



## Direct and Indirect Effects of Nitrogen Supply and Disease Source Structure on Rice Sheath Blight Spread

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### ABSTRACT

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Effects of nitrogen and type of disease source on focal expansion of rice sheath blight (caused by *Rhizoctonia solani*) were studied in two field experiments. Four disease source types, represented by inoculated individual hills at the center of experimental plots, were obtained by combining two inoculum doses with two heights of inoculation in the canopy. Compared with the low inoculum dose, there was a small but significant increase of disease severity on source hills with the higher inoculum dose, and no disease developed on noninoculated controls. Severity on the sheaths was higher on hills inoculated at the sheath level than on hills inoculated at the leaf level. Conversely, leaf severity was

higher on hills inoculated at the leaf level than on hills inoculated at the sheath level. Increased nitrogen supply increased host plant tissue contacts (leaf-to-leaf and leaf-to-sheath), increased the capacity of the canopy to retain moisture, and increased the leaf nitrogen content in both experiments. The size of sheath blight foci was much larger in the rainy (3,000 to 30,000 cm<sup>2</sup>) than in the dry (250 to 1,100 cm<sup>2</sup>) season. In both experiments, foci expanded most rapidly in the high nitrogen supply (120 kg per ha) level compared with medium (80 kg per ha) and no nitrogen supply. Positioning of inoculum in the upper layer of the canopy (leaf level) on source hills resulted in faster spread of foci than positioning in the lower layer (sheath level). Multiple regression and path coefficient analyses suggested that nitrogen drives focal expansion in sheath blight essentially via indirect effects: increased tissue contacts in the canopy and higher leaf wetness.

Rice sheath blight, caused by *Rhizoctonia solani* Kühn, anastomosis group 1 (7,22) is an increasing concern for rice production in South and Southeast Asia, especially in intensified production systems. These intensified systems rely heavily on the use of high-yielding cultivars, high crop densities, and high rate of fertilizer, especially nitrogen. All these factors have been considered in more or less detail as contributing to increased disease problems (7,21,22).

There are relatively few reports that specifically indicate that sheath blight can be reduced with resistant cultivars (22). The pathogen has an extremely wide host range, and no source of complete resistance to the pathogen has been found so far (7,22). To a large extent, the linkage between increased disease intensity and the use of high-yielding varieties seems to be attributable to corresponding changes in cropping practices and habitat for the pathogen.

High crop density, either through increased sowing rate in direct-seeded rice (20) or higher planting density in transplanted rice (16) has long been advocated as a major factor favoring increase of sheath blight. In Japan, the increased prevalence of the disease over the period 1955 to 1975 is largely attributed to increased planting density (21).

Reports of increased disease intensity associated with increased nitrogen fertilizer supply are also numerous (e.g., 13,15). Like stand density, increased growth caused by higher nitrogen supply results in denser and more humid canopies, and more intercepted light early in the season. Direct changes in the host's susceptibility with higher nitrogen supply have also been postulated, but this is still controversial (13,36,37).

Separating direct and indirect effects of nitrogen supply is difficult. Their influence on the dynamics of the pathogen via crop growth, crop physiology, and crop microclimate are confounded. While these difficulties are common in epidemiological field studies, they are compounded in the case of rice sheath blight by the influence of canopy growth on disease spread and the difficulty of accurately measuring the disease.

The objective of this study was to develop techniques to describe quantitatively the spread and increase of sheath blight epidemics, to evaluate how sheath blight is affected by nitrogen supply, and to assess hypotheses to explain the epidemiological response of sheath blight to nitrogen.

### MATERIALS AND METHODS

**Crop establishment.** Experiments were established at the International Rice Research Institute, Philippines, during a rainy (experiment 1) and during a dry (experiment 2) season. Nurseries of IR72, a short-cycle, high-yielding rice cultivar, were established on 15 July 1992 and 29 January 1993 for experiments 1 and 2, respectively. Seedlings were transplanted at a rate of 6 to 8 per hill, with a spacing of 20 cm between hills within rows and 20 cm between rows.

**Inoculum.** A *Rhizoctonia solani* anastomosis group AG1-1a isolate obtained from infected rice was maintained in culture tubes containing potato-dextrose agar medium. Prior to inoculation, the fungus was grown in petri dishes containing the same medium and incubated for 10 days at room temperature (20 to 27°C). Plugs of growing hyphae were then transferred to bottles containing an autoclaved substrate consisting of rice grain/rice

hull mixture (1:5, wt/wt) (26) and incubated at room temperature for 2 weeks.

