DISRUPTION OF SEX PHEROMONE COMMUNICATION IN THE RICE STEM BORER MOTH, *Chilo suppressalis* WALKER (LEPIDOPTERA: PYRALIDAE), WITH SEX PHEROMONE COMPONENTS AND THEIR RELATED ANALOGUES

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Introduction

The rice stem borer, *Chilo suppressalis* Walker, is one of the most serious pests of the rice plants throughout the Far-East and Southeast Asia. Larval stem boring results in extensive damage and crop loss. A large quantity of insecticides is used annually for its control in these areas, but the use of insecticides can lead to insect resistance and environmental problems. Identification and synthesis of the female sex pheromone complex was undertaken in the hope that the synthetic pheromones would be of value for population monitoring and for direct control by mating disruption techniques. The female sex pheromone complex of this insect consists of two olefinic aldehydes, Z-11-hexadecenal (Z-11-HDAL) and Z-13-octadecenal (Z-13-ODAL) (Nesbitt *et al* ., 1975: Ohta *et al.*, 1976). It was confirmed that the synthetic Z-11-HDAL and Z-13-ODAL acted as attractants when they were mixed together in ratios of 1:1 to 20:1, in particular the ratios of 3:1, 5:1 being most effective (Tatsuki *et al.*, 1977).

As reviewed by Roelofs and Carde (1977), the sex pheromone complex consists of multiple components in many lepidopterous species, and each of the pheromone components as well as the sex pheromones and their analogues has been used as "attraction disruptants". We therefore undertook a research program aimed at applying the sex pheromone components and their structurally related compounds to the direct control of *Chilo suppressalis*. We synthesized the sex pheromone components and 12 analogues including compounds having the same alkyl moieties as the pheromone components and other functional end groups, i.e. alcohol, acetate and methyl groups.

The present paper deals with the disruptive effect of the sex pheromone components and their related compounds on the sex pheromone communication of *Chilo suppressalis*.

Disruption of sex pheromone communication

1 Preliminary screening tests to identify possible disruptants

1) Disruption of male orientation to virgin female-baited traps by each compound placed in the traps

Disruptive activities of the sex pheromone components and their 12 structurally related compounds against male orientation to female-baited traps were tested in paddy fields in Niigata Prefecture, Japan, in June, 1977. One mg of each test compound applied on a cotton wick was placed in a trap containing two virgin females. The effect of each compound was analyzed by comparing the total number of males caught in the treated traps with those in control traps.

As shown in Table 1, all the compounds tested had considerable disruptive effect on male orientation to virgin females. Among them, the sex phermone components, as well as their E-isomers and alcohols, acetates and methyl substitutes were very effective. It was of great interest to observe that Z-5-hexadecene (Z-5-HD) and Z-5-octadecene (Z-5-OD), although having much less polarity than the pheromone components and the other derivative groups, could disrupt male orientation effectively.

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Table 1 Disruptive effect of the sex pheromone components and 12 related compounds placed in female-baited traps on male orientation to the traps[a]

Compound	Total no. of males caught[b]	% Reduction[c]
Z–11–hexadecenal	0	100.0 c
Z–13–octadecenal	15	89.0 c
E–11–hexadecenal	7	94.9 c
E–13–octadecenal	7	94.9 c
Hexadecenal	37	72.8 b
Octadecenal	26	80.9 bc
Z-5–hexadecene	13	90.4 c
Z–5–octadecene	1	99.3 c
E–5–hexadecene	32	76.5 b
E–5–octadecene	35	74.3 b
Z–11–hexadecenol	3	97.8 c
Z–13–octadecenol	2	98.5 c
Z–11–hexadecenyl acetate	1	99.3 c
Z–13–octadecenyl acetate	7	94.9 c
control	136	– a

a. Data from Kanno *et al.* (1978)

b. Two replicates for 14 successive nights. Treatments were applied every other day.

c. Percentage followed by the same letter was not significantly different at the 5% level with Duncan's multiple range test from original data.

2) Disruption of male orientation to virgin female traps by each compound around the traps

It is known that in some Lepidoptera non-pheromone chemicals that were disruptive against male orientation to traps baited with virgin females when dispensed simultaneously from the traps had little or no disruptive effect when dispensed over a rather wide area surrounding the traps (e.g., Mitchell *et al.*, 1974; Rothschild, 1974). We therefore conducted another field test in which the compounds were applied over a rather wide area.

