

Mechanisms of resistance: a major gap in understanding planthopper-rice interactions

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Host-plant resistance (HPR) has been a valuable tool in the management of planthoppers. However, experience with the brown planthopper, *Nilaparvata lugens* (Stål), indicates that field resistance is limited because planthoppers can quickly overcome resistance genes. As new approaches to improving the durability of resistant rice varieties are being developed, there is a need to better predict the outcomes of gene deployment in terms of planthopper virulence responses. However, as this paper points out, major gaps still exist in our understanding of resistance mechanisms and of how these mechanisms relate to identified resistance genes. These knowledge gaps will hinder the future success of resistance breeding strategies and varietal deployment. Using the brown planthopper as an example, this paper reviews planthopper-HPR research since the 1970s, links available information on brown planthopper-rice interactions, and calls for increased attention to hypothesis-based research that will improve our capacity to manage field resistance and planthopper virulence.

Knowledge is essential for the efficient management of natural and derived ecosystems. To best predict the outcome of agricultural interventions, this knowledge should be based on an understanding of the processes underlying ecological patterns (Romesburg 1981). In the absence of manipulative experimentation, management predictions are based only on sets of observations that are directly related, but never tested, or perceived to be related, but the relations are indirect or there is no relation at all (Romesburg 1981). As knowledge is accumulated, information usually shifts from observations of patterns (descriptive studies) to an understanding of processes (manipulative studies with hypothetical-deductive (HD)-hypothesis testing) (Romesburg 1981). This paper suggests that our current understanding of interactions between planthoppers and the rice plant is largely based on descriptive studies. However, to improve planthopper management through host-plant resistance, increased attention should focus on the processes underlying resistance and on planthopper adaptations to resistant varieties.

Nearly 50 years of research has produced a considerable body of information on planthopper-rice interactions, including extensive information on levels of host-plant

resistance in rice (Kaneda et al 1981, Jung-Tsung et al 1986, Khush and Virk 2005), planthopper population and behavioral responses to resistant varieties (Padgham 1983, Velusamy and Heinrichs 1986, Padgham and Woodhead 1988, Kimmins 1989, Bing et al 2007), and, particularly in recent years, the genes associated with observed resistance (Yamasaki et al 2000, Huang et al 2001, Kawaguchi et al 2001, Murata et al 2001, Yang et al 2002, Jairin et al 2007). This research has had a major impact on rice production with the deployment of planthopper-resistant rice varieties throughout South and Southeast Asia (Khush and Virk 2005). However, despite the continued interest in rice resistance, successful management of planthoppers has been limited and recent major outbreaks have occurred in parts of India, Bangladesh, Thailand, Vietnam, China, and the Philippines (personal communications between national agricultural research institutes and IRRI).

Using resistance to brown planthopper (BPH) (*Nilaparvata lugens* Stål) as an example, this paper highlights major gaps in our understanding of planthopper-rice interactions. It suggests that our understanding of the functioning of resistance genes has been hindered by a lack of manipulative experimentation during the elucidation of mechanisms, and therefore that the effective deployment of resistance genes, the management of planthoppers, and the management of the viruses they transmit are limited by knowledge constraints. This paper suggests that reductionist experimentation, particularly during the screening of breeding materials and lines, has restricted our understanding of resistance to planthopper feeding responses and has therefore led to an overemphasis on nutrient-based and anti-feeding resistance mechanisms. Finally, a change in research direction is proposed to increase the future efficiency of varietal development for resistance, and to improve gene deployment at regional and farm levels.

A brief history of planthopper-rice research

Research into planthoppers increased dramatically in the early 1970s when Asian farmers began to experience extensive and sustained BPH outbreaks caused, largely, by an overuse of insecticides (Heinrichs 1994, Gallagher et al 1994). The scale of the problem during the 1970s is indicated by the rapid increase in scientific output at that time (Fig. 1). By 1973, the first resistant rice variety, IR26, was released in Asia. IR26 contained the dominant *Bph1* gene for resistance that became associated with initial dramatic declines in BPH populations. However, within 2 years, *Bph1* had “broken down” and planthopper densities began to increase again. In 1976, scientists responded with the release of IR36 and other varieties that contained the recessive *bph2* resistance gene. However, in the late 1980s and early 1990s, *bph2* also began to break down. IR56 and other varieties containing the *Bph3* resistance gene have been deployed since 1982 but these have also broken down in many regions (Gallagher et al 1994).

Studies into host-plant resistance have continued as a major focus of research on planthoppers, albeit with a lull in research output during the 1990s. Many early studies examined insect behavioral responses using electrical penetration graph (EPG)

