Effect of Rice Leafminer (Diptera: Ephydridae) Feeding on Early Growth of the Rice Plant[1]

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ABSTRACT One, two, and four eggs of the rice leafminer, *Hydrellia griseola* (Fallen), were transferred on the single third, fourth, and fifth leaves of rice seedlings, and two eggs were transferred on multiple-leaf combinations of the fourth, fifth, and seventh leaves. Plant weight and root weight showed significant differences among the treatments at a 5% level at 18 to 26 days after infestation in all the experiments. Total leaf area of a specific leaf mined ranged from 19 to 100%. Feeding effects on younger leaves were more pronounced than on older leaves. Larvae mining more than one leaf caused a greater effect on plant growth than an equal number of larvae mining a single leaf. Mining in leaves restrained on the water surface showed a greater effect on the plant growth than infestations in upright leaves.

The rice leafminer, *Hydrellia griseola* (Fallen), is a multivoltine, polyphagous species. The larvae feed on several plant species of different families, but graminaceous plants are the predominant hosts (Kuwayama 1956, Grigarick 1959, Deonier 1971). The effect of mining activity on the plant varies with age and sequence of the leaf, as well as with the extent and nature of injury to tissues.

The first serious leafminer infestation of rice in California was reported by DeOng in 1922. Lange et al. (1953) reported an outbreak of this pest in practically all the rice-growing areas of California during 1953 that resulted in a ca. 10 to 20% crop loss. Kuwayama (1956) reported an outbreak of this miner in Japan during 1954 and 1956. The crop loss was 39,000 kl from 75,000 ha of rice in Hokkaido, Japan, in 1956.

The rice leafminer is an early-season pest of rice in California, where rice is grown in continuously flooded paddies. Adults are quite mobile and oviposit throughout the rice field. The female prefers to lay eggs on the upper surface of leaves lying prostrate on the water surface. Larvae mine and feed on leaf mesophyll. They may move from leaf to leaf or to the leaf sheath and stem (Grigarick 1959, Lange and Miller 1970), depending on larval density and food availability. Deep water and especially cool temperatures during the early part of the growing season reduce plant vigor (Lange et al. 1953, Kuwayama 1956, Grigarick 1963). These conditions cause the leaves to lie on the water surface, which results in increased oviposition and subsequent mining. If the weather is warm and growing conditions are favorable for upright leaf growth, the infestation may end rapidly, due to egg mortality on leaves and larval mortality within leaves (Grigarick 1959).

There are several reports on effects of natural and artificial defoliations of rice. In 1974, Navas concluded from studies in Panama on upland rice that plants were capable of tolerating heavy damage from the fall armyworm and perhaps other foliage-feeding insects, particularly during the first few weeks of growth. Taylor (1972), using artificial defoliations, indicated that upland rice plants could recover and compensate for loss of foliage, particularly when this occurred before and during tillering. However, Bowling (1978) showed significant differences in yield when rice plants were artificially defoliated during the seedling and tillering stages of growth before permanent flooding. Studies with artificial infestations of the whorl maggot on rice in the Philippines (Anonymous 1981) showed a reduction in plant height and a 2-week delay in maturity. However, the damage sometimes stimulated tillering, and yield was not decreased. It was emphasized by Hering (1951) that larval feeding on leaves during an early stage of plant development caused the maximum effect on plant growth. No quantitative information has been reported on the effect of the rice leafminer on rice growth. Our research was undertaken to determine the effect of varying leafminer larval densities on different early stages of flooded rice seedlings.

Materials and Methods

Experiments were conducted in a greenhouse at Davis, Calif., during the summer of 1981. Rice variety 'M 301' was grown in plastic Amoco pots (height, 12 cm; top diameter, 11 cm; bottom diameter, 9 cm). Each pot was filled three-fourths full with sterilized soil. Ammonium sulfate (21% N) was applied at the rate of 561 kg/ha. Four seeds, treated with Captan at the rate of 1.5 g of AI/kg of seeds, were placed in each flooded pot. The seeds were soaked in water for 24 h before planting. After the rice seedlings emerged from the water, they were thinned to one per pot. Algal growth was controlled with copper sulfate at the rate of 11 kg/ha. All pots were then transferred to a large pan (198 by 117 by 20 cm deep) filled with water and maintained to a height of 10 cm above the soil surface in the pots throughout the test.