The pheromone components and pheromone-related compounds that showed relatively potent disruption in the previous test were used. Twelve polyethylene capsules each containing 100 mg of the respective compounds were evenly placed in two concentric circles of 1 and 2 m radius (Test A) or one circle of 5 m radius (Test B) with a virgin female-baited trap at the center. Polyethylene capsules containing only the solvent were used as controls. The plots were designed at intervals of 200 m in two parallel straight lines, for duplication test, about 300 m apart. Both tests A and B were run for 5 nights without renewing the capsules. The numbers of males caught in the traps each night were counted the next day.

The results are shown in Table 2. In Test A, relatively good disruption of male orientation could be achieved with most of the compounds tested when the compounds were dispensed in the same air currents that were carrying natural pheromone. The pheromone components, Z-5-HD and Z-11-hexadecenol (Z-11-HDOL) almost completely disrupted male orientation. E-isomers of the pheromone were also highly effective as attraction disruptants. In Test B, in which the dispensers were placed around the traps more sparsely than in Test A, considerable disruptive effect was also

Table 2 Effect of the sex pheromone components and 8 related compounds surrounding female baited traps on male orientation to the traps[a]

Compound	Test A: No. of males caught[b]	Test A: Percentage of disruption[c]	Test B: No. of males caught[b]	Test B: Percentage of disruption[c]
Z–11–hexadecenal	1	99.3 a	12	90.8 a
Z–13–octadecenal	3	98.0 a	35	73.3 b
E–11–hexadecenal	17	88.9 ab	45	65.6 b
E–13–octadecenal	15	90.2 ab	15	88.5 a
Z–5–hexadecene	1	99.3 a	8	93.9 a
Z–5–octadecene	50	67.3 c	39	70.2 b
Z–11–hexadecenol	1	99.3 a	28	78.6 b
Z–13–octadecenol	42	72.5 bc	37	71.8 b
Z–11-hexadecenyl acetate	71	53.6 c	44	66.4 b
Z–13–octadecenyl acetate	148	3.3 d	91	30.5 bc
control	153	– d	131	– c

a. Data from Kanno *et al.* (1980)

b. Total of two replicates for 5 nights

c. Percentage followed by the same letter within a column was not significantly different at the 5% level with Duncan's multiple range test from original data.

obtained with most of the compounds. However, Z-11-hexadecenyl acetate (Z-11-HDA) and Z-13-octadecenyl acetate (Z-13-ODA) which were very effective when set in the traps with virgin females in the previous test had little or no effect when placed around the traps. (Table 2).

2 Suppression of mating with sex pheromone components and hydrocarbons

To verify if mating suppression could occur simultaneously with the disruption of male orientation under similar conditions to those used in the previous test, the sex pheromone components and the hydrocarbons, Z-5-HD and Z-5-OD, were tested in fields in Niigata in June of 1979.

Ten mg of each compound was soaked in a polyethylene capsule, which was then heat-sealed to prevent the compound from leaking out rapidly. Solvent only was used in a capsule for the control. Twelve sealed capsules of each compound were set in a circle of 3 m radius at about 30 cm above the rice plants, and in a concentric 2 m circle 15 virgin females each tethered with a cotton thread to a plastic rod were also evenly distributed. The moths were exchanged for new ones every day during the test period and the collected ones were then dissected to verify whether mating had been successful by the observation of spermatophore formation. The plots were also designed at about 200 m intervals in duplication. The locations of the compounds were changed every other night to reduce the possibility of error due to location. Release rates of the compounds from the capsules were determined by direct weighing using a micro-balance.

The effect of each compound on the successful mating is shown in Table 3. All of the four compounds tested significantly suppressed the mating, especially Z-11-HDAL and Z-5-HD had a highly inhibitory effect on mating. Among them, each C-16 compound was more effective than the corresponding C-18 compound in this case as well. This difference in effectiveness might also reflect the difference in release rate. It was also shown that the release rate of each hydrocarbon was several times greater than that of the corresponding aldehyde as shown in Fig. 1. (Table 3 and Fig. 1).

