JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

ORIGINAL CONTRIBUTION

The effect of leaffolder *Cnaphalocrocis medinalis* (Guenee) [Lepidoptera: Pyralidae] injury on the plant physiology and yield loss in rice

Ch. Padmavathi[1], G. Katti[1], A. P. Padmakumari[1], S. R. Voleti[2] & L. V. Subba Rao[3]

1 Entomology Division, Directorate of Rice Research, Hyderabad, Andhra Pradesh, India
2 Plant Physiology Division, Directorate of Rice Research, Hyderabad, Andhra Pradesh, India
3 Plant Breeding Division, Directorate of Rice Research, Hyderabad, Andhra Pradesh, India

Keywords
chlorophyll, flag leaf damage, leaffolder, photosystem II, physiological parameters, yield parameters

Correspondence
Chintalapati Padmavathi (corresponding author), Entomology Division, Directorate of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh 500 030, India. E-mail: chintalapatipadmavathi68@gmail.com

Received: March 20, 2012; accepted: May 24, 2012.

doi: 10.1111/j.1439-0418.2012.01741.x

Abstract

Influence of leaffolder feeding on chlorophyll, PS II activity and plant–water relations, effect of larval density on leaf damage and time course studies on larval feeding behaviour on altered physiological changes in TN1 rice culture were studied. Quantification of yield losses in the field caused by leaffolder was also assessed. Leaffolder damage resulted in 57% reduction in chlorophyll content, 23% reduction in PS II activity and 23% reduction in relative water content in comparison with control. Rice leaffolder larva folds the leaf and scrapes the green tissue from within the fold resulting in scorching and drying of the leaves. Larval density had differentially influenced effective leaf area of rice crop. Larval densities of more than 3 larvae per hill at maximum tillering stage resulted up to 20% unfilled grains, 28–57% reduction in PS II activity and 23% reduction in relative water content in comparison with the control. At flowering stage, flag leaf area damage of above 25% resulted in more than 50% unfilled grains over control, indicating direct effect of yield reduction in rice. Thus, a cumulative effect of loss in chlorophyll, reduced photosynthate availability and altered water relations caused by the leaffolder injury to flag leaf lead to greater yield loss in rice.

Introduction

Leaffolder is widely distributed in the rice-growing tracts of 29 humid tropical and temperate countries in Asia, Oceania, Australia and Africa between 48°N and 24°S latitude and 0°E to 172°W longitude (Khan et al. 1988). Once considered as a minor pest, it has now attained a major pest status with the spread of high-yielding rice varieties, continuous availability of rice crop in double cropping areas (Loevinsohn et al. 1993) and the accompanying changes in cultural practices like increased application of fertilizers and use of broad-spectrum insecticides (Bautista et al. 1984).

Colonization of leaffolder occurs throughout the rice crop, but infestations are generally greater in the reproductive and ripening stages (Litsinger et al. 2006). The larva folds the leaves longitudinally by stitching the leaf margins and feeds by scraping the green mesophyll tissue from within the folded leaves. This feeding causes linear pale white stripe damage resulting in membranous patches. First and early second-instar larvae are gregarious in nature. Late second-instar onwards, larva folds the leaves and becomes solitary. Each larva is capable of destroying several leaves by its feeding during the development. Because of many folded leaves, heavily infested fields appear scorched. *C. medinalis* takes about

24 days for the complete development from egg to adult passing through five larval instars (Padmavathi et al. 2006). Because of the leaffolder feeding, the general vigour and photosynthetic ability of an infested rice plant become greatly reduced. The infested plants are also predisposed to bacterial and fungal infection (Bashir et al. 2004). Leaffolder injury also induces rice sheath rot, difficulty in heading and massive yield loss at the reproductive stage (Xiao 1990).