Maximum and minimum air and water temperatures during the tests were 34.4 and 15.0°C, and 32.2 and 16.1°C, respectively. Overall mean maximum and minimum air and water temperatures were 30.0 and 17.2°C, and 26.6 and 19.4°C, respectively.

Experiments A to E represented varying infestation levels, on different leaves or combinations of leaves at

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different plant ages. Five treatments within each experiment were arranged in a randomized complete block design. Each treatment was replicated 5 to 15 times. The details of each experiment are summarized below and in Fig. 1.

All rice leafminer eggs used in the tests were collected on the margins of Putah Creek, ca. 27 km west of Davis. Graminaceous weed leaves containing eggs were picked and transported in a plastic bag containing water. The egg-infested leaves were maintained on the water surface in pans in the laboratory for 2 to 3 days while the eggs incubated. Eggs were examined with a microscope to determine their suitability for transfer to experimental rice plants. Change in color from white to light pink indicated egg maturity. Because the female adheres its eggs to the leaf surface, it was necessary to cut out a section (2 by 4 mm) containing individual eggs before transferring them. Leaf sections were coated with wheat flour paste at both ends and attached to the leaf surface (egg down) of the experimental rice plants. Transfers of one and two eggs per leaf were made to one or both sides of the midrib at the center of the length of the leaf. Treatments of four eggs were made similarly in two pairs equidistantly along the leaf. The attached leaf sections were covered with pieces of wet paper towel (1.5 by 1.5 cm) to prevent drying of leaf sections and eggs. About 24 h later the paper towelling was removed and leaves were checked for mining. If eggs failed to hatch or larvae were missing, the desired density levels were obtained by transferring newly hatched larvae from a culture maintained separately.

The third, fourth, and fifth leaves in experiments A, B, and C, respectively, and the fourth, fifth, seventh leaves in experiment D were restrained on the water surface to provide a suitable environment for continued mining. Leaves were held prostrate on the water with a piece of string that was affixed on both sides of the pan with binder clips. In experiment D, two pieces of string were affixed 6 cm apart to hold the three infested leaves of each plant. Two uninfested checks were included in experiments A, B, and C. Both checks had the experimental leaf restrained on the water surface. One of them also had two blank leaf sections attached to the experimental leaf to determine if experimental procedure affected plant growth. In experiment D, one of the two uninfested checks had the three experimental leaves restrained on the water surface and the other allowed the leaves to remain upright. A single uninfested check for experiment E had the experimental leaf restrained on the water surface.

Larvae mined for ca. 7 to 8 days and then pupated inside the mine. The plants were maintained an additional 10 days, and the percent mined on each leaf was visually estimated to the nearest 5 to 10%. At the termination of each experiment, plants were removed from the pots and all soil was washed from the roots. Plants were placed in plastic bags and refrigerated for subsequent measurement of growth characteristics. Before measurement plants were blot dried with paper towels. The following characteristics were measured: (1) plant wet weight—entire plant; (2) root dry weight—roots cut from the stem at the crown and air dried for 2 weeks; (3) plant height—from the tip of the longest leaf to the base of the crown; (4) root length—from the tip of the mode of the longer roots to the base of the crown; (5) number of tillers per plant—each separate stem with unfurled leaves; (6) number of leaves per plant—all leaves longer than 5 cm. The leaf sequence terminology was based on Chang and Bardenas (1965).

The percent reductions due to the treatments in all experiments were calculated based on the standard check. The data were analyzed by two-way analysis of variance, and the treatment means were compared by using LSD ($P = 0.05$).

