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A feeding test to identify rice varieties resistant to the leaf folder, *Cnaphalocrocis medinalis* (Guenee)

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Abstract. Dynamics of leaf feeding by the larvae of rice leaf folder *Cnaphalocrocis medinalis* (Guenee) was studied under greenhouse conditions to develop a feeding test to identify varietal resistance. Area of leaf damaged by larvae during their development indicated that first 3 instars accounted for only 8·06% and V instar alone for 66·3% of the total feeding. In view of the highest feeding rate and the longest feeding duration, V instar was chosen for feeding test. No significant differences in area of leaf damaged were recorded when V instar larvae fed for 48 h on 30, 45 and 60 days old plants or when leaf nitrogen content varied from 2–3·4%. Inherent variability among individual larvae in feeding rate could be maintained within acceptable limits with 5 replications.

The proposed feeding test involved caging of individual V instar larvae for 48 h on 30–45 days old plants of test varieties and recording area of leaf damage. The test revealed varietal differences in area of leaf damaged by the leaf folder and displayed consistency over time. Based on feeding test, 19 rice varieties have been identified as resistant against leaf folder.

Keywords. Rice leaf folder; feeding test; varietal resistance; *Cnaphalocrocis medinalis.*

1. Introduction

The rice leaf folder, *Cnaphalocrocis medinalis* (Guenee), is an important pest in almost all of the rice growing countries of Asia. Pest outbreaks and consequent damage to the crop have been reported from Japan (Wada *et al* 1980), Nepal (Pradhan and Shahi 1983) and other countries (Heinrichs *et al* 1985). In India, severe damage due to this pest has been recorded from Maharashtra (Dorge *et al* 1971), Madhya Pradesh (Gargav *et al* 1971), Orissa (Yadava *et al* 1972), Gujarat (Upadhyay *et al* 1975), West Bengal (Chatterjee 1979), Uttar Pradesh (Verma *et al* 1979) and Haryana (Ram 1986).

Economic injury to the crop is related to the extent of leaf damage and period during crop growth when the damage is inflicted (Bautista *et al* 1984; Miyashita 1985). Varietal differences in the extent of damage by the leaf folder have been extensively noted based mainly on the natural incidence and leaf damage, as feeding is principally restricted to green mesophyll tissue (Fraenkel *et al* 1981). Non-uniform pest pressure and unpredictable buildup limit field evaluation as a reliable technique to assist host-plant resistance breeding programmes. As an alternative, greenhouse screening of rice varieties has been suggested by Yadava *et al* (1972), Waldbauer and Marciano (1979) and Heinrichs *et al* (1985). These methods, generally require prolonged rearing of the pest on test entries and recording insect survival and leaf damage. On the other hand, Das and Nair (1974) outlined a simple method of directly recording area of leaf feeding by individual larvae. In the

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present studies, we have attempted to validate this approach by studying dynamics of leaf feeding by *C. medinalis* larvae on selected test varieties and propose a feeding test to rapidly identify resistant varieties in the greenhouse.

2. Materials and methods

2.1 *Rearing of the insect*

The rice leaf folder, *C. medinalis*, is being reared in the greenhouse at the Directorate of Rice Research since 1979. Larvae were reared on rice variety, TN 1 (susceptible) while adults were provided with 10% sucrose solution. Eggs were collected by caging 10–15 pairs of adults on 20–25 days old potted rice plants. Temperatures in the greenhouse ranged from 20–36°C and humidity 50–85% RH. Once in a year, field collected adults were mixed with the greenhouse reared insects to avoid inbreeding.

2.2 *Measurement of larval feeding*

Strips of leaves with black head stage eggs were kept in Petri-dishes containing wet cotton wool. Neonate larvae (25) from these were caged individually at 1 larva/plant on 30 days old plants. Larval survival and moultings were noted. Larvae were transferred daily to new plants. Width of head capsule in each instar was measured with an occular micrometre under a 30X binocular microscope. Area of leaf damaged by the larva was measured by holding the leaf against mm^2 graph paper and counting the mm squares visible through the translucent scraped area when held against light. Means were computed to determine the amount of feeding on successive days and during specific larval instars.