**Experimental design.** Both experiments had a split-plot design with eight replications, in which nitrogen levels represented the whole plots and inoculation treatments represented the subplots. The individual subplot size was 3.4 × 3.4 m (17 × 17 hills). Within a main plot, subplots were separated from each other by a three-hill-wide border, and main plots by a 0.8-m path including a levee. Three nitrogen treatments were used:  $N_0$  (no nitrogen supply),  $N_1$  (40 kg per ha nitrogen as urea applied at seedling stage and 40 kg per ha nitrogen applied at maximum tillering), and  $N_2$  (as  $N_1$ , with an additional 40 kg per ha nitrogen applied when plants were in the boot stage).

Five inoculation treatments were introduced by varying the canopy position where inoculum was placed and the amount of colonized substrate. Treatment  $S_5$  was 5 g of colonized substrate applied at sheath level,  $L_5$  was 5 g at leaf level,  $S_{15}$  was 15 g at sheath level,  $L_{15}$  was 15 g at leaf level, and  $C$  was a noninoculated

control. Inoculations were done at 50 days after transplanting (i.e., at maximum tillering/panicle initiation). Prior to inoculation, the central hill of each plot was tagged. Inoculation (26) consisted in inserting in a tagged rice hill a specified amount of the rice grain/rice hull mixture colonized by the sheath blight pathogen.

**Disease measurement.** The establishment of disease in a subplot was assessed as the percentage of sheath and leaf area covered by sheath blight lesions on five tillers chosen at random on the inoculated hill at its center 12 days after inoculation. The shape of a sheath blight focus was assumed to be elliptical, and its area calculated as  $a = \pi (x \cdot y) / 4$ , where  $x$  and  $y$  are the length and width of the focus, respectively. The variables  $x$  and  $y$  were measured as the distance (cm) between the two most distal lesions in a subplot, on both sides of an inoculated hill within and across rows, respectively. Focal area,  $a$ , was assessed six times throughout the cropping season, starting 12 days after inoculation. A mean rate of increase of focal area ( $R$ ) over the six assessments was calculated as

$$R = 1/(n-1) \left[ \sum_{i=1}^{n-1} (a_{i+1} - a_i) / (t_{i+1} - t_i) \right]$$

where  $n = 6$  is the total number of observations,  $i$  the current observation number, and  $t$  the time since inoculation.

**Host and environmental factors.** Leaf wetness was assessed on nine mornings (6 a.m.) during the cropping season with a visual four-point rating scale (Fig. 1). On each morning observations were made for three layers of the crop canopy on each of ten hills chosen at random in each main plot. An average wetness rating  $w_n$  was calculated for each of the nine assessment dates as the average score over 10 hills and three layers in each whole plot (nitrogen treatment). The variable  $w_n$  was taken as a measure of the potential of a canopy to retain moisture before it evaporates. Canopy wetness was considered to reflect both this potential and rainfall patterns prevailing in the two experiments. The two seasons differed so much in rainfalls that the product  $W = w \times r$ , where  $r$  is the mean daily rainfall during an experiment, and  $w$  is the mean of nine average wetness ratings, was used to produce a simplified representation of canopy wetness throughout the cropping season.

Counts of leaf-to-leaf and leaf-to-sheath contacts between neighboring plants were made at three development stages of the crop: panicle initiation, booting, and ripening. One hill was chosen at random in each subplot, and counts were made from visual observation of such contacts between this hill and its eight neighbors. Areas under leaf-to-leaf and leaf-to-sheath contact progress curves were calculated, as well as areas under cumulative (leaf-to-leaf and leaf-to-sheath) contacts ( $C$ ).

The nitrogen contents of rice leaves were measured at the same development stages as were the tissue contacts. One hill was sampled at random in the noninoculated plot of each main plot (nitrogen level) in each block. The nitrogen concentration of the leaves was determined with the Kjeldahl mineralization method (33). Areas under leaf nitrogen content progress curves ( $N$ ) were computed.

**Data analysis.** Sheath blight severities on the leaves ( $l$ ) and on the sheaths ( $s$ ) on the source hills at 12 days after inoculation were analyzed with analysis of variance (14,24). The subplot factor, inoculation, was partitioned into effects of inoculum amount and height (8,14). Analyses of variance of the area of foci ( $a$ ) were done for each observation date in both experiments. The average leaf wetness data ( $w$ ) per observation date were analyzed across nitrogen levels with a one-way analysis of variance procedure. Log-transformed areas under leaf-to-leaf and leaf-to-sheath contact progress curves were also analyzed using a split-plot design. The leaf nitrogen content was analyzed in both experiments using a one-way analysis of variance across nitrogen levels.

