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Effects of Salicylic Acid on Probing and Oviposition of the Rice Plant- and Leafhoppers (Homoptera: Delphacidae and Deltocephalidae)

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Although the rice plant- and leafhoppers attempted only a superficial probing to distilled water, they probed deeply and repeatedly into the rice plant sap, consequently a number of elongated and branched stylet sheaths were produced. Salicylic acid provoked a significantly stimulated probing of *N. lugens*, but did not elicite remarkable probing responses of the other species. *N. lugens* oviposited preferably in the salicylic acid media, while only a few eggs were laid in the salicylic acid-free control medium. On the contrary, the salicylic acid media were not preferred for oviposition of *L. striatellus* to the control medium which a considerable number of eggs were deposited in. The other species laid few or no eggs in neither salicylic acid media nor the control medium.

INTRODUCTION

The rice plant- and leafhoppers belonging to Delphacidae and Deltocephalidae are known to ingest the phloem or xylem saps inserting their stylets into the vascular bundles of rice plants (SŌGAWA, 1973). It has been considered that these insects locate their sucking sites within the host plant by means of a random exploratory probing. It has been indicated that the exploratory probing of *Nilaparvata lugens* is activated by the special gustatory stimuli present in the rice plant sap; and a phenol-carboxylic acid, salicylic acid, has been demonstrated to act as a probing stimulant for this species (SŌGAWA, 1974). One of the purpose of the present investigation is to compare the probing responses of the five species of plant- and leafhoppers which feed similarly on the rice plant for distilled water, rice plant sap, and salicylic acid solutions, and to evaluate the probing stimulatory effect of salicylic acid for these insects. This paper also deals with the influence of salicylic acid on ovipository responses of these insects.

MATERIALS AND METHODS

Insects: Female adults of the species given below were used, which were taken from their stock colonies reared successively on the rice seedlings in our laboratory.

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Delphacidae

Nilaparvata lugens (STÅL), the brown planthopper

Sogatella furcifera (HORVÁTH), the white-back planthopper

Laodelphax striatellus (FALLÉN), the smaller brown planthopper

Deltocephalidae

Nephotettix cincticeps (UHLER), the green rice leafhopper

Inazuma dorsalis (MOTSCHULSKY), the zigzag-striped leafhopper

Apparatus: The probing and ovipository responses of the plant- and leafhoppers were examined using an apparatus shown in Fig. 1. It is composed of a glass ring (27 mm in inner diameter, 20 mm in height), whose top and bottom are covered with tetoron gause with a hole through which the insects are introduced and a sheet of stretched parafilm membrane respectively, and a watch glass. The glass ring is placed on the watch glass containing a test medium, so as to enclose the medium between the parafilm membrane and watch glass.

Media: The following three kinds of media were prepared for the experiments:

(1) Distilled water

(2) Rice plant sap; the crude sap obtained by pressing the aerial parts of rice plants (var. Taichung (native)-1, tillering stage) was centrifuged at 16,000 rpm for 20 min at 2°C, and its supernatant was filtrated through a collodion bag under a reduced pressure. The translucent yellowish filtrate was submitted to the experiments.

(3) Salicylic acid solution; Salicylic acid was dissolved in 2% sucrose solution at concentrations of 0.001, 0.002 and 0.004 M. As a control, 2% sucrose solution was used.

Bioassay for probing response: Since the plant- and leafhoppers produce the so-called stylet sheath in the probing media by secreting a coagulable saliva along with the stylet penetration (SŌGAWA, 1971), their probing responses could be evaluated by measuring the stylet sheaths produced. For each test, ten planthoppers or five leafhoppers were introduced in the apparatus which was confined in a plastic container with moistened filter papers to keep a high humidity, and allowed to probe the test medium through a parafilm membrane for two days at 25°C under continuous lighting. At the end of this period, the stylet sheaths deposited through the parafilm