Number of publications

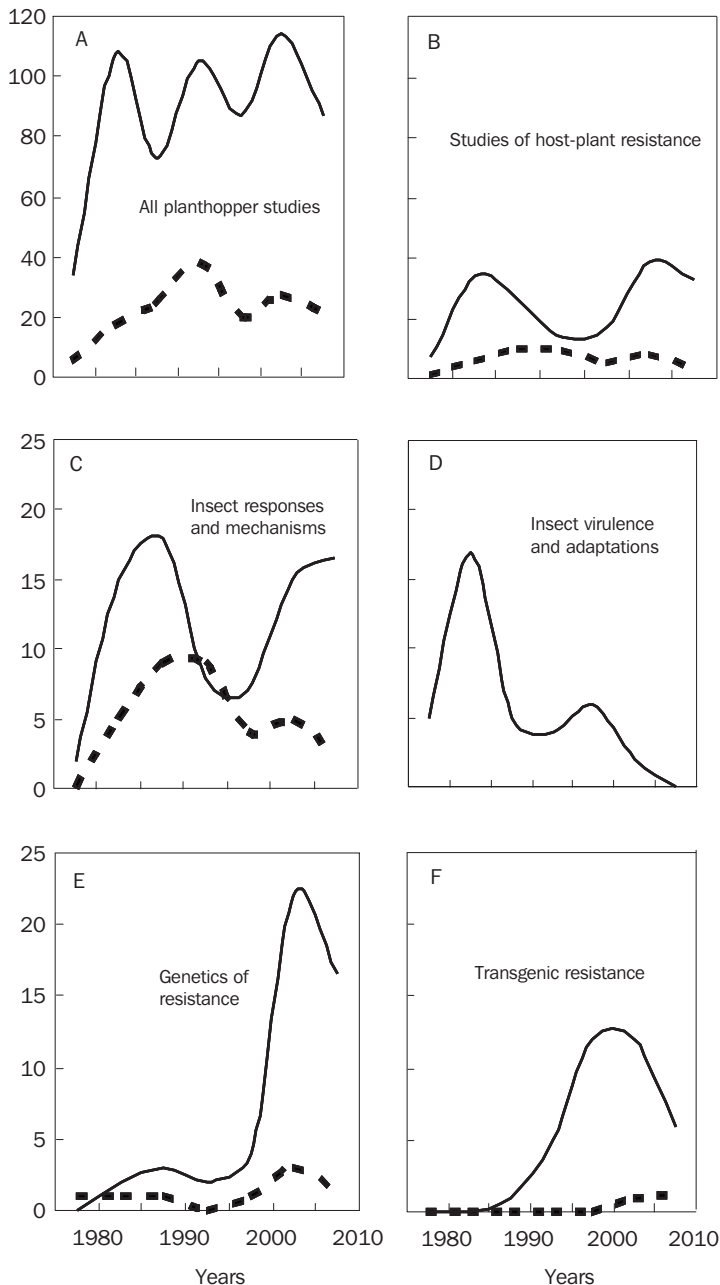


Fig. 1. Papers published on planthoppers (BPH [solid line] and WBPH [broken line]) between 1974 and 2008 as cited on ISI-Web of Knowledge (searches = *Nilaparvata lugens* or brown planthopper and *Sogatella furcifera* or white-backed planthopper). (A) All hopper papers, (B) papers concerning rice varietal resistance, (C) papers that quantified insect responses to resistant rice varieties and investigated resistance mechanisms (including induced mechanisms), (D) papers concerning “biotypes” or otherwise investigating insect selection on resistant rice varieties, (E) papers that employed genetic techniques or investigated the genetics behind constitutive resistance to hoppers, and (F) papers that investigated hopper responses to transgenic rice with genes for hopper damage reduction. Graphs on the same row have the same y-axes.

technology and measured fitness losses incurred on resistant varieties (Velusamy and Heinrichs 1986, Padgham and Woodhead 1988, Kimmins 1989). Furthermore, because of the increasing virulence of BPH populations against the *Bph1*, *bph2*, and *Bph3* genes, studies of “biotype” responses were a prevalent feature in the 1970s and 1980s; however, an inability to adequately define biotypes or to apply the biotype concept (Heinrichs 1994) may have led to an almost complete neglect of virulence issues in the past 10 to 15 years (Fig. 1). Advances in molecular techniques since the mid-1990s have led to an increase in our understanding of resistance and the identification of about 21 (some tentative) genes and various quantitative trait loci (QTLs) linked to resistance in established varieties and wild rice species (Yamasaki et al 2000, Huang et al 2001, Khush and Virk 2005, Myint et al 2009). Biotechnological advances have also led to an increased interest in engineered resistance (Tang et al 2001a,b, Saha et al 2006).

Overall, research since the 1970s has produced a number of resistant varieties, some with known resistance genes, and a clear indication of planthopper responses to some modern resistant and differential varieties (i.e., varieties [often landraces or traditional] generally possessing known resistance genes). However, there has been a relatively minor gain in the understanding of resistance mechanisms such that, to date, there is still no clear mechanistic link between major resistance genes and observed planthopper behavioral or fitness responses. Nevertheless, QTLs have been identified and linked to the ovicidal response (see Sogawa et al, this volume, and below) and a suite of genes has been linked to induced defenses (Wang et al 2004, Yang et al 2006, Hao et al 2008).

Planthopper responses to resistant varieties

Reductionist experimentation is essential in understanding plant-insect interactions. However, experiments and bioassays should be set in a holistic framework that incorporates plant and insect phenologies and adequately accounts for adaptation and genetic diversity in target organisms. Figure 2 is a schematic diagram of the planthopper life-behavior cycle broken down into key “behaviors” (or activities)—indicated by rectangles—(dispersal, feeding, probing, etc.) and major “behavior options”—indicated by diamonds. Behaviors are depicted as simple motor responses elicited through sensory evaluation of environmental cues. Options, which lead to specific behaviors, are influenced by plant volatiles, secondary chemicals, chemical taste, wax composition, and/or other plant characteristics, and are determined by the physiological state of the insect. In reductionist experimentation, observed behaviors can sometimes be determined by insect physiological drive alone. For example, in no-choice trials, Zaheruddeen and Prakasa Rao (1988) found BPH to oviposit on 72 host plants, including common weeds, wild rice species, and crops (wheat [*Triticum aestivum* L.], oats [*Avena sativa* L.], and barnyard millet [*Echinochloa frumentacea* (Roxb.) L.]); however, BPH, a monophagous insect, is unlikely to oviposit on most of these plants when not confined or when given a host-plant choice (i.e., Nagata and Hayakawa 1998, Hattori 2001).