Five variables— $N$ ,  $C$ ,  $W$ ,  $s$ , and  $l$  (Table 1)—were used to describe variation of focal expansion rates ( $R$ ) in inoculated plots, in

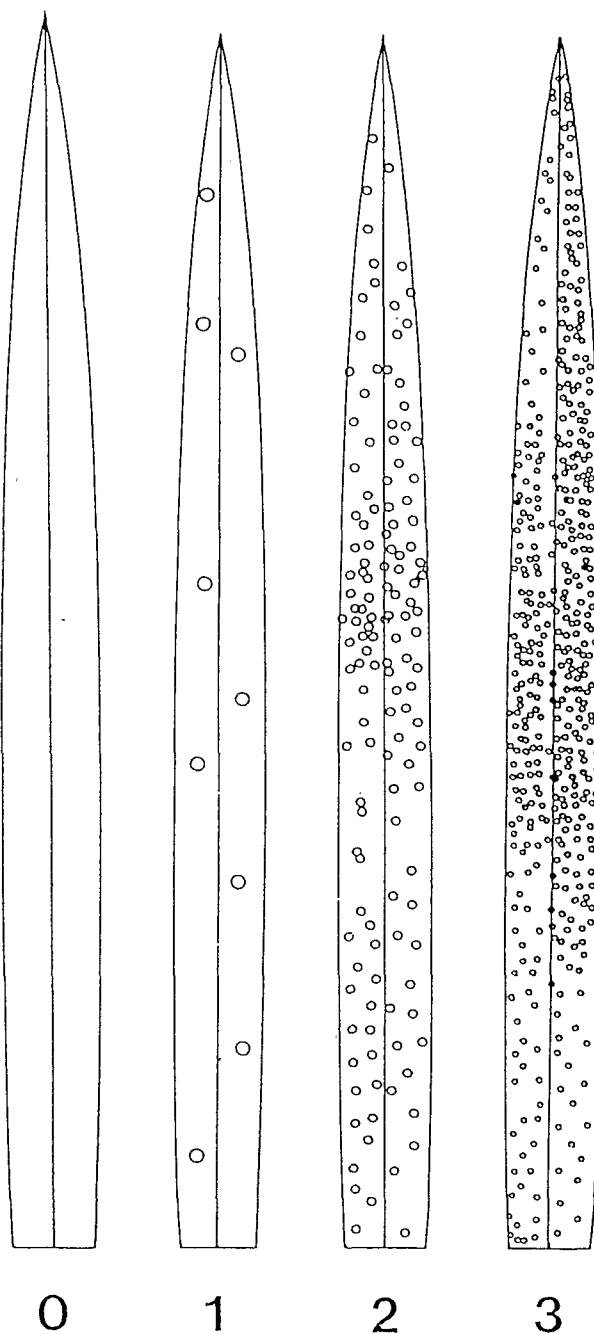


Fig. 1. Diagrammatic scale used to assess leaf wetness of the canopy.

a stepwise, forward-selection, regression analysis. The regressions were performed on subplot means averaged over the eight replications (8), with data combined from the two experiments. The dependent variable was focal expansion rate transformed as natural logarithm ( $\ln(R + 1)$ ) prior to regression to reduce variance heterogeneity (6). Several regression models with predetermined combinations of predictors ( $W$ ,  $C$ ,  $s$  and  $l$ ;  $W$  and  $C$ ;  $s$  and  $l$ ;  $N$ ,  $W$  and  $C$ ;  $N$ ,  $s$  and  $l$ ) were also tested. The resulting equations were assessed by aptness of residual plot, coefficient of determination, and  $F$  test (25).

Correlation analysis indicated colinearity among predictors. Path coefficient analysis (2,5,9,12,29-31) was used to assess the direct and indirect contributions of multiple, often correlated factors, on focal expansion. Results provided by this technique included (i) a descriptive synthesis of regression equations, and (ii) determination of direct and indirect effects of experimental factors.

## RESULTS

**Disease intensity on source hills.** Mean sheath blight severity on the leaves was higher on source hills inoculated at the leaf level (for  $L_5$  and  $L_{15}$ ,  $34 \pm 2$  and  $47 \pm 2\%$ , respectively, in experiment 1, and  $24 \pm 2$  and  $31 \pm 3\%$ , respectively, in experiment 2) than on source hills inoculated at sheath level (for  $S_5$  and  $S_{15}$ ,  $17 \pm 2$  and  $23 \pm 2\%$ , respectively, in experiment 1, and  $13 \pm 1$  and  $19 \pm 2\%$ , respectively, in experiment 2). Disease severity on the sheaths was higher on the hills inoculated at sheath level (for  $S_5$  and  $S_{15}$ ,  $16 \pm 2$  and  $18 \pm 2\%$ , respectively, in experiment 1, and  $7 \pm 1\%$  and  $10 \pm 2\%$ , respectively in experiment 2) than on hills inoculated at leaf level (for  $L_5$  and  $L_{15}$ ,  $3 \pm 1$  and  $9 \pm 2\%$ , respectively, in experiment 1, and  $6 \pm 1$  and  $6 \pm 1\%$ , respectively, in experiment 2). Severity of sheath blight increased significantly with increasing amounts of inoculum (Table 2). There was also a small but significant increase of severity on the sheath with increased inoculum amounts. The noninoculated controls had small to neg-

