Effects of nitrogen on egg-laying inhibition and ovicidal response in planthopper-resistant rice varieties

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ABSTRACT

A series of experiments was set up to examine the effects of nitrogen on rice (Oryza sativa L.) resistance against Nilaparvata lugens (Stål) and Sogatella furcifera (Horváth). Egg laying by N. lugens was reduced on the indica variety IR60. Nymph biomass (N. lugens and S. furcifera) was also lower on IR60: this was associated with low honeydew production and a high proportion of xylem-derived honeydew in N. lugens but not in S. furcifera. Nitrogen increased egg-laying by S. furcifera and increased N. lugens nymph biomass on all varieties tested. Oviposition and egg mortality in both planthopper species were examined on plants at 15, 30 and 45 days after sowing (DAS). Sogatella furcifera laid more eggs on plants at 15 DAS, but laid few eggs during darkness; N. lugens continued to lay eggs on older rice plants (30 DAS) and during darkness. Egg mortality was high on cv. Asiminori, highest at 45 DAS, and higher for S. furcifera than for N. lugens. Mortality of S. furcifera eggs was associated with lesions around the egg clusters. These were more common around clusters laid during the day and suggested induction by Asiminori of an ovicidal response. Egg mortality declined under higher soil nitrogen levels. Results are discussed in the light of improving rice resistance against planthoppers and reducing rates of planthopper adaptation to resistance genes.

1. Introduction

The brown planthopper, Nilaparvata lugens (Stål), and white-backed planthopper, Sogatella furcifera (Horváth), are major pests of rice (Oryza sativa L.) in Asia (Cheng, 2009; Bottrell and Schoenly, 2012). In recent years (since about 2000), outbreaks of both species have been recorded with increasing frequency throughout the region (Fujita et al., 2013). S. furcifera is particularly problematic in hybrid rice largely because of high cytoplasmically inherited susceptibility among Chinese hybrid varieties (Cheng, 2009; Horgan et al., 2016a). N. lugens is a serious pest under intense rice production in lowland irrigated farms where outbreaks are linked to high nitrogen and pesticide inputs (Gallagher et al., 1994; Heinrichs, 1994; Bottrell and Schoenly, 2012).

Host plant resistance has been the principal focus of public research for management of both planthopper species for the last several decades. Currently about 50 resistance gene loci and several Quantitative Trait Loci (QTLs) have been identified against N. lugens (36 genes) and S. furcifera (14 genes) (Fujita et al., 2013; Horgan et al., 2015). Among the notable resistance QTLs are those associated with the induced ovicidal response of some japonica rice varieties, and particularly cv. Asominori (Yamasaki et al., 2000). The ovicidal response was first noted during field observations in Japan as discoloured spots surrounding egg clusters on mature japonica rice plants (Sogawa, 1991). Since then, the physiological and genetic mechanisms behind the ovicidal response have been well described (Seino et al., 1996; Suzuki et al., 1996; Seino and Suzuki, 1997; Yamasaki et al., 2000). There have been few further observations of ovicidal response in varieties other than Reiho and Asominori (but see cv. Natsuhikari in Yang et al., 2013). Indeed, there are very few published accounts of any antixenotic defenses against planthopper oviposition or further antibiotic mechanisms acting on

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planthopper eggs in rice (Fujita et al., 2013).

Fertilizers are known to increase planthopper fitness on rice (Sogawa, 1970; Cheng, 1971; Preap et al., 2001; Crisol et al., 2013; Horgan et al., 2016a). Furthermore, fertilizers have been shown to reduce antibiotic defenses of rice against planthopper nymphs (Salim and Saxena, 1991; Lu et al., 2004; Vu et al., 2014; Srinivasan et al., 2015; Horgan et al., 2016b). In contrast to several research papers on the instability of antibiotic defenses against nymphs, there have been few evaluations of stability in antixenotic defenses (but see Lu et al., 2004) and no studies on the stability of the ovicidal response against planthopper eggs under high nitrogen fertilization.

In the present study, we examine the effects of nitrogenous fertilizer on egg laying and ovicidal response in susceptible and resistant rice varieties. We selected two resistant rice varieties that have been noted to either inhibit egg-laying (i.e., IR60: Peñalver Cruz et al., 2011) or to exhibit ovicidal-like responses (i.e., Asiminori, this paper). Because the former represents an *indica* rice variety and the latter a *japonica* rice variety, we included IR22 and T65 as susceptible *indica* and *japonica* lines, respectively, for comparison. We also examined the time of egg-laying by the two planthopper species (day/night) and on plants of different ages. We discuss our results in the light of improving farm management of resistant rice varieties to optimize the effects of resistance and prevent planthopper adaptation to resistance genes.