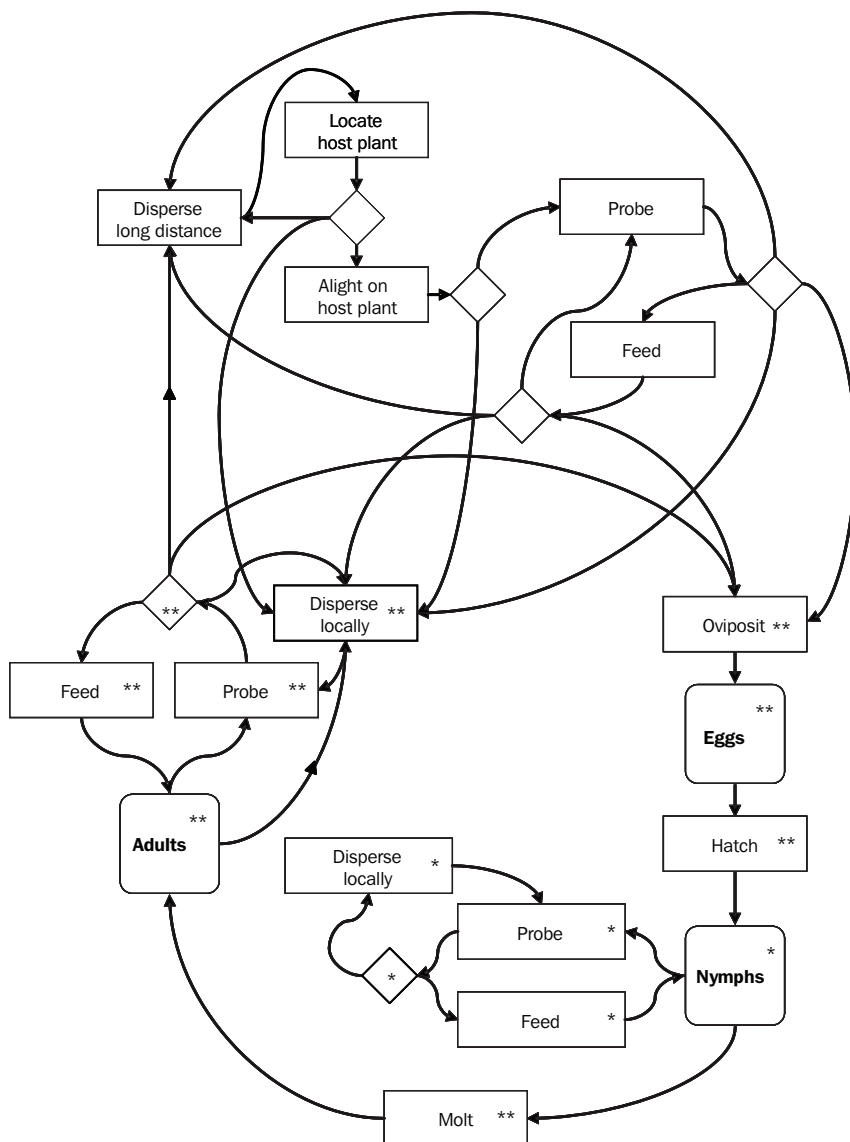


Fig. 2. Schematic depiction of the planthopper life-behavior cycle. The cycle indicates the three major life-history stages (bold squares) and their associated behavioral interactions with the rice plant (rectangles). Diamonds indicate key behavior options that are governed by internal (insect physiological) and external cues (plant kairomones, volatiles, etc.). A single asterisk indicates life-behavior entities incorporated into the SSST and MSST; a double asterisk indicates entities incorporated into the MSST alone. Field testing in hotspots can encompass the entire cycle.

Current knowledge of planthopper-rice interactions has been heavily influenced by the prevalence of a single reductionist screening method, the standard seedbox screening test (SSST), that continues to be widely used in the development of resistant varieties (personal communications between national agricultural research institutes and IRRI). In the SSST, seeds of each cultivar (usually >15 cultivars per test) are sown in a single row in a seedbox of about 60 × 40 × 10 cm. Suitable susceptible and resistant checks are sown in similar rows in the same box. Seven days after sowing, seedlings are thinned to about 20 plants per row and infested with about eight second-instar nymphs per seedling. When susceptible checks are killed (usually after about 1 week), plants are rated for damage on a 0–9 scale, where 0–3 is classified as resistant, 4–6 as moderately resistant, and 7–9 as susceptible (Velusamy et al 1986). Different rice-breeding centers and laboratories sometimes vary minor details of the protocol (Kaneda et al 1981).

A second method, the modified seedbox screening test (MSST), was designed to overcome certain inconsistencies in the SSST and better detect “field resistance,” that is, when resistance is maintained or increases as plants age. In the MSST, seeds are sown and thinned as in the SSST, but infested at 20 days after sowing with four second-instar nymphs per plant. Plants are evaluated at the time that susceptible checks are killed, using the same scale as in the SSST. With the MSST, usually mortality of the susceptible check is caused by F₁ BPH, that is, the original nymphs mature and reproduce in the seedbox, and their offspring kill the plants (Velusamy et al 1986). Although the MSST is an improvement on the SSST, because of its higher cost and longer turnover time, it is seldom used in bulk screening (personal communications between national agricultural research institutes and IRRI).

These two methods have been extremely useful for rapid, high-throughput, and inexpensive screening of the necessarily large amounts of material required to find and isolate resistance genes. Furthermore, they incorporate “choice,” that is, the target insects can choose between a variety of options before initiating feeding (SSST and MSST) or oviposition (MSST) responses. However, as Figure 2 indicates, the SSST, the most widely used screening method, is only capable of evaluating nymphal feeding responses to the test plants. The MSST improves on the SSST by allowing nymphs to develop to adults, when they can oviposit, presumably in response to cultivar suitability for nymphal development (Fig. 2). When cultivars have different levels of resistance, nymphs will disperse between plants, the degree of movement (activity) being negatively correlated with feeding. Movement between plants is assumed to simulate field responses; however, it is largely governed by push-pull dynamics in the experimental arena, the strength of which is determined by the idiosyncrasies, combinations, and relative positions of the cultivars under testing, and is therefore unstable between successive tests. As Figure 2 indicates, four key behavior options that determine levels of field infestation are never considered when using the SSST or MSST. These are (1) the option to alight on a host plant after the host plant has been located, (2) the option to probe or disperse locally (within or between plants) on a host plant after alighting, (3) the option to disperse (long or short distances) or feed following probing (and possibly also to oviposit), and (4) the option to disperse

(long or short distances) or oviposit following feeding. Only field trials are capable of evaluating these responses.

Field screening of cultivars in BPH hotspots may include all aspects of the BPH life-behavior cycle; however, field studies are subject to varying planthopper densities at a series of nested scales. Published results from field screening studies are rare. Ōya and Fukamachi (1987) examined planthopper settlement on rice varieties (14 to 40 days after transplanting) in the field in Japan. They found that immigrant adult females alighted equally on plots of resistant and susceptible cultivars; however, the planthoppers did not stay on the resistant cultivars for more than 1 or 2 days; therefore, immigrants were stimulated to disperse from resistant cultivars, but the stimuli to settle on rice were similar among susceptible and resistant cultivars. Although the resistance mechanisms were likely related to anti-feeding, the prevalence of the SSST suggests that the resistant varieties used in these field trials were selected and developed precisely because of their anti-feeding effects. Therefore, prescreening and cultivar selection using the SSST may cause an overall bias toward feeding-related mechanisms even in field trials, further restricting our understanding of the available resistance mechanisms inherent to rice.

During the 1980s and early 1990s, a large number of studies were conducted to examine the responses of planthoppers to resistant rice varieties and to explain patterns emerging from SSSTs (Fig. 3). Differential varieties such as IR46 and Mudgo, which contain the *Bph1* gene, ASD7 with the *bph2* gene, IR62 and Rathu Heenati with the *Bph3* gene, and Babawee with the *bph4* gene were a prominent feature in these studies; however, aspects of planthopper response have been published for more than 100 resistant varieties or wild rice accessions (see references in Figs. 3 and 4). Perhaps unsurprisingly, because of the prevalence of the SSST in developing resistant varieties and because of converging experimental protocols (i.e., plants < 35 days old, planthoppers at a nymphal stage, and one variety, usually TN1, used both to rear planthoppers and as a susceptible check during experimentation), all planthopper responses can be related either directly or indirectly to nymphal feeding behavior (Fig. 3). Although the planthopper responses can be linked to specific genes by assessing behavior on differential varieties, they are for the most part general responses to resistance for which the underlying mechanisms are still largely unknown. Little attention has been given to any other types of host-plant-elicited behavioral responses that might determine susceptibility or resistance in the field. For example, there is no documented evidence to indicate that fit females (i.e., those unaffected by a poor diet) will avoid settling or reduce oviposition on resistant plants in the field. Such considerations are useful because viral transmission may occur during planthopper probing (Cabauatan et al, this volume), but is effectively avoided if planthoppers are deterred from settling on the plants. The logistics of determining field responses or developing resistant varieties with novel resistance mechanisms (i.e., not related to postprobing avoidance) are obviously considerable. What is important to note here is that, through our methodology, such mechanisms are rarely detected and have not been generally incorporated into resistant varieties.

