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Studies on the Feeding Habits of the Brown Planthopper, *Nilaparvata lugens* (STÅL) (Hemiptera : Delphacidae) V. Probing Stimulatory Effect of Rice Flavonoid

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The probing stimulatory effect for feeding of *N. lugens* was found in the cases of four kinds of rice flavonoids, tricin-5-glucoside, glucotricin, orizatin and homoinetin; and out of the other 13 flavonoids, luteorin showed the effect as strong as that of the rice flavonoids.

INTRODUCTION

Among botanically restricted substances with significant roles in the feeding process of aphids, phlorizin and sinigrin were recognized for their functions as stimulant for the stylet penetration of certain aphid species (KLINGAUF, 1971; NAULT and STYER, 1972). The stylet probing of planthoppers and leafhoppers was also stimulated by chemical factors existed in their host plants (SEKIDO and SŌGAWA, 1976), and salicylic acid was found to be a probing stimulant for the brown planthopper, *Nilaparvata lugens* (SŌGAWA, 1974; SEKIDO and SŌGAWA, 1976).

In the present experiment, attempts were made to detect the probing stimulatory effect of the host species-specific flavonoid compound for feeding of *N. lugens* other than salicylic acid.

MATERIALS AND METHODS

Insect. Female adults of the brown planthopper, *Nilaparvata lugens* which were collected from the stock culture maintained on rice seedlings in the laboratory were used.

Preparation of test solution. Four kinds of flavone glycosides, tricin-5-glucoside, glucotricin, orizatin (Fig. 1–1) and homoinetin (Fig. 1–2), isolated from the rice leave, and other 13 kinds of related flavonoid compounds were submitted to bioassay for the effect of the probing stimulant dissolved in 2% sucrose solution at the given concentrations, or being saturated with the solution when their solubilities were lower than the given concentrations. Homoinetin dissolved in 20% sucrose solution containing radioactive ^{32}P-H_3PO_4 was also tested for its effectiveness on the sucking response of *N. lugens*. Sucrose solution without flavonoids was used as the control. All flavonoids tested here were supplied by Assistant Professor Dr. Shozo KUWATSUKA, Faculty of Agriculture, Nagoya University.

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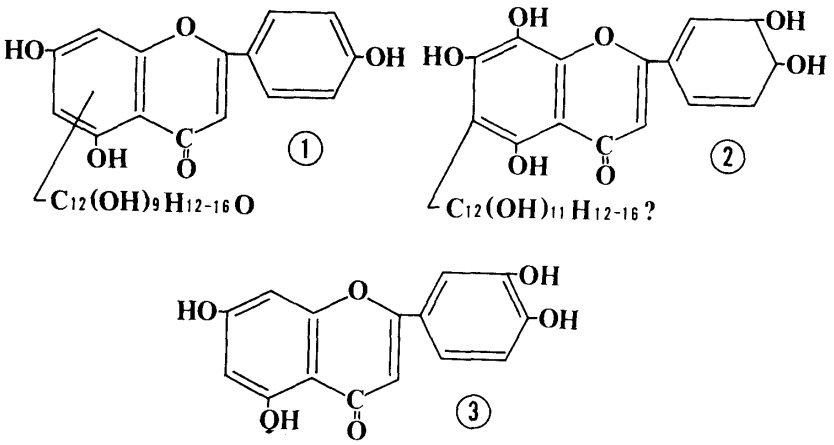

Fig. 1. Chemical structures of probing stimulatory flavonoids. 1, orizatin; 2, homoinetin; 3, luteorin.

Procedure. Probing and sucking responses of *N. lugens* for tested compounds were evaluated by means of the same methods essentially as those of SEKIDO and SŌGAWA (1976) and SAKAI and SŌGAWA (1976), respectively.

