Antifeedant of Barnyard Grass Against the Brown Planthopper, *Nilaparvata lugens* (STÅL) (Homoptera: Delphacidae)

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Barnyard grass (var. *oryzicola*) showed no apparent damage in paddy fields where hopperburn occurred by a large number of *N. lugens*. The causes of barnyard grass resistance to this insect and the insect-host interrelations of three species of planthoppers (*N. lugens*, *L. striatellus* and *S. furcifera*) between rice plant and barnyard grass were studied. Nymphs of *N. lugens* could not grow by feeding barnyard grass without regard to its leaf stage and died within a few days, whereas those grew well by feeding rice plant. On the contrary, nymphs of the other two species of planthoppers grew up normally to the adult stage even on barnyard grass as well as on rice plant. Extract of barnyard grass showed an antifeedant effect against *N. lugens* when it was added to a sucrose solution. It seems that resistance of barnyard grass to this insect is due mainly to the presence of antifeedant(s).

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (STÅL), the smaller brown planthopper, *Laodelphax striatellus* (FALLEN), and the white-back planthopper, *Sogatella furcifera* (HORVÁTH) are serious insect pests on rice plants in Asian countries. The brown planthopper often outbreaks in a large number and causes serious damage, referred to as "hopperburn", on rice plants during the ripening season. In paddy fields where hopperburn occurred, it has occasionally been observed that barnyard grass *Echinochloa crus-galli* (Linn.) Beauv. var. *oryzicola* (Vasing.) Ohwi survives without any damage. Barnyard grass is, however, known to be one of host plants for *L. striatellus* and *S. furcifera*.

The present report concerns barnyard grass resistance to *N. lugens* in relation to the insect-host interrelations of three species of planthoppers.

MATERIALS AND METHODS

Insects: Three species of planthoppers (*N. lugens*, *L. striatellus* and *S. furcifera*) collected in September, 1969, in the paddy field of the Kyushu Agricultural Experiment Station, Fukuoka Prefecture, have been reared successively in rearing bottles (480 cm³ in volume) at 25°C, 14 hr illumination in our laboratory. Rice seedlings (var. Kinmaze) served as food and also oviposition sites.

Plants: Rice plant (var. Kinmaze) and barnyard grass (var. *oryzicola*) at the 5 to 7 leaf stage were grown in a green house at 25—30°C. Barnyard grass used for extracting antifeedant(s) against *N. lugens* was collected in August, 1970 in the paddy field of

Fig. 1. Apparatus for collecting honeydew. A, seedling of each species of plants; B, absorbent cotton; C, plastic cage; D, filter paper; E, glass plate; F, polyethylene cup.

the Experimental Farm, Kyoto University, Osaka Prefecture, where pesticides applications have not been conducted.

Rearing experiments on plants: Seedlings or stem parts of each plant species, their cut ends wrapped with moistened polyurethane, were inserted into an end of a glass tube (17 cm $\times$ 1.5 cm); the open end of the glass tube was covered with cotton gauze. Ten 3rd-instar nymphs of each planthopper species were introduced into the glass tube and allowed to feed on the plant under laboratory conditions. The plant in the glass tube was renewed every other day. Survival of the insects was observed every day.

Experiments on honeydew: According to Sôgawa's procedure (Sôgawa, 1970), ten female adults of the macropterous form of each planthopper species were introduced into a plastic cage as shown in Fig. 1 and allowed to feed on the plants for 24 hr at 27°C, under continuous illumination. The amount of honeydew excreted from the insects on the filter paper was estimated by color intensity following treatment with ninhydrin reagent.