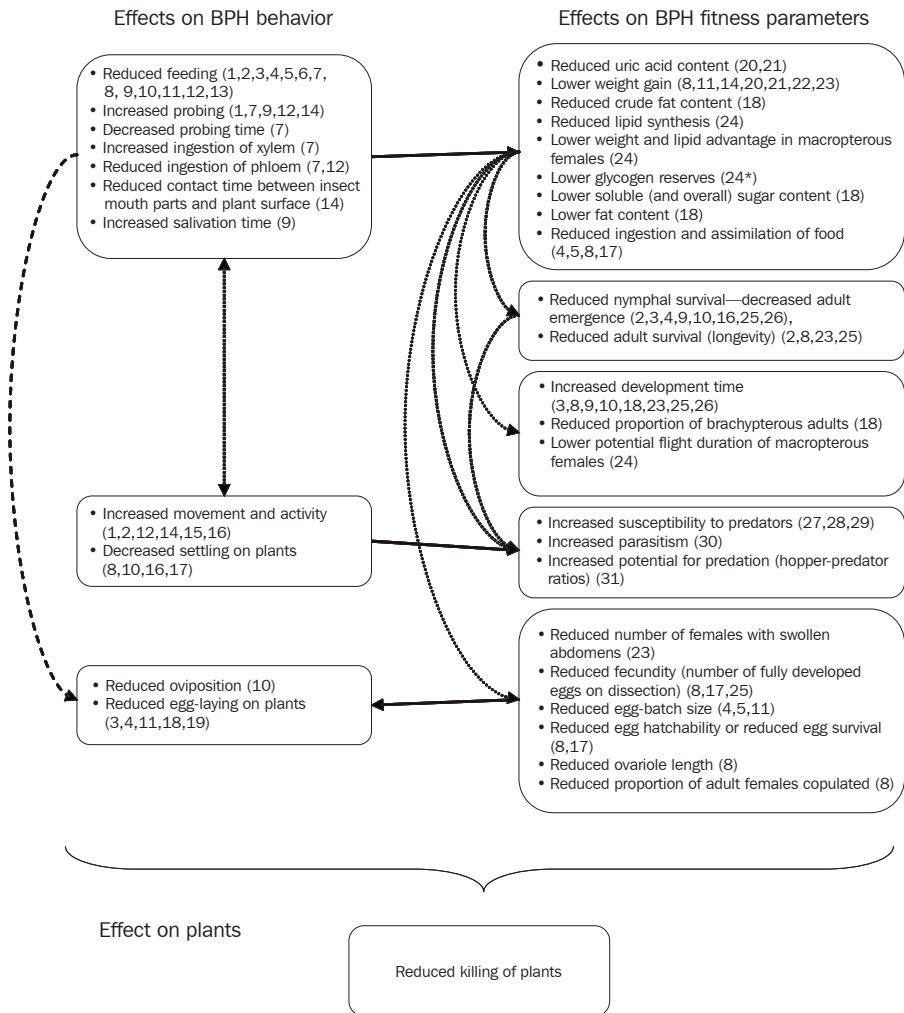
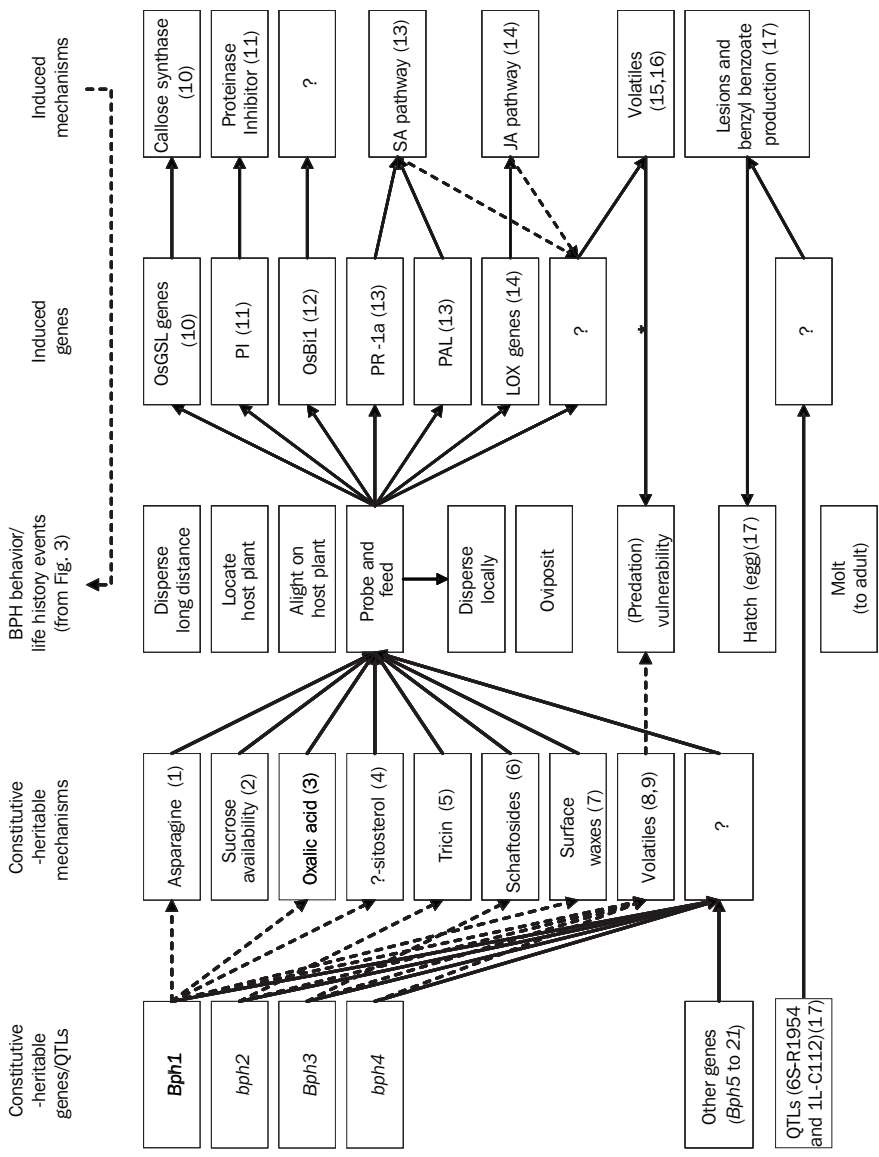