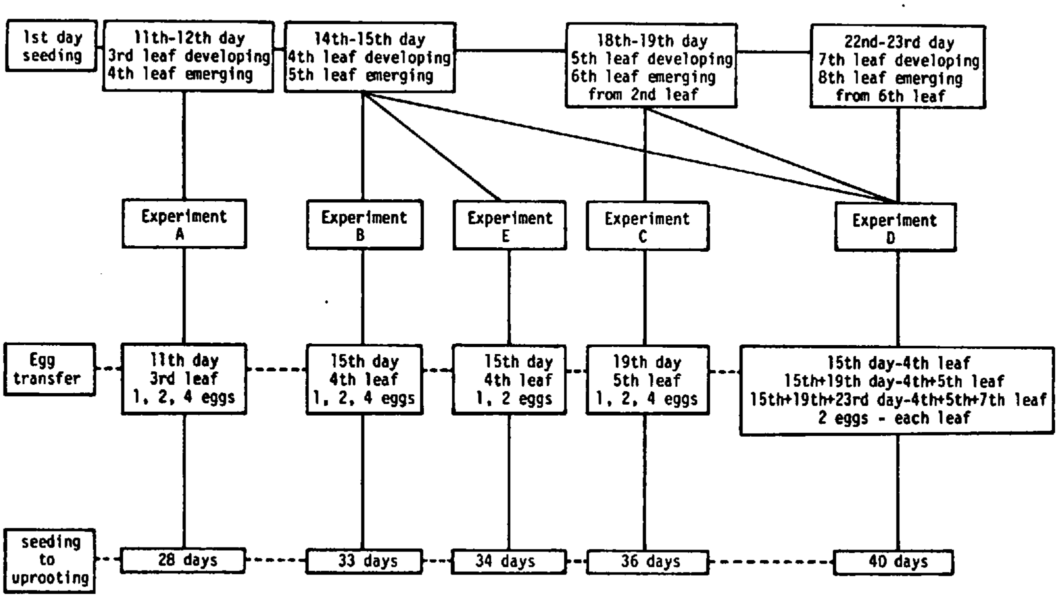

FIG. 1. Leaf growth sequence and procedure for experiments A through E.

Results

The average amount of foliage mined ranged from 19 to 100% in experiments A, B, and C, depending on leaf size and number of larvae per leaf (Table 1). The third (smallest) leaf was completely or near completely mined at all larval infestation levels. Available leaf tissue at this age and size of leaf supported a maximum of 1 larva per leaf to pupation. A wider range in the percentage of leaf area mined resulted from infestations of the fourth, fifth, and seventh leaves (experiments B through E). Infestations of the third leaf with four larvae occasionally killed seedlings due to larval movement to and feeding on the rice stem. These plants were also included in plant growth measurements. The amount of mining on the fourth and fifth leaves by one larva per leaf was 33 and 19% of the leaf, respectively. The percentages of leaf area mined increased with an increase in larval density (Table 1). For the same level of infestation, the percentage of leaf area mined decreased with an increase in leaf size (leaves 4, 5, and 7 in experiments B, C, and D). Experiment E demonstrated the same trend with respect to infestation levels and leaf areas mined, but showed a sharp difference in the percent of leaf area mined between leaves infested in the upright position and infested leaves on the water surface. The latter were mined to a greater extent at the same level of infestation.

When the third leaf was infested in experiment A, there was a definite increase in the percent reduction of all plant growth characteristics with an increase in the level of infestation (Table 2). Differences between two and four larvae were not significant. All growth characteristics at two and four larvae showed significant negative effects compared to the standard check. Significant differences from the standard check and one larva were found for characteristics involving weight and number of tillers and leaves, but not plant height or root length. Some plants with four larvae died due to migration of these larvae to other plant parts. The data on the plant wet weight showed considerable variation, ranging from 0.44 to 2.57 g, because of plant mortality.

The infestation of the fourth leaf (experiment B) showed the same general trend with respect to plant growth measurements (Table 3) as was observed for the third leaf, such as a decrease in growth with an increase in infestation. However, significant differences from the standard check were recorded only with measurements including weight. The percent reduction in the growth characteristics for an infestation of the fourth leaf was far less than that recorded for the third leaf at all three levels of infestation.

The infestation of the fifth leaf (experiment C) showed the same trend as experiments A and B. Plant growth decreased with an increase in the number of larvae per leaf (Table 4). Significant differences in measurements of growth from the standard check were noted for a greater number of growth characteristics than for the fourth-leaf infestation. The percent differences of these characters from the standard check for the fourth and fifth leaves were of a much closer magnitude than for the third leaf.