Table 3 **Effect of the sex phenomone components and the hydrocarbons on mating suppression of tethered females[a]**

Compound	No. of females collected[b]	Percentage of mating[c]	Percentage of mating suppression
Z–11–hexadecenal	212	8.0 a	86.0
Z–13–octadecenal	209	26.3 bc	54.1
Z–5–hexadecene	223	17.9 b	68.8
Z–5–octadecene	221	31.7 c	44.7
control	218	57.3 d	–

a. Data from Kanno *et al.* (1980)

b. Total of two replicates for 8 nights

c. Percentage followed by the same letter was not significantly different at the 5% level with Duncan's multiple range test from original data.

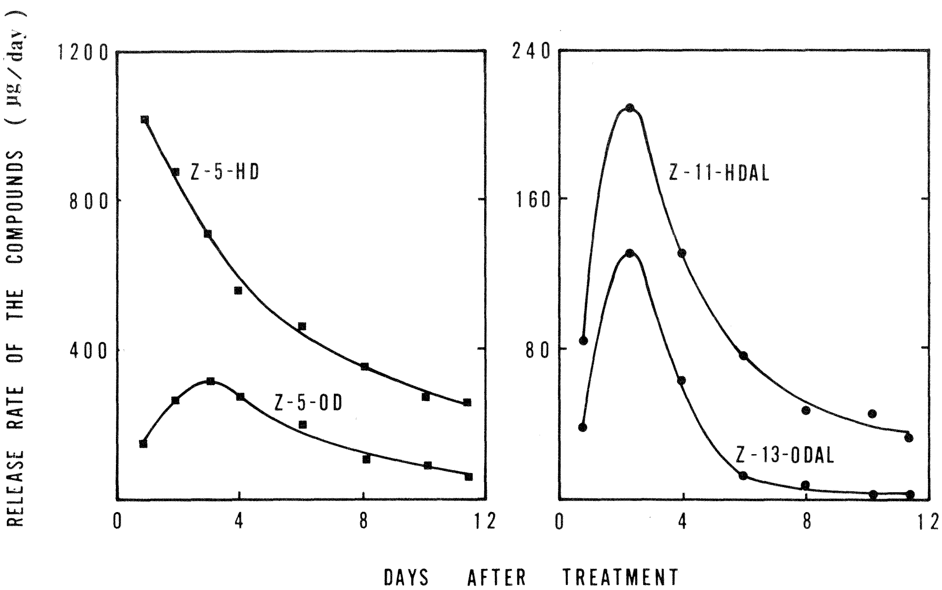

Fig. 1 Variation in release rates of the sex pheromone components and of the hydrocarbons from the polyethylene capsule

3 Evaluation of disruptive activity of Z-11-HDAL and Z-5-HD by comparative field test

The disruptive activities of Z-11-HDAL and Z-5-HD were compared in the fields in Niigata in June of 1979. Half a ml of the hexane solution containing 0.1, 0.3, 1, 3 and 10 mg of each respective compound was dispensed on a rubber septum. For the control, the solvent alone was applied on a septum. Six rubber septa treated with each compound at the concentrations listed above were placed in a circle 1 m in radius with a virgin female-baited trap in the center. The plots were separated from each other by a 200 m "buffer" zone, and were designed in duplication. The locations of the compounds and of the control were also changed every other night for re-randomization. This test was run for 8 nights and the number of males caught in the traps each night were counted the next day.

Fig. 2 shows the results of this test. The disruptive effect of Z-11-HDAL and Z-5-HD on male orientation was affected by the amount of the compound in the rubber septum. The plot treated with 10 mg of Z-11-HDAL per septum showed a marked disrupting effect. Also plots treated with 10 mg of Z-5-HD exhibited highly disrupting effect on male orientation. However, these effects decreased gradually with the decrease in the amount of compound in a septum. It was also observed that the disruptive effect of Z-11-HDAL was apparently higher than that of Z-5-HD, though the release rate of Z-11-HDAL was lower than that of Z-5-HD, as shown in the other tests described previously. Nevertheless, the usefulness of Z-5-HD was ascertained in this test for the following reason; although Z-5-HD was shown to be ca. 10 to 30 times less effective with respect to the dosage used than Z-11-HDAL, such shortcoming was compensated by its chemical stability and the simplicity of its synthesis. (Fig 2).