Yield losses because of leaffolders ranging from 63% to 80% were reported in rice, with high-yielding or hybrid rice varieties being more susceptible (Teng et al. 1993). In India, among the four species of leaffolders reported, *C. medinalis* emerged as the major and regular pest of rice (Padmavathi et al. 2006). Murugesan and Chellaiah (1983) reported that approximately 10% flag leaf damage (FLD) by leaffolder reduced grain yields by 0.13 g per tiller and the number of fully filled grains by 4.5% in a glasshouse study, while in simulation studies, yields were not affected (Heong et al. 1998). Shanmugam et al. (2006) identified leaffolder as one of the most serious productivity constraints responsible for yield gap of rice in Tamil Nadu, India, accounting for ~11.18% losses. However, in field, larval stage and density as well as stage of the crop are the major factors that determine the quantum of larval feeding and yield reduction (Heong 1990). Although a number of studies reported the losses caused by leaffolder, effect of its feeding on the physiological status of leaf was not studied in detail. Therefore, field and greenhouse studies were undertaken to study the effects of leaffolder infestation on some of the physiological parameters related to photosynthesis and also to establish the relationship between FLD and grain yield in rice. This information is also useful for developing damage thresholds at flowering stage for integrated management of this pest.

Materials and Methods

A total of four experiments were conducted during wet (*Kharif*) and dry (*Rabi*) seasons in 2006 and 2007.

Test insects

Leaffolder *C. medinalis* colony was maintained on Taichung Native 1 (TN 1) plants under greenhouse conditions (25 ± 5°C temperature and 60 ± 10% relative humidity) at Directorate of Rice Research, Rajendranagar, Hyderabad. Ten pairs of adults were released separately for oviposition on 20- to 25-day-old TN 1 plants covered with cylindrical mylar cage (45 cm height and 14 cm diameter). Recommended fertilizer dose of 100 kg N, 80 kg P_2O_5 and 60 kg K_2O/ha was calculated per pot basis taking soil weight into account, and TN 1 plants were grown. Honey solution (20%) was provided as a source of food for adults. After pre-oviposition period of 3 days, egg laying was observed. The potted TN 1 plants with eggs were separated and kept for further development. Fresh plants were provided as and when necessary for the transfer of larvae. Neonate and third-instar larvae used in the present experiments were taken from this stock culture.

Influence of leaffolder on chlorophyll, PS II activity and water relations

In an attempt to understand the physiological response of rice flag leaves to leaffolder feeding, experiments were conducted in the greenhouse on Taichung Native 1 (TN1) plants grown in clay pots (26 cm in diameter and 28 cm in height) at 25 ± 5°C temperature and 60 ± 10% relative humidity. In each pot, only 10 tillers were maintained. Once the plants attained flag leaf stage, five flag leaves (50% tillers) were infested by restricting a single third-instar leaffolder larva per flag leaf using a thin-perforated polyethylene sheet, while remaining five flag leaves without larva served as control. Five flag leaves from each pot constituted one replication, and there were four such replications. After larval pupation (7–8 days of feeding), visual flag leaf damage (FLD) was scored as per cent area fed by the larva. The whole flag leaf area of TN 1 plants was considered as 100%, and if one-fourth area was damaged, it was considered as 25%, and if half the flag leaf area was damaged, it was considered as 50% and so on. Observations were recorded on physiological parameters such as chlorophyll content (SPAD 502 meter; Minolta, Tokyo, Japan), photosystem II (PS II) activity of dark-adapted leaves by Chlorophyll Fluorimeter (ADC FM 1500, Herts, UK), (Ranganathan et al. 2006) and relative water content (Barrs and Weatherly 1962). SPAD meter readings were taken from three places on a leaf, that is, top, middle and bottom portions, and mean was taken as one reading. For the estimation of osmotic potential, leaf samples from control and infested leaves were freeze-dried and stored at −20°C. Prior to measurement, samples were thawed, cell sap was extracted using a plastic syringe. Vapour pressure osmometer (Vapro 5520, Wescor, Logan, UT) was used to

measure the osmotic potential of the extracted cell sap (Borras et al. 2003; Nageswara Rao 2010).

Effect of larval density on leaf damage and yield

In another pot experiment, in order to study the effect of larval density on leaf damage and yield, TN 1 plants grown in pots as in experiment 1 were used at the maximum tillering stage. Neonate leaffolder larvae were released at varying levels, that is, 0, 1, 3 and 5 larvae in each pot and covered with polyethylene (mylar) cage. There were four replications for each treatment. Larvae were allowed to feed and pupate, and after 14 days, pupae were collected for observations on adult emergence in the laboratory. Observations on damaged leaves were taken at 7 days and 15 days after the release of larvae. Data on grain yield, total number of grains and unfilled grains were recorded at maturity.