2.3 *Factors influencing leaf feeding*

2.3a *Plant age*: Plastic pots containing 30, 45 and 60 days old plants of TN 1 were selected for this study. Fifth instar larvae were individually caged on these plants for 48 h. Area of leaf damage was measured as described earlier. Replications for each age group were 7 or 8.

2.3b *Level of nitrogen in leaf*: Potted TN 1 plants were raised with and without nitrogen fertilization. In nitrogen applied pots urea top dressing was given at 100 kg N/ha in two equal splits on 5 and 20 days after sowing. For each level of fertilizer application, 5 pots each containing ten 30 days old plants were used. Fifth instar larvae were individually caged on each set of plants for 48 h. Prior to release, only mother tillers of 5 plants per pot were retained by cutting off other tillers. Second and third leaves from the top of the primary tillers of the remaining 5 plants were pooled for estimating nitrogen after oven drying. While 13–17 replications were maintained for estimating leaf damage by larvae, 3 replications were kept for nitrogen estimation by micro kjeldahl digestion method.

2.4 *Variability in leaf feeding*

Several sets of observations over a period of 24 months, each with varying number of replications were considered to note the variability in amount of leaf feeding in different batches of V instar larvae. Each observation consisted of caging one larva on 30–45 days old TN 1 plants for 48 h and recording leaf area damaged. Variability was expressed in terms of SE as per the formula:

$$\text{Variability index} = \frac{\text{SE}}{\text{Mean}} \times 100.$$

Based on this index, the optimum number of replications was determined.

2.5 *Varietal evaluation*

On the basis of results obtained in the above investigations, rice varieties were evaluated by caging V instar larvae individually on 30–45 days old plants of test varieties for 48 h. Damaged leaf area was measured at the end of this period as described. Varieties were screened in sets of 5–10 entries along with the susceptible check TN 1. After initial sets of results, PTB 12 was used as resistant check. Four to ten replications were maintained. Average values of leaf area damaged in test entries were statistically compared with that for susceptible check variety in the corresponding set. After several sets of screening, consistency in values against susceptible and resistant checks was tested.

3. Results

3.1 *Measurement of larval feeding*

Area of leaf scraped by larvae on each successive day is presented in figure 1. While 10 of the initial 25 larvae survived till pupation, active feeding was observed up to

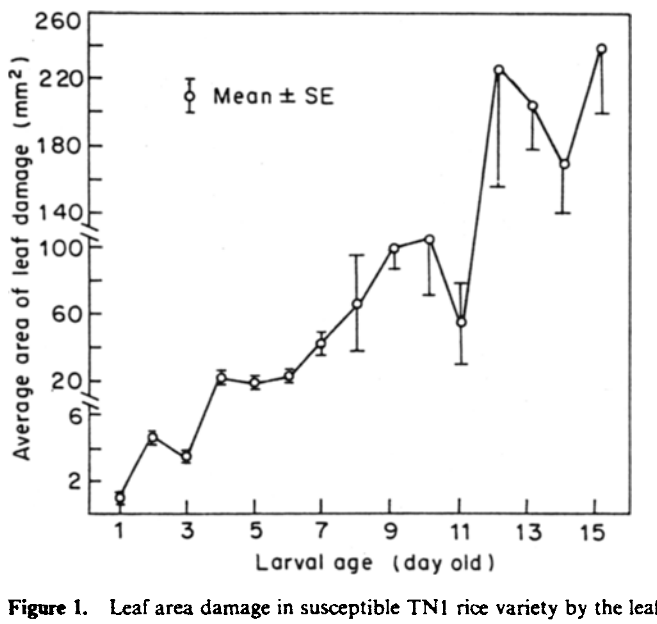

Figure 1. Leaf area damage in susceptible TN1 rice variety by the leaf folder, *C. medinalis* larvae.