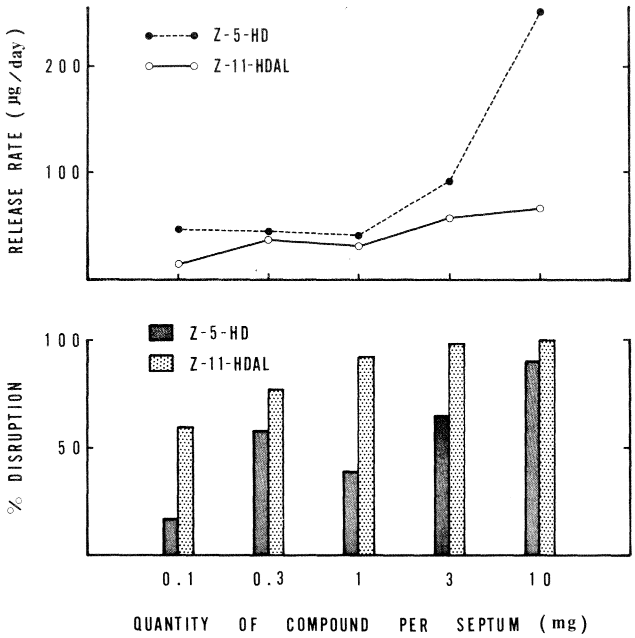

Fig. 2 Comparison of disruptive activities on male orientation and of release rate from the rubber septum between Z-11-hexadecenal and Z-5-hexadecene

4 Large scale communication disruption test with Z-5-HD

The practical usefulness of Z-5-HD as disruptant was demonstrated through the results of several tests as described previously. Moreover, we attempted a large scale disruption test to determine whether the crop damage by the larvae of the next generation could be decreased with Z-5-HD. This test was also conducted in the paddy fields of Niigata in the summer of 1979. For the test, six experimental blocks were used with two of them treated with the compound and the other four as controls. Blocks were nearly 2000 m^2 each and 200 to 300 m apart. The two treated areas were separated from each other by a distance of ca. 600 m. The compound was incorporated in synthetic rubber films (40% Z-5-HD in styrene-isoprene copolymer, 60μ thickness), with one side covered with polypropylene films (25μ thickness) as a barrier. The laminated films were cut into 1 × 4 cm strips to use as dispensers. The dispensers were attached to fishing-rods at 1 m intervals. The rods were strung over the test fields at 1.5 m intervals, so that the dispensers were suspended at 1 × 1.5 m intervals 20 to 30 cm above the plants. The total numbers of the dispensers used in each treated area were 1221 for block I (2002 m^2) and 1120 for block II (1999 m^2), respectively. The treatment was applied from July 31 to August 25. This period almost coincided with the second moth flight usually from late July to late August with a peak period around August 10. No insecticides were sprayed on the fields during and after the experiment.

The results are shown in Table 4. The data showed significantly less injury of the plants in both Z-5-HD treated areas than in the control areas. These results suggest that the mating frequency in the treated areas was decreased by the treatment with this compound, although the area treated was relatively small. We had also intended to monitor the disruptive effect in using female-baited traps during the test period, but such test could not be completed because of the extremely low number of males caught even in the control traps. One of the factors could possibly be ascribed to a low population density. Total amount of Z-5-HD that evaporated in the test areas during the test period was estimated at approximately 35 g (87.5 g/ha). (Table 4).

Table 4 Effect of Z–5–hexadecene on injury of rice plants by larvae of *Chilo suppressalis*[a]

Treatment	Block	Degree of injury in stubbles[b]	
		percentage of injured stubbles[c]	percentage of injured stems[c]
Z–5–hexadecene	I	14.2 a	1.01 a
	II	12.2 a	1.04 a
Untreated	III	32.4 c	2.60 x
	IV	25.8 b	2.17 c
	V	25.2 b	1.54 b
	VI	31.6 bc	2.77 d

a. Data from Kanno *et al.* (1980)

b. For evaluation, 500 stubbles were randomly sampled from each block.

c. Percentage followed by the same letter within a column was not significantly different at the 5% level with Duncn's multiple range test from original data.

5 Additional laboratory tests on mating disruption

1) Laboratory bioassay of disruptant chemicals

To observe the male sexual behaviour induced by the female sex pheromone, a wind tunnel (45 × 45 × 240 cm, air flow rate: 0.3 – 0.4 m/sec.) was designed in the laboratory. When a cage containing a virgin female was suspended near the up-wind end, male moths released in the tunnel showed the following pheromone behavioural responses: up-wind orientation flight including casting and hovering, landing near the female (on the cage), and wing fanning while walking round the cage (mating dance).