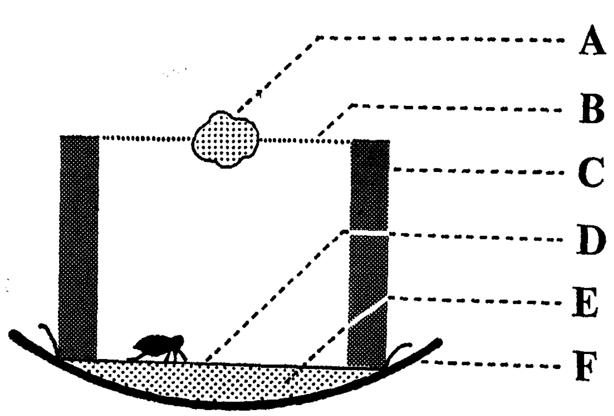

Fig. 1. Apparatus to assay the probing and ovipository responses of the female adults of the rice plant- and leafhoppers for the test media. A, cotton plug; B, tetoron gauze; C, glass ring; D, parafilm membrane; E, medium to be tested; F, watch glass.

membrane were detected by staining with 0.1% rhodamine B aqueous solution and observed microscopically. The average length and percentages of elongated and branched sheaths were recorded as parameters for evaluation of the probing responses. The average value of stylet sheath length was calculated by sampling 50 single tubular sheaths from each apparatus at random. Significant difference between them was analyzed by *t*-test after square-root transformation (SŌGAWA, 1974). The elongated sheath was defined the longer sheaths which were formed fewer than 1% in distilled water. The experiments for each medium were replicated three times.

Bioassay for ovipository response: The same apparatus and procedures as above were applied to this bioassay. Matured female adults were selected and used. The eggs laid in the test medium through a parafilm membrane were counted under a binocular microscope. The egg numbers recorded were transformed to logarithmic values in order to stabilize the variance, and then subjected to the *Q*-test (SNEDECOR and COCHRAN, 1967).

RESULTS

Stylet sheaths formed in distilled water

The stylet sheaths produced by the three species of planthopper were mostly short, 50 to 60 μ long, and were not branched as shown in Table 1. The sheaths longer than 160 μ produced by *N. lugens*, 130 μ by *S. furcifera*, and 150 μ by *L. striatellus* were recorded as low as 1%, which were termed the elongated sheath as mentioned above. The both species of leafhoppers were scarcely formed the stylet sheaths, although they tried to puncture the parafilm membrane by the stylets.

Stylet sheaths formed in rice plant sap

The planthoppers produced a large number of elongated sheaths in the rice plant sap. Their average length was nearly trebled as compared with that in distilled water. The elongated sheaths accounted for more than 50% of all sheaths detected. The percentages for the branched sheaths were ranged from 30 to 50% according to the species. The leafhoppers deposited also the stylet sheaths in this media. However, they were generally shorter, although irregularly thicker, than those of the planthoppers (Fig. 2). The branching was not so definite as that in the sheaths of the planthoppers. It seems possible that the maxillary stylets of the leafhoppers are inserted further beyond the tip of the stylet sheaths formed as have been indicated by POLLARD (1968). *N. cincticeps* tended to form shorter and branched sheaths as compared with *I. dorsalis*. The data obtained were assembled in Table 2.

Table 1. THE PROBING RESPONSES OF THE FEMALE ADULTS OF THE RICE PLANTHOPPERS FOR DISTILLED WATER

Species	Mean length of stylet sheath		Critical length of elongated sheath (μ)	Branched sheath (%)
	Measured value (μ)	Transformed value (±S.E.)		
N. lugens	61	7.56±1.91	160	1.3
S. furcifera	48	6.67±1.83	130	8.8
L. striatellus	61	7.59±1.83	150	1.9

Table 2. THE PROBING RESPONSES OF THE FEMALE ADULTS OF THE RICE PLANT- AND LEAFHOPPERS FOR THE RICE PLANT SAP

Species	Mean length of stylet sheath		Flongated sheath (%)	Branched sheath (%)
	Measured value (μ)	Transformed value (±S.E.)		
N. lugens	190	13.57±0.20	66.0	53.5
S. furcifera	147	11.86±0.21	58.7	33.1
L. striatellus	161	12.54±0.16	58.7	40.1
N. cincticeps	62	7.70±0.13	—	50.6
I. dorsalis	72	8.32±0.14	—	11.6