15 days. All larvae underwent 5 instars. Area of leaf damaged by each of the 5 instars, duration of larval instars along with width of the head capsule is given in table 1. It is evident that 3 initial larval instars accounted for only 8·06% of the total area of the leaf fed, while V instar alone contributed to 66·3% of the total feeding. Width of the head capsule in each instar registered distinct difference and instars could be visually identified. It is thus logical to expect any differences in the amount of feeding by larvae to be clearly manifested when V instar larvae are involved.

In another study, neonate larvae during a period of 5 days did 4·2% of total feeding, while 10 days old larvae accounted for 69·7% (table 2). These results generally agreed with the earlier study since 10 days old larvae tended to be in V instar and these instars fed most voraciously. Based on these experiments only V instar larvae were used in the subsequent studies.

3.2 *Factors influencing leaf feeding*

3.2a *Plant age*: Leaf area damaged by V instar larvae in 48 h when caged on 30, 45 and 60 days old TN 1 plants is given in table 3. Though slightly higher amount of feeding was noted on 30 days old plants, no significant differences were evident with respect to plant age in the range tested.

3.2b *Level of nitrogen in leaf*: Leaf feeding by V instar larvae on TN 1 plants raised under two different levels of nitrogen fertilizer is presented in table 4. Though level of nitrogen in leaf in these two sets of pots was significantly different from each other, leaf area damaged by the larvae did not differ significantly.

Table 1. Width of head capsule, duration and leaf area scraped by different instars of *C. medinalis*.

Larval instar	Width of head capsule (mm) Mean ± SE	Larval duration (days) Mean ± SE	Leaf area scraped (mm^2) Mean ± SE	Average leaf area (mm^2) scraped/ day	Percentage of total area scraped
I	0·23 ± 0·001 (10)	2·29 ± 0·18	7·06 ± 1·24 (23)	3·08	0·59
II	0·36 ± 0·002 (15)	2·14 ± 0·14	30·11 ± 4·96 (18)	14·07	2·50
III	0·53 ± 0·006 (11)	2·20 ± 0·20	59·44 ± 10·82 (15)	27·01	4·97
IV	0·81 ± 0·007 (10)	3·75 ± 0·25	306·00 ± 68·30 (13)	81·60	25·60
V	1·06 ± 0·013 (10)	5·25 ± 0·25	793·00 ± 123·80 (11)	151·05	66·32
Total	—	15·00 ± 0·40	1195·6	—	100

Numbers in parentheses are replications.

Table 2. Leaf feeding by different aged larvae of *C. medinalis*.

Age of larvae	Leaf area scraped in 5 days (mm^2) Mean ± SE	Total feeding (%)
Neonate	46·9 ± 7·5 (10)	4·2
5 days old	449·8 ± 28·7 (10)	26·1
10 days old	807·0 ± 63·4 (10)	69·7

Numbers in parentheses are replications.

Table 3. Influence of plant age on leaf feeding by V instar *C. medinalis* larvae.

Plant age	No. of replications	Leaf area scraped in 48 h (mm^2) Mean ± SE
30 days old	7	298·8 ± 35·7^a
45 days old	8	276·0 ± 21·5
60 days old	7	211·3 ± 25·8^b

Comparison of means (*t* test). *a–b*, Not significant.

Table 4. Influence of level of nitrogen in leaf on feeding by V instar *C. medinalis* larvae.

Treatment	Level of nitrogen in leaf (% dry wt.) Mean ± SE	Leaf area scraped in 48 h (mm^2) Mean ± SE
Low nitrogen*	2·00 ± 0·17^a(3)	242·2 ± 30·5^c(13)
High nitrogen**	3·40 ± 0·06^b(3)	254·5 ± 28·5^d(17)

Comparison of means (*t* test): *a–b*, $P < 0·01$; *c–d*, Not significant; Numbers in parentheses are replications.
*Plants raised in puddled soil with no application of N fertiliser.
**Plants raised with two top dressings of N at 50 kg N/ha on 5th and 20th day after sowing.