Effect of Z-11-HDAL and Z-5-HD on the male behaviour was examined. Each compound was applied on five filter paper tips (100 μg each) suspended in the wind tunnel ca. 10 cm down-wind from the female cage, to enable the pheromone plumes to become permeated with the compound. Ten to 15 male moths were then introduced into the wind tunnel from an inlet hole near the down-wind end. Male behaviour was observed and recorded for the first 5 min. in the presence of the filter paper, and for further 5 min. after the removal of the compound.

Table 5 shows that both Z-11-HDAL and Z-5-HD compounds completely inhibited any pheromone behavioural response of male moths. The male behaviour in the presence of either compound was hardly distinguishable from that in the control I, i.e spontaneous activities. The difference between Z-11-HDAL and Z-5-HD in the behaviour was observed after the removal of the compounds; in the case of Z-5-HD, the pheromone behavioural response was initiated within a few minutes, while in the case of Z-11-HDAL, the effect of the treatment persisted during the observation period. In the latter, however, pheromone behavioural responses were seldom observed 10 min. or more later.

These data suggest that the mechanism involved in the behavioural disruption by Z-5-HD is similar to that by Z-11-HDAL. Furthermore, it is also suggested that habituation of the moths to Z-5-HD, if it exists, is not so effective in the sex pheromone perception of this species as that to Z-11-HDAL. (Table 5).

Table 5 Effect of the sex pheromone component, Z–11-hexadecenal, and the hydrocarbon, Z–5–hexadecene, on male pheromone behaviour in laboratory wind tunnel

Grade of male response[a]	Male responses[b]							
	Control I[c]		Control II[d]		Z–5–HD		Z–11–HDAL	
++	0[e]	0	6	6	0	3	0	0
+	0	0	0	0	0	2	1	0
–	6	6	0	0	6	1	5	6

a. ++ : 2 or more males simultaneously performed "mating dance", +: individual male(s) performed "mating dance", –: no pheromone behaviour was observed

b. No. of tests in which each response grade was observed; 6 replicates.

c. Solvent and black cage.

d. Left column in each treatment represents first 5 min. of observation and the right the second 5 min. (see text)

2) Electroantennogram (EAG) experiment

To evaluate the olfactory activities with each of the four compounds, namely the sex pheromone components Z-11-HDAL and Z-13-ODAL, and the hydrocarbons Z-5-HD and Z-5-OD, dose-response curves were obtained from male antennae by EAG technique. As shown in Fig. 3, the two pheromone components induced apparently higher EAG activites than the hydrocarbons and Z-5-HD which was much more active than Z-5-OD, was approximately 10 times less active than the pheromone component Z-11-HDAL. It was conspicuous that the level of differences in the EAG activity between Z-5-HD and Z-11-HDAL coincided well with that in the disruptive effect on male orientation obtained in the field test (see II-C). (Fig. 3)

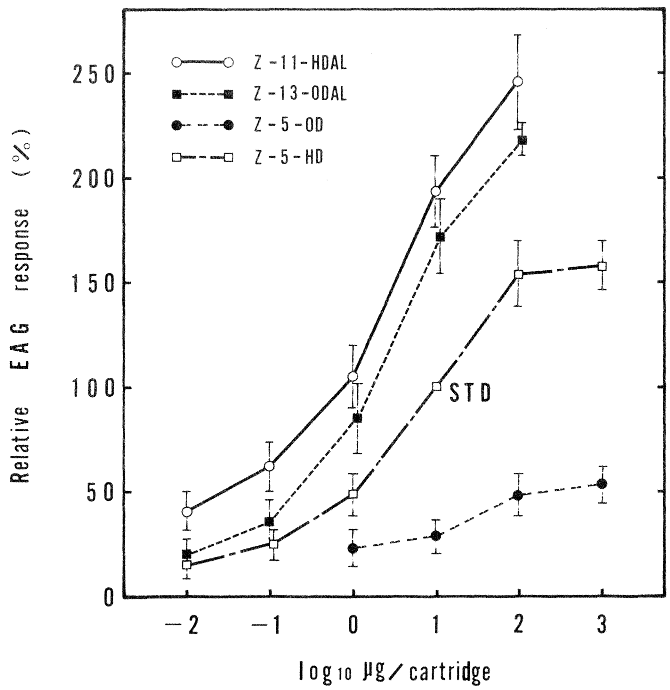

Fig. 3 EAG responses from male antennae of *Chilo suppressalis* to various concentrations of the sex pheromone components and the hydrocarbons. 10 μg of Z-5-hexadecene was used as the standard

