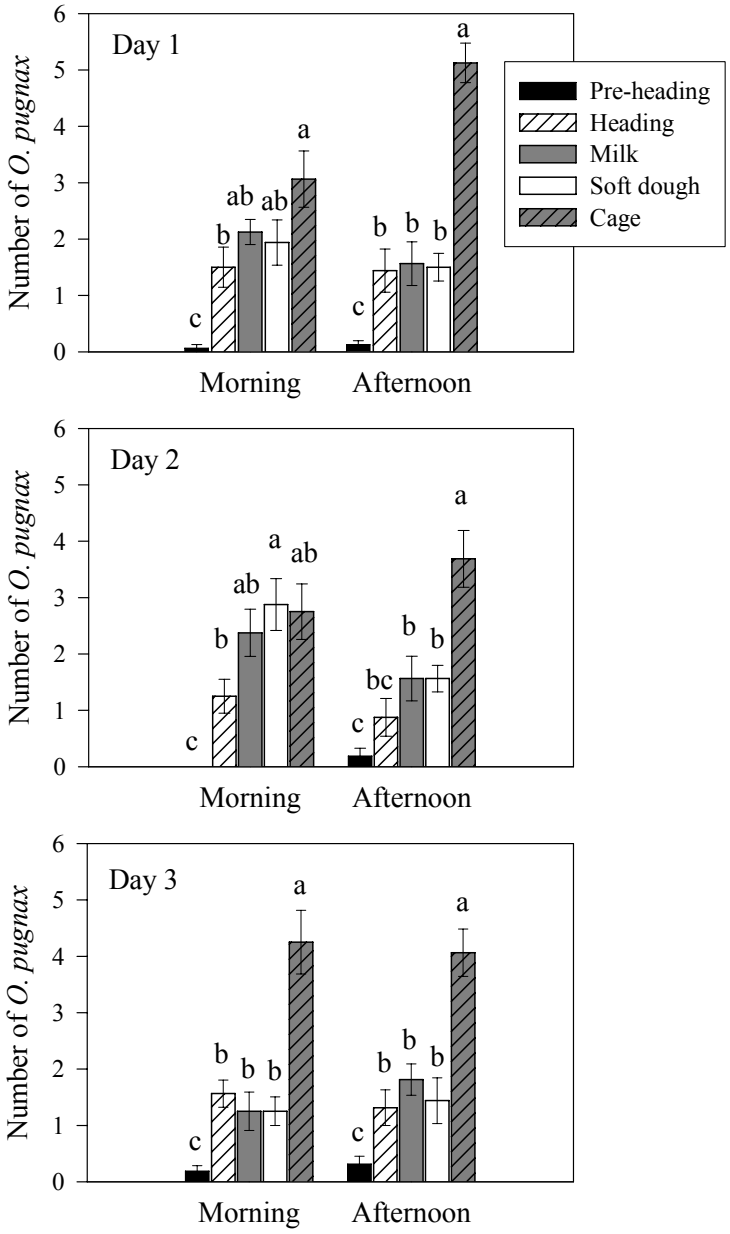

Fig. 5.3. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections on days 1, 2 and 3. Beaumont, TX, 2004. For each day and inspection time, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

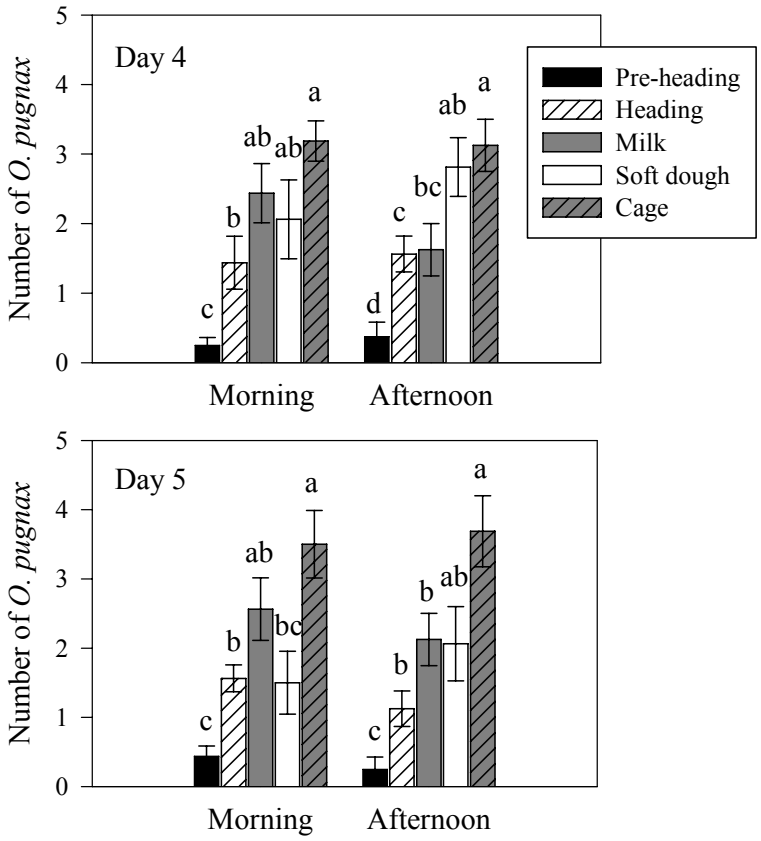

Fig. 5.4. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections on days 4 and 5. Beaumont, TX, 2004. For each day and inspection time, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

significantly different from heading or soft dough plants. During the afternoon inspection, significantly more *O. pugnax* were observed on heading, milk and soft dough than pre-heading plants. On day 4, during the morning inspection significantly more *O. pugnax* were observed on heading, milk and soft dough than pre-heading plants, while during the afternoon inspection significantly more insects were observed on soft dough than pre-heading or heading plants. Number of insects observed on milk plants was not significantly different from heading or soft dough plants. On most days, the number of insects observed on the cage surface was significantly higher than on plants. For all days, significantly fewer insects were observed on pre-heading than on any other plant stage during both morning and afternoon inspections. When comparing the mean number of *O. pugnax* observed during morning and afternoon inspections per location and day in 2004, no significant differences were detected on days 3 and 5. On day 1, significantly more insects were observed on the cage surface during the afternoon than the morning inspection ($P = 0.014$). On day 2, significantly more insects were observed during the morning than the afternoon inspection on milk and soft dough plants ($P = 0.009$ for milk plants, $P = 0.012$ for soft dough plants). On day 4, significantly more insects were observed on soft dough plants during the afternoon than the morning inspection ($P = 0.043$).

Fig. 5.5 shows the mean number of male and female *O. pugnax* per location during morning and afternoon inspections for 2004. During morning inspections, significantly more males were observed on milk and soft dough than on pre-heading or heading plants, while no difference was found in the number of females observed on

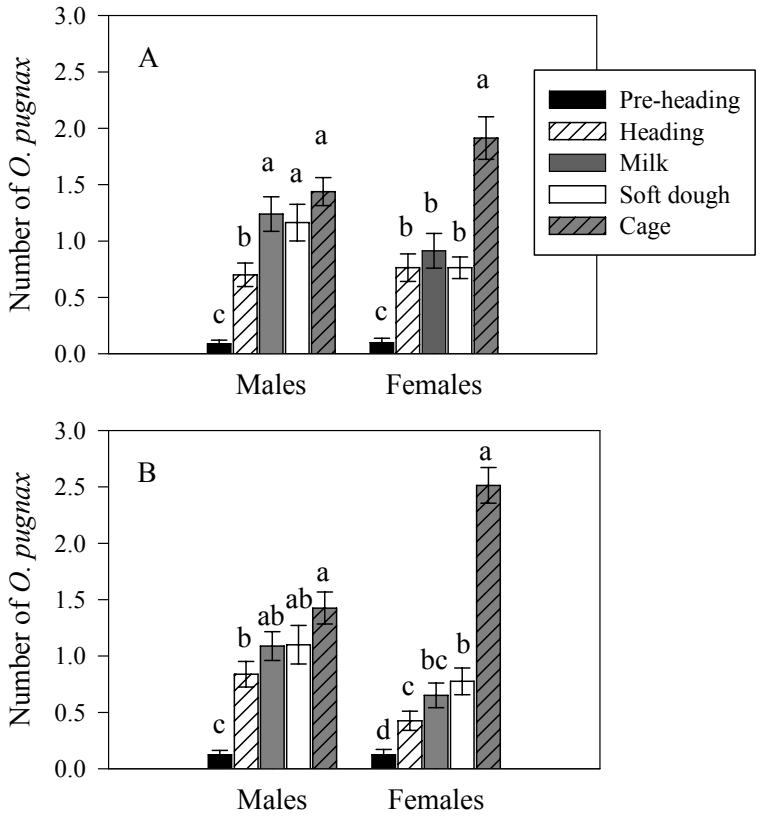

Fig. 5.5. Mean number of male and female *O. pugnax* ± SEM (n = 80) per location on rice at different stages of development during morning (A) and afternoon (B) inspections. Beaumont, TX, 2004. For each inspection time and sex group, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

heading, milk or soft dough plants. During afternoon inspections, no difference was found in the number of males observed on heading, milk and soft dough plants, while significantly more females were observed on soft dough than pre-heading or heading plants. Number of female *O. pugnax* observed on milk plants was not significantly different from numbers observed on heading or soft dough plants. Significantly fewer *O. pugnax* were observed on pre-heading plants than on the other plant stages during both morning and afternoon inspections. When comparing the mean number of male and female *O. pugnax* observed per location, during morning inspections, significantly more males than females were found on milk and soft dough plants ($P = 0.037$ for milk, $P = 0.001$ for soft dough), and during afternoon inspections significantly more males than females were found on heading, milk, and soft dough plants ($P = 0.002$ for heading, $P = 0.005$ for milk, $P = 0.029$ for soft dough). During both inspection times, significantly more females than males were found resting on the cage surface ($P = 0.044$ for morning and $P < 0.001$ for afternoon).

Fig. 5.2 and table 5.3 show the mean number of *O. pugnax* observed per location across days for morning and afternoon inspections in 2005. During morning inspections, number of *O. pugnax* observed on pre-heading and milk plants and on the cage surface remained relatively constant across days. Number of *O. pugnax* observed on heading plants increased, while number of insects on soft dough plants decreased across days. During afternoon inspections, number of *O. pugnax* observed on all plant stages and the cage remained relatively constant across days. Likewise, a similar trend was detected during morning inspections for heading and soft dough plants.

Table 5.3. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections for 5 days. Beaumont, TX, 2005

Day	Morning				
	Pre-heading	Heading	Milk	Soft dough	Cage
1	0.313 ± 0.149ab	0.563 ± 0.253cd	2.500 ± 0.538c	4.750 ± 0.533c	1.563 ± 0.272a
2	0.125 ± 0.088ab	0.188 ± 0.108d	3.625 ± 0.582ab	4.188 ± 0.592c	0.438 ± 0.165b
3	0.063 ± 0.063b	1.188 ± 0.352bc	3.125 ± 0.508abc	3.813 ± 0.461bc	1.188 ± 0.449ab
4	0.125 ± 0.088ab	1.688 ± 0.325ab	3.813 ± 0.355a	2.250 ± 0.415a	1.313 ± 0.282a
5	0.375 ± 0.153b	2.438 ± 0.377a	2.813 ± 0.317bc	2.500 ± 0.372ab	0.938 ± 0.231ab
Day	Afternoon				
	Pre-heading	Heading	Milk	Soft dough	Cage
1	0.063 ± 0.063a	0.438 ± 0.231ab	2.438 ± 0.509ab	3.688 ± 0.337a	2.500 ± 0.418b
2	0.313 ± 0.157a	0.188 ± 0.108b	2.625 ± 0.375a	2.438 ± 0.419b	3.625 ± 0.402ab
3	0.125 ± 0.088a	0.750 ± 0.184a	2.500 ± 0.492ab	2.125 ± 0.280b	3.938 ± 0.480ab
4	0.250 ± 0.102a	0.813 ± 0.242a	2.563 ± 0.449ab	1.813 ± 0.410b	3.938 ± 0.466ab
5	0.313 ± 0.120a	1.188 ± 0.277a	1.313 ± 0.282b	1.875 ± 0.357b	4.625 ± 0.427a

Means within the same column followed by the same letter are not significantly different ($P > 0.05$; LSD).

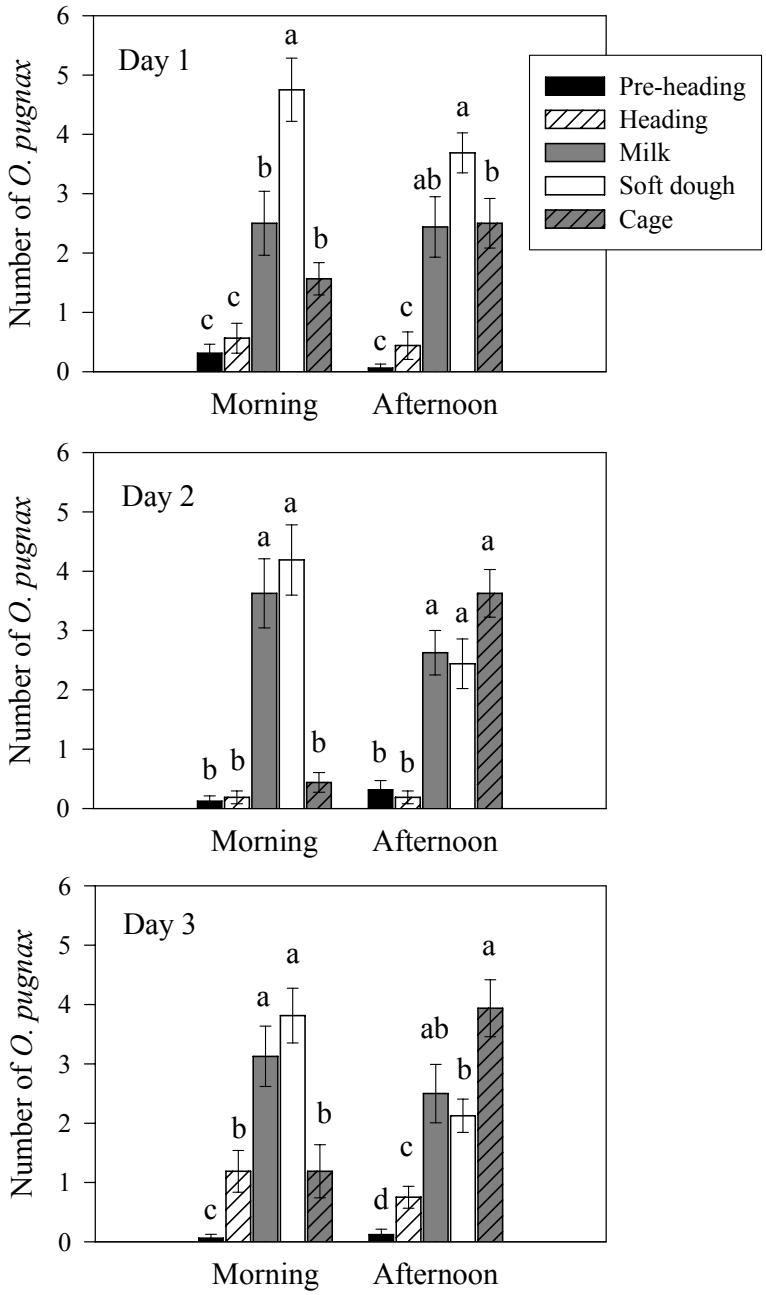

Fig. 5.6. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections on days 1, 2 and 3. Beaumont, TX, 2005. For each day and inspection time, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