3.3 *Variability in leaf feeding*

Data of several sets of observations with 4–12 replications indicated leaf feeding variability index to be negatively correlated with the number of replications ($r = -0·959$). It could be computed from this regression that 5 replications would result in 11·4% variability, whereas in practice sets of 5 replications produced variability indices in the range of 10–12 which can be considered as fairly acceptable.

3.4 *Varietal evaluation*

A total of 100 rice germ plasm accessions were evaluated on the basis of leaf area fed by the V instar larvae in 48 h of caging. The leaf damage in test varieties (table 5) ranged from 70·8 ± 6·2 mm^2 (Choorapundy) to 402·9 ± 25·7 mm^2 (Company Chittari). Statistical comparisons within given set of testing (*t* test) indicated all the varieties with leaf damage less than 200 mm^2 to generally differ significantly from the susceptible check TN 1.

Consistency in area of leaf damage in susceptible and resistant checks in different sets of evaluation is presented in table 6. Though mean values for each check variety varied slightly among the different sets of evaluations, these values did not differ significantly. Thus, other environmental factors like temperature, humidity, photoperiod etc. could not have drastically affected the feeding by the V instar

Table 5. Performance of selected rice varieties against the leaf folder damage in greenhouse*.

Variety	Leaf area damage (mm^2) Mean ± SE
Choorapundy	70·8 ± 6·2
Gorsa	94·6 ± 17·2
Ptb 33	113·8 ± 22·2
Ptb 19	118·3 ± 10·2
T 10	125·0 ± 16·1
ARC 10660	129·4 ± 11·6
RP 2068-18-4-5	138·6 ± 25·6
Kula Peruvela	148·2 ± 16·1
Balam	151·2 ± 26·7
Ptb 12	160·9 ± 33·3
Chempan	163·5 ± 12·3
IET 5742	167·4 ± 21·6
W 1263	167·5 ± 12·4
Chemban	170·4 ± 21·3
Co 29	172·0 ± 40·1
Darukasail	181·8 ± 35·6
ARC 7064	187·0 ± 17·6
MO I	189·6 ± 25·4
T 1406	191·8 ± 26·6
IET 5741	268·3 ± 22·2
ARC 6605	280·3 ± 28·3
Badhsabhog	310·8 ± 35·1
ARC 10744	323·2 ± 26·6
ARC 10840	360·8 ± 40·9
Company Chittary	402·9 ± 25·7
TN 1 (susceptible check)	330·0 ± 26·7

*No. of replications varied from 4 to 10.

Table 6. Leaf area damage recorded in susceptible and resistant varieties by V instar *C. medinalis* larvae in different sets of feeding tests.

			Leaf area damage (mm^2) in	
			TN 1 (susceptible check)	PTB 12 (resistant check)
Set	Month	Year	Mean ± SE	Mean ± SE
1.	March	1985	299·8 ± 14·4^a (5)	—
2.	August	1985	341·5 ± 34·0 (8)	—
3.	April	1986	358·7 ± 27·6^b (9)	207·0 ± 27·9^c (5)
4.	August	1986	330·0 ± 26·7 (8)	152·0 ± 29·7 (9)
5.	April	1987	316·8 ± 30·0 (12)	116·3 ± 27·1^d (9)
6.	May	1987	315·0 ± 32·9 (7)	170·0 ± 34·0 (6)
	Average		326·9	161·3

Numbers in parentheses are replications.
Comparison of means: Resistant check differed significantly from susceptible check in each set of testing ($P < 0·05$).
a–b, *c–d*, Not significant (*t* test).

larvae during the brief period of test. Based on area of leaf damage, either in terms of absolute values or more reliably on relative terms with reference to the susceptible check variety, the test varieties can be rated for their resistance to the leaf folder.

4. Discussion

Leaf feeding in different larval instars noted in this study indicated highest amount and rate of feeding by V instar larvae. All the larvae observed underwent 5 instars prior to pupation. Though 6 instars were exceptionally observed in this insect by Rajamma and Das (1969) and Vyas *et al* (1981), other workers have reported 5 instars (Lingappa 1972; Velusamy and Subramanian 1974; Wada 1979).