Fig. 3. Effects of resistant rice varieties on BPH behavior and fitness parameters. Note that feeding effects can be linked to all behavior and parameters (associated arrows). Numbers indicate examples as follows: 1—Padgham and Woodhead (1988); 2—Saxena and Okech (1985); 3—Cohen et al (1997); 4—Bing et al (2007); 5—Hattori (2001); 6—Shigematsu et al (1982); 7—Kimmins (1989); 8—Jung-Tsung et al (1986); 9—Velusamy and Heinrichs (1986); 10—Senguttuvan et al (1991); 11—Baqui and Kershaw (1993); 12—Hao et al (2008); 13—Velusamy et al (1986); 14—IRRI (1985); 15—Woodhead and Padgham (1988); 16—Velusamy et al (1990); 17—Velusamy (1988); 18—Yin et al (2008); 19—Zaheruddeen and Prakasa Rao (1988); 20—Hongoh and Ishikawa (1997); 21—Sasaki et al (1996); 22—Stevenson et al (1996); 23—Myint et al (2009); 24—Padgham (1983); 25—Cheng et al (2001); 26—Pathak and Kalode (1980a); 27—Kartohardjono and Heinrichs (1984); 28—Rapusas et al (1996); 29—Senguttuvan and Gopalan (1990); 30—Wang et al (2008 a); 31—Heinrichs et al (1986). Asterisk indicates that no difference was observed.

Fig. 4. Current information on mechanisms of resistance to BPH in rice, indicating relations between known genes, resistance mechanisms, insect responses, and induced responses. Solid arrows indicate reliable evidence for proposed links, broken arrows indicate links suggested through indirect evidence. Asterisk indicates a general effect occurring in susceptible and resistant rice. Numbers indicate sources as follows: 1—Sagawa and Pathak (1970); 2—Jung and Im (2007); 3—Yoshihara et al (1980); 4—Shigematsu et al (1982); 5—Bing et al (2007); 6—Stevenson et al (1996); 7—Woodhead and Padgham (1988); 8—Saxena and Okech (1985); 9—Velusamy et al (1990); 10—Hao et al (2008); 11—Weng et al (2003); 12—Wang et al (2004); 13—Xu et al (2003); 14—Wang et al (2008b); 15—Rapusas et al (1996); 16—Luo et al (2006); 17—Yamasaki et al (2000).



Constitutive-heritable defenses in rice

Manipulative experiments are required to test hypotheses concerning resistance mechanisms. This is because mechanisms are processes and therefore cannot be determined by simple descriptive studies (Romesburg 1981). However, in spite of this requirement, few studies have ever manipulated aspects of planthoppers or rice plants to gain an understanding of their interactions (but, see Saxena and Okech 1985, Shigematsu et al 1982, Woodhead and Padgham 1988, Stevenson et al 1996). Nevertheless, the small number of studies that have conducted manipulative experiments have given concrete evidence of some of the mechanisms behind heritable defenses (Fig. 4). These mechanisms can be grouped as (1) diet-related, (2) volatile or (secondary) chemical related, and (3) related to plant-surface characteristics.

Diet-related mechanisms

The ability to manipulate sugar concentrations and amino acid levels in artificial planthopper diets has helped determine possible diet effects on planthopper behavior and fitness. Feeding by planthoppers on susceptible rice varieties reduces stored sugar content (Loka Reddy et al 2004). Planthoppers require this sucrose for successful development and, on artificial diets, have maximum survival and moderate nymphal development times when fed 25% sucrose solution. Glucose, fructose, and maltose can also function as nutrients in the presence of sucrose (Koyama 1985). On examining the nutritional components of resistance in the Korean variety Cheongcheongbyeo, Jung and Im (2005) found that phloem of this resistant variety had sugar contents similar to those of the susceptible variety Taebaegbyeo; however, the amounts ingested and excreted were different between the varieties, with significantly less occurring in planthopper excreta from the resistant variety. This suggests that anti-feedants acted on insect taste sensilla, or that secondary chemicals disturbed the digestive or feeding processes of the insect, but that the resistant variety Cheongcheongbyeo was otherwise nutritionally adequate for planthopper feeding (Jung and Im 2005).

As early as the 1970s, the amino acid asparagine was noted to have a stimulatory effect on brown planthopper feeding (Sogawa and Pathak 1970). On artificial diets, the lack of single amino acids does not cause planthopper mortality; however, the loss of each of three sulfur-containing amino acids (cysteine, histidine, and methionine) increased nymphal development time and reduced survival; furthermore, the lack of all three sulfur-containing amino acids caused mortality of first instars (Koyama 1986). BPH survival and longevity increase when amino acids are combined with sucrose (5%) (Pathak and Kalode 1980a). In preference tests, asparagine, arginine, leucine, and valine strongly enhanced planthopper feeding on sucrose (5%). Vitamins also improved planthopper survival and enhanced settling (Pathak and Kalode 1980a).

Mechanisms involving volatiles and secondary chemicals

Saxena and Okech (1985) extracted rice plant volatiles as steam distillates from a range of susceptible (TN1) and resistant (Mudgo, ASD7, Rathu-Heenati, Babawee, Ptb33, and ARC6650) rice varieties. In a series of experiments in which distillates

were applied to the susceptible TN1, mixed with sugar solutions or topically applied to nymphs, they found steam distillate from resistant varieties to significantly decrease female settling and feeding activity, while it increased the mortality of adults and nymphs. Velusamy et al (1990) produced similar effects when they applied steam distillates (at 1,000 ppm and above) of *Oryza officinalis* in similar experiments. Using column chromatography, Bing et al (2007) isolated the flavonoid 5,7,4'-trihydroxy-3',5'-dimethoxyflavone (tricin) from IR36 (with the *bph2* gene). In experiments, successive levels of tricin reduced feeding by nymphs and reduced the numbers maturing to adults on an artificial diet; furthermore, tricin reduced feeding and egg-laying by adults on tricin-treated versus tricin-untreated rice seedlings. Unfortunately, in their experiments, neither Velusamy et al (1990) nor Bing et al (2007) presented results from controls, that is, volatiles (steam distillates) or solvent fractions (containing secondary compounds) from susceptible materials. Feeding inhibitors can occur in susceptible varieties: for example, silicic acid, which inhibits BPH sucking, is a "general inhibitor" that is found in the parenchyma of both susceptible and resistant rice plants (Yoshihara et al 1979).