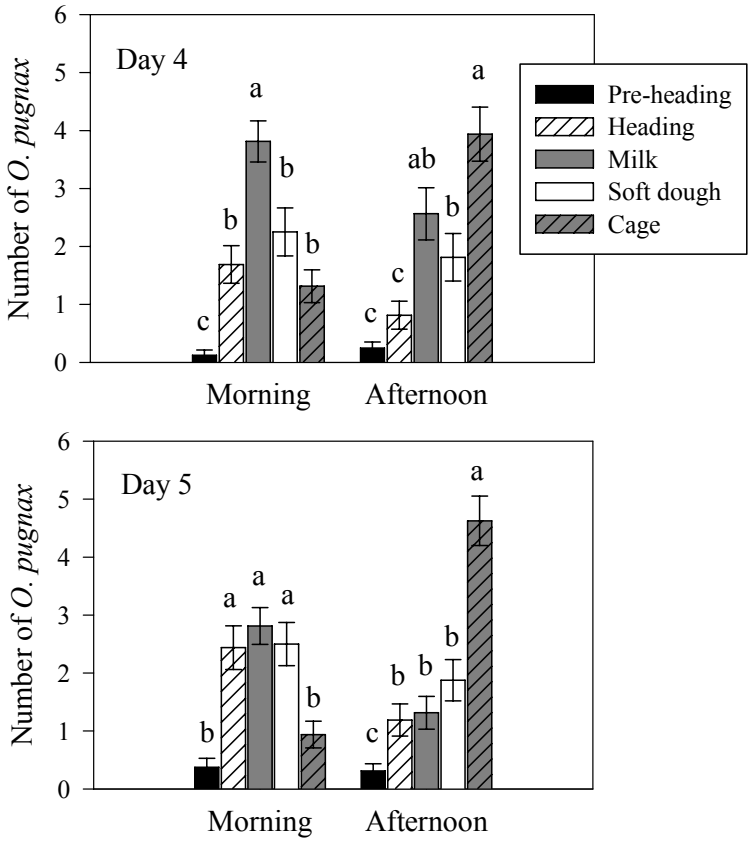

Fig. 5.7. Mean number of *O. pugnax* ± SEM (n = 16) per location on rice at different stages of development during morning and afternoon inspections on days 4 and 5. Beaumont, TX, 2005. For each day and inspection time, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

Figs. 5.6 and 5.7 show the mean number of *O. pugnax* observed per location during morning and afternoon inspections on different days for 2005. On day 1, during morning and afternoon inspections, more *O. pugnax* were observed on soft dough than on pre-heading, heading or milk plants. On days 2 and 3, during both morning and afternoon inspections, a similar number of *O. pugnax* was observed on soft dough and milk plants, and significantly more on these than on pre-heading or heading plants. No difference was detected in the number of *O. pugnax* observed on pre-heading and heading plants on days 1 and 2, but on day 3 significantly more insects were observed on heading than on pre-heading plants. On day 4, during morning and afternoon inspections, more *O. pugnax* were observed on milk than on pre-heading, heading or soft dough plants. However, during afternoon inspections, number of *O. pugnax* observed on milk and soft dough plants was not significantly different. During the morning inspection, significantly more *O. pugnax* were observed on heading than on pre-heading plants, while during the afternoon inspection no difference was detected in the number of insects observed on pre-heading and heading plants. On day 5, during morning and afternoon inspections, no differences were found in the number of *O. pugnax* observed on heading, milk or soft dough plants, while significantly fewer insects were observed on pre-heading than on other plants.

Number of *O. pugnax* observed on the cage surface varied considerably among dates when compared to the number of insects on plants. More *O. pugnax* were observed on the cage surface during afternoon than morning inspections, and only on day 1 was this difference insignificant ($P < 0.001$ for day 2, $P = 0.003$ for day 3, $P < 0.001$ for day

4, $P < 0.001$ for day 5). More *O. pugnax* were observed on plants during morning than afternoon inspections, although in some cases the difference was not significant ($P = 0.013$ for day 2, soft dough plant; $P = 0.006$ for day 3, soft dough plant; $P = 0.015$ for day 4, milk plant; $P = 0.017$ for day 5, heading plant; $P = 0.01$ for day 5, milk plant).

Fig. 5.8 shows the mean number of male and female *O. pugnax* per location during morning and afternoon inspections for 2005. During morning and afternoon inspections, significantly more male and female *O. pugnax* were observed on milk and soft dough than on heading or pre-heading plants. Significantly fewer male or female insects were observed on pre-heading than on any other plants. When comparing males and females, no differences were detected in the numbers observed on any of the plants during morning inspections. During afternoon inspections, significantly more males than females were observed on soft dough plants only ($P = 0.001$). During both inspection times, significantly more females than males were observed on the cage surface ($P = 0.005$ for morning and $P < 0.001$ for afternoon).

Discussion

In 2004, 45% of insects observed were on panicles, 14% on foliage and 41% on the cage surface. In 2005, 65% were on panicles, 9% on foliage and 26% on the cage surface. Normal feeding, mating and oviposition were observed in the cages throughout the experiments. Viator et al. (1983) infested caged wheat plants with *O. pugnax* and observed that feeding on panicles was reduced when insects were in protected areas

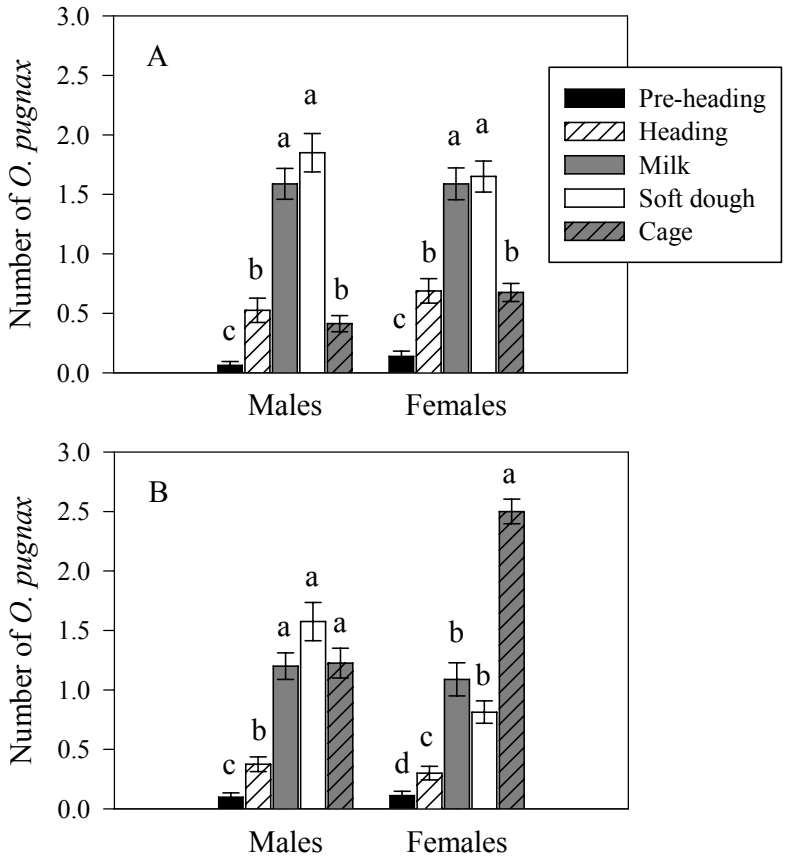

Fig. 5.8. Mean number of male and female *O. pugnax* ± SEM (n = 80) per location on rice at different stages of development during morning (A) and afternoon (B) inspections. Beaumont, TX, 2005. For each inspection time and sex group, bars followed by the same letter are not significantly different ($P > 0.05$, LSD).

within the cage or the foliage. Other researchers have found reduced mite and insect survival when using clip cages (Crafts-Brandner and Chu 1999). In the current experiments, mean percentage *O. pugnax* mortality per cage per inspection per day was 9.6 and 7.4 for 2004 and 2005,respectively. Insect mortality was expected because insects were field collected and their age and condition were unknown. Although collection and transport of insects from field to greenhouse were carefully conducted, these activities also may have affected survival of insects.

The attractiveness of different stages of panicle development did not change considerably through time in 2004 (Fig. 5.1, Table 5.2). In 2005, during morning inspections, mean number of insects on plants with panicles at the heading stage increased from 0.563 on day 1 to 2.438 on day 5, while mean number of insects on plants with panicles in the soft dough stage decreased from 4.75 to 2.5 (Fig. 5.2A, Table 5.3). During afternoon inspections, mean number of insects on plants with panicles in the heading stage increased from 0.438 on day 1 to 1.188 on day 5, while mean number of insects on plants with panicles at the soft dough stage decreased from 3.688 to 1.875 (Fig. 5.2B, Table 5.3). Differences between 2004 and 2005 experiments may be due to differences in the ages of plants. In 2004, soft dough plants were 6 days older than milk plants and these were 9 days older than heading plants. In 2005, soft dough plants were 20 days older than milk plants, and these were 10 days older than heading plants. Soft dough plants in 2005 were more mature with respect to milk plants than soft dough plants in 2004; hence, during the 5 days of observation, grains continued to mature turning into hard dough, a stage less suitable for *O. pugnax* feeding (Douglas and Tullis

1950, Odglen and Warren 1962, Patel et al. 2006). Insects may have moved from the more mature plants to heading or milk plants.

The general trend was milk and soft dough stages of panicle development were more attractive to *O. pugnax* than heading or pre-heading stages. Pre-heading plants were the least attractive to the insect, which is confirmed by previous field observations of very low numbers of *O. pugnax* detected in commercial fields of pre-heading rice (Ingram 1927, Douglas 1939, Douglas and Tullis 1950, Odglen and Warren 1962, Jones and Cherry 1986, Rashid et al. 2006). In 2004, averaging across inspection times and days, 0.219 ± 0.053, 1.363 ± 0.175, 1.944 ± 0.234, and 1.9 ± 0.239 *O. pugnax* were observed on pre-heading, heading, milk and soft dough plants, respectively. In 2005, mean *O. pugnax* numbers were 0.206 ± 0.043, 0.944 ± 0.089, ± 2.731 ± 0.195 and 2.944 ± 0.185 for pre-heading, heading, milk and soft dough plants, respectively.

Number of insects on the cage surface tended to be similar to or higher than the number of insects observed on milk and soft dough plants and on many days, higher during afternoon than morning. More female than male insects were observed on the cage surface, while males were more numerous on plants (Figs. 5.5 and 5.8). The presence of fewer female insects on plants and more on the cage surface may indicate females are more actively involved in dispersion, possibly searching for oviposition substrates after feeding and mating. Competition with other insects for food and oviposition substrates also may play a role in increased female *O. pugnax* movement. Todd (1989) reported that dispersion of the southern green stink bug, *Nezara viridula* (Linnaeus), in rice fields in Japan is achieved by females. Female *N. viridula* have been

found to disperse up to 1000 m per day by flight in search of oviposition and feeding sites. Also, *N. viridula* females again disperse soon after egg laying and spend less time on the same plant than non-ovipositing females or males. Sampling of rice fields at different times of day has shown differences in population levels within the same fields. This has been attributed to vertical movement of the insects within plants during the day, especially during periods of higher temperature (Rashid et al. 2006, Way et al. 2006). Dispersion of female *O. pugnax* also may contribute to differences in population levels detected in rice fields at different times of day.

Rashid et al. (2006) suggested that *O. pugnax* rely on visual, odor and other cues to disperse from alternate host weeds to heading rice. At close range, insects obtain more olfactory information. Contact chemoreceptors located on the antennae, mouth parts or tarsi also may be involved in gathering host plant information (Panda and Khush 1995). During feeding, factors such as chemical composition of food and presence of olfactory and gustatory feeding stimulants or deterrents may influence the acceptance of a host plant (Panda and Khush 1995). Results described herein indicate that panicles in the milk and soft dough stages may produce stimuli that increase attractiveness to *O. pugnax*. Identification of these stimuli may be a first step to designing new approaches to rice stink bug management, such as disruption of orientation, attraction to toxic baits or development of less insect-attractive cultivars (Metcalf 1994).

O. pugnax management guidelines indicate that rice fields should be monitored from heading to harvest (Way et al. 2006). Insecticide applications are recommended only when insect populations reach economic thresholds. Current rice production

practices sometimes include fungicide applications during the late boot stage. In an effort to reduce application costs, some producers tank-mix fungicides with insecticides for *O. pugnax* control (M. O. Way, personal communication). Our results indicate that application of insecticides with little or no residual activity directed at *O. pugnax* before heading are unnecessary. The reduced attractiveness of pre-heading rice to *O. pugnax*, combined with the short residual activity of currently labeled insecticides for *O. pugnax* control (Way and Wallace 1990), make this practice questionable.

CHAPTER VI

DETERMINATION OF RICE STINK BUG SPATIAL PATTERN AND DEVELOPMENT OF VISUAL SAMPLING METHODS AND POPULATION SAMPLING PLANS

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), is a serious pest of rice, *Oryza sativa* L., in the southern United States (Way 2003) attacking the crop from flowering to grain maturity. This insect is responsible for reductions of rough and head rice yields, and grain quality (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Harper et al. 1993, Tindall et al. 2005, Patel et al. 2006) by feeding on developing kernels, introducing pathogenic microorganisms and causing a discoloration of the grain known as "peck" for which growers are penalized.

O. pugnax overwinters as an adult in grassy areas, woodland trash and ground litter (McPherson and McPherson 2000), and emerges during the spring to feed on wild hosts and rice when they become available. Rice panicles can be attacked at any time by immigrating adults (Way and Bowling 1991) and field populations can increase dramatically due to movement from nearby grasses and harvested sorghum fields (Douglas 1939, Way 2003).