Experiment D (Table 5) compared infestations of two larvae each for the fourth, fourth and fifth, and fourth, fifth, and seventh leaves. The same trends as experiments A, B. and C were observed when the infestation per plant was increased, but experiment D differed in that the infestation per leaf was kept constant. All growth characteristics showed significant differences at all infestation levels from the standard check. The percent reductions for most of the growth characteristics were greater for the multiple-leaf infestation when compared with the same number of larvae on a comparable single leaf (fourth and fifth leaves in Tables 3 and 4). Percent reductions in tillering and number of leaves (Table 5) were higher than in experiments B and C, which probably reflects the stress on these older plants at tillering.

Experiment E tested the effect of leafmining (fourth leaf) on plant growth in relation to leaf position above or on the water surface. A consistent trend in reduction in plant growth was apparent for both one and two larvae per leaf only when the leaf was restrained on the water

Table 1. Estimated percent leaf area mined with varying numbers of rice leafminer larvae infesting rice leaves of different ages

No. of larvae	Leaf position	Avg % of leaf area mined			
		3rd leaf Expt A	4th leaf Expt B	5th leaf Expt C	7th leaf
1	Water surface	93	33	19	—
2	Water surface	98	66	41	—
4	Water surface	100	98	76	—
			Expt D		
2	Water surface	—	68	—	—
2	Water surface	—	68 +	43	—
2	Water surface	—	71 +	46 +	33
			Expt E		
1	Water surface	—	28	—	—
1	Upright	—	6	—	—
2	Water surface	—	60	—	—
2	Upright	—	6	—	—

Table 2. Comparison of means of different growth characteristics resulting from varying numbers of rice leafminer larvae infesting the third leaf, and percent reduction as compared with the standard check

Treatment	Means[a]/plant of 15 replications in expt A					
	Plant wet wt (g)	Root dry wt (g)	Plant height (cm)	Root length (cm)	No. of tillers	No. of leaves
1 Larva	2.47b	0.05b	58.2a	15.0a	2.9bc	7.8bc
	(22.4)[b]	(23.3)	(1.7)	(6.4)	(17.3)	(20.4)
2 Larvae	1.85c	0.03c	53.8b	13.4b	2.5cd	7.3cd
	(41.8)	(48.3)	(9.0)	(16.7)	(28.9)	(25.9)
4 Larvae	1.73c	0.03c	53.8b	13.1b	2.3d	6.4d
	(45.6)	(48.3)	(9.1)	(18.3)	(32.7)	(34.7)
Check with 2 leaf sections	3.16a	0.06a	59.5a	15.7a	3.1ab	8.7b
	(0.6)	(0.0)		(1.9)	(9.5)	(11.6)
Check—standard	3.18a	0.06a	59.1a	16.0a	3.5a	9.8a

[a]Means followed by the same letter are not significantly different at the 5% level, by the least significant difference (LSD) test.
[b]Values in parentheses represent percent reduction from the standard check.

Table 3. Comparison of means of different growth characteristics resulting from varying numbers of rice leafminer larvae infesting the fourth leaf, and percent reduction as compared with the standard check

Treatment	Means[a]/plant of 12 replications in expt B					
	Plant wet wt (g)	Root dry wt (g)	Plant height (cm)	Root length (cm)	No. of tillers	No. of leaves
1 Larva	16.23ab	0.48a	60.7a	22.4a	7.1a	24.8a
	(3.4)[b]	(0.6)	(0.4)	(3.8)	(5.6)	(2.3)
2 Larvae	15.08bc	0.44a	60.6a	21.8a	6.9a	24.8a
	(10.2)	(9.3)	(0.5)	(6.3)	(7.9)	(2.6)
4 Larvae	14.31c	0.37b	60.4a	21.6a	6.8a	24.6a
	(14.8)	(23.6)	(0.8)	(7.2)	(10.0)	(3.3)
Check with 2 leaf sections	16.63a	0.48a	61.9a	22.6a	7.1a	25.5a
	(1.0)	(0.0)		(3.0)	(5.3)	
Check—standard	16.80a	0.48a	60.9a	23.3a	7.5a	25.4a

[a]Means followed by the same letter are not significantly different at the 5% level, by the least significant difference (LSD) test.
[b]Values in parentheses represent percent reduction from the standard check.