Toxic secondary chemicals also play a role in resistance. For example, when BPH feeds on the resistant Chinese variety B5 (containing *Bph14* and *Bph15* genes), a gene (*Y342*) encoding for P450 is activated. In insects, P450s metabolize hormones and pheromones, but they are best known for their role in the metabolism of insecticides and host-plant chemicals (Yang et al 2006); however, it is unknown whether the specific toxic chemicals involved in B5 resistance are constitutive or induced. Oxalic acid in Mudgo (*Bph1*) inhibits BPH sucking in parafilm bioassays; however, there is still no evidence to indicate that oxalic acid content is higher in the phloem of Mudgo compared with susceptible varieties (TN1) (Yoshihara et al 1980). Stevenson et al (1996) found that the phloem of resistant rice varieties (Ratthu Heenati, BG300, and BG379/2) had higher individual concentrations of schaftoside, isoschaftoside, and total apigenin-C-glycosides (all are C-glycosidic flavonoids) than susceptible varieties (BG380, BG94/1). By altering the concentration of schaftoside in parafilm feeding sachet tests (with 20% sucrose solution), they found that BPH mortality increased as schaftoside concentrations increased from 250 to 500 $\mu\text{g mL}^{-1}$. The mechanism behind all three resistant varieties was derived from Ratthu Heenati (*Bph3* gene) and Stevenson et al (1996) suggest that this is anti-feeding rather than toxic. Using a pair of isogenic japonica rice lines, 80R (resistant) and 74S (susceptible), developed through repeated selection of F_{11} through F_{19} plants from an F_2 (Hoyoku \times Mudgo) \times Kochikaze cross (therefore containing the *Bph1* gene), Shigematsu et al (1982) determined that aerial plant parts of 80R contained beta-sitosterol, stigmasterol, and campesterol in larger quantities than 74S. Furthermore, honeydew collected from planthoppers feeding on 80R had cholesterol and beta-sitosterol, which caused a sucking inhibitory effect: in parafilm tests, 50 ppm of beta-sitosterol and 15% sucrose caused total inhibition of sucking. Other sterols showed similar effects: 80R also had about one-fifth of the asparagine content of 74S (this varied between plant parts and was measured in leaves, but not in the stem or sheath); asparagine also stimulates BPH feeding (Shigematsu et al 1982).

Mechanisms involving the plant surface

Evidence for plant-surface effects on planthopper behavior is limited to a single study (Woodhead and Padgham 1988). A lack of confirmed mechanisms involving the plant surface is perhaps largely due to limited research attention to rice surface features and their effects. Woodhead and Padgham (1988) extracted epicuticular waxes from IR22 (susceptible), IR46 (*Bph1* gene), and IR62 (*Bph3* gene) and manipulated plants by switching wax applications between varieties. They found wax composition to affect feeding; specifically, they suggest that a high ratio of long to short carbon-chain compounds in IR46 and the presence of shorter chain hydrocarbons in IR22 largely determined planthopper feeding responses. Plant-surface effects are also suggested from a study by Zhang et al (2004) comparing planthopper feeding on resistant variety B5 and susceptible variety MH63. They found more saliva sheaths on the upper part of stems of B5 plants, whereas those left in MH63 plants were mainly on the lower part of the stems. However, varying amounts of “general inhibitors” such as silicic acid can also determine the location of sucking sites (Yoshihara et al 1979).

Planthopper-induced defenses in rice

Planthopper attack causes a suite of responses in the rice plant, some of which ultimately lead to symptoms of hopper burn. Many of these responses, in both resistant and susceptible varieties, involve differential gene regulation related to such diverse functions as metabolism, energy, cell-cycle and DNA processing, transcription, protein synthesis, cellular transport, development, biogenesis of cellular components, subcellular localization (Wang et al 2008a), and starch breakdown (Loka Reddy et al 2004, Hao et al 2008). Plants infested by BPH also emit inducible volatiles (i.e., linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, indole) that are not detected in healthy or mechanically damaged plants (Xu et al 2002). Overall, feeding induces senescence in rice: genes involved in macromolecule degradation and plant defenses against stresses are generally up-regulated, whereas those involved in photosynthesis and cell growth are down-regulated (Yuan et al 2005). Using relatively new molecular techniques, particularly microarray analyses, considerable information has recently been gained concerning induced rice defenses to planthopper attack (i.e., Zhang et al 2004, Yuan et al 2005, Yang et al 2006, Hao et al 2008)

Attack-related elicitors, such as β -glucosidase, present in the saliva of BPH, have been linked to salicylic acid (SA), hydrogen peroxide, and ethylene production (Wang et al 2008b). Ethylene in turn, can induce the expression of the *OsBi1* gene (*O. sativa* BPH-induced gene of unknown function), particularly in tissues around the bundle sheath, including vascular tissue, stomium, and tapetum of rice stems (Wang et al 2004). BPH damage also induces expression of the *PR-1a* gene (acidic pathogen-related protein 1), and chitinase (Pr-3) and *PAL* (phenylalanine ammonia-lyase), both of which are involved in the SA signaling pathway (Xu et al 2003). Whereas most evidence indicates that the plant response to BPH attack is similar to pathogen-induced responses, that is, through the SA pathway, there is some evidence that the jasmonic acid (JA) pathway is also activated (Zhang et al 2004, Wang et al 2008a). This is

mainly through expression of LOX genes, of which *OsLOX1* (*O. sativa* lipoxygenase 1) is likely to be the main LOX involved in the response to biotic and wounding stress. OsLOX1 protein, which builds up slowly during at least 48 h after BPH attack, is involved in JA and (Z)-3-hexenal synthesis (Wang et al 2008a). BPH infestation also induces expression of the Bowman-Birk *PI* gene (mainly in parenchyma of rice leaves and stems) to produce PI (proteinase inhibitor). This low-molecular-weight protein combines with proteinase to inhibit digestion in insects (Weng et al 2003).

Recently, Hao et al (2008) have demonstrated that callose is deposited on the sieve plates in BPH-infested rice. Where planthopper stylets had been inserted, callose deposition increased during the first 3 days of infestation in B5 (resistant) and TN1 (susceptible) rice. However, prolonged BPH feeding caused callose deposition to decrease in TN1 (Hao et al 2008). Callose deposition is coordinated through the activities of callose synthase and the callose-hydrolyzing enzyme β -1,3-glucanase; transcripts of four callose synthase encoding genes (*OsGSL1*, *OsGSL3*, *OsGSL5*, and *OsGSL7*) were detected following planthopper attack (Hao et al 2008). The expression patterns in six β -1,3-glucanase genes were also investigated. Three of these, *Osg1*, *Gns5*, and *Gns6*, were up-regulated following planthopper attack. A further gene, *Gns4*, appears to be constitutively expressed in B5, but induced in TN1 (Hao et al 2008).