Time course studies on larval feeding behaviour on chlorophyll and PS II activity

In this experiment, six treatments were included in which third-instar larva was restricted to feed on the flag leaf for varying periods of time in mud pots having 10 tillers. Treatments were imposed at the booting stage. The treatments included T1 = third-instar larva restricted to feed on flag leaf for 24 h; T2 = third-instar larva restricted to feed on flag leaf for 48 h; T3 = third-instar larva restricted to feed on flag leaf till pupation; T4 = full flag leaf removed; T5 = half flag leaf removed; T6 = control. Each pot represented one replication, and there were five replications per each treatment. Flag leaf damage was scored as per cent area fed by the larva. SPAD readings and PS II activity were recorded in the flag leaf and penultimate leaf (just below the flag leaf) in each tiller of all the treatments.

Quantification of yield losses caused by leaffolder - seasonal variation

A field experiment was carried out in 270-m^2 area during both wet (*kharif*) and dry (*Rabi*) seasons in 2006 and 2007, with TN 1 plant as test variety. Recommended fertilizer dose of 100 kg N, 80 kg P_2O_5 and 60 Kg K_2O was applied in the field. Total P_2O_5 and K_2O and 50% N were applied as basal, while remaining 50% N was applied in two split doses, at maximum tillering and panicle-initiation stages. The field area was divided longitudinally into three equal plots with channels in between, and each plot was considered as a replication. The natural incidence of leaffolder was high in the field. Fifty damaged hills (each hill consists of 8–10 tillers) were tagged at flowering stage in each replication. From the marked hills, panicles along with the corresponding flag leaves were harvested individually. The damaged area of the flag leaf of each panicle was quantified in all the samples. On the basis of the FLD levels, panicles were grouped into five categories, *viz.* control/healthy, <25%, 26–50%, 51–75% and >75% FLD. Data on various yield components such as grain yield per tiller, total number of grains per panicle, number of filled and unfilled grains were recorded.

Data analysis

T-test was performed between damaged and control leaves in the first experiment for various physiological parameters. One-way ANOVA was conducted with larval densities and feeding periods as treatments for all the variables, and mean separation procedures were conducted when F-tests were significant ($P \leq 0.05/0.0001$) by using Fisher's least significant difference (LSD) procedures. Repeated-measure analysis was conducted for the data on the number of damaged leaves at 7 days and 15 days using SAS system, version 9.2 in the experiment on the effect of larval density on leaf damage. Covariate analysis was conducted to know the effect of the season on leaffolder damage and in turn on grain yield. Correlations and simple linear regressions were performed with FLD as independent variable and yield components as dependent variables using SAS, version 9.2 (SAS Institute Inc., 2008).

Results

Influence of leaffolder on chlorophyll, PS II activity and water relations

Flag leaf area damage varied from 0% to 80% depending on the activity of feeding by the larvae. There was a general decline in chlorophyll content with an increase in damage. As a result of leaffolder damage, there was a significant reduction in SPAD values in damaged leaves (13.92) compared to control (32.76), indicating 57% reduction in chlorophyll content (table 1). A significant negative correlation ($r = -0.766$, $P \leq 0.0003$) was observed between per cent flag leaf area damage and chlorophyll content. Similarly, the Fv/Fm values were also high in control (0.7251) than in damaged

Table 1 Flag leaf–water relations and chlorophyll as affected by leaffolder, *C. medinalis* damage

Parameter	Control leaves	Damaged leaves	d.f.	t-stat	P
Chlorophyll (SPAD values)	32.76 ± 3.55	13.92 ± 1.46	13	4.36	0.0007
PS II activity (Fv/Fm)	0.7251 ± 0.024	0.555 ± 0.052	16	2.82	0.0124
RWC (%)	84.024 ± 2.394	64.756 ± 3.144	9	5.21	0.0005
Osmotic potential (MPa)	−1.705 ± 0.145	−1.065 ± 0.085	3	4.45	0.0210

All values are mean ± SE. RWC, relative water content.

leaves (0.5550), indicating 23% reduction in PS II activity because of leaffolder feeding (table 1). Also, leaffolder damage appeared to interfere with leaf–water relations measured in terms of relative water content (RWC) by 23% reduction, whereas osmotic potentials slightly increased resulting in change in leaf turgor.