RESULTS

The probing stimulatory effects of four kinds of rice flavonoids, tricin-5-glucoside, glucotricin, orizatin and homoinetin, were examined at the concentration of 500 ppm except for tricin-5-glucoside saturated with the solution because of its low solubility. All these compounds markedly stimulated the probing response of *N. lugens* in terms of average length of stylet sheaths and percentage of elongated sheaths (120 μ or longer) and branched ones (Table 1). Length of the non-branched sheath formed in the rice flavonoid solution was in the range of 100 to 150 μ in average, while that in the flavonoid-free control one was only about 60 μ. Consequently it was recognized that percentage of the elongated sheath was recorded in the former solution. This evidence was further emphasized in the cases of branched sheath being apparently and more frequently produced in each flavonoid solution. The branched sheath was usually much longer, in the range of 150 to 200 μ in average, as compared with the non-branched sheaths produced even in the same flavonoid solution as well as in the control. These figures of stylet sheaths formed in the rice flavonoid solution clearly indicated that *N. lugens* performed repeatedly deeper stylet incertion into the solution in response to these rice falvonoids. Orizatin and homoinetin were effective as the probing stimulant at the concentration of 100 to 1000 ppm (Table 2). On the other hand, homoinetin was neutral to the sucking response of *N. lugens* at this concentration (Table 3).

Among the various flavonoids tested, only luteorin (Fig. 1–3), similar to the aglycones of oryzatin and homointin in the chemical structure, showed a probing stimulatory activity as strong as that of said rice flavonids (Table 4). Catechin, distylin, isoquercitrin, wogonin, and kaempferol were also effective, to some extent, while flavonoid glycosides, such as robinin, myricitrin, hesperidin and quercitrin, containing rhamnose moiety showed no or weak, if any, probing stimulatory function.

Table 1. PROBING RESPONSES OF *N. lugens* FOR RICE FLAVONOIDS[a]

Compound	Type of sheath	No. of sheath observed	Average length of sheath: Measured value (μ)	Average length of sheath: Transformed value[b] (±S. E.)	Elongated sheath[c] (%)	Sheath with indicated number of branch (%): 1	2	3	≥4
Tricin-5-glucoside	Non-branched	118	114	3.26±0.08 b	40	80	—	—	—
	Branched	28	191	4.27±0.17 c	79	—	10	6	3
Glucotricin[d]	Non-branched	153	124	3.42±0.07 b	41	74	—	—	—
	Branched	54	187	4.25±0.11 c	85	—	13	8	5
Orizatin[e]	Non-branched	207	151	3.83±0.05 c	73	69	—	—	—
	Branched	92	171	4.09±0.07 c	84	—	16	7	8
Homoinetin	Non-branched	90	96	3.02±0.07 b	26	49	—	—	—
	Branched	93	149	3.77±0.09bc	59	—	19	16	16
Control	Non-branched	103	58	2.36±0.05 a	3	91	—	—	—
	Branched	10	—	—	—	—	4	3	2

[a] They were dissolved in 2% sucrose solution at 500 ppm, excepting tricin-5-glucoside saturated in it because of its low solubility.

[b] The measured values were transformed to logarithmic values ($\sqrt{1/10\mu}$) in order to stabilize the variance: Values followed the same letter are not significantly different at the 0.1% level of probability.

[c] 120 μ or longer.

[d] Slightly contaminated with tricinin.

[e] Slightly contaminated with homooryzatin.

TABLE 2. PROBING STIMULATORY EFFECT OF ORIZATIN AND HOMOINETIN FOR *N. lugens* AT DIFFERENT CONCENTRATIONS

Compound	Concentration (ppm)	No. of sheath observed	Average length of sheath[a]: Measured value (μ)	Average length of sheath[a]: Transformed value[b] (±S. E.)	Elongated sheath[c] (%)	Branched sheath (%)
Orizatin[d]	1000	100	127	3.42±0.10 b	41	30
	500	84	118	3.37±0.06 b	48	57
	100	34	83	2.76±0.13 a	21	56
	50	80	77	2.70±0.06 a	15	20
	10	100	65	2.48±0.06 a	7	18
	0	100	75	2.66±0.06 a	13	6
Homoinetin[e]	5000	113	88	2.90±0.05 b	13	58
	1000	110	113	3.25±0.08 c	42	61
	500	160	108	3.22±0.04 c	34	32
	100	115	107	3.18±0.09 c	26	33
	50	128	82	2.79±0.05 b	17	8
	10	139	86	2.86±0.05 b	16	23
	0	150	57	2.33±0.03 a	3	4

[a] Average length of non-branched sheaths.