Table 4. Comparison of means of different growth characteristics resulting from varying numbers of rice leafminer larvae infesting the fifth leaf, and percent reduction as compared with the standard check

Treatment	Means[a]/plant of 15 replications in expt C					
	Plant wet wt (g)	Root dry wt (g)	Plant height (cm)	Root length (cm)	No. of tillers	No. of leaves
1 Larva	17.89ab	0.53a	63.9a	24.0b	8.6a	25.9ab
	(0.7)[b]	(4.0)	(0.3)	(1.8)	(2.9)	(3.0)
2 Larvae	17.48b	0.50a	62.2b	23.8b	8.5a	25.7ab
	(3.0)	(10.3)	(2.9)	(2.6)	(4.5)	(3.7)
4 Larvae	15.44c	0.43b	62.0b	22.0c	8.3a	25.1b
	(14.3)	(23.0)	(3.3)	(9.8)	(6.8)	(6.3)
Check with 2 leaf sections	19.18a	0.54a	64.3a	25.3a	8.7a	26.9a
		(1.8)			(2.2)	
Check—standard	18.02ab	0.55a	64.1a	24.4ab	8.9a	26.7a

[a]Means followed by the same letter are not significantly different at the 5% level, by the least significant difference (LSD) test.
[b]Values in parentheses represent percent reduction from the standard check.

surface (Table 6). These reductions were significant for plant and root weight measurements and also root length. Plant growth characteristics were somewhat larger for upright mined leaves than for plants with restrained unmined leaves, but the differences were only significant for root length. The differences between one and two larvae per leaf for restrained leaves were not significant in this test.

Measurements of growth of checks frequently showed a slight reduction for those checks with attached leaf sections in experiments A and B, but not significantly so, and this trend was reversed in experiment C. The

Table 5. Comparison of means of different growth characteristics resulting from varying numbers of rice leafminer larvae infesting the fourth, fifth, and seventh leaf, and percent reduction as compared with the standard check

Treatment	Means[a]/plant of eight replications in expt D					
	Plant wet wt (g)	Root dry wt (g)	Plant height (cm)	Root length (cm)	No. of tillers	No. of leaves
2 Larvae on 4th leaf	12.35b	0.36bc	63.4b	23.9bc	6.5bc	20.1bc
	(21.1)[b]	(31.1)	(2.1)	(5.5)	(17.4)	(14.4)
2 Larvae each on 4th and 5th leaf	10.76c	0.29cd	62.9b	23.7c	5.8cd	18.8c
	(31.3)	(45.1)	(2.9)	(6.4)	(26.9)	(20.2)
2 Larvae each on 4th, 5th, and 7th leaf	9.73c	0.25d	61.6c	23.2c	5.5d	18.6c
	(37.9)	(51.3)	(4.8)	(8.4)	(30.1)	(20.8)
Check—4th, 5th, and 7th leaves on water surface	14.88a	0.40b	63.8b	25.0ab	7.0ab	21.5ab
	(5.0)	(23.0)	(1.5)	(1.2)	(11.4)	(8.5)
Check—all leaves upright (standard)	15.66a	0.52a	64.8a	25.3a	7.9a	23.5a

[a]Means followed by the same letter are not significantly different at the 5% level, by the least significant difference (LSD) test.
[b]Values in parentheses represent percent reduction from the standard check.