Effect of larval density on leaf damage and yield

When 1, 3 and 5 larvae were released on a hill at maximum tillering stage and allowed to develop, it was observed that a single larva caused damage in five leaves during its development. An average of 17 damaged leaves was observed after pupation in the treatment with 5 larvae resulting in 57% damaged leaves per pot as against 27% and 15% damaged leaves per pot in the treatments with 3 larvae and 1 larva, respectively, although the effective larval density was 3 larvae per pot. Total grain number per panicle varied from 160 to 266 with mean number of unfilled grains increasing with increased levels of larval density. A corresponding decrease in grain weight (2.88–1.60 g) was observed with increase in the number of larvae per hill. There was an increase of 13.13–20.39% unfilled grains in the pots released with larvae compared to control (table 2).

Time course studies on larval feeding behaviour on chlorophyll and PS II activity in flag leaf

The flag leaf area damage in different treatments varied from 20% to 50%. SPAD readings ranged from 18.48 to 39.13 in the flag leaves and 22.63 to 41.58 in the penultimate leaves. Damaged flag leaves had low chlorophyll content as compared to undamaged flag leaves, indicating a per cent reduction of 28–41% over control. The photochemical efficiency of PS II (Fv/Fm) ranged from 0.6352 to 0.7966 in the flag leaves and 0.7357 to 0.7845 in the undamaged penultimate leaves, suggesting 10–20% reduction over control (table 3).

Quantification of yield losses caused by leaffolder seasonal variation

There were significant and negative correlations between FLD and grain weight/panicle as well as number of filled grains/panicle while being positive for % unfilled grains during wet season (tables 4 and 5). Similarly, during dry season also, the correlations were significantly negative in relation to grain weight/panicle and filled grains per panicle and significantly positive with % unfilled grains. There was a reduction in yield and yield components at different FLD levels, and the reduction was higher at

Table 2 Influence of leaffolder larval density on leaf damage, insect development and grain yield

	No. of leaves damaged after insect release	% insects reaching adulthood	Total grains (No)	Grain weight (g)	% unfilled grains
Treatments					
Control	0 ± 0	0 ± 0	266 ± 10.18	3.28 ± 0.93	19.54 ± 0.65 (0)
1 larva	3.62 ± 0.84	100 ± 0	235 ± 9.54	2.88 ± 1.44	32.67 ± 3.26 (13.13)
3 larva	7.37 ± 1.16	66.67 ± 13.61	210 ± 19.43	2.20 ± 0.82	39.93 ± 4.07 (20.39)
5 larva	17.5 ± 1.89	65 ± 5.0	160 ± 6.51	1.60 ± 0.76	38.55 ± 3.47 (19.01)
d.f.	3,16	3,12	3,12	3,12	3,12
F	22.35	33.32	13.14	10.56	8.73
P	0.05	≤0.0001	0.0004	0.05	0.0024
LSD	5.30	22.34	38.19	1.02	9.70

All values are mean ± SE. Values in parenthesis are % unfilled grains over control.

Table 3 Time course studies on larval feeding behaviour on chlorophyll and PS II activity (Fv/Fm)

Treatments	% FLD area	Flag leaf		Normal penultimate leaf	
		Chlorophyll (SPAD values)	Fv/Fm	Chlorophyll (SPAD values)	Fv/Fm
T1 – larval feeding for 24 h	20 ± 3.54	22.7 ± 1.91	0.6967 ± 0.026	26.57 ± 0.78	0.7357 ± 0.015
T2 – larval feeding for 48 h	42 ± 8.45	19.78 ± 1.84	0.7202 ± 0.03	24.65 ± 0.79	0.7375 ± 0.007
T3 – larval feeding till pupation	50 ± 11.73	18.48 ± 4.67	0.6352 ± 0.13	41.58 ± 1.99	0.7845 ± 0.007
T4 – full flag leaf removed	100 ± 0			22.63 ± 0.78	0.7593 ± 0.008
T5 – half flag leaf cut	50 ± 0	39.13 ± 1.75	0.7347 ± 0.013	34.7 ± 1.54	0.7573 ± 0.018
T6 – control	0 ± 0	31.62 ± 1.57	0.7966 ± 0.009	29.02 ± 2.97	0.7648 ± 0.016
F	31.01	11.25	0.96	17.76	2.11
P	≤0.0001	≤0.0001	0.4510	≤0.0001	0.0985
LSD	17.74	7.74	0.17	4.92	0.04
d.f.	5,24	4,20	4,20	5,24	5,24