Table 3. THE PROBING RESPONSES OF THE FEMALE ADULTS OF THE RICE PLANT- AND LEAFHOPPERS FOR SALICYLIC ACID SOLUTIONS

Species	Concentration of salicylic acid (M)	Mean length of stylet sheath[1]		Elongated sheath (%)	Branched sheath (%)
		Measured value (μ)	Transformed value (±S.E.)		
N. lugens	0.004	102	9.71±0.23 a	17.3	27.5
	0.002	113	10.22±0.23 a	18.0	32.9
	0.001	104	9.80±0.22 a	17.3	8.2
	0	71	8.30±0.12 b	0.0	2.3
S. furcifera	0.004	77	8.32±0.22 a	13.3	16.1
	0.002	60	7.44±0.17 b	4.7	11.0
	0.001	70	8.01±0.19ab	13.3	6.3
	0	67	7.84±0.19ab	8.0	3.8
L. striatellus	0.004	90	9.06±0.23ab	19.3	27.0
	0.002	99	9.57±0.22 a	20.7	22.6
	0.001	81	8.75±0.17 b	6.7	8.2
	0	85	8.79±0.23 b	12.7	8.0
N. cincticeps	0.004	55	7.27±0.13 a	—	36.3
	0.002	51	7.00±0.11ab	—	33.8
	0.001	47	6.73±0.11 b	—	28.9
	0	56	7.36±0.11 a	—	22.5
I. dorsalis	0.004	63	7.72±0.15 a	—	27.7
	0.002	55	7.12±0.16 b	—	35.4
	0.001	48	6.70±0.15 b	—	19.0
	0	48	6.80±0.15 b	—	44.4

[1] Means followed by the same letter(s) are not significantly different at the 5% level of confidence in *t*-test.

Stylet sheaths formed in salicylic acid media

N. lugens produced significantly longer sheaths in the media containing salicylic acid than did in the control. About 20 % of the sheaths formed in the salicylic acid media were of the elongated ones, but no such sheaths appeared in the salicylic acid-free control medium. Moreover, about 30% of the sheaths deposited in 0.002 and 0.004 M salicylic acid media had branches. Generally, heavily branched sheaths

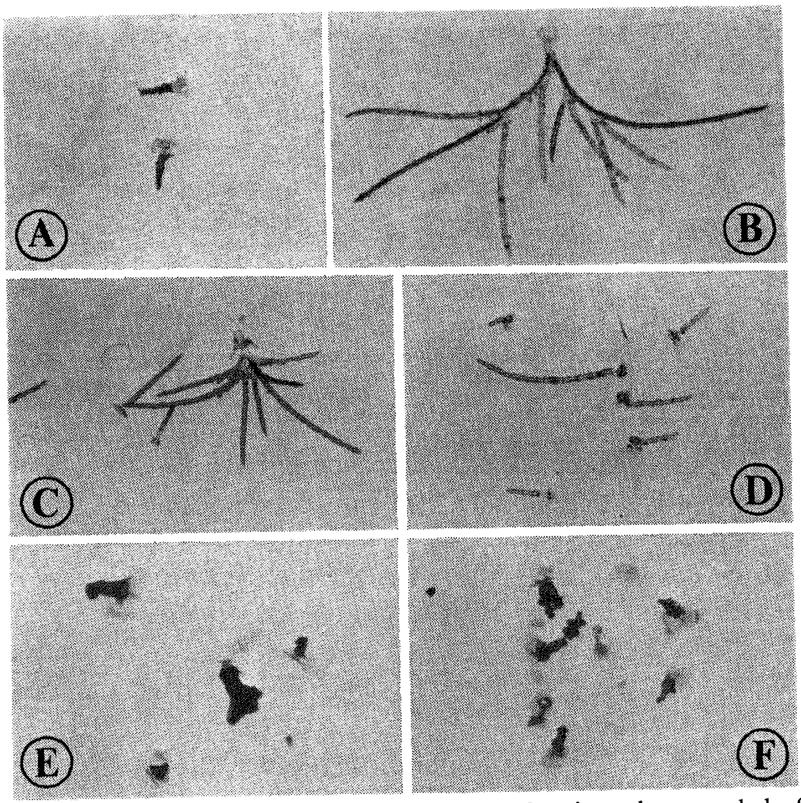

Fig. 2. The stylet sheaths (×96) produced by the rice plant- and leafhoppers. A, *N. lugens* (2% sucrose solution=control); B, *N. lugens* (0.004 M salicylic acid solution); C, *S. furcifera* (0.004 M salicylic acid solution); D, *L. striatellus* (0.004 M salicylic acid solution); E, *N. cincticeps* (0.002 M salicylic acid solution); F, *I. dorsalis* (0.002 M salicylic acid solution).

were more frequently observed in the media containing of higher concentration of salicylic acid. The most densely branched sheath had 13 branches, which was found in 0.004 M salicylic acid medium. On the other hand, no remarkable differences were recognized between the mean length of the stylet sheaths of *S. furcifera* or *L. striatellus* formed in the salicylic acid media and the control medium, excepting a strong tendency that both the planthoppers produced more frequently the branched sheaths in 0.002 and 0.004 M salicylic acid media than did in the control medium. Two species of leafhoppers gave almost identical responses against the media containing salicylic acid of different concentrations as well as the control medium. The data obtained were assembled in Table 3.