[b] See Table 1 : Values followed by the same letter are not significantly different at the 5% level.

[c,d,e] See Table 1.

Table 3. Effect of Homoinetin[a] on Sucking Response of *N. lugens*

Concentration (ppm)	Radioactivity in insect body	
	cpm/insect[b]	Ratio[c]
1000	193	1.6
500	160	1.5
100	114	1.1
50	259	2.4
10	124	1.2
0	106	1.0

[a] Dissolved in 20% sucrose solution.
[b] Average of three replications, each containing five insects.
[c] Ratio against control (0 ppm).

Table 4. Probing Responses of *N. lugens* for Various Flavonoids

Compund	No. of sheath observed	Average length of non-branched sheath (μ)	Elongated sheath (%)	Branched sheath[c] (%)	Chemical structure
Luteorin[a]	71	162	72	25	3', 4', 5, 7-OH
Catechin[b]	191	116	41	7	3, 3', 4', 5, 7-OH
Distylin[b]	121	112	38	13	3, 3', 4', 5, 7-OH
Wogonin[a]	66	104	29	17	5, 7-OH, 8-OCH_3
Kaempferol[a]	80	102	28	8	3, 4', 5', 7-OH
Myricetin[a]	55	98	23	15	3, 3', 4', 5, 5', 7-OH
Fustin[b]	149	95	19	34	3, 3', 4', 7-OH
Isoquercitrin[a]	125	112	34	7	Quercetin-3-glucoside
Lutin[a]	55	94	26	22	Quercetin-3-rutinoside
Quercitrin[a]	144	86	18	3	Quercetin-3-rhamnoside
Hesperidin[a]	68	75	15	12	Hesperetin-7-rhamnoglucoside
Myricitrin[a]	45	68	2	16	Myricetin-3-rhamnoside
Robinin[b]	97	51	1	6	Kaempferol-7-rhamnoside-3-galactrhamnoside
Control	127	68	10	6	

[a] Saturated. [b] 500 ppm. [c] see Table 1.

DISCUSSION

Kuwatsuka (1962) has investigated the constituent flavonoid in the rice plant, and disclosed the chemical structure of the flavone glycosids used in the present experiment. These characteristic flavonoids were found to have stimulatory effects on the probing of *N. lugens* at the concentration of 0.01 to 0.1%. As the total flavonoid content in the rice plant is about 0.1 to 0.3% (Kuwatsuka, 1962), it is quite possible that the flavonoid acts as natural probing stimulant for feeding of *N. lugens*.

Similar roles of secondary plant substances for aphid feeding have been reported by Klingauf (1971) and Nault and Styer (1972), as well. Phlorizin, a phenol glucoside existed on the apple genus *Malus*, has been indicated to have a positive effect on probing behavior of *Rhopalosiphum insertum* and *Aphis pomi* at the concentration of 0.05% (Klingauf, 1971). In the above experiments, the effect of phlorizin on the aphid

probing has been evaluated, based on the frequency and duration of probes, however, it should be noticed that those behavioral responses were seemingly influenced not only by the probing stimulants but also the sucking ones. The depth of probes seems to be more skillful criteria for the probing responses. NAULT and STYER (1972) have demonstrated more clearly that sinigrin, a mustard oil glucoside restricted to *Cruciferae*, stimulates the phloem seeking probe fo *Hyadaphis erysimi*. This is based on the evidence that the aphid produced many long stylet sheath (12 μ or longer) in 1% sinigrin solution five times or more in comparison with the control.

It has been generally pointed out that those probing stimulants for aphids and salicylic acid which has previouly reported as a probing stimulant for feeding of *N. lugens* show a neutral or even inhibitory effect on the sucking response of the insect (MOON, 1967; WEARING, 1968; MONTGOMERY and ARN, 1974; SŌGAWA, 1974). Likewise, homoinetin, one of the probing stimulatory flavonoid did not induce any significant sucking response in case of *N. lugens*. This seems to support an idea that the highly localized feeding manner of the planthoppers and leafhoppers as well as aphids is mediated principally by two independent sets of appropriate gustatory stimuli, *i.e.*, the probing stimulant and the sucking one (SŌGAWA, 1973).

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