All values are mean ± SE. FLD, flag leaf damage (%).

51–75% FLD. Total grains per panicle varied from 133 to 74 under control and FLD conditions, respectively. The per cent unfilled grains were 20.14% and 26.83% more than the control (23.21%) in 51–75% and >75% FLD categories, respectively (table 4), during wet season, while it was slightly lower during dry season. Pooled analysis (fig. 1) of per cent unfilled grains of both seasons revealed a significant regression ($R^2 = 0.7689$, $P \leq 0.0001$) with FLD. Covariate analysis revealed that the season had no effect on FLD (t value 3.55 at 1 d.f., $P \leq 0.0004$).

Table 4 Yield and yield components as influenced by leaffolder at different damage levels

Flag leaf damage	Total grains/ panicle	Filled grains (%)	Unfilled grains (%)
Wet season (*Kharif*)			
Control n = 50	133 ± 2.07	76.79 ± 0.63	23.21 ± 0.63
<25% n = 55	104 ± 2.89	73.96 ± 0.48	26.04 ± 0.58
26–50% n = 33	101 ± 3.61	63.66 ± 1.22	36.34 ± 1.23
51–75% n = 28	74 ± 3.55	56.65 ± 1.04	43.35 ± 1.04
>75% n = 19	101 ± 1.32	49.97 ± 1.44	50.04 ± 1.44
Dry season (*Rabi*)			
Control n = 64	122 ± 1.43	79.03 ± 0.35	20.97 ± 0.35
<25% n = 56	95 ± 1.33	76.08 ± 0.43	23.92 ± 0.43
26–50% n = 32	84 ± 1.26	67.24 ± 0.69	32.76 ± 0.69
51–75% n = 27	69 ± 2.04	59.29 ± 0.81	40.71 ± 0.81
>75% n = 18	99 ± 1.48	53.84 ± 0.88	46.15 ± 0.88
d.f.	350	366	366
t-stat	3.198	3.250	3.254
P	0.0015	0.0012	0.0012

All values are mean ± SE. 'n' represents the number of panicles.

Discussion

Because of the leaffolder feeding, the general vigour and photosynthetic ability of an infested plant are greatly reduced. In the present investigation, this is apparent through the reduction in chlorophyll values under restricted larval feeding and during time course studies (tables 1 and 3). Evidence of chlorophyll loss because of feeding behaviour of larva was confirmatory in the present investigation, as there was no reduction in chlorophyll values in half-cut leaves as well as control leaves. These results were further corroborated with the reduction in PS II activity as measured by dark-adapted leaves.

Before feeding, larva folds the leaves causing a reduction in leaf area that is available for photosynthesis. In the present study, the per cent reduction in the leaf area because of folding varied from 4% to 36% depending on the length of the fold and total leaf area. The efficiency of PS II (Fv/Fm) was found low in damaged leaves. Larval feeding seems to influence the water relations of plants as seen by the reduction in the relative water content of damaged leaves (table 1), although the vascular bundles are left intact by the feeding larvae as reported by Fraenkel et al. (1981). Therefore, the disruption of transport to and from remaining mesophyll cells does not seem to be a plausible explanation for reduced photosynthesis in damaged leaves. In the present study, the reduction in photosynthesis in green leaf area was not associated with the extent of leaf area lost because of feeding.