Sampling can be classified as population or decision sampling. In population sampling the interest lies in determining, with a certain level of reliability, the insect population density. In decision sampling, also known as commercial sampling, the goal is to classify a population above or below a certain threshold, so that a decision

regarding a management action can be made (Ruesink 1980, Buntin 1994, Wilson 1994). To design a sampling program, the determination of the spatial pattern of the insect is essential (Kuno 1991, Wilson 1994). Previously, Foster et al. (1989) reported that the spatial pattern of *O. pugnax* in Florida rice fields was aggregated; however, the sample unit size they employed was different from the sample unit size currently employed in Texas. In the present study, the spatial pattern of *O. pugnax* in Texas rice fields was determined and used to develop population sampling plans for this insect.

Currently, the only recommended method to sample for *O. pugnax* in Texas is the sweep net (SN) (Way et al. 2006). Rice fields should be sampled once or twice a week from 50% heading to harvest. A 38 cm diameter net is swept from side to side with each step while walking through the field, making sure the top of the net is flush with the top of the panicles. After 10 consecutive sweeps, the number of adult rice stink bugs is recorded. This constitutes one sample unit. A total of 10 sample units per management area is recommended to arrive at a population estimate. This fixed sample size has been recommended since the 1960s (Bowling 1962, 1969). However, the reliability of this sampling plan or the optimum sample size for *O. pugnax* population sampling has not been determined. Other sampling methodologies have been investigated recently (Rashid et al. 2006). Visual and SN counts in grassy margins and yellow pyramid traps have been used in an effort to predict *O. pugnax* populations in rice fields; however, rice stink bugs were observed or caught only before and after rice panicle development and maturation, limiting the utility of these methodologies.

Many rice producers in Texas have not adopted the SN (Harper et al. 1990) and rely on non-standardized, subjective, visual observations of *O. pugnax* populations. Although this "sampling technique" is common, it is not based on scientific criteria. In this study, the performance of the SN method was assesed, and visual sampling methods were compared to the SN method in an attempt to facilitate *O. pugnax* population estimation in rice fields.

Materials and Methods

Data collection. Data were collected during 2003 and 2004 from commercial rice fields located in Chambers, Colorado, Fort Bend, Jackson and Jefferson Cos., TX. Seven fields were sampled in 2003 and 10 in 2004. Stages of panicle development during sampling were heading, milk and dough. Heading was considered to begin at panicle exertion. Milk was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was milky and panicles began to bend downward due to weight of developing grains. Dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was dough (not liquid) and hulls turned from green to tan. A field was considered in heading, milk or dough when 75% of the panicles in the field reached one of these stages of development. Planting method (drilled or broadcast seeded) of sampled fields also was recorded. Most fields in Texas are drill seeded with well defined rows. However, occasionally fields are replanted; these fields do not have well defined rows but have the appearance of a broadcast seeded field. If rows were easily visible and allowed relatively easy movement in the field, the

field was considered drill seeded. If rows were not visible, the field was classified broadcast seeded.

Selected fields were divided into parallel transects 18 m apart. Transects were selected and samples taken every 18 m, starting 9 m from the field margin. The number of sampling points in each transect and transects per field varied with field size. At each sampling point, SN and visual samples were taken in adjacent areas but spaced enough to avoid interference among methods. Fields were sampled only once during each season, or, if sampled more than once, they were sampled at different stages of panicle development. Sampling was conducted between 1000 – 1200 and 1400 – 1700 h CDT. Sampling before 1000 h CDT was hampered by the presence of dew on foliage, which interfered with SN sampling. Sampling between 1200 and 1400 h CDT was avoided due to high temperatures. In 2003, all visual sampling methods were performed by the same operator, while SN samples were taken by different operators. In 2004, all sampling methods were performed by each of three operators, and time to complete each sampling method was recorded.

Visual sampling methodologies. Three visual methods were developed and compared to the SN. For the first visual method, a "T-tool" (TT) (Fig. 6.1), a common device used to sample for the fungal disease sheath blight caused by *Rhizoctonia solani* Kuhn, was evaluated. The TT consists of two polyvinyl chloride (PVC) pipes in the form of a T, one a handle (1.25 m long) and the other (0.65 m long) attached perpendicular to the handle. The operator walked 4.5 m in 20 s using the TT to lightly push through the panicles to disturb the insects. Adult *O. pugnax* observed on or flying from panicles in

Fig. 6.1. *O. pugnax* visual sampling using the T-tool (TT).

the area disturbed by the TT were counted. For the second visual method, a "sweep stick" (SS) (Fig. 6.2) made of a 1 m long PVC pipe (2 cm diameter) was used by the operator to lightly disturb rice panicles, sweeping 180 degrees from one side to the other with each step. Only adult *O. pugnax* observed on or flying from the panicles in the area determined by the last 0.38 m (diameter of the SN net) of the SS were recorded. A total of five consecutive sweeps was performed and the number of *O. pugnax* observed after each sweep was recorded. Number of *O. pugnax* after one (SS1), two (SS2), three (SS3), four (SS4) and five (SS5) sweeps of the SS was compared to the number of insects caught with the SN. For the third visual method, a "long stick" (LS) (Fig. 6.3) made of a 1.5 m long PVC pipe (2 cm diameter) was used to gently disturb the rice panicles while sweeping 180 degrees in front of the operator. The number of adult *O. pugnax* observed on or flying from the disturbed panicles along the entire length of the LS was recorded. SN samples were taken following the procedures described in the 2006 Rice Production Guidelines (Way et al. 2006).

Effect of location of sample and time of day on SN sampling. SN samples taken nearest the field margin (9 m) were labeled "perimeter" samples, while all other samples were labeled "within field" samples. For each field, perimeter and within field samples were compared using analysis of variance (ANOVA) with factors being field and location of sample.

For each sampling date, numbers of adult *O. pugnax* caught with the SN during morning and afternoon hours were compared using ANOVA with factors being sampling date and time of day. Sampling date was preferred over field as a factor because some

Fig. 6.2. *O. pugnax* visual sampling using the sweep stick (SS).

Fig. 6.3. *O. pugnax* visual sampling using the long stick (LS).

fields were sampled during the course of more than one day, and weather conditions sometimes changed drastically during different days. Mean numbers of *O. pugnax* caught at different times of day on each sampling date were compared using Fisher's Least Significant Difference (LSD) test.

Spatial pattern. Taylor's model relating variance and mean is one of the best models to describe spatial aggregation (Taylor et al. 1978, 1980, Taylor 1984). The variance corresponding to different population means can be estimated using the variance-mean relationship developed by Taylor (1961)

$$s^2 = ax^b \tag{6.1}$$

where s^2 is the sample variance, x is the sample mean, and a and b are Taylor's coefficients. Taylor's coefficients are usually estimated by log-log transformation of equation (6.1), but this method can overestimate s^2 at low densities. For this reason, coefficients for the sampling methods included in this study were calculated by nonlinear regression of variance versus mean *O. pugnax* aggregated counts (see comparison between SN and visual sampling) (Wilson et al. 1983b, Binns and Nyrop 1992, Wilson 1994).

Comparison between SN and visual sampling. Three criteria were used to evaluate the visual methods used in this study. First, a good correlation must exist between SN and visual counts. Second, the relationship between SN and visual counts should not be affected by planting type, panicle stage, time of day, or operator. Third, the visual methods must optimize cost-reliability. The first two criteria were evaluated

by comparing SN and visual sample units; relative cost-reliability was determined for the visual methods with respect to the SN method.

SN sampling is a relative method which does not yield an absolute population estimate per unit area of habitat (Southwood 1978). The visual sampling methods in the present study also are relative methods. Only Bowling (1969) attempted to determine the absolute number of *O. pugnax* in rice; however, cultivars used at the time and their spatial arrangement in the field (row and plant spacing) have changed considerably, making this determination irrelevant for present conditions. Since no absolute method to sample *O. pugnax* populations in rice is available, the visual methods described in this study were calibrated to the SN method. To calibrate sampling methods, paired samples should be taken and compared, but achieving a high correlation is difficult when comparing single observations (Todd and Herzog 1980). Because of this, *O. pugnax* visual and SN counts were aggregated by sampling date, panicle developmental stage (heading, milk or dough), location of sample in the field (perimeter or within field), time of day of sampling (morning or afternoon), and type of planting (drill or broadcast seeded). Analyses were performed on the mean of the aggregated counts.

*Correlation between SN and visual counts***.** Linear regression analyses were performed to determine the level of correlation between SN and visual counts. Mean SN counts were regressed against mean TT, LS and SS counts, and linear regression equations estimated.

Effect of factors on the SN and visual methods correlations. Type of planting, stage of panicle development, time of day and operator can influence the relationship

between SN and visual sampling. The purpose of the present study was to identify a visual method(s) least affected by these factors allowing reliable sampling under a variety of conditions. Number of observed adult *O. pugnax* was analyzed using analysis of covariance (ANCOVA) with factors (categorical variables) being planting type, stage of panicle development and time of day. Number of adult *O. pugnax* caught with the SN served as the covariate (continuous variable). ANCOVA allows comparison of intercepts (main effects) and slopes (interactions) of the regression lines generated between SN and visual counts for different factors. For a visual method, if intercepts and slopes for different levels of a factor are not significantly different, the relationship between SN and visual counts is not affected by the factor; however, if intercepts or slopes are significantly different, the relationship between counts changes with changing levels of the significant factor. Only in 2004 were all sampling methods performed by each of the three operators. For that reason, the effect of the operator was determined only with 2004 data. In this case, the number of *O. pugnax* for each visual sampling method was analyzed using ANCOVA, with operator as random factor and number of adult *O. pugnax* caught with the SN as covariate.

Cost-reliability. Wilson (1994) defines relative cost-reliability as the ratio of the costs of two sampling methods expressed as:

$$C_v / C_{sn} = n_v(\theta_v + \phi_v) / n_{sn}(\theta_{sn} + \phi_{sn}) \qquad (6.2)$$

where C_v and C_{sn} are the cost per sample in time for a given level of reliability for the visual and SN sampling methods, respectively; n_v and n_{sn} are the number of sampling units required for an estimate for a given level of reliability with the corresponding

sampling method; θ_v and θ_{sn} are the times required to examine an individual sample unit using the corresponding sampling method; and Φ_v and Φ_{sn} are the times required to move between sample units for the corresponding sampling method.

Equation (6.2) calculates the relative cost-reliability of a visual method with respect to the SN method based on the number of samples units and time required to reach an estimate for a given level of reliability. However, equation (6.2) does not consider the physical effort necessary for each sampling method to reach an estimate. Scouts may prefer the sampling method that is less physically demanding. An advantage of the visual methods tried in this study is that they are less strenuous than sweeping rice using the SN.

Assuming that the probability of adoption of a sampling method is inversely proportional to the physical effort required to sample, the physical effort required to sample an insect population using the ith sampling method, E_i, can be expressed as:

$$E_i = \varepsilon / p_i \tag{6.3}$$

where p_i is the probability of adoption of the ith sampling method and ε is a constant relating E_i to p_i. Incorporating E_i in equation (6.2), one obtains:

$$C_v / C_{sn} = n_v(\theta_v + \phi_v)E_v / n_{sn}(\theta_{sn} + \phi_{sn})E_{sn} \tag{6.4}$$

and replacing E_i in (6.4) with (6.3),

$$C_v / C_{sn} = n_v(\theta_v + \phi_v)p_v^{-1} / n_{sn}(\theta_{sn} + \phi_{sn})p_{sn}^{-1} \tag{6.5}$$

where C_v / C_{sn} is the relative cost-reliability that incorporates probability of adoption, p_{sn} is the probability of adoption of the SN method and p_v is the probability of adoption of the visual method. Equation (6.5) can be used to determine the relative cost-reliability of

a visual method with respect to the SN considering not only sample size and sampling time but also sampling effort. To determine the probability of adoption of the visual methods, 20 potential users of the novel visual methods (growers, Crop Consultants and County Agents) were interviewed. The probability of adoption of the SN was obtained from Harper et al. (1990).

During the collection of samples, the time required to count the number of insects caught with the SN increased as the number of insects caught increased. To incorporate this time variation into the cost-reliability analysis, the time needed to examine a sample unit at different mean population densities was estimated by linear regression analysis.

The sample size (n) required to obtain a population estimate with a given level of reliability can be determined using the formula presented by Karandinos (1976) and modified by Wilson and Room (1983)

$$n = t^2_{\alpha/2} D_x^{-2} s^2 x^{-2} \tag{6.6}$$

where $t_{\alpha/2}$ is the standard normal variate for a two-tailed confidence interval; D_x is a proportion of the mean equivalent to half the desired confidence interval, a measure of reliability; and x is the mean population density. Substituting s^2 in equation (6.6) with equation (6.1), we obtain

$$n = t^2_{\alpha/2} D_x^{-2} a x^{b-2} \tag{6.7}$$

Substituting n in equation (6.5) with equation (6.7), and including the linear regression equation relating SN and visual counts, one obtains:

$$C_v / C_{sn} = a_v (A + Bx)^{(b_v - 2)} (\theta_v + \phi_v) p_v^{-1} [(a_{sn} x^{(b_{sn} - 2)})(\theta_{sn} + \phi_{sn}) p_{sn}^{-1}]^{-1} \qquad (6.8)$$

where C_v/ C_{sn} is the relative cost-reliability of the visual method with respect to the SN method for population sampling; a_v and b_v are Taylor's coefficients for the visual method; a_{sn} and b_{sn} are Taylor's coefficients for the SN method; A and B are the intercept and slope, respectively, of the linear regression equation relating visual to SN counts; and x is mean population density expressed as numbers of adult *O. pugnax* caught per 10 SN sweeps. Equation (6.8) was used to determine the relative cost-reliability of visual methods compared to the SN method for a given level of reliability.

Optimum sample size. Using equation (6.7), optimum sample sizes for the SN and the most appropriate visual methods were calculated to obtain estimates with 90% confidence ($\alpha = 0.1$) within 10, 20 or 30% of the mean ($D_x = 0.1$, 0.2 or 0.3). The reliability of a parameter estimate for the SN method for different insect population densities for the currently recommended sample size ($n = 10$) also was calculated. For all statistical analyses, when assumptions of normality of residuals and constant variances were not met, data were transformed before applying ANOVA or ANCOVA. The Box-Cox procedure was used to determine the best transformation (Kutner et al. 2005). All statistical analyses were performed using the SPSS package (SPSS Inc. 2005) at an α level of 0.05.

Results

Location, sampling dates, number of sample units, cultivar, panicle developmental stage, and planting type per sampled field are presented in Table 6.1.