Though leaf feeding gradually increased in amount as larvae developed, daily fluctuations were noted when feeding was monitored continuously (figure 1). Decline in feeding prior to moulting and its subsequent increase may have caused such fluctuations. Thus, the inter-moult period would give higher consistency in feeding rate. In view of the highest feeding rate (151·1 mm^2/day) and the longest feeding duration (5·25 ± 0·25 days) V instar larvae were considered most suitable for the feeding test. Larval feeding for 48 h duration was taken as optimal as it allowed sufficient time for larvae to settle down and feed actively while not prolonged enough for attaining prepupal quiescence.

In studies to note factors influencing leaf feeding, V instar larvae recorded no significant differences in area of damage while feeding on 30, 45 and 60 days old TN 1 plants. Nevertheless, there was a trend of decreased feeding on older plants. Younger host plants appeared to be more suitable for this insect as larvae reared on rice leaves at ripening stage showed prolonged development (Wada and Kobayashi 1980) and underwent 6 larval instars (Wada 1979). However, the exposure period in the present study being brief in terms of total larval duration, variations in amount of feeding were not significant. This fact would allow greater flexibility in adopting the feeding test because it would permit 3 to 4 weeks time to carry out the evaluation. Plants younger than 30 days old age presented practical problem in measuring the area of leaf damage. Either V instar larvae totally fed on the leaves without leaving even the epidermis and vascular bundles or damaged leaves succumbed and wilted before measurements could be made.

The feeding test with V instar larvae on TN 1 variety plants raised at two different levels of nitrogen fertilization also did not indicate significant differences in amount of damage. However, leaf nitrogen level in these two batches of plants differed significantly. Many reports are available indicating higher buildup of leaf folder in fields receiving higher levels of nitrogenous fertilizer (see Upadhyaya *et al* 1981). This would imply greater amount of feeding by larvae on plants with greater leaf nitrogen content. Probably, the brief exposure period again might have limited the full expression of such effects in this feeding test.

Besides host plant age and leaf nitrogen content, ambient temperature is most likely to influence feeding by larvae. No separate studies were made at different constant temperatures as the feeding test was contemplated to be used to evaluate a set of varieties at one time and only the relative differences with the check variety are to be considered. Nevertheless, several sets of evaluations conducted during different months of the year with varying maximum and minimum temperatures did show relative consistency with reference to the check varieties (table 6).

Inherent variability among individual V instar larvae in feeding rate was acceptable with variability index in the range of 10–15 with only 5 replications. Though 10 replications would be ideal, with 5 replications more test varieties can be handled.

It is important to note that the proposed feeding test which was insensitive to plant age and leaf nitrogen level could bring out varietal differences distinctly. Area of leaf damage among test varieties in the feeding test encompassed a wide range. These values showed statistically significant differences among the test and check varieties and were also fairly consistent over a period of 24 months. PTB 12, identified as resistant check in this study also performed well against the pest in field evaluation at different test locations in India under the coordinated programme (DRR 1986). Further, 4 of the varieties viz. Choorapundy, Gorsa, Darukasail and Balam with damage scores 1 and 3 in greenhouse evaluation at IRRI (Heinrichs *et al* 1985) also registered low feeding damage in our test. We thus propose the larval feeding test as an alternative method of greenhouse evaluation of varieties against leaf folder *C. medinalis*.

Based on values obtained in the present screening of varieties, entries could be grouped as those displaying less than 100 mm^2 damage, 100–150, 150–200 and above 200 mm^2 damage. Only the latter group of varieties, in general, did not significantly differ from the susceptible check TN 1 in set-wise comparisons. However, we do not propose to use the absolute values of leaf damage in rating varieties as such values are not universally constant. However relative basis of damage can be considered to score varieties. The principle of feeding test proposed can also be adopted with suitable modifications for evaluation of varieties against other leaf scraping rice pests like rice hispa, *Dicladispa armigera* (Oliv.) and caseworm *Nymphula depunctalis* (Guenee).

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