Table 5 Regression between flag leaf damage levels and yield components

	Regression equation	R^2	t-value	P
Wet season (*Kharif*)				
FLD (*X*) & total grains/panicle (*Y*)	$Y = 120.72 - 0.4931X$	0.3147	9.14	≤0.0001
FLD (*X*) & grain weight/panicle (*Y*)	$Y = 2.3988 - 0.0212X$	0.5241	14.16	≤0.0001
FLD (*X*) & filled grains/panicle (*Y*)	$Y = 92.08 - 0.6519X$	0.5962	16.39	≤0.0001
FLD (*X*) & unfilling percentage (*Y*)	$Y = 23.58 + 0.3049X$	0.7324	22.44	≤0.0001
d.f. = 182				
Dry season (*Rabi*)				
FLD (*X*) & total grains/panicle (*Y*)	$Y = 110.3 - 0.4352X$	0.3855	11.17	≤0.0001
FLD (*X*) & grain weight/panicle (*Y*)	$Y = 2.4866 - 0.0218X$	0.5708	16.27*	≤0.0001
FLD (*X*) & filled grains/panicle (*Y*)	$Y = 86.977 - 0.5903X$	0.6451	19.03*	≤0.0001
FLD (*X*) & unfilling percentage (*Y*)	$Y = 21.592 + 0.2882X$	0.9368	33.28*	≤0.0001
d.f. = 199				

FLD, flag leaf damage (%).

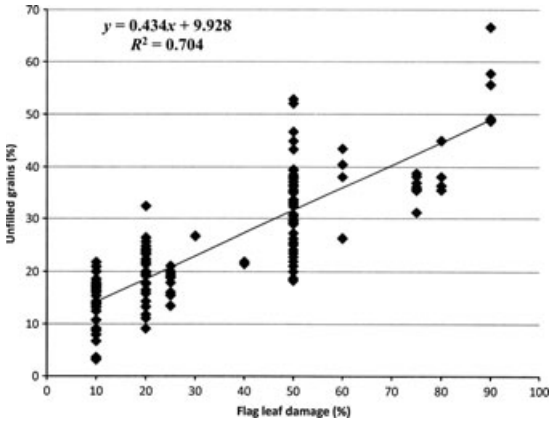

Fig. 1 Relationship between flag leaf damage (%) and unfilled grains across the seasons.

Hence, the absence of this correlation suggests that the reduction in photosynthesis may be a result of the folding of the leaf, but not of the feeding activity. De jong (1992) reported a significant reduction in net photosynthesis per unit green leaf area in folded leaves, and this reduction in total photosynthesis was attributed to the combined effect of reduction in the maximum rate of net photosynthesis per unit green leaf area because of folding and reduction in the green leaf area because of feeding. Watanabe (1999) observed that the effect of leaffolder injury caused by the reduction in photosynthetic rate was larger than the effect of leaffolder injury caused by the reduction in leaf area because of folding. He estimated that a 10% loss in leaf area resulted in 14–19% reduction in net photosynthesis.

On infestation, leaffolder initiates folding followed by feeding from central region of the lamina and proceeds towards tip or basal end of the leaf. Hence, in most cases of damage, the undamaged leaf segments of both apical and bottom regions of the leaf folds remained green. On the other hand, under severe situations of high larval density, complete leaf damage occurs. The photosystem II activity (Fv/Fm) was slightly reduced in these regions indicating that the insect feeding results in reducing the efficiency of PS II similar to other abiotic stress situations. It is also pertinent to note that the reduction in net photosynthesis may be not only due to the effect of leaffolder feeding and folding but also due to factors such as a reduction in opening of stomatal aperture and a reduction in photosynthetic activity in the mesophyll tissue. Reports also indicate that herbivore feeding damage leads to the desiccation at the site of feeding (Brito et al. 1986; Morrill et al. 1995; Haile and Higley 2003).

Effect of larval density on leaf damage and yield parameters revealed that a single larva can cause damage in five leaves during its development (table 2). Earlier study by Hafeez et al. (2010) has clearly established a negative correlation between per cent leaf infestation and yield reduction (0.15 g/tiller). At larval densities of 3 or 5, 20% unfilled grains over control were observed. Nevertheless, in our study, larval densities never exceeded five or more than 5 larvae per hill under field conditions. Time course studies on larval feeding resulted in the reduction of 28–57% chlorophyll content, 10–23% PS II activity and 22% relative water content over control in damaged flag leaves. Fv/Fm values were found high in penultimate leaf as compared to damaged flag leaf. These studies were performed in a greenhouse, but rice plants were not covered with mylar cage throughout the crop-growth period but only for the period mentioned as

per the treatment. In case of the first experiment, the mylar cage was kept for 7–8 days. In the second experiment, the mylar cage was kept for about 15–18 days, that is, until pupation. In the third experiment, it varied from 1 to 8 days. Later, the mylar cage was removed, and plants were allowed in the greenhouse till the maturity of grains. So, solar radiation could not be a limiting factor in these experiments.