Table 6.1. Location, sampling dates, cultivar, panicle developmental stage and planting type of sampled rice fields, TX, 2003 and 2004

Field	Location	County	Sampling Dates	Cultivar	Panicle stage	Planting type
1	Beaumont	Jefferson	24,26 June 2003	Sierra	Heading	Drill
2	Ganado	Jackson	01 July 2003	Cocodrie	Heading	Drill
3	Nome	Jefferson	03, 07, 08 July 2003	Cocodrie	Milk	Broadcast
4	Winnie	Chambers	17, 18 July 2003	Cocodrie	Milk	Broadcast
5	China	Jefferson	22, 23, 25 July 2003	CL 161	Heading	Drill
6	China	Jefferson	05, 06, 08 Aug. 2003	CL 161	Dough	Drill
7	China	Jefferson	19, 20 Aug. 2003	Cocodrie	Dough	Drill
8	China	Jefferson	05, 06 July 2004	CL 161	Heading	Drill
9	China	Jefferson	09, 12 July 2004	CL161	Heading	Drill
10	Rosenberg	Fort Bend	22 July 2004	Chenniere	Heading	Drill
11	Nome	Jefferson	26 July 2004	Cocodrie	Milk	Broadcast
12	Beaumont	Jefferson	29 July 2004	Cocodrie	Dough	Broadcast
13	Eagle Lake	Colorado	30 July 2004	CLXL8	Dough	Drill
14	Nome	Jefferson	05, 06 July 2004	CL 161	Milk	Drill
15	China	Jefferson	11 Aug. 2004	CL 161	Dough	Drill
16	China	Jefferson	12 Aug. 2004	CL 161	Dough	Drill
17	China	Jefferson	13 Aug. 2004	CL 161	Dough	Drill

Table 6.2 shows the total number of sample units taken for each sampling method, the average number of adult *O. pugnax* caught or observed and the range of counts.

Effect of location of sample and time of day on SN sampling. Location main effect was significant ($F = 24.2$; df = 1, 1002; $P < 0.001$), indicating a significant difference in the number of *O. pugnax* caught between perimeter and within field samples. Significantly more insects were caught in perimeter samples (6.465 ± 0.274) than in within field samples (5.127 ± 0.147). Field main effect was significant ($F = 50.959$; df = 16, 1002; $P < 0.001$), indicating significant differences in number of *O. pugnax* among fields. The interaction between field and location of sample in the field was not significant.

For 17 of 29 sampling dates, samples were taken during both morning and afternoon hours in the same field. Although ANOVA resulted in a significant interaction between sampling date and time of day ($F = 2.304$; df = 16, 987; $P = 0.003$), Table 6.3 shows that more *O. pugnax* were caught during morning than afternoon hours in 15 of 17 dates. Time of day main effect was significant ($F = 10.707$; df = 1, 987; $P = 0.001$), indicating that, across all sampling dates, more insects were caught during morning (4.941 ± 0.164) than afternoon hours (4.326 ± 0.197).

Table 6.2. Total number of sample units taken by sampling method, mean number of adult *O. pugnax* caught or observed ± SEM, and range of counts, TX, 2003 and 2004

Sampling method[a]	Sample units	Mean *O. pugnax* caught or observed	Range
SN	1033	5.32 ± 0.221	0 – 48
TT	919	2.92 ± 0.127	0 – 23
LS	645	2.92 ± 0.102	0 –13
SS1	1025	1.16 ± 0.051	0 – 10
SS2	1025	2.32 ± 0.960	0 – 20
SS3	1025	3.55 ± 0.139	0 – 27
SS4	1025	4.70 ± 1.810	0 – 35
SS5	1025	5.83 ± 0.221	0 – 42

[a] SN, 10 sweep net sweeps; TT, one T-tool pass (4.5 m in 20 s); LS, one long stick sweep; SS1, one sweep stick sweep; SS2, two sweep stick sweeps; SS3, three sweep stick sweeps; SS4, four sweep stick sweeps; SS5, five sweep stick sweeps.

Table 6.3. Mean number of adult *O. pugnax* per 10 sweep net (SN) sweeps ± SEM caught during morning and afternoon hours on different sampling dates, TX, 2003 and 2004

Sampling date	Mean adult *O. pugnax* per 10 SN sweeps	
	Morning (*n*)	Afternoon (*n*)
26 Jun 03	1.6 ± 0.6 (38)	1.4 ± 0.7 (25)
01 Jul 03	27.9 ± 0.5 (49)	22.4 ± 0.7 (29)
08 Jul 03	1.7 ± 0.8 (20)	0.7 ± 0.6 (30)
22 Jul 03	4.4 ± 0.8 (20)	3.0 ± 0.8 (20)
05 Aug 03	3.5 ± 1.1 (10)	2.9 ± 0.8 (18)
08 Aug 03	3.1 ± 1.2 (9)	4.6 ± 0.8 (20)
19 Aug 03	5.5 ± 0.8 (20)	3.8 ± 0.6 (30)
20 Aug 03	3.8 ± 0.8 (20)	3.0 ± 0.6 (30)
05 Jul 04	1.5 ± 1.1 (10)	1.0 ± 0.6 (30)
06 Jul 04	2.5 ± 1.1 (10)	0.7 ± 1.1 (10)
09 Jul 04	2.3 ± 0.6 (40)	2.2 ± 0.8 (20)
22 Jul 04	6.6 ± 0.9 (16)	4.9 ± 0.6 (34)
26 Jul 04	8.7 ± 0.8 (20)	7.8 ± 0.6 (30)
29 Jul 04	6.4 ± 1.1 (10)	3.3 ± 0.8 (20)
05 Aug 04	5.6 ± 1.1 (10)	5.3 ± 2.0 (3)
06 Aug 04	2.5 ± 1.1 (10)	4.2 ± 0.8 (18)
11 Aug 04	5.6 ± 0.8 (20)	5.0 ± 0.8 (20)

Spatial pattern. Figs. 6.4 and 6.5 show how the relationship between variance and mean changes with density. For all sampling methods, the variance is larger than the mean at most densities, suggesting an aggregated spatial pattern (Davis 1994). The figures show differences in the degree of aggregation for different sampling methods. Insects are perceived as more aggregated with the SN and SS5, and less with the SS1 and TT.

Comparison between SN and visual sampling. All regression analyses associating visual and SN counts were significant (Table 6.4). R-squared values were high and ranged from 0.639 for LS to 0.825 for SS3. Results of ANCOVA for LS and SS2 show no significant differences in the intercepts or slopes of the lines (Table 6.5); therefore, a single line was used to describe the relationship between the SN and the visual methods. No differences were found in the intercepts for the rest of the visual methods; however, differences were found in the slopes for time of day of sampling. ANCOVA showed that the relationship between SN and visual methods was not affected by the use of different operators. Intercepts and slopes were not significantly different for operators sampling with any of the visual methods ($P > 0.05$).

Cost-reliability. The linear regression between SN counts and time (in seconds) required to take and examine a SN sample unit was significant ($F = 11.974$, $P = 0.001$, $r^2 = 0.255$, $n = 36$). Intercept and slope for this relationship were 17.370 ± 2.311 and 1.616 ± 0.467, respectively. Linear regression between visual counts and time required

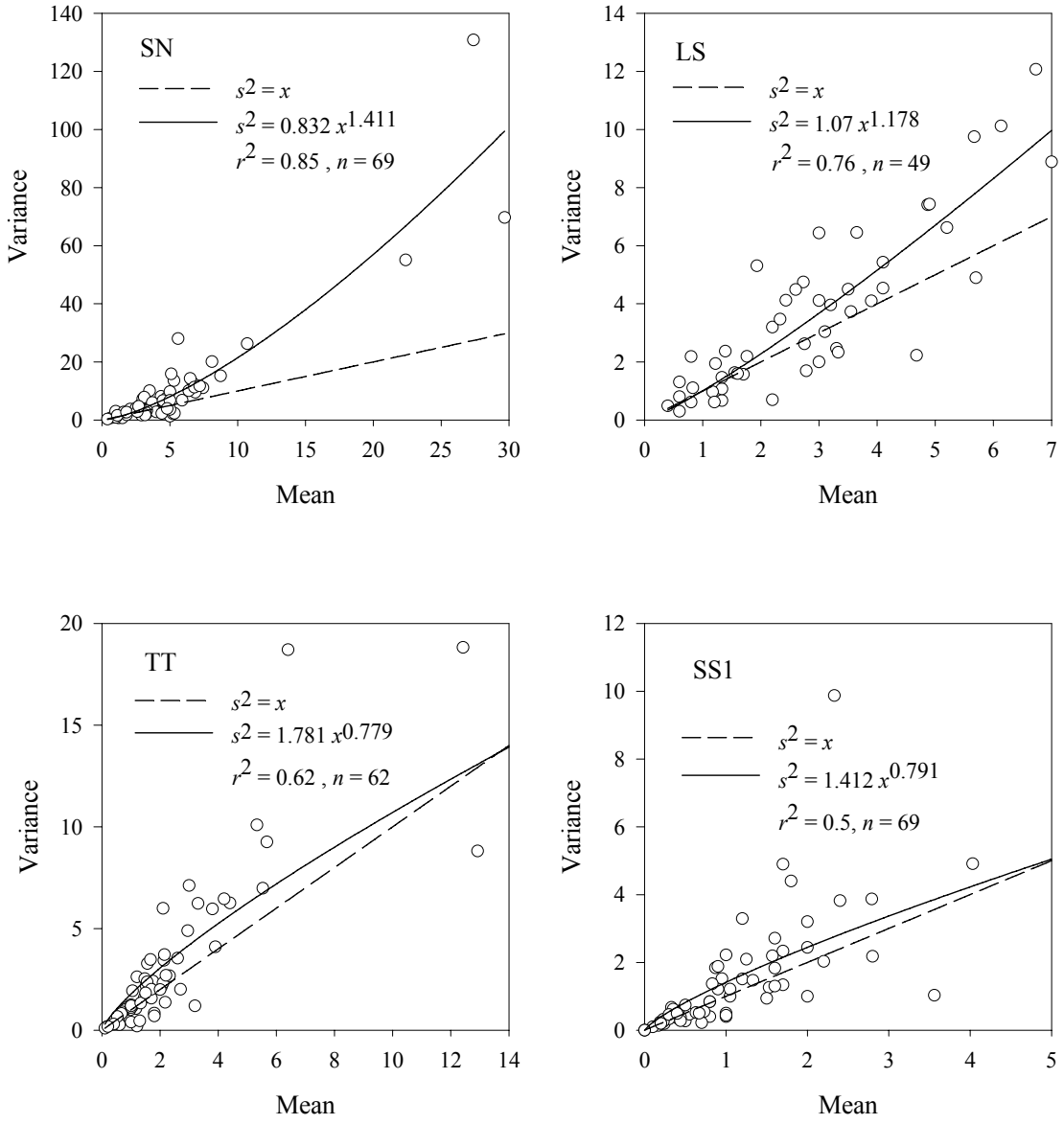

Fig. 6.4. Taylor's variance–mean relationships ($s^2 = a\ x^b$) for *O. pugnax* when sampling using 10 sweep net sweeps (SN), one long stick sweep (LS), one T-tool pass (TT) and one sweep stick sweep (SS1) in Texas rice fields.

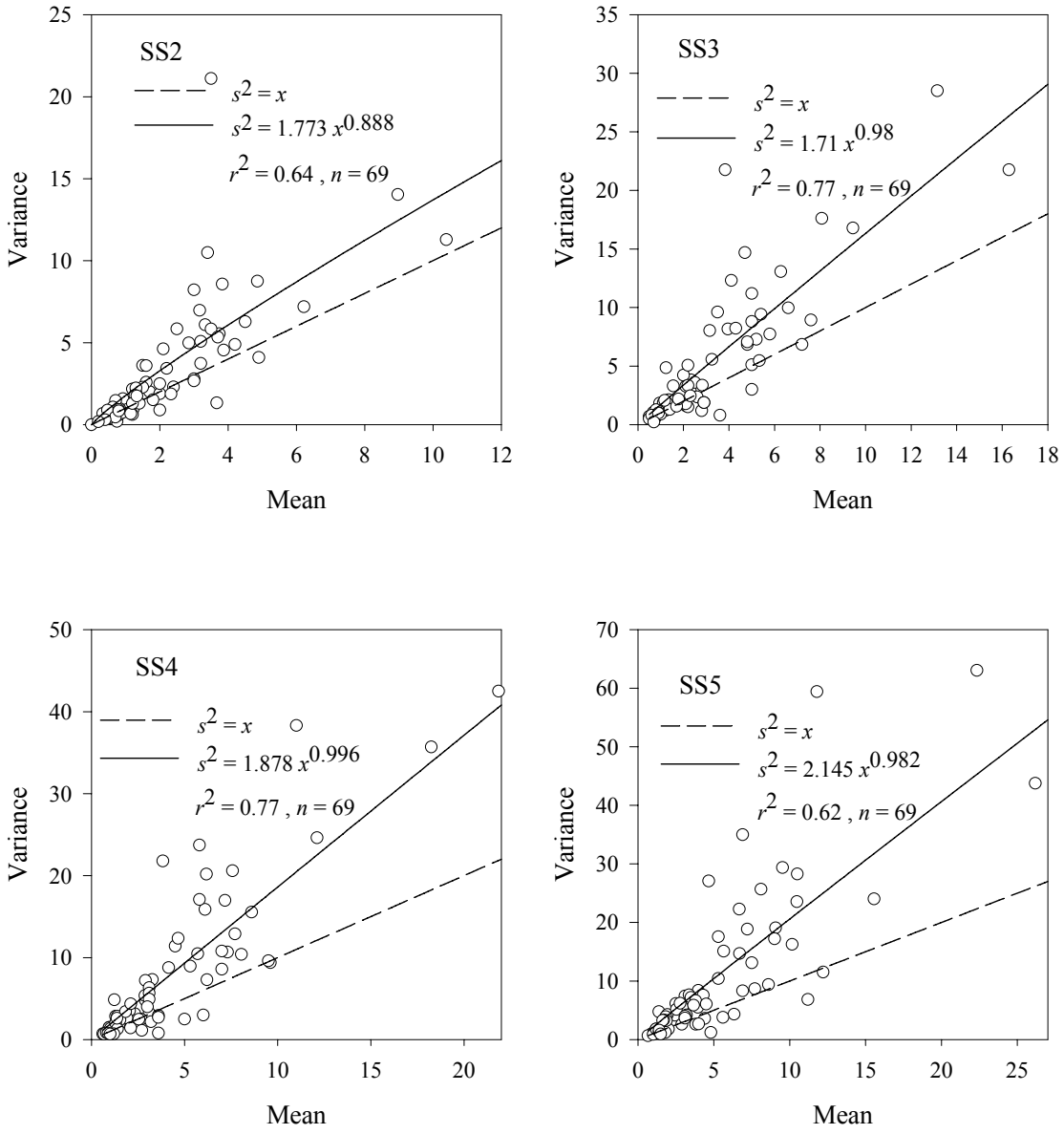

Fig. 6.5. Taylor's variance–mean relationships ($s^2 = a\ x^b$) for *O. pugnax* when sampling using two (SS2), three (SS3), four (SS4) and five (SS5) sweep stick sweeps in Texas rice fields.