As we gain information on the processes behind induced responses to planthopper attack, several features are emerging. First, rice responds to BPH attack predominantly, but perhaps not uniquely, through the SA pathway (Wang et al 2004, 2008b, Xu et al 2003). Second, the mechanisms involved in induced rice responses are secondary to constitutive defenses; therefore, continuous prolonged feeding on susceptible varieties induces defensive responses to a greater degree than in resistant varieties. For example, when comparing B5 (resistant) and MH63 (susceptible) rice varieties, PI is actually expressed at higher levels in the susceptible variety (Zhang et al 2004). In contrast, callose deposition was higher in B5 than in TN1 (Hao et al 2008); however, this could simply be a direct response to increased probing activity by planthoppers on resistant varieties (see Fig. 3), in the same way that β -1,3-glucanase breakdown of callose may be more apparent in TN1 because of increased salivation and extended feeding by planthoppers on this susceptible variety. A difficulty in controlling for planthopper activities on resistant and susceptible varieties (i.e., ensuring quantitatively equal amounts of probing, salivation, and feeding) prevents adequate comparisons of induced responses in resistant and susceptible varieties at micro-analytical scales. Furthermore, many induced responses are general responses to BPH that occur in both resistant and susceptible varieties; therefore, they appear to have little utility in defending rice against this planthopper, which, as part of its monophagous nature, may have overcome these specific defenses (i.e., by inducing β -1,3-glucanase [Hao et al 2008], or up-regulating the B-subunit of PP2A in response to plant PP2A production [Yang et al 2006]). Finally, except for the ovicidal response in japonica rice varieties (see below), induced defenses have not been linked to the known major resistance genes or resistance QTLs (Fig. 4), and there is no evidence to link planthopper-induced responses to any single resistant rice variety.

Although the influence of natural enemies is seldom considered when developing resistant varieties (the SSST and MSST are laboratory based and exclude natural enemies), interactions with natural enemies represent a further mechanism by which resistant rice varieties decrease BPH damage. Increased predator efficiency is often the result of poor herbivore fitness (as described below) and cannot be regarded as a true resistance mechanism. However, natural enemies are attracted to induced volatiles emitted during herbivore attack (Rapusas et al 1996, Xu et al 2002, Luo et al 2006). In a study by Rapusas et al (1996), resistant plants infested with BPH nymphs or eggs attracted significantly more *Cyrtorhinus lividipennis* Reuter (a mirid egg predator) and *Micraaspis hirashimai* (Sasaji) (a predatory coccinellid) than uninfested plants (however, the authors did not include a susceptible variety in their experiments). The authors indicate that some of the resistant rice plants emitted volatiles in response to BPH attack, which attracted these predators. In contrast, Luo et al (2006) indicate that the parasitoid *Anagrus nilaparvatae* Pand et Wang was attracted more to susceptible varieties than to resistant varieties.

Ovicidal response: linking induced and constitutive defenses

The japonica rice ovicidal response to planthopper oviposition (Sogawa 1991, Suzuki et al 1996, Seino et al 1996, Kiyonaga et al 1997, Yamasaki et al 2000) is different from other known rice resistance mechanisms for a number of reasons (Fig. 4). First, it was not selected through the SSST or MSST, but directly observed in the field: because it occurs in older plants (Suzuki et al 1996), it is overlooked by both screening methods. Second, because the response is quantifiable, it has been clearly linked to a series of QTLs (Yamasaki et al 2000, Sagawa et al, this volume). Third, it includes a heritable (Yamasaki et al 2000, Sagawa et al, this volume) induced component to defense that is linked to an established biochemical pathway (Seino et al 1996, Seino and Suzuki 1997).

The ovicidal response was first identified by Sogawa (1991) when he noted that dark brown discoloration of rice leaf sheaths in response to oviposition by whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth), was associated with early-stage egg mortality. Suzuki et al (1996) describe the reaction in detail: two distinct responses can occur. These they described as “watery” and “nonwatery” lesions. In nonwatery lesions, discoloration is restricted to the epidermal area around the region of egg insertion, whereas, in watery lesions, within 12 hours after oviposition, up to 11 rows of air spaces, including those containing the egg, become fully or partially filled with fluid. The formation of watery lesions causes necrosis of parenchymal cells in the lesion with the epidermis around the area of egg insertion gradually turning dark brown and often with eventual senescence of the entire leaf sheath (Suzuki et al 1996). BPH also suffers high egg mortality if a watery lesion is formed, but, unlike the plant response to WBPH, the response to BPH does not include discoloration of the leaf sheath (Suzuki et al 1996). The formation of a watery lesion is associated with up to 80% mortality of WBPH, whereas nonwatery lesions are associated with only about 12% mortality (Suzuki et al 1996). Benzyl benzoate, identified in extracts from watery lesions, has

been shown to cause ovicidal activity against WBPH at concentrations above 6.4 ppm (Seino et al 1996).

Using GC-MS (gas chromatography-mass spectrometry), Seino and Suzuki (1997) showed that C6-ring benzoic acid was used in biosynthesis of benzyl benzoate in watery-lesion tissues. They suggest that the benzyl benzoate biosynthesis pathway in watery lesions may include a series of intermediate reductions, including benzaldehyde and benzyl alcohol (Seino and Suzuki 1997). This pathway is apparently up-regulated in response to planthopper oviposition. Benzyl benzoate may cause direct egg mortality or may act indirectly by affecting planthopper symbionts (symbiont-free eggs cannot complete embryonic development, Schwemmler 1994 in Seino et al 1996). Ovicidal response to BPH is of a lower intensity than to WBPH; nevertheless, the grade of watery lesion can be associated with BPH egg mortality (Kiyonaga et al 1997, Yamasaki et al 2000). Using a set of 71 rice recombinant inbred lines (F₁₁) derived from a cross between Asominori (with ovicidal response) and IR24 (indica variety without ovicidal response), Yamasaki et al (2000) have linked the response to QTLs on chromosomes 1 and 6. Both these QTLs were located in the same chromosomal regions as two of the 10 known QTLs for ovicidal response to WBPH (Yamasaki et al 2000). The ovicidal response depends on plant age and is most intense at the maximum tillering stage, but negligible in small tillers (Suzuki et al 1996). Heritability and other aspects of ovicidal response are described in detail in Sogawa et al (this volume).

Resistance interactions with the rice ecosystem

Success in the deployment of resistant rice varieties depends on a series of factors inherent to the rice ecosystem. Whereas the genetic composition of resistant varieties is maintained through inbreeding or directed crosses (in hybrid varieties), herbivores, and their associated symbiotic gut flora (Sasaki et al 1996; Chen, this volume), respond through selection to the presence and availability of resistant material. For this reason, resistance breakdown has been a major feature in the history of BPH-resistant rice (Heinrichs 1994, Gallagher et al 1994). However, resistance is also influenced by farm management practices that affect soil nitrogen levels (Pathak and Kalode 1980b, Kajimura et al 1993, Visarto et al 2001), water availability (Baqui and Kershaw 1993b), and the density of natural enemies (Senguttuvan and Gopalan 1990, Heinrichs et al 1986, Gallagher et al 1994, Rapusas et al 1996, Cuong et al 1997, Luo et al 2006) or herbivore competitors (Cheng et al 2001).