Under field conditions, weather fluctuations in wet and dry seasons have profound impact on plant–insect interactions, thereby influencing yield. From the data, the grain number varied in the panicles either as a natural developmental process or as a result of insect damage. In natural conditions, also in TN1 rice cultivar, about 21–23% unfilled grains in dry and wet seasons were recorded. Subtracting these per cent values of control from those of leaf area infestation levels (<25%, 26–50%, 51–75% and >75%) indicated expected yield loss because of leaffolder. On this basis, there was not much difference between the seasons in grain loss in terms of per cent chaff/unfilled grains, which is in the range of 3% (<25% FLD), 12% (26–50% FLD), 20% (51–75% FLD) and 25% at >75% FLD. However, >25% FLD resulted in more than 50% unfilled grains over control. It is clearly evident (fig. 1) that for every 10% increase in FLD, there is a 3% increase in per cent unfilled grains. As flag leaf contributes to maximum grain filling (45–50%) in rice (Yoshida 1981; Voleti 2003), the damage to flag leaf is very critical. Earlier, Murugesan and Chellaiah (1983) reported 70% yield reduction when FLD exceeded 75% during flowering stage in greenhouse. For every 1% increase in the number of infested leaves, the grain yield was reduced by 0.95% (Murugesan and Chellaiah 1986). In a simulation study, Samalo et al. (1996) also found that the high level of FLD (45–70% of the area removed) resulted in significant yield losses. Miyashita (1985) reported yield reduction in proportion to the ratio of the damaged area of the two upper-most leaves. The ratio of yield reduction (Y) increased in proportion to the square of the ratio of injured leaf (X) as represented by the regression equation $Y = 1.55 + 0.0067X^2$ ($r = 0.9925$). A significant yield reduction was observed at heading stage with a leaf injury level higher than 25%. A simulation model by Graf et al. (1992) estimated yield reduction by 66% at a density of 3 fourth- and fifth-instar larvae at booting and 8 fourth- and fifth-instar larvae at heading. On the basis of field-based regression model, mean yield loss of 0.21–1.36 t/ha was estimated because of leaffolder injury in China (Kun et al. 2010). Moreover, leaffolder infestation at each of the reproductive and ripening stages showed additivity in terms of damage or yield loss when infestation was combined with stem borer dead heart or white ear head damage (Litsinger et al. 2011). On the basis of the present results, FLD of >25% can be considered as critical for yield loss; hence, FLD of <25% should be considered as threshold level for taking up any control measure.

Field damage at tillering stage varied between 0% to 20% damaged leaves. Earlier field study (Padmavathi et al.2004) on the quantification of yield losses because of leaffolder damage revealed a non-significant negative correlation between leaf damage and grain yield ($r = -0.3317$, $P \leq 0.5$) at tillering stage. Heong et al. (1998) also reported that the rice plant can tolerate damage up to 20% without any economic yield loss helped by compensatory mechanism. However, data in the present study indicated that, under controlled conditions, with increase in larval density from 1 to 5 per pot, there is an increase in leaf damage and in per cent unfilled grains (table 2).

Overall, a cumulative effect of loss in chlorophyll, reduced photosynthate availability and altered water relations interfered by the leaffolder lead to greater yield losses. Negative correlations with grain weight and filled grains in both seasons and positive correlation with unfilled grains (%) under the field conditions are a result of physiological imbalance caused by leaffolder damage.

Acknowledgements

Authors are thankful to Dr. BC Viraktamath, Project Director, Dr IC Pasalu and Dr JS Prasad, Principal Scientists and Head, Crop Protection, for their encouragement during the course of the study.

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