contacts were slightly more frequent in the noninoculated controls of experiment 2 ( $C = 1,037 \pm 122$ ,  $L_5 = 841 \pm 93$ ,  $S_5 = 944 \pm 113$ ,  $L_{15} = 889 \pm 112$ ,  $S_{15} = 948 \pm 111$  contacts per day;  $P < 0.01$ ), probably because of the growth-reducing effect of the disease. Similar results were obtained for leaf-to-sheath contact curves, with a strong nitrogen effect ( $N_0 = 118 \pm 11$ ,  $N_1 = 271 \pm 19$ ,  $N_2 = 219 \pm 14$  contacts per day in experiment 1;  $N_0 = 72 \pm 11$ ,  $N_1 = 189 \pm 12$ ,  $N_2 = 191 \pm 12$  contacts per day in experiment 2) which was significant in both experiments ( $P < 0.01$ ). Leaf-to-sheath contacts were slightly less frequent in some of the inoculation treatments of experiment 2, probably due to reduction in canopy growth by disease in these treatments ( $C = 193 \pm 21$ ,  $L_5 = 139 \pm 14$ ,  $S_5 = 149 \pm 18$ ,  $L_{15} = 121 \pm 16$ ,  $S_{15} = 151 \pm 18$  contacts per day;  $P < 0.01$ ). There was a significant variation ( $P < 0.01$ ) in both leaf-to-leaf and leaf-to-sheath contacts among replications in experiment 1 due to heterogeneity in crop growth over the experimental area.

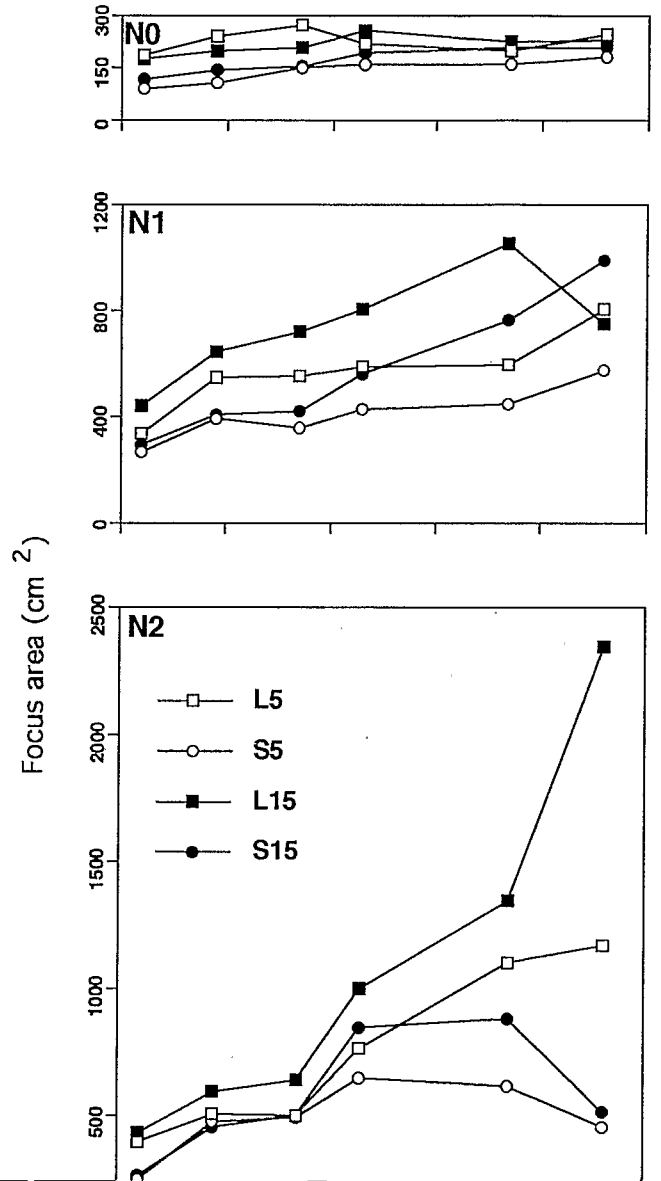