Rearing experiments on plant extracts: Five 3rd-instar nymphs of *N. lugens* were introduced into the apparatus shown in Fig. 2, and allowed to feed by sucking the test solution (0.4 ml) through stretched parafilm (American Can Company) under labo-

Fig. 2. Bioassay apparatus for feeding responses to plant extracts.

ratory conditions. The test solution was renewed every other day. In order to survey the feeding inhibitory effect of barnyard grass to *N. lugens*, the following procedure was made. To the test solution, a small amount of $^{32}\text{P}\text{-H}_3\text{PO}_4$ was added such that radioactivity was 400 cpm per μl . Ten 3rd-instar nymphs of *N. lugens* were introduced into the apparatus and allowed to suck the solution for 24 hr at 27°C , under continuous illumination. The insects were decomposed with concentrated nitric acid by heating with an infrared lamp and radioactivity was measured with a windowless gas flow counter (Aloka JDC-311 2 π).

RESULTS

Survival rate on rearing with two plants: Rearing experiments of the 3rd instar nymphs of three species of planthoppers were carried out with 10-to 15-day-old seedlings or rice plant and barnyard grass. As shown in Fig. 3, *N. lugens* reared on barnyard grass died

Fig. 3. Survival of the 3rd-instar nymphs of three species of planthoppers on each species of plant seedlings. o-o, rice plant; x-x, barnyard grass.

Fig. 4. Survival of the 3rd-instar nymphs of *N. lugens* on rice seedlings after feeding on seedlings of barnyard grass for 1-2.5 days. o-o, rice plant; x-x, barnyard grass.

Fig. 5. Honeydew excreted onto filter paper by three species of planthoppers fed on each plant species.

within 3 days. On the contrary, *L. striatellus*, and *S. furcifera* grew up normally even on barnyard grass as well as on rice plant. The same results were also obtained when the 1st-instar nymphs of each planthopper species was reared on stem parts of each plant species at the 5 to 7 leaf stage in stead of seedlings.

In order to discern the reason(s) for *N. lugens* mortality within a few days after feeding on barnyard grass, the following experiments were made. The 3rd-instar nymphs of the planthopper were reared on 10- to 15-day-old seedlings of barnyard grass. Upon termination of the given rearing periods as shown in Fig. 4, they were transferred to rice seedlings. The survival rate decreased on barnyard grass as a function of increasing the rearing period. The survivals, however, grew up normally on rice seedlings just after changing their food plant.

As shown in Fig. 5, *N. lugens* fed on rice plant excreted a large amount of honeydew, while those on barnyard grass excreted a small quantity. In the case of the other two species of planthoppers, however, no remarkable difference in the amount of honeydew was observed between feeding on rice plant and on barnyard grass.

These experimental results suggest that barnyard grass does not contain substances which are toxic to *N. lugens*, but does appear to contain some antifeedant(s) which inhibits feeding of the insect and results in death by starvation.

Extraction of antifeedant(s) from barnyard grass: The extraction was made as follows: a methanol extract of barnyard grass (4 kg, fresh weight) was dissolved in water (3 liter) and washed successively with hexane (1 liter $\times$ 3), chloroform (1 liter $\times$ 3), and then ethyl acetate (1 liter $\times$ 3). The aqueous solution was evaporated to dryness to give a brown oil (106.2 g), which was tentatively called as the water extract of barnyard grass. Rice plant was also extracted by the same procedure. Rearing experiments with a 15% sucrose solution containing the water extract of barnyard grass or rice plant at a concentration of 5% were carried out according to the procedure described above using the 3rd-instar nymphs of *N. lugens*. The results are summerized

in Fig. 6. The mortality did not increase when insects were allowed to feed on the 15% sucrose solution or on 15% sucrose plus a 5% water extract of rice plant; however, a value of 100% mortality was attained within 5 days on 15% sucrose plus a 5% water extract of barnyard grass which was similar to the case for water alone. This fact suggests that mortality observed for *N. lugens* may be attributed to an antifeedant(s) contained in the barnyard grass extract.