Table 6.4. Parameter estimates ± SEM of linear regression analyses between 10 sweep net sweeps and visual adult *O. pugnax* counts, TX, 2003 and 2004

Sampling method[a]	Intercept	Slope	*F*	*P*	r^2	n
TT	0.098 ± 0.212	0.475 ± 0.029	270.364	< 0.001	0.818	62
LS	-0.156 ± 0.358	0.675 ± 0.074	83.343	< 0.001	0.639	49
SS1	0.279 ± 0.094	0.184 ± 0.013	190.923	< 0.001	0.740	69
SS2	0.407 ± 0.163	0.396 ± 0.023	294.314	< 0.001	0.815	69
SS3	0.541 ± 0.242	0.611 ± 0.034	316.901	< 0.001	0.825	69
SS4	0.754 ± 0.322	0.786 ± 0.046	696.575	< 0.001	0.816	69
SS5	0.972 ± 0.399	0.956 ± 0.057	285.642	< 0.001	0.810	69

[a]TT, one T-tool pass (4.5 m in 20 s); LS, one long stick sweep; SS1, one sweep stick sweep; SS2, two sweep stick sweeps; SS3, three sweep stick sweeps; SS4, four sweep stick sweeps; SS5, five sweep stick sweeps.

Table 6.5. Results from ANCOVA for number of adult *O. pugnax* observed with different visual methods, TX, 2003 and 2004

Factors[a]	Sampling method[b]											
	TT			LS			SS1			SS2		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
S	0.524	2, 52	0.595	0.130	2, 39	0.879	1.892	2, 59	0.160	1.178	2, 59	0.315
D	1.369	1, 52	0.247	0.234	1, 39	0.631	0.837	1, 59	0.364	0.0003	1, 59	0.985
PT	0.103	1, 52	0.750	1.231	1, 39	0.274	0.838	1, 59	0.364	0.776	1, 59	0.382
SN	25.371	1, 52	< 0.001	63.523	1, 39	< 0.001	77.455	1, 59	< 0.001	67.224	1, 59	< 0.001
S x SN	0.034	2, 52	0.967	1.304	2, 39	0.283	1.863	2, 59	0.164	0.323	2, 59	0.725
D x SN	5.410	1, 52	0.024	1.405	1, 39	0.243	7.216	1, 59	0.009	3.179	1, 59	0.080
PT x SN	0.078	1, 52	0.782	1.977	1, 39	0.168	0.517	1, 59	0.475	0.523	1, 59	0.472

Table 6.5. Continued

Factors[a]	Sampling method[b]								
	SS3			SS4			SS5		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
S	0.798	2, 59	0.455	0.694	2, 59	0.503	1.072	2, 59	0.349
D	0.188	1, 59	0.666	0.700	1, 59	0.406	1.418	1, 59	0.238
PT	0.562	1, 59	0.457	0.292	1, 59	0.591	0.341	1, 59	0.562
SN	74.376	1, 59	< 0.001	71.504	1, 59	< 0.001	74.729	1, 59	< 0.001
S x SN	0.128	2, 59	0.880	0.076	2, 59	0.927	0.273	2, 59	0.762
D x SN	5.968	1, 59	0.018	9.359	1, 59	0.003	12.676	1, 59	0.001
PT x SN	0.514	1, 59	0.476	0.508	1, 59	0.479	0.829	1, 59	0.366

[a]S, panicle stage; D, time of day of sampling; PT, planting type; SN, 10 sweep net sweeps counts.

[b]TT, one T-tool pass (4.5 m in 20 s); LS, one long stick sweep; SS1, one sweep stick sweep; SS2, two sweep stick sweeps; SS3, three sweep stick sweeps; SS4, four sweep stick sweeps; SS5, five sweep stick sweeps.

to take a visual sample unit was not significant ($P > 0.05$) for any visual methodologies, indicating that time required to take a visual sample unit was not affected by the number of insects observed. Average time, in seconds, required to take a sample unit for each visual method was 33.37 ± 0.93, LS; 13.28 ± 0.62, SS1; 27.32 ± 1.19, SS2; 42.02 ± 1.78, SS3; 55.79 ± 2.42, SS4; and 69.82 ± 3.11, SS5. Time required for the TT method was always 20 seconds. Time required to move between sample units was 18.72 ± 0.323 seconds, based on samples taken during 2005 throughout the Texas Rice Belt. This time was assumed to be the same for all sampling methodologies. Time for the operator to record data while sampling was not included for any of the methods. Scouts do not record data while sampling. Usually, data recording is done once sampling a management area is finished (M. O. Way, personal communication).

A survey of 20 potential users revealed that 18 of them would adopt a visual sampling method for *O. pugnax*. Adoption probability of the novel visual methods (p_v) was calculated to be 0.9. Harper el al. (1990) conducted mail surveys among Texas rice producers during 1986 and 1987 and calculated the probability of adoption of the sweep net (p_{sn}) to be 0.4.

Relative cost-reliability values for visual sampling methods relative to the SN method are shown in Fig. 6.6.Values larger than one indicate the SN method is more cost-reliable than a specific visual method, while values smaller than one indicate the converse. For all visual methods, as insect populations increased, relative cost-reliability

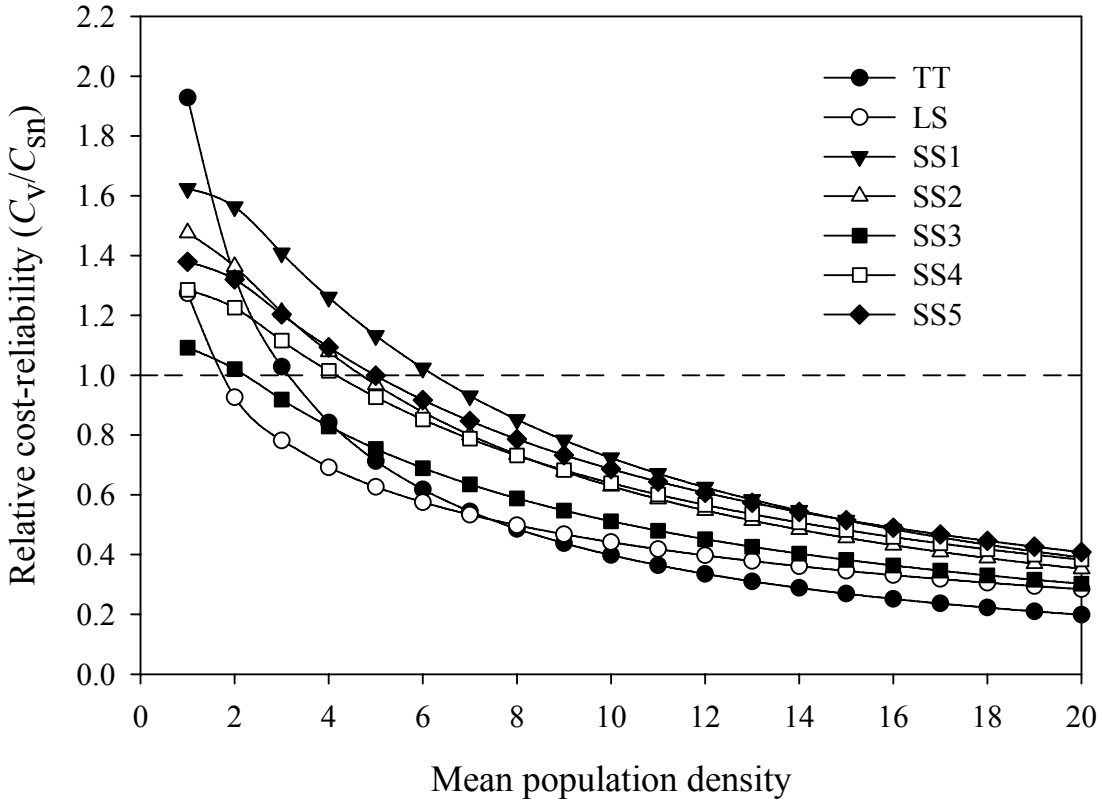

Fig. 6.6. Relative cost-reliability for the T-tool (TT), long stick (LS), one (SS1), two (SS2), three (SS3), four (SS4) and five (SS5) sweep stick sweeps with respect to the sweep net (SN). Mean population density is expressed in number of adult *O. pugnax* per 10 SN sweeps. The dashed straight line represents a value of one, meaning the SN and visual methods have the same cost reliability. For values above this line, the SN is more cost reliable, and below it, the visual methods are.

decreased. The visual methods were more cost-reliable than the SN at most *O. pugnax* densities. LS and SS3 were more cost-reliable than the SN for densities of two adult *O. pugnax* or more per 10 SN sweeps; TT, for densities of three or more; SS4, for densities of four or more; SS2 and SS5, for densities of five or more; and SS1, for densities of six or more.

Optimum sample size. LS and SS2 correlated well with the SN and these correlations are not affected by stage of panicle development, time of day, type of planting, or operator. Table 6.6 shows the number of sample units required to arrive at an estimate with a given level of reliability for different population densities expressed as number of adult *O. pugnax* per 10 SN sweeps, LS and SS2. For the same density, to obtain an estimate within 10% of the mean ($D_x = 0.1$), the number of sample units required is large (> 100) for all methods, especially at low population levels. To obtain an estimate within 30% of the mean ($D_x = 0.3$), the number of sample units is considerably smaller. The SN and LS methods require a similar number of sample units at populations higher than five adult *O. pugnax* per 10 SN sweeps, but at lower populations the LS method requires more sample units than the SN method. At all densities, SS2 requires more sample units than the SN or LS to reach an estimate with the same level of reliability.

Using equation (6.4), the reliability of taking 10 sample units with the SN method for different insect population densities was calculated (Fig. 6.7). As population density increases, the reliability of an estimate obtained by taking 10 sample units increases (D_x becomes smaller).

Table 6.6. Optimum sample size required to obtain a population estimate within 10, 20 and 30% of the mean for the sweep net (SN), long stick (LS) and two sweep stick sweeps (SS2) for *O. pugnax* in rice

Population density[a]	Reliability (D_x)								
	10%			20%			30%		
	SN	LS	SS2	SN	LS	SS2	SN	LS	SS2
1	227	469	615	59	119	155	27	54	70
2	152	238	394	40	61	100	19	29	46
3	120	165	288	32	43	74	16	21	34
4	102	129	225	27	34	58	13	16	27
5	90	107	185	24	28	48	12	14	23
6	81	91	156	22	25	41	11	12	19
7	74	81	135	20	22	36	10	11	17
8	69	72	119	19	20	31	10	10	15
9	64	66	106	18	18	28	9	9	14
10	60	60	95	17	17	26	9	9	13
11	57	56	87	16	16	24	9	8	12
12	54	52	80	15	15	22	8	8	11
13	52	49	74	15	14	20	8	8	10
14	50	46	68	14	13	19	8	7	10
15	48	44	64	14	13	18	8	7	9

[a] Number of adult *O. pugnax* per 10 SN sweeps.

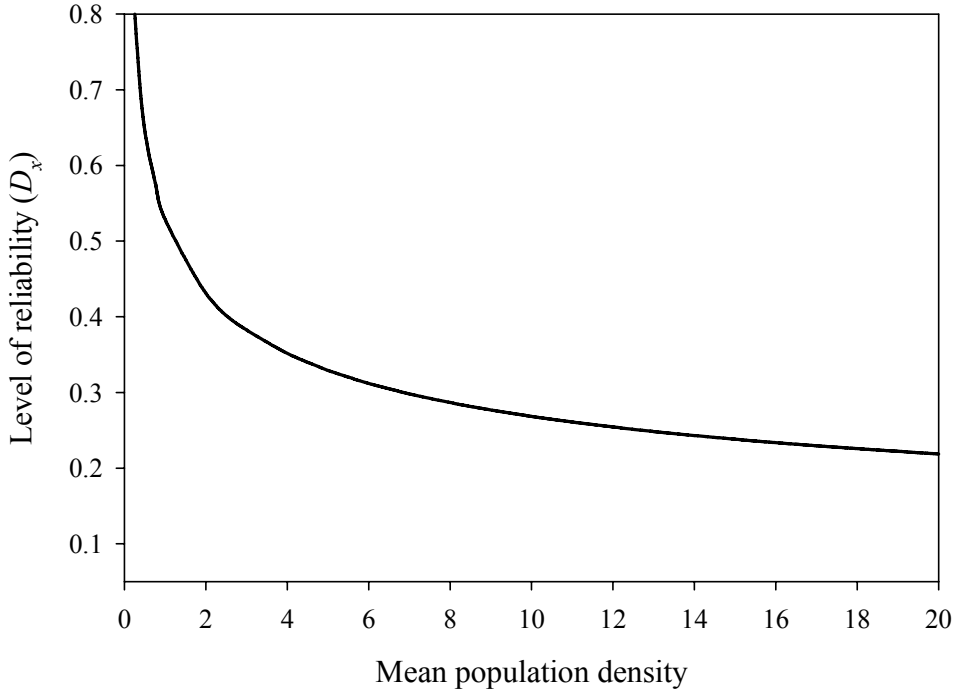

Fig. 6.7. Level of reliability, expressed as a proportion of the mean (D_x), of using a fixed sample size of n = 10 for population sampling of *O. pugnax* in rice using the sweep net (SN) at different population densities. Mean population density is expressed as number of adult *O. pugnax* per 10 SN sweeps.

Discussion

Populations sampled included a wide range of insect densities (Table 6.2). SN counts ranged from 0 to 48 adult *O. pugnax* per 10 SN sweeps; however, most counts ranged from three to 15 adults. In Texas, damaging populations are considered to be 3 to 15 adults per 10 SN sweeps (Harper et al. 1993, Way et al. 2006); so, the data include population levels that are economically relevant.

Current *O. pugnax* recommendations suggest avoiding field margins when sampling (Way et al. 2006). In many cases, field margins are weedy and growth pattern and stage of rice plants can be different compared to the remainder of the field. Presence of grassy weeds has been found to be a factor favoring *O. pugnax* infestation (Tindall et al. 2004, Cherry and Bennett 2005). Vegetation surrounding a field also may influence insect populations near the margins of a field (Cherry and Bennett 2005). Results of the current study show that SN samples taken 9 m from the field margin caught significantly more *O. pugnax* than SN samples taken within the field. To avoid border effects and obtain unbiased estimates of population density, SN samples should be taken farther than 9 m from the field border. Past research has found that SN samples taken 50 m from field margins provide a good estimate of *O. pugnax* populations (Foster et al. 1989).