2. Materials and methods

2.1. Plant materials

We used two *O. sativa* subsp. *japonica* lines (one variety, one landrace) and two *O. sativa* subsp. *indica* varieties in our experiments. T65 (Ac79) is a japonica variety first released in Taiwan about 1923. The variety is highly adaptable but has high susceptibility to planthopper damage (De Datta, 1981). Asiminori (Ac39942) is a *japonica* landrace. The variety has some noted allelopathy to weeds (Kim and Shin, 1998). We found moderate to high levels of egg mortality in *S. furcifera* on Asiminori during greenhouse trials in the Philippines (Horgan unpublished). Examination of the responses by the plant indicated a high proportion of eggs and egg clusters with fluid filled lesions similar to those described for Asominori (Yamasaki et al., 2000). Furthermore, the occurrence of lesions was strongly influenced by ambient light conditions (Supplementary materials: Table S1; Fig. S1) as described by Yang et al. (2013) for ovicidal response in cv. Natsuhikari. The relation between Asiminori and Asominori is unclear, as restrictions on the movement of rice germplasm (Asominori is not available at the International Rice Research Institute [IRRI]) prevented us from conducting a phylogenetic comparison of the two varieties.

IR22 is an *indica* variety released by IRRI in 1969. The variety has been widely distributed in Asia, Africa and Latin America. It is known to be highly susceptible to planthoppers and possesses no known resistance genes (Khush and Virk, 2005). IR60 is an *indica* variety released by IRRI in 1983 that is thought to possess the *Bph3* gene for resistance against *N. lugens* (Khush and Virk, 2005) together with other, unidentified resistance sources (Peñalver Cruz et al., 2011). The variety has not been adopted beyond ca 10% by famers in the Philippines, but has been recommended for parts of Mindanao (South Philippines) affected by rice tungro disease (Khush and Virk, 2005; Peñalver Cruz et al., 2011).

Seed of IR22, IR60 and T65 were acquired from the Plant Breeding, Genetics and Biotechnology (PBGB) Division at IRRI in Los Baños, Laguna, Philippines. Seed of Asiminori were acquired through the Rice Germplasm Collection at IRRI. All experiments were conducted using potted plants in a greenhouse at IRRI. During the experiments, temperatures ranged between 26 °C and 37 °C and no artificial lighting was used (12:12h, day:night [D:N]). Plants were monitored daily and pots were watered and weeded as necessary. Unless otherwise stated, plants received no fertilizers and were not treated with any pesticides.

2.2. Planthopper colonies

In our experiments, we used *N. lugens* and *S. furcifera* from research colonies held at IRRI. The colonies were initiated in 2004 using wild-caught individuals from rice fields in Laguna (14°10′N, 121°13′E) with periodic introgressions of wild caught individuals from the same location each year. Previous studies using these same colonies have indicated that they are virulent against *Bph1*, *bph2*, *BPH25*, and *BPH26*. *S. furcifera* from the same region were also virulent against *Wbph2*, *Wbph3* and *wbph4* (Srinivasan et al., 2015; Horgan, unpublished). The insects were reared continuously on the susceptible variety TN1 (≥30-day old rice plants) in wire mesh cages of 91.5 × 56.5 × 56.5 cm (H × L × W) under greenhouse conditions (26−45 °C, 12:12 D:N).

2.3. Planthopper responses to varieties and nitrogen levels

A series of bioassays that were each replicated six times in a randomized block design was conducted in the greenhouse (26−36 °C, 12:12 D:N) to examine planthopper responses to the rice varieties under zero added fertilizer (N0) and with the equivalent of 60 kg/ha added fertilizer (N1). Plants were used in the bioassays 25 days after sowing (DAS). These were maintained in size-0 pots (5 × 2.5 cm: H × R) with paddy soil under acetate insect cages (45 × 2.5 cm, H × R). We selected three non-choice bioassays to assess potential antixenotic and antibiotic defenses among the rice varieties. Egg-laying and nymph weight gain have been noted elsewhere as sensitive planthopper response parameters; development time is closely correlated with nymph weight (see Peñalver Cruz et al., 2011) and was not recorded in the study.

In a no-choice, egg-laying bioassay, single gravid females were placed on each of the rice plants and allowed to feed and lay eggs for 3 days. After 3 days, the females were removed and the plants left for a further 3 days to allow the eggs to develop. After a total of 6 days, the plants were dissected under a stereomicroscope (20 × magnification) to count the eggs.

To examine nymph survival and weight gain, newly emerged nymphs (ten nymphs on each plant) of each of the two planthopper species were placed separately on the rice plants. Nymphs were allowed to feed and develop for 15 days after which the number of survivors was recorded. The survivors were then killed and dried at 60 °C in a forced draft oven and weighed. The plants were cut above the soil and were also dried and weighed.

Honeydew excreted by planthoppers was quantified using the method of Pathak and Heinrichs (1982). Planthoppers that had been starved for 24 h were confined to within 5 cm of the base of the plants in specially prepared plastic chambers. The chambers were placed on top of filter paper, neatly fitted around the plant shoot. The filter papers had been treated with bromocresol green. Bromocresol green indicates the nature of the honeydew as coming from the phloem or xylem (see Ferrater et al., 2015). The area of excreted honeydew spots on the bromocresol-treated filter paper was measured using Image J software version 1.48 (National Institutes of Health, USA). The insects used in the honeydew feeding test were collected, oven-dried at 60 °C for 3 days, and weighed. Honeydew production by each planthopper was standardized to the weight of the planthopper.