The presence of an antifeedant(s) in barnyard grass was more clearly confirmed by the following experiment. The 3rd instar nymphs of *N. lugens* were reared with a 15% sucrose solution containing a 5% water extract of rice plant or barnyard grass tagged with $^{32}\text{P}\text{-H}_3\text{PO}_4$. After feeding had proceeded for 24 hr, the radioactivity in whole bodies of the planthoppers was counted. Counts were high in those planthoppers which were fed on the sucrose solution containing the water extract of rice plant, while it was almost negligible in those insects fed on the sucrose solution containing the water extract of barnyard grass (Fig. 7). From the results, it is concluded that the water extract of barnyard grass contains some potent antifeedant(s) which inhibits feeding of *N. lugens* on barnyard grass.

DISCUSSION

Considerable information is available concerning the insect-plant relationship for chewing insects, but is rather limited with regard to that for plant sucking insects. There are three species of planthoppers, *N. lugens*, *L. striatellus* and *S. furcifera*, which are known to be serious pests of the rice plant by sucking the plant sap. Among them, outbreaks of *N. lugens* occasionally occurred in paddy fields during the autumn and caused severe damage to rice plants. Although the bionomics and population dynamics of this insect have been studied in relation to the forecasting of outbreaks by many investigators, few have studied relationship between insect and host plant, except for varietal resistance of rice plant to the insect.

Several varieties of barnyard grass including var. *oryzicola* have been reported as host plants of *L. striatellus* and *S. furcifera*. In the case of *N. lugens*, however, some varieties of barnyard grass were reported as one of the host plants for this insect (for instance of var. *crus-galli*, HASEGAWA, 1955, ISHIZAKI, *et al.*, 1957, NASU, *et al.*, 1965) while

Fig. 6. Effect of the water extract of each plant species on survival of the 3rd instar nymphs of *N. lugens*. S, sucrose; R, the water extract of rice plant; B, the water extract of barnyard grass.

Fig. 7. Mean ^{32}P intake of the 3rd instar nymphs of *N. lugens* from each test solution at 27°C for 24 hr. S, 15% sucrose; R, 15% sucrose plus 5% water extract of rice plant; B, 15% sucrose plus 5% water extract of barnyard grass.

KISIMOTO (1965) reported that nymphs of this insect could not develop by feeding on this variety (*crus-galli*) and died before maturation.

According to our observations in paddy fields where barnyard grass survived without any damage to outbreaks of *N. lugens* together with the results of laboratory rearing experiments described in this paper, it can be concluded that barnyard grass (var. *oryzicola*) is not available as a host plant for this insect.

Several experiments were conducted to examine the cause of resistance of barnyard grass (var. *oryzicola*) to *N. lugens* since it is curious that the plant is accepted as a host for two other species of planthoppers. From the results of the feeding experiments with rice plant and barnyard grass given in Fig. 4, and of honeydew excreted from the insects fed on these plants given in Fig. 5, it is suggested that some antifeedant(s) contained in barnyard grass may play an important role in the resistance of barnyard grass to *N. lugens*. The presence of an antifeedant(s) in barnyard grass was confirmed by feeding experiments with the water extract of rice plant or barnyard grass in which $^{32}\text{P}\text{-H}_3\text{PO}_4$ had been added. The mean ^{32}P intake of insects fed on a barnyard grass extract was almost negligible compared with those fed on a rice plant extract (Fig. 7). It was concluded that barnyard grass resistance to *N. lugens* is based mainly on the presence of some potent antifeedant(s) for the insect. Isolation and characterization of the antifeedant(s) are now in progress and the results will be presented in a subsequent paper.

In addition to the present experiments, our preliminary experiments on host preference of three species of planthoppers indicated that some attractant(s) seem to be contained in rice plant (unpublished data). The presence or absence of attractant(s) and antifeedant(s) in the plants may participate in the insect-host plant relationship of three species of planthoppers. It is most important that the chemical nature of these

substances be clarified not only to better understand the insect-host relationship but also for practical application of these substances for control of insects.

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