Previous research also has found significant effects of time of day in SN catches. Rashid (2006) found that during hot, sunny days in Arkansas, samples taken at 1330 h CDT had fewer *O. pugnax* than earlier or later sampling times. Results of the present study consistently show that more insects were caught with the SN between 1000 and 1200 than between 1400 and 1700 h CDT (Table 6.3). During the hottest time of day

insect movement to lower parts of the canopy may cause this difference. When comparing *O. pugnax* populations over time, samples should be taken during the same time of day. Other workers determined time of day is not a significant factor in the number of *O. pugnax* caught with the SN (Douglas 1939, Cherry and Deren 2000); however, the sample unit size in these experiments was much larger

The variance-mean ratio can be used to classify the spatial pattern of a species as aggregated ($s^2 > x$), random ($s^2 = x$) or uniform ($s^2 < x$) (Davis 1994, Wilson 1994). Because spatial aggregation is density dependent (Taylor et al. 1978, Taylor 1984), the spatial pattern of an insect can change from aggregated to random to uniform as density decreases (Wilson 1994). Several factors other than density also can influence the spatial pattern of an insect. For example, Wilson and Room (1983) found that as cotton arthropods aged, the spatial pattern of the insects became less clumped, producing smaller values of Taylor's coefficients *a* and *b*. The use of different sampling methods and sample unit sizes produce differences in the perceived spatial pattern of insects. Results from the current study show the spatial pattern of *O. pugnax* in Texas rice fields was aggregated ($s^2 > x$) at most densities for all sampling methods (Figs. 6.4 and 6.5). Degree of aggregation varied depending on the sampling method used. *O. pugnax* was perceived as highly aggregated when using the SN method, and less so when using visual methods. Likewise, Foster et al. (1989) determined that the spatial pattern of *O. pugnax* in rice fields in Florida was aggregated.

All visual sampling methodologies correlated well with the SN method (Table 6.4) but only LS and SS2 relationships with the SN were unaffected by time of day of

sampling (Table 6.5). The other visual sampling methodologies were significantly affected by time of day of sampling which indicates that a single linear regression equation does not accurately describe the relationship with the SN method; thus, two functions are needed, one for morning and another for afternoon.

Operator did not affect the relationship between visual and SN sampling, which is probably due to operator training prior to sampling. Training was required for the operators to become familiarized with the sampling methods and learn to visually distinguish *O. pugnax* from other insects. *O. pugnax* possibly can be confused with nabids and other hemipterans of similar size but these insects usually are not found in densities comparable to *O. pugnax*. Past research suggests that visual sampling in rice is possible. Bowling (1969) correlated *O. pugnax* visual counts in small rice plots with SN counts taken from the same plots and also found a high correlation between them. Ferrer and Shepard (1987) found a strong correlation between absolute counts of *Scotinophara coarctata* F., a pentatomid pest of rice in various parts of Asia, and visual counts in the field, and developed a sampling plan based on visual counts.

Previous research reported the physical aspects of SN sampling can be a factor discouraging the adoption of this method (Harper et al. 1990). Sampling for *O. pugnax* occurs in flooded fields with dense rice canopies during summer months when temperatures and humidity in Texas typically are very high. Under these conditions, performing 10 consecutive sweeps with a SN is an arduous task. Although a SN is relatively inexpensive ($ 23.50, BioQuip Products Inc., Rancho Dominguez, CA), some farm managers may not possess one when needed. Also, nets frequently need replacing

due to the abrasive nature of rice plants. All these factors may explain why growers, Crop Consultants and County Agents have a strong preference for visual sampling methods. An advantage of the visual sampling methods evaluated in this study is that they require less physical effort than the SN. It is difficult to quantify directly the amount of sampling effort required to reach a population estimate. Traditionally, relative cost-reliability only considers the number of sample units needed and time required to sample. By including the probability of adoption of a sampling method (as a measure of sampling effort), relative cost-reliability is broadened and presents a better comparison of sampling methods.

For most densities, the visual methods described in this study are more cost-reliable than the SN (Fig. 6.6). Of the visual methods, SS5 required the fewest sample units to reach an estimate, which was expected since this method intercepted the most canopy area. However, when considering time, SS5 was the most expensive per sample unit. In the same manner, while SS1 and SS2 required less time than LS to inspect a sample unit, the fewer sample units required for LS made it more cost-reliable than SS1 or SS2. TT was the least cost-reliable method at low insect densities, but its relative cost-reliability decreased rapidly and approached one for populations equivalent to three adult *O. pugnax* per 10 SN sweeps. The disadvantage of TT is a great deal of standardization (walking time and distance while taking the sample) is required, making it somewhat impractical. The cost-reliability of two sampling methods, as calculated in our study, is independent of the reliability level (D_x) and the error rate (α), but is dependent on

sampling time, insect density, spatial pattern and the probability of adoption of the sampling methods being compared, as shown in equation (6.8) (Wilson et al. 1982).

Among the visual methods evaluated in the present study, LS and SS2 appear to be the most appropriate for field use. Both methods correlate well with the SN and are not affected by stage of panicle development, time of day of sampling, type of planting, or operator. The number of insects observed with these visual methods can be converted into SN counts using the equations $LS = -0.156 + 0.675\ SN$ and $SS2 = 0.407 + 0.396\ SN$, where *SN* is the number of adult *O. pugnax* caught with 10 SN sweeps, *LS* is the number of adults *O. pugnax* per one long stick sweep and *SS2* is the number of adult *O. pugnax* per two sweep stick sweeps (Table 6.4).

In Texas, 10 sample units are recommended to estimate *O. pugnax* density using the SN. Using this fixed sample size for population sampling purposes, the reliability of a SN estimate is within 53% of a mean of one insect per 10 sweeps, within 33% of a mean of five insects per 10 sweeps, and within 27% of a mean of 10 insects per 10 sweeps (Fig. 6.7). Considering a minimum level of reliability of 30%, for a sample size of $n = 10$, the reliability of the SN method at densities lower than six *O. pugnax* is poor. For a population estimate to be within 30% of the mean when populations are as low as one insect per 10 sweeps, a sample size of $n = 27$ is required (Table 6.6). This indicates that the number of sample units needed to arrive at a population estimate using the SN should be increased when populations are low. If the desired level of reliability is higher, a greater number of sample units are needed (Table 6.6).

Population sampling of *O. pugnax* may require a larger investment of time and effort than commercial sampling. In commercial sampling the objective is to classify an insect population above or below a threshold, while in population sampling the objective is to obtain a parameter estimate with a certain level of reliability. Parameter estimation of *O. pugnax* may be important in certain situations, such as insect-plant interaction and ecological studies, regional monitoring efforts and evaluation of potential management practices. Results of the present studies may be used by researchers, county extension agents, consultants and farm managers to facilitate sampling and improve reliability of *O. pugnax* estimates for research purposes.

CHAPTER VII

DEVELOPMENT OF SEQUENTIAL SAMPLING PLANS FOR THE RICE STINK BUG

Introduction

The rice stink bug, *Oebalus pugnax* (Fabricius) (Hemiptera: Pentatomidae), is a serious pest of rice, *Oryza sativa* L., in the southern United States (Way 2003) attacking the crop from flowering to grain maturity. This insect is responsible for reductions of rough and head rice yields, and grain quality (Douglas and Tullis 1950, Swanson and Newsom 1962, Bowling 1963, Harper et al. 1993, Tindall et al. 2005, Patel et al. 2006) by feeding on developing kernels, introducing pathogenic microorganisms and causing a discoloration of the grain known as "peck" for which growers are penalized.

Monitoring field populations is the basis for successful management of *O. pugnax* in rice. Due to its high mobility (Douglas 1939), wide host range (McPherson and McPherson 2000), and preference to feed on rice (Naresh and Smith 1983, Rashid et al. 2006), populations can increase rapidly to damaging levels. Currently, the only recommended method to sample for *O. pugnax* in Texas is the sweep net (Way et al. 2006). Rice fields should be sampled once or twice a week from 50% heading to harvest. A 38 cm diameter net is swept from side to side with each step while walking through the field, making sure that the top of the net is flush with the top of the panicles. After 10 consecutive sweeps, the number of adult rice stink bugs is recorded. This constitutes one sample unit. A total of 10 sample units per management area is recommended. Current economic thresholds for *O. pugnax* in Texas vary from an average of three to 15 adults

per 10 sample units depending on the stage of the crop, expected yield and rice price, planting date, and cost of insecticide application (Harper et al. 1993, Harper et al. 1994, Way et al. 2006).

The objective of sampling can be to determine a parameter estimate (population sampling) or to classify an insect population as exceeding or not exceeding damaging levels (commercial monitoring) (Ruesink 1980, Buntin 1994, Wilson 1994). In commercial monitoring, the interest of the sampler does not lie in estimating the mean population level with a defined reliability, but in classifying insect populations above or below the economic threshold with the ultimate goal of determining if a management action is needed. Using sequential sampling plans for commercial monitoring have generated savings in sampling time in many crops when compared to fixed sample size plans (Rothrock and Sterling 1982a, b, Ferrer and Shepard 1987, Hoffmann et al. 1991), particularly when populations are much lower or higher than the economic threshold. Also, reduction in the number of insecticide applications has resulted from the implementation of such plans (Shepard 1980). Sequential sampling allows relatively rapid information collection at the lowest possible cost with a high level of reliability.

Many rice producers in Texas have not adopted the sweep net (Harper et al. 1990), consequently they do not use economic thresholds in their decision making process. Instead, they make treatment decisions based on non-standardized, subjective, visual observations of *O. pugnax* populations. Availability of visual sequential sampling plans may encourage producers to incorporate monitoring and the use of economic thresholds in their management practices.

In the present study, sequential plans to sample *O. pugnax* using the sweep net were developed and compared to the currently used fixed sample size plan. Also, sequential plans for alternative visual sampling methods were developed and their relative cost efficiency with respect to the sweep net method determined.

Materials and Methods

Data collection. Data were collected during 2003 and 2004 from commercial rice fields located in Chambers, Colorado, Fort Bend, Jackson and Jefferson Cos., TX. Seven fields were sampled in 2003 and 10 in 2004. Stages of panicle development during sampling were heading, milk and dough. Heading was considered to begin at panicle exertion. Milk was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was milky and panicles began to bend downward due to weight of developing grains. Dough was considered to begin when consistency of the caryopsis of at least 50% of the grains on a panicle was dough (not liquid) and hulls turned from green to tan. A field was considered in heading, milk or dough when 75% of the panicles in the field reached one of these stages of development.

Selected fields were divided into parallel transects 18 m apart. Transects were selected and samples taken every 18 m, starting 9 m from the field margin. The number of sampling points in each transect and transects per field varied with field size. At each sampling point, SN and visual samples were taken in adjacent areas but spaced enough to avoid interference among methods. Fields were sampled only once during each season,

or, if sampled more than once, they were sampled at different stages of panicle development. Time required to complete each sampling method was recorded.

Sampling methodologies. The sweep net (SN) and two visual sampling methods, the "long stick" (LS) and the "sweep stick" (SS), were used to develop sequential sampling plans. The LS, a 1.5 m long PVC pipe (2 cm diameter), was used to gently disturb the rice panicles while sweeping 180 degrees in front of the operator. The number of adult *O. pugnax* observed on or flying from the disturbed panicles along the entire length of the LS was recorded. The SS is a 1 m long PVC pipe (2 cm diameter) used by the operator to gently disturb rice panicles, sweeping 180 degrees from one side to the other with each step. After two sweeps of the SS, the number of adult *O. pugnax* observed on or flying from disturbed panicles along the last 0.38 m (diameter of the SN) of the SS was recorded. One sample unit for the SN, LS and SS methods consisted of 10 consecutive sweeps, one sweep and two sweeps, respectively.

Sequential sampling. Equation (7.1) (Karandinos 1976, Wilson 1994) was used to develop a sequential sampling plan for the SN, LS and SS:

$$n = t^2_{\alpha \text{ or } \beta} \mid x - T \mid^{-2} a\, x^b \qquad (7.1)$$

where n is sample size; t is the standard normal variate for a one tailed confidence interval; α is the error of determining that the insect population is above the economic threshold when it is not, also known as type I error; β is the error of determining that the population is below the economic threshold when it is not, also known as type II error; x is the mean population density; T is the economic threshold, and a and b are Taylor's coefficients. Taylor's coefficients for each sampling method were calculated in Chapter

VI. Economic thresholds used for the SN method were five adult *O. pugnax* per 10 sweeps for heading and milk stages of panicle development, and 10 adult *O. pugnax* per 10 sweeps for the dough stage of panicle development (Way et al. 2006). To determine the economic thresholds for the visual methods, linear regression equations obtained in Chapter VI relating SN to visual methods were employed. Error rates used to develop the sequential plans were $\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$.

Cost-reliability. When comparing sampling methods for commercial monitoring, the best method is one that provides the most reliable classification of the insect population for a given cost. The costs of making a management decision with a given level of reliability for two sampling methods can be compared using relative cost-reliability (Wilson 1994), expressed as:

$$C_v / C_{sn} = n_v(\theta_v + \phi_v) / n_{sn}(\theta_{sn} + \phi_{sn}) \qquad (7.2)$$

where C_v and C_{sn} are the cost per sample in time for a given level of reliability for the visual and SN sampling methods, respectively; n_v and n_{sn} are the number of sampling units required to classify an insect population as above or below the economic threshold for a given level of reliability with the corresponding sampling method; θ_v and θ_{sn} are the times required to examine an individual sample unit using the corresponding sampling method; and Φ_v and Φ_{sn} are the times required to move between sample units for the corresponding sampling method.

Equation (7.2) calculates the relative cost-reliability of a visual method with respect to the SN method based on the number of sample units and time required to classify a population as above or below the economic threshold for a given level of

reliability. However, equation (7.2) does not consider the physical effort necessary for each sampling method. Scouts may prefer the sampling method that is less physically demanding. An advantage of the visual methods tried in this study is that they are less strenuous than sweeping rice using the SN.

Assuming that the probability of adoption of a sampling method is inversely proportional to the physical effort required to sample, the physical effort required to sample an insect population using the ith sampling method, E_i, can be expressed as:

$$E_i = \varepsilon / p_i \tag{7.3}$$

where p_i is the probability of adoption of the ith sampling method and ε is a constant relating E_i to p_i. Incorporating E_i in equation (7.2), one obtains:

$$C_v / C_{sn} = n_v(\theta_v + \phi_v)E_v / n_{sn}(\theta_{sn} + \phi_{sn})E_{sn} \tag{7.4}$$

and replacing E_i in (7.4) with (7.3),

$$C_v / C_{sn} = n_v(\theta_v + \phi_v)p_v^{-1} / n_{sn}(\theta_{sn} + \phi_{sn})p_{sn}^{-1} \tag{7.5}$$

where C_v / C_{sn} is the relative cost-reliability that incorporates probability of adoption, p_{sn} is the probability of adoption of the SN method and p_v is the probability of adoption of the visual method. Equation (7.5) can be used to determine the relative cost-reliability of a visual method with respect to the SN considering not only sample size and sampling time but also sampling effort. To determine the probability of adoption of the visual methods, 20 potential users of the novel visual methods (growers, Crop Consultants and County Agents) were interviewed. The probability of adoption of the SN was obtained from Harper et al. (1990).

During the collection of samples, the time required to count the number of insects caught with the SN increased as the number of insects caught increased. To incorporate this time variation into the cost-reliability analysis, the time needed to examine a sample unit at different mean population densities was estimated by linear regression analysis.

Substituting *n* in equation (7.5) with equation (7.1), and including the linear regression equation relating SN and visual counts (Table 7.1), one obtains:

$$C_v / C_{sn} = B^{-2} a_v (A + Bx)^{b_v} (\theta_v + \phi_v) p_v^{-1} [(a_{sn} x^{b_{sn}})(\theta_{sn} + \phi_{sn}) p_{sn}^{-1}]^{-1} \qquad (7.6)$$

where C_v / C_{sn} is the relative cost-reliability of the visual method with respect to the SN method; a_v and b_v are Taylor's coefficients for the visual method; a_{sn} and b_{sn} are Taylor's coefficients for the SN method; A and B are the intercept and slope of the linear regression equation relating visual to SN counts; and x is mean population density expressed in number of adult *O. pugnax* caught per SN sample unit (10 sweeps). Equation (7.6) was used to determine the relative cost-reliability of commercial sampling visual methods with respect to the SN method for a given level of reliability.

Comparison between fixed and sequential SN sampling plans. A comparison was made between the currently recommended SN fixed sample size plan and the SN sequential sampling plans developed. Thus, 27 SN samples of size $n = 10$ were taken during the 2005 growing season between 29 June and 8 August. Fields in different stages of panicle development, with different cultivars and in different locations were sampled. Each sample corresponded to a different field, or if a field was sampled more than once,

Table 7.1. Equations relating visual [long stick (LS) and sweep stick (SS)] to sweep net (SN) counts of *O. pugnax* and economic thresholds used for development of sequential sampling plans

Sampling method	Linear regression equation	Economic thresholds	
		Heading	Milk and Dough
SN	-	5	10
LS	$LS^{a} = -0.156 + 0.675\ SN^{b}$	3.2	6.6
SS	$SS^{c} = 0.407 + 0.396\ SN$	2.4	4.4

[a] Number of adult *O. pugnax* observed per one LS sweep.

[b] Number of adult *O. pugnax* caught per 10 SN sweeps.

[c] Number of adult *O. pugnax* observed per two SS sweeps.

samples were taken at different stages of panicle development. Samples were taken randomly following the Texas Rice Production Guidelines recommendations (Way et al. 2006). During the sampling process, the number of adult *O. pugnax* caught after each sample unit was recorded. Numbers were later compared to the corresponding sequential sampling tables developed for the SN method for both error rates used ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$) and sample size and decision reached (control action needed, control action not needed, continue sampling) recorded. Mean sample size for each sequential plan was calculated and compared using a paired-samples *t*-test. Fixed and sequential sampling plans sample size were compared using a one sample *t*-test. Percentage of sample size reduction for sequential plans with respect to the fixed sample size plan also was calculated. Statistical analyses were performed using the statistical software SPSS (SPSS Inc. 2005).

Results

Sequential sampling. Equations relating visual to SN sampling methods and the economic thresholds used in developing the sequential sampling plans are shown in table 7.1. SN sequential sampling plans are presented in Fig. 7.1; LS sequential plans in Fig. 7.2, and SS sequential plans in Fig. 7.3. To use the sequential sampling plan, select the appropriate figure corresponding to the sampling method to be used, the economic threshold relevant to the stage of the crop, and the desired error rate. If the cumulative number of adult *O. pugnax* for the corresponding sample unit number falls in the "Stop sampling, control action not needed" area, a management intervention is not required

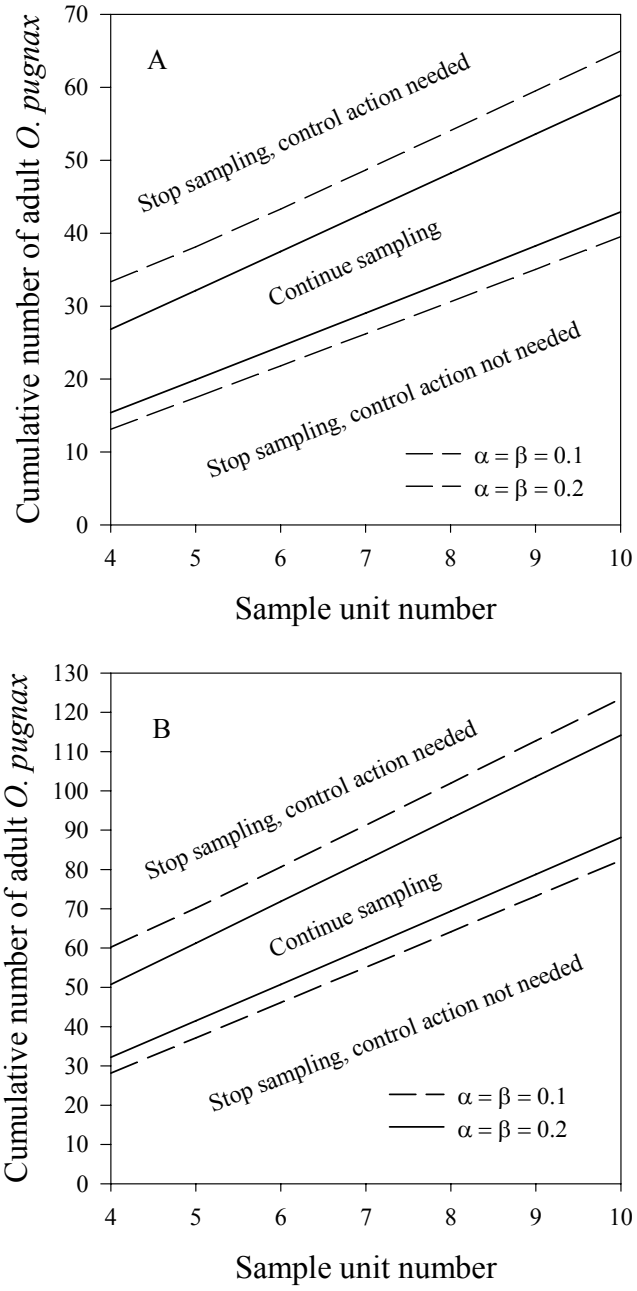

Fig. 7.1. Sequential sampling plan for *O. pugnax* using the sweep net (SN) method for (A) economic threshold of five adults per 10 sweeps (heading and milk stages) and (B) economic threshold of 10 adults per10 sweeps (dough stage) and two error rates ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$). A sample unit is 10 sweeps of the SN.

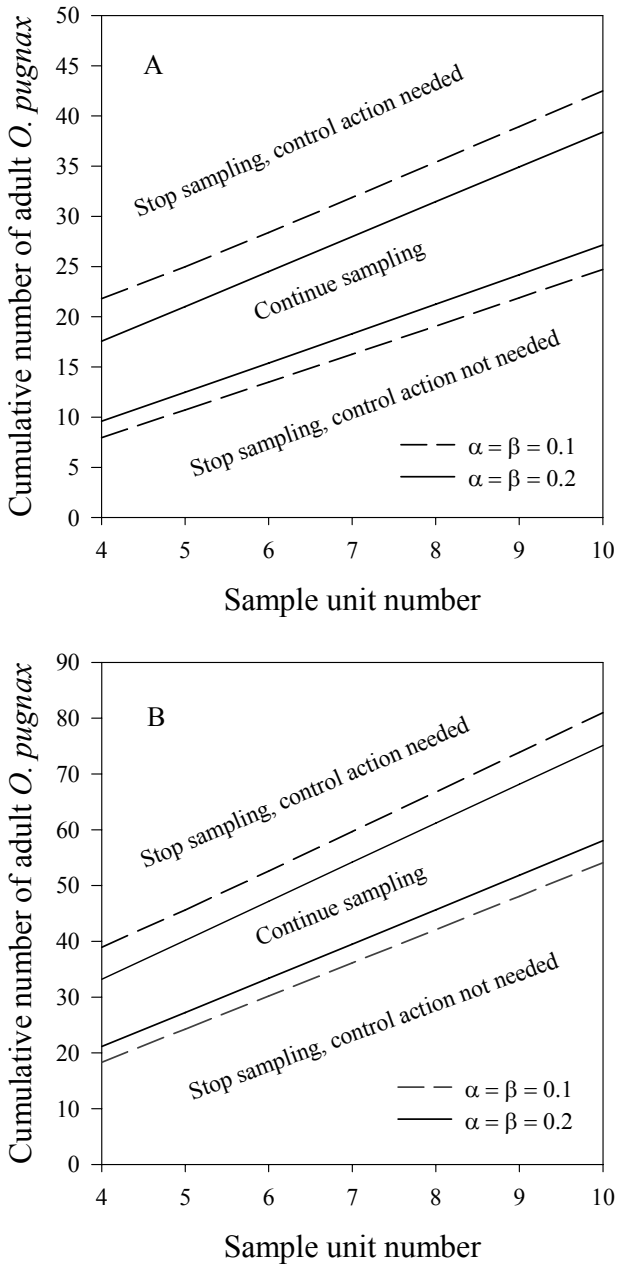

Fig. 7.2. Sequential sampling plan for *O. pugnax* using the long stick (LS) method for (A) economic threshold of 3.2 adults/long stick sweep (heading and milk stages) and (B) economic threshold of 6.6 adults per long stick sweep (dough stage) and two error rates ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$). A sample unit is one sweep of the LS.

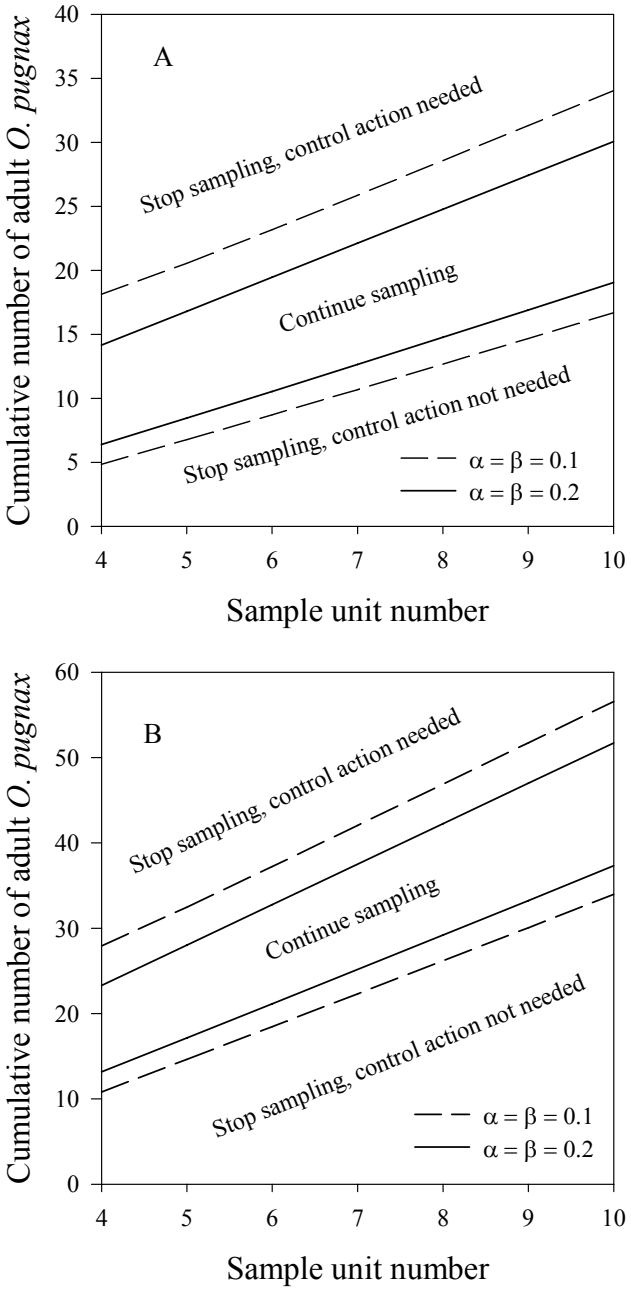

Fig. 7.3. Sequential sampling plan for *O. pugnax* using the sweep stick (SS) method for (A) economic threshold of 2.4 adults per two sweep stick sweeps (heading and milk stages) and (B) economic threshold of 4.4 adults per two sweep stick sweeps (dough stage) and two error rates ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$). A sample unit is two sweeps of the SS.

and sampling can stop. Likewise, if the cumulative number of adult *O. pugnax* falls in the "Stop sampling, control action needed" area, a management intervention is required and sampling can stop. If the cumulative number falls in the "Continue sampling" area, more samples are needed until a decision is reached or the maximum number of sample units (n = 10) is reached. If the maximum number of sample units has been taken and a decision has not been reached, the field should be sampled again in 2 or 3 days. Four sample units were arbitrarily selected as the minimum necessary before comparing the cumulative number of insects caught or observed with the sequential sampling plans. This number was selected to avoid unrealistic estimates based on sample sizes that are too small. Shepard (1980) also recommended a minimum of four sample units when sampling arthropods in soybeans. Ten was selected as the maximum number of sample units because this is the number of sample units scouts currently employ for *O. pugnax* sampling using a SN (Way et al. 2006).

Cost reliability. The linear regression between SN counts and time (in seconds) required to take and examine a SN sample unit was significant (F = 11.974, P = 0.001, r^2 = 0.255, n = 36). Intercept and slope for this relationship were 17.370 ± 2.311 and 1.616 ± 0.467, respectively. Average time in seconds required to take a sample unit for each visual method was 33.37 ± 0.93 for LS and 27.32 ± 1.19 for SS. Time required to move between sample units was 18.72 ± 0.323 seconds, and was estimated from samples taken during 2005 in different fields throughout the Texas Rice Belt. This time was assumed to be the same for all sampling methodologies. Time the operator spent recording data after taking a sample unit was not included in our analysis.

A survey of 20 potential users revealed that 18 of them would adopt a visual sampling method for *O. pugnax*. Adoption probability of the novel visual methods (p_v) was calculated to be 0.9. Harper el al. (1990) conducted mail surveys among Texas rice producers during 1986 and 1987 and calculated the probability of adoption of the sweep net (p_{sn}) to be 0.4.

Relative cost-reliability values for the visual sampling methods with respect to the SN method are shown in Fig. 7.4. Values > 1 suggest that the SN method is more cost-reliable than the visual method being compared, while values < 1 suggest the converse. The LS is more cost-reliable than the SN method, while the SS is less cost-reliable than the SN method at densities less than eight adult *O. pugnax* per 10 SN sweeps and becomes more cost-reliable than the SN only at higher densities.

Comparison between fixed and sequential SN sampling plans. Sample size required to reach a decision using the 0.1 and 0.2 error rate sequential sampling plans was significantly smaller than the currently used fixed sample size ($t = 20.396$, df = 26, $P < 0.001$ for the 0.1 error rate plan; $t = 50.187$, df = 26, $P < 0.001$ for the 0.2 error rate plan) (Table 7.2). The mean number of sample units taken with the 0.1 error rate sequential plan was significantly larger than the number taken with the 0.2 error rate sequential plan ($t = 2.508$, df = 26, $P = 0.019$). On average, the use of sequential sampling reduced the number of sample units required to reach a decision 55% with respect to the fixed sample size of $n = 10$. A summary of decisions reached with all sampling methods also is presented in Table 7.2. The fixed sample size plan yielded two "Control action needed" and 25 "Control action not needed" decisions. The 0.1 error rate

Table 7.2. Comparison of mean sample size (± SEM) required to reach a management decision for the sweep net method using the sequential sampling plan versus the fixed sample size plan

Error rate	Mean sample size	% reduction	Decisions				
			Fixed		Sequential		
			No action	Action	No action	Action	Continue sampling
$\alpha = \beta = 0.1$	4.67 ± 0.26	53.3	25	1	25	1	1
$\alpha = \beta = 0.2$	4.26 ± 0.11	57.4			26	1	0

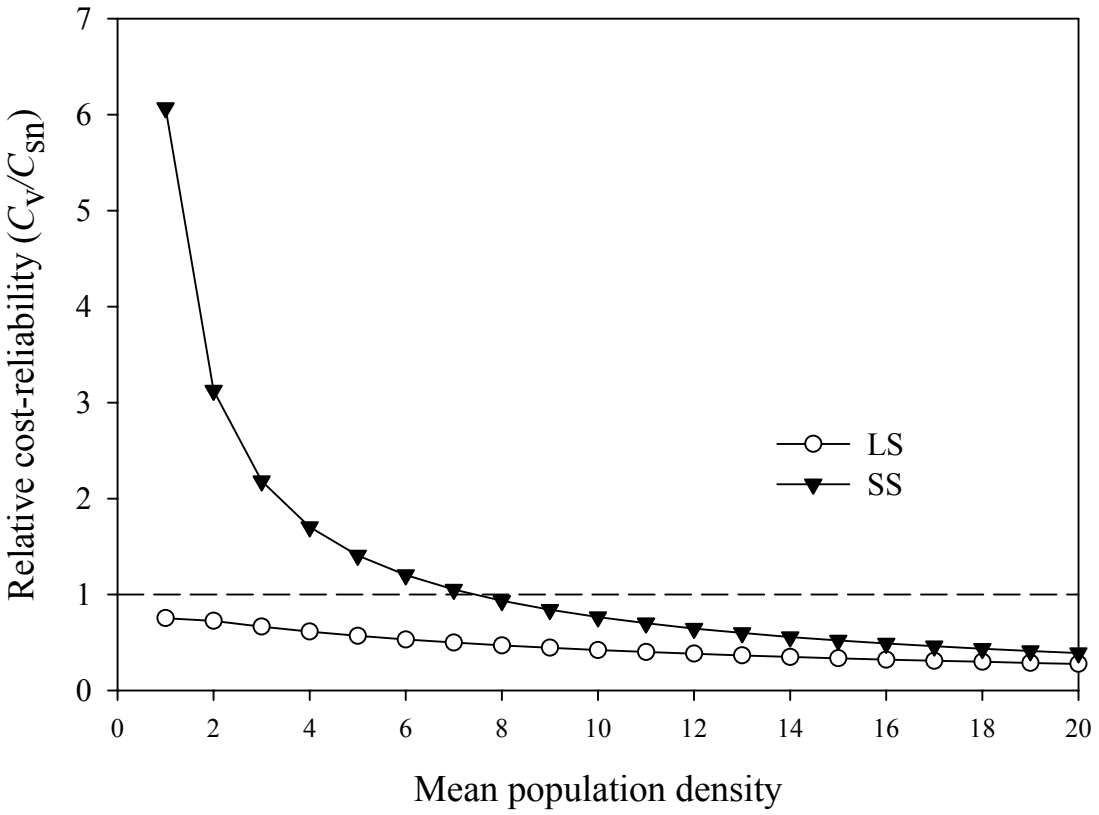

Fig 7.4. Relative cost-reliability for long stick (LS) and sweep stick (SS) commercial sampling plans with respect to the sweep net (SN) commercial sampling plan. Mean population density is expressed as number of adult *O. pugnax* per SN sample unit (10 sweeps). The dashed straight line represents a relative cost-reliability value of one, where the SN and visual methods have the same cost-reliability; above this line, the SN method is more cost-reliable, and below it, the visual methods are.

sampling plan yielded one “Control action needed”, 25 “Control action not needed”, and one “Continue sampling” decisions. The 0.2 error rate sampling plan yielded one “Control action needed” and 26 “Control action not needed” decisions. Out of the 27 samples, only on one occasion was the decision reached by the 0.1 and 0.2 error rate sequential plans different from the decision reached by the fixed sample size plan. In this field, rice was in the milk stage and the mean population density was 5.3 adult *O. pugnax* per SN sample unit (10 sweeps). A decision could not be reached only in one field when using the 0.1 error rate sequential plan; rice in this field was in the heading stage and had a mean population density of 4.5 adult *O. pugnax* per SN sample unit. In most sampled fields, *O. pugnax* populations were lower than the economic threshold, requiring only four sample units to reach a decision

Discussion

Sequential sampling was originally developed by Wald (1945), and since then has been used in pest sampling in many crops. Sequential sampling serves to classify populations above or below a threshold, rather than to provide estimates of population parameters. Current economic thresholds for *O. pugnax* in Texas vary from three to 15 adults per 10 SN sweeps. However, in practice, most growers and consultants use a threshold of five adult *O. pugnax* per 10 SN sweeps during heading and milk stages of panicle development, and 10 adult *O. pugnax* per 10 SN sweeps during the dough stage of panicle development. These thresholds, and their equivalents for the visual sampling

methodologies employed in this study, were used to develop the sequential sampling plans.

As mentioned above, α is the probability of determining that the insect population is above the economic threshold when in reality it is not. The consequence of this type of error is that a management action will be initiated when in reality it is not needed. Usually this translates to unnecessary insecticide applications. Because of the crop's short period of susceptibility to *O. pugnax* and the insect's ability to rapidly infest fields, management of this pest relies heavily on insecticides. Beta (β) is the probability of deciding the population is below the economic threshold when in reality it is not, prompting the scout to falsely conclude that a management action is not needed. An acceptable level of α is determined by minimizing the cost of sampling and the cost of unnecessary control actions (insecticide application for example), and for β by minimizing the cost of sampling and the cost of damage due to lack of necessary pest control (Wilson et al. 1983b, Wilson 1994). Many sequential sampling programs developed for pest insects have used α and β error rates of 0.1, meaning that for every 10 decisions, one may be wrong (Shepard 1980, Wilson et al. 1983a). The error rates used in the present study assign equal importance to minimizing costs associated with unnecessary applications (α) and minimizing potential damage to the crop due to lack of control when control is needed (β). Alpha (α) not only takes into consideration direct costs of unnecessary insecticide applications, but also the cost of other consequences of pesticide use such as insecticide resistance development, secondary pest outbreaks, resurgence, and environmental degradation. Unfortunately, these costs are difficult to

quantify. In Texas, *O. pugnax* has not developed resistance to insecticides (Drees and Plapp 1986, Way et al. 2006); however, resistance development is a concern since many of the insecticides (pyrethroids) used in rice production have a similar mode of action. By assigning the same value to α and β, we want to convey that if resistance were to develop due to unnecessary insecticide applications, severe economic consequences would ensue.

Plans developed with error rates of 0.1 produced stop lines that are farther apart than lines from plans developed with error rates of 0.2. Widely separated lines delimit a larger area of indecision, implying that more sample units are required to reach a decision as the insect population approaches the economic threshold. The two error rates provided (0.1 and 0.2) give the scout the alternative to choose a more or less conservative plan. Lower risk (lower α and β) will require more sample units, and an increase in sampling time is possible when using the more conservative sampling plan which can be preferable when the value of the crop is high or when the cost of control is elevated. For example, when comparing the number of sample units necessary to reach a decision in Texas rice fields, it was found that on average, significantly more sample units were necessary using the 0.1 error rate sampling plan (Table 7.2).

The sample size currently recommended for *O. pugnax* using the SN is 10 sample units. Comparison of the fixed sample size plan with the sequential sampling plans developed here shows that considerable savings in time are possible when using sequential sampling plans (Table 7.2). This is especially true when populations are well above or below the economic threshold. The greater the difference in the mean

population and the economic threshold, the fewer sample units required to determine if the population is above or below the threshold. Likewise, as the population density approaches the economic threshold, a greater number of sample units is necessary to classify the population (Wilson et al. 1983b). In the present experiment, in fields sampled with both fixed and sequential sampling plans, a 55% reduction in sampling time was found when using the sequential sampling plans. Prior research for cotton pests reported similar results. Rothrock and Sterling (1982b) found sequential sampling reduced sample size 86% compared to fixed sample size plans. Decisions reached by both sampling plans were not significantly different. In rice, Ferrer and Shepard (1987) reduced sampling time for the Malayan black bug, *Scotinophara coarctata* (F.), a pest of rice in the Philippines and other parts of Asia, by 50% using sequential sampling plans. In the same manner, Hoffmann et al. (1991) reported a reduction in sample size of 62% when using sequential sampling plans for eggs of the tomato fruitworm, *Helicoverpa zea* (Boddie) in California. Other examples are reported in the literature (Shepard 1980, Wilson 1994).

When comparing the visual methods in this study with SN sampling, relative cost-reliability indicates the LS is the most cost-reliable method for sampling *O. pugnax* using a sequential plan. Only at densities higher than eight adult *O. pugnax* per 10 SN sweeps was the SS more cost-reliable than the SN. As shown in equation (7.6), relative cost-reliability is independent of the economic threshold and error rates used, as long as they are the same for the sampling methods being compared, but is dependent on the

sampling costs, the insect density, the corresponding variance and the probability of adoption of the sampling methods.

In the present study, we have shown sequential sampling for *O. pugnax* in rice has the potential to significantly reduce the number of sample units necessary to reach a management decision, which decreases overall sampling cost. The LS is the most cost reliable method to sample for *O. pugnax* using sequential sampling. The development of sequential sampling plans and visual sampling methods serve to increase the frequency and reliability of monitoring *O. pugnax* populations in commercial rice production.

CHAPTER VIII

SUMMARY

Damage Assessment and Sampling of the Rice Stink Bug in Rice in Texas

Relative susceptibility of stages of rice panicle development to rice stink bug: whole plant greenhouse and field experiments. No differences were found in weight of rough, brown or milled rice among caged plants infested with *O. pugnax* during different stages of panicle development. Number and weight of filled grains per cage were not affected by *O. pugnax* infestation. However, number and weight of empty grains tended to be higher in treatments infested during heading. *O. pugnax* feeding may have caused an increase in the number of empty grains, but plants may have compensated for this injury by filling more grains or increasing the movement of photosynthates to grains not fed on by *O. pugnax*. Adults and nymphs caused peck during all stages of panicle development. Adult *O. pugnax* caused higher percentage peck than nymphs. Averaging across all greenhouse experiments, adults caused 2.25, 1.79, and 2.24 times more peck than nymphs during heading, milk, and soft dough, respectively. Higher percentage peck was found in grain from cages infested during milk and soft dough than in grain from cages infested during heading. In three of four experiments, *O. pugnax* feeding did not affect percentage whole kernels. This may be explained by low peck incidence during 2006, optimum rice drying temperatures and careful milling of samples. An inverse relationship was found between percent peck and percent whole grain weight only in one of four experiments.

Relative susceptibility of stages of rice panicle development to adult female and male rice stink bug feeding: Number of grains, percentage filled grains, weight of filled grains and percentage whole kernels per panicle were not significantly affected by infestation of single panicles for 48 h with one adult *O. pugnax* at heading, milk, soft dough or hard dough. No significant differences were found in the percentage peck caused by male or female *O. pugnax* at any stage of panicle development. When comparing percentage peck produced by infestation of *O. pugnax* during different stages of panicle development in 2005, percentage peck was significantly lower in uninfested panicles and panicles infested during hard dough than in panicles infested during heading, milk or soft dough. No differences were found in percentage of peck caused by *O. pugnax* during heading, milk or soft dough. In 2006, no significant differences were found in percentage peck produced by *O. pugnax* in panicles infested during heading, milk, soft dough or hard dough. Higher percentage of peck during the hard dough stage during this year may have been the result of late drainage of the field.

Attractiveness of stages of rice panicle development to rice stink bug: greenhouse experiments. Observation of male and female *O. pugnax* caged on rice plants at different stages of panicle development showed that attractiveness of *O. pugnax* to plants with panicles at milk and soft dough stages was greater than plants at the pre-heading or heading stage. Pre-heading plants were the least attractive to the insects, confirming field observations. More females were observed on the cage surface, and more males on plants. This may indicate that females are more involved in dispersion

than males. Results show that application of insecticides with little or no residual activity during the pre-heading stage are likely ineffective.

Determination of rice stink bug spatial pattern and development of visual sampling methods and population sampling plans. Significant differences were found between perimeter and within field sweep net samples, indicating that samples taken 9 m from the field margin overestimate within field *O. pugnax* populations. More *O. pugnax* were consistently caught with the sweep net during the morning than afternoon. For all sampling methods evaluated during this study, *O. pugnax* was found to have an aggregated spatial pattern at most densities. When comparing sweep net with visual sampling methods, one sweep of the "long stick" and two sweeps of the "sweep stick" correlated well with the sweep net. This relationship was not affected by time of day of sampling, stage of panicle development, type of planting or operator. Relative cost-reliability that incorporates probability of adoption indicates that the visual methods are more cost-reliable than the SN to sample *O. pugnax* for research purposes in Texas rice fields.

Development of sequential sampling plans for the rice stink bug. Sequential sampling plans were developed for the sweep net, the "long stick" and the "sweep stick". Relative cost-reliability that incorporates probability of adoption showed that sampling using the long stick is more cost-reliable than sampling using the sweep net. Two passes of the sweep stick was less cost-reliable than the sweep net method at low densities, and became more cost-reliable than the sweep net at densities of eight or more adult *O. pugnax* per 10 sweep net sweeps. For sweep net sampling, comparison of the currently

used fixed sample size plan (n = 10) and sequential sampling showed that sequential sampling reduced the number of sample units required to reach a decision by 56% with respect to the fixed sample size plan.

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