Oviposition in salicylic acid media

While *S. furcifera*, *N. cincticeps* and *I. dorsalis* laid few, if any, eggs in the test media, *N. lugens* and *L. striatellus* showed interesting ovipository responses for salicylic acid. The total number of eggs deposited by *N. lugens* increased as increase of salicylic acid concentration in the medium, whereas only a few were laid in the salicylic acid-free control medium. On the contrary, *L. striatellus* laid preferably eggs in the control medium, and a reduced number of eggs were recorded in the media containing salicylic acid. These data were shown in Table 4.

Table 4. OVIPOSITION BY *N. lugens* AND *L. striatellus* IN SALICYLIC ACID SOLUTIONS

Species	Concentration of salicylic acid (M)	No. of eggs deposited[a]					Mean	
		I	II	III	IV	V	Measured value	Transformed value
N. lugens	0.004	7	58	4	20	53	28.4	1.28550
	0.002	11	50	23	26	4	22.8	1.25946
	0.001	4	17	3	0	0	4.8	0.51126
	0	3	0	0	5	0	1.6	0.27604
	D[a]							0.84252
L. striatellus	0.004	0	11	18	17	19	13.0	0.98285
	0.002	4	3	3	27	7	8.8	0.85067
	0.001	26	27	25	25	11	22.8	1.35753
	0	21	43	77	51	15	41.4	1.55962
	D[b]							0.63928

[a] Fifteen insects were used for each replication.

[b] The least significant difference between means at 5% level of confidence in *Q*-test.

DISCUSSION

It is much difficult and time-consuming to observe the probing responses of the plant- and leafhoppers, because the probing is carried out within a dietary substrate. However, the shape and size of the stylet sheaths produced during the probing represents an available information in their probing responses. In general, short and nonbranched sheaths indicate a shallow and single thrust of the stylets. Therefore, the probing in distilled water was regarded to be of superficial or to be interrupted soon after penetration of the parafilm membrane in most cases. On the other hand, the elongated and branched sheaths are considerable to be due to deep and repeated probing. The stylet sheaths produced in the rice plant sap were mostly of this type, and were similar to those formed within the rice plant tissues. Such features of the stylet sheaths indicated the presence of certain factors which stimulate the probing in the rice plant sap. In a previous paper, it has been reported that salicylic acid, which had been isolated from the extracts of rice plants (ISHII, et al., 1962), functions as a probing stimulant for *N. lugens*, and its optimum effect has been recorded when 0.001 to 0.004 M salicylic acid was present together with 2% sucrose (SŌGAWA, 1974). This evidence was reconfirmed in this experiment. Salicylic acid did not elicite so significant probing responses of *S. furcifera* and *L. striatellus* as those of *N. lugens*, and had no effect on the probing of *N. cincticeps* and *I. dorsalis*. It is, therefore, able to concluded that salicylic acid was a specific probing stimulant for *N. lugens*. It is also undoubted that more potential probing stimulants other than salicylic acid are present in the rice plant sap, because the plant- and leafhoppers exhibited more activated probing responses for it than for the salicylic acid media.

MITSUHASHI (1970) and MITSUHASHI and KOYAMA (1975) have reported that *L. striatellus* lay a considerable number of eggs in 5 to 10% sucrose solution, while the other species of plant- and leafhoppers including *N. lugens*, *S. furcifera* and *N. cincticeps* lay only a negligible number of eggs in it. This agrees with our present results. Among the insects tested, only *L. striatellus* oviposited actively in 2% sucrose solution used as a control. The ovipository activity of this species was inhibited in 2% sucrose solution containing salicylic acid. It is of particular interest that salicylic acid stimulated not only the probing but also the oviposition of *N. lugens*. In this connection it seems valuable to refer to an evidence that the ovipository behaviours of the potato leafhopper, *Empoasca fabae*, are always initiated following the stylet probing, and the stimuli which induce the oviposition are perceived by means of the stylet probing (CARLSON and HIBBS, 1970).

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