Fertilizers

Excess fertilizer increases planthopper fitness on resistant rice varieties (IR72 and Mudgo), therefore compromising resistance (Pathak and Kalode 1980b, Visarto et al 2001). Excess nitrogen is known to increase the total free amino acids in Mudgo, but has no effect on soluble sugars (Pathak and Kalode 1980b). Kajimura et al (1993) found that BPH densities in organically farmed paddies were lower than in chemically fertilized and poultry-manured plots. This was not due to altered reproductive rates or differences in densities of natural enemies. However, the amino acid content in rice

differed between organic and chemically grown rice plants (Kajimura et al 1995): Kajimura et al (1995) found asparagine (1 of 20 amino acids analyzed) as the only amino acid that was significantly lower in organic rice than in chemically fertilized rice. Asparagine is a known BPH feeding stimulant and may have led to increased feeding and higher survival on the chemically fertilized plants (Kajimura et al 1995). It is still unknown how different rice varieties (including resistant and susceptible varieties) respond to the form and amount of nitrogen applied to fields.

Water

In a study by Baqui and Kershaw (1993), BPH honeydew excretion (linked to feeding), weight gain, and the number of eggs per egg-batch were reduced in TN1 (susceptible), but also in Mudgo (*Bph1*), ASD7 (*bph2*), Rathu-Heenati (*Bph3*), and Babawee (*bph4*) when these varieties were water stressed. In contrast, there was no effect of water stress on Ptb33 (with possibly two unknown resistance genes). However, overall, BPH did poorly on Ptb33 even under optimal water regimes.

Insecticides and natural enemies

The magnitude effect of natural enemies on BPH populations in the rice ecosystem has been determined through a number of field studies (see Way and Heong 1994 and references therein). The overuse and poor management of insecticides are often associated with planthopper outbreak (Cuong et al 1997, Heinrichs 1994). Insecticides may also decrease the durability of resistance in the field (Gallagher et al 1994) because BPH mortality is ultimately determined by interactions between varietal resistance and natural enemies (Karthhardjono and Heinrichs 1984, Senguttuvan and Gopalan 1990, Rapusas et al 1996, Cuong et al 1997, Luo et al 2006). Because of poor nutrition or intoxication, BPH developing on resistant varieties may be sluggish during evasive responses to their natural enemies; also, they move more frequently to find suitable feeding sites and have longer development times (Fig. 3). Although not specifically tested, these factors are suggested to increase BPH vulnerability to predators and parasitoids, causing observed higher predation on resistant compared with susceptible varieties (Karthhardjono and Heinrichs 1984, Senguttuvan and Gopalan 1990).

In a field study from Vietnam, BPH-predator ratios were also generally higher on resistant (IR64, IR62032-189) compared with susceptible (Jasmine) varieties in insecticide-free rice plots. However, insecticide-treated plots had higher BPH-predator ratios and the predator advantage of the resistant varieties was reduced (Cuong et al 1997). The success of early-maturing rice varieties in preventing BPH population buildup has also been linked to an increased efficiency of predators and observed lower BPH-predator ratios when compared with late-maturing varieties (Heinrichs et al 1986). The effects of insecticides on parasitoids have not been examined in the context of resistant rice varieties. Although parasitoids play a role in BPH population regulation, no evidence suggests that parasitoids more efficiently parasitize BPH on resistant varieties; rather, the opposite may occur. Luo et al (2006) found that the parasitoid *A. nilaparvatae* was less attracted to JA-treated resistant (IR26 and IR64)

than JA-treated susceptible varieties (TN1, B97-59, XS63) in paired (laboratory) and multichoice (field) tests.

Interestingly, Cheng et al (2001) have shown that rice resistance to BPH can break down after previous attack by planthoppers of a different species. In experiments with BPH and WBPH, the effects of feeding by one species increased fitness of the second species feeding on the same plant. This was not observed after feeding by a single planthopper species on the same plant. The mechanisms behind these observations have not yet been addressed.

Conclusions

The SSST and MSST have been extremely successful in identifying resistant phenotypes and leading to the large number of resistant rice varieties now available. However, in most modern varieties, BPH resistance is predominantly feeding-related. Novel resistance mechanisms may be required to improve durability, and this will require new screening methods. The pervasiveness of a single reductionist screening bioassay (the SSST) underlies many of the difficulties we now face concerning deployment for durable resistance in rice. New approaches to attaining durable resistance now confront these issues: strategies for pyramiding genes should depend on knowledge of gene function and, therefore, the related resistance mechanisms; however, apart from the ovicidal response, we have little understanding of how any of the major resistance genes or QTLs function. The management of BPH-transmitted viruses could also be improved by knowledge of varietal resistance mechanisms. Furthermore, predictions on the consequences of insecticide and nutrient management for gene deployment and durability would benefit from a greater understanding of these mechanisms.

Most of our current knowledge on resistance consists of identified resistance genes and planthopper responses to resistant varieties. The majority of these responses confirm that anti-feeding mechanisms underlie resistance in most modern varieties. This is probably not due to any lack of alternative mechanisms in the rice genome, but rather because of convergent protocols in most resistance studies and during varietal development. A small number of manipulative studies indicate that amino acids (some of which act as feeding stimulants) and secondary chemicals (many of which are anti-feedants) determine feeding responses. Certain volatiles and surface waxes can deter both feeding and settling. Only one mechanism unrelated to feeding behavior has been described: the ovicidal response. Unsurprisingly, this mechanism was first detected during field observations and is independent of the SSST. During the past 10 years, there has been increased attention to induced responses to planthopper attack on rice. Induced mechanisms are likely independent of the major resistance genes and act secondarily to constitutive defenses. Their value for rice breeding needs to be re-examined. Finally, resistant varieties are inserted into complex and diverse rice ecosystems that vary in soil nutrients, climatic regimes, landscape structure, and associated flora and fauna. Nutrient levels, determined by fertilizer regimes, and the density of natural enemies, determined by landscape features and insecticide usage,

interact with deployed varieties to determine the field success and durability of resistance.

In the face of this evidence, new screening techniques should be developed to identify genotypes with novel mechanisms that can be combined with known feeding-based mechanisms when pyramiding defenses. Further manipulative experimentation is required to clearly determine the mechanisms behind resistance. These and emerging mechanisms should be linked back to known, tagged resistance genes. Furthermore, attention should be given to issues of field resistance, that is, a stable resistance over the life-time of the plant. Finally, effective deployment of resistant varieties should be considered in the context of planthopper adaptations to genes and mechanisms, and the accelerating effects of fertilizers and pesticides on breakdown of the resistance genes that are currently available.

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Notes

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