Table 6. Comparison of means of different growth characteristics resulting from one and two larvae of the rice leafminer on the fourth leaf, both on the water surface and upright, and percent differences as compared with the check

Treatment	Means[a]/plant of five replications in expt E					
	Plant wet wt (g)	Root dry wt (g)	Plant height (cm)	Root length (cm)	No. of tillers	No. of leaves
1 Larva—leaf on water surface	4.97b	0.11bc	62.5a	19.6bc	3.2a	11.4a
	(10.6−)[b]	(15.6−)	(0.0)	(2.5−)	(5.9−)	(6.6−)
1 Larva—leaf upright	6.02a	0.15a	62.6a	21.0a	3.4a	12.8a
	(8.4+)	(7.4+)	(0.2+)	(4.5+)	(0.0)	(4.9+)
2 Larvae—leaf on water surface	4.80b	0.11c	61.6a	19.2c	3.0a	11.4a
	(13.7−)	(17.8−)	(1.4−)	(4.5−)	(11.8−)	(6.6−)
2 Larvae—leaf upright	5.86a	0.15a	62.6a	21.5a	3.6a	12.2a
	(5.4+)	(8.2+)	(0.2+)	(7.0+)	(5.9+)	(0.0)
Check—leaf on water surface	5.56a	0.14ab	62.5a	20.1b	3.4a	12.2a

[a]Means followed by the same letter are not significantly different at the 5% level, by the least significant difference (LSD) test.
[b]Values in parentheses represent percent differences from the check.

experimental technique of restraining three leaves on the water surface for one of the checks of experiment D consistently showed a reduction of growth over the check with all leaves upright. These reductions were significant for root dry weight and plant height.

Discussion

Either the third or fourth leaves of field grown rice in California are the first to emerge through the water surface, depending on multiple factors such as water depth, temperature, and growth rate. This study showed that the greatest effect on plant growth caused by rice leafminer larvae was on the third leaf, i.e., the youngest leaf infested. The reduction in or loss of plant growth that was recorded after this leaf was infested was the result of both growth retardation and death of the plant. The amount of leaf area mined ranged from 93 to 100% for the third leaf. This resulted in the occasional death of a seedling due to larval migration to other tissues, such as the stem, in search of food. Infestations of the third leaf at two and four larvae per leaf each resulted in a 13% plant mortality. Migration of larvae to the stem seemed to be fortuitous, because some larvae sank to the bottom and did not reestablish on the host. The significant difference in number of leaves between the two checks (Table 2) may be the result of the mechanics of placement of the blank leaf sections on the third leaf compared with the unmanipulated standard check.

The percent total leaf tissue mined on the fourth, fifth, and seventh leaves of test plants was less, and the effect on the plant was limited to retardation of growth. As expected, reduction in growth was greater as the level of infestation increased. This study also demonstrated that a larval infestation on more than one leaf had a greater effect on plant growth than did an equal number of larvae on a single leaf. Murata (1961) reports that the most important factor determining dry-matter production during the early growth stage is generally the leaf area rather than the photosynthetic capacity of the leaves. Tanaka (1958) and Murata and Matsushima (1975) consider the photosynthesis of lower leaves to be a very important factor in supplying assimilates to the root system and maintaining the root activity.

The effects on early plant growth can be overcome to a certain extent during later growth stages (Taylor 1972). However, the degree of recovery from loss of leaf tissue may not be fully compensated when compared with that of a healthy plant. Since the effects of larval mining during early plant growth can be significant, even though plants may recover to some extent during later growth stages, there could be a delay in growth and uneven maturity within a field (Grigarick 1963, Lange and Miller 1970), with an ultimate loss of grain yield (Bowling 1978).

In our experiments, grain yield could not be assessed because of long-term atypical conditions of the greenhouse. Field-grown rice plants are susceptible to various chewing and sucking insects after the normal period of attack by the rice leafminer, so the effects of early injury may be compounded.

The final experiment (E) investigated the relationship of leafmining to leaf position of the young, emerging leaves. The extent of mining was greatly reduced, and larvae did not complete development in upright leaves, as compared with infested leaves lying on the water surface. The reasons for upright leaves not being a suitable habitat for larval mining are not known, but temperature, moisture, larval behavior patterns, and plant physiology may all affect leaf acceptance by the larvae.

This research indicates that, in a pest management program for control of the rice leafminer, practices that promote rapid plant growth should be encouraged. Most important is water management during emergence of the third and fourth leaves, because proper water regulation (below 10 cm) will help rice leaves remain upright, thus making the leaves less desirable for oviposition and mining.

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