Concluding remarks

It is well known that the sex pheromones in many insect species are composed of multiple components. In such cases, air permeation with an individual component can often disrupt the sexual communication between male and female (Yushima *et al.*, 1975; Tamaki *et al.*, 1975). The effect of the related compounds of the sex pheromones as disruptants has also been investigated in many species (McLaughlin *et al.*, 1972; Shorey *et al.*, 1974; Cameron *et al.*, 1975; Mitchell *et al.*, 1975; Rothschild, 1975).

Our present study also enabled to demonstrate that the sex pheromone components as well as numerous pheromone-like compounds disrupt male orientation in *Chilo suppressalis*. The results of this study emphasized that all the test compounds having the same carbon chain length (C-16 and C-18) with a Z-olefinic linkage at the same position from the methyl terminal as that of the pheromones showed a potent disruptive effect. Among these compounds, Z-11-HDAL as the major pheromone component and Z-5-HD were very effective in inhibiting male orientation and mating. It was also shown that the disruptive effect of Z-11-HDAL was apparently higher than that of Z-5-HD. Although Z-5-HD was shown to be ca. 10 to 30 times less effective with respect to the dose applied than Z-11-HDAL its chemical stability and the simplicity of its synthesis compensated such shortcoming. Actually, the application of Z-5-HD in the fields decreased injury of the plants by the larvae of *Chilo suppressalis*.

For disruption of sex pheromone communication in *Chilo suppressalis*, other pheromone-related compounds such as Z-9-tetradecenyl formate and Z-11-hexadecenyl formate, have been investigated as potential disruptants (Beevor *et al.*, 1977; Beevor and Campion, 1979). Experiments will be conducted to determine the extent of Z-5-HD disruptive activity compared with that of the formates.

Moreover, it is interesting to point out that hydrocarbons like Z-5-HD can be applied to the sex pheromone communication of other species that utilize aldehydes as pheromone components, such as *Heliothis* spp., *Plutella xylostella*, etc.

Disruptive effect of mixtures of the pheromonal components or of the other related C_{16} and C_{18} compounds on male orientation and mating are also of interest. Such investigations are under way in our laboratory.

Abstract

In the rice stem borer moth, *Chilo suppressalis* Walker, the effect of the sex pheromone components Z-11-HDAL and Z-13-ODAL, and their 12 structurally related compounds on sex pheromone communication between male and female was studied under field conditions. Relatively good disruption of male orientation could be achieved with most of the test compounds except acetates. Among these compounds, Z-11-HDAL and Z-5-HD were most effective as disruptants on male orientation. In the mating suppression test, all the four test compounds, sex pheromone components and hydrocarbons, significantly suppressed the mating and Z-11-HDAL and Z-5-HD in particular had a highly inhibitory effect on mating. It was also shown that the disruptive effect of Z-11-HDAL on male orientation was apparently higher than that of Z-5-HD in comparative field test. Nevertheless, it seems that Z-5-HD is more suitable for practical use than Z-11-HDAL, because of its chemical stability and the simplicity of its synthesis. Z-5-HD decreased the injury of the plants when applied over wide areas. This observation suggests that the mating frequency in the treated areas was decreased by the treatment with this compound. The experiments conducted in the laboratory such as wind tunnel and EAG experiments, also supported the field data.

Acknowledgement

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Discussion

Yoshimeki, M. (Japan): Did you consider the possible role of wind velocity and changes in direction in the attraction/ disruption effect of the sex pheromone complex?

Answer: We changed the locations of the compounds as often as possible so as to minimize the possible error due to factors such as wind velocity and changes in directions.

Yoshimeki, M. (Japan) **Comment**: I still have some reservations about the validity of such experiments.

Dyck, A. V. (International Rice Research Institute, the Philippines): 1) What is the meaning of "percent injured stems"? 2) Did you measure yield differences among the treatments?

Answer: 1) The term "percent injured stems" refers to the percentage of rice hills infested with the larvae. 2) Yield differences between the treated and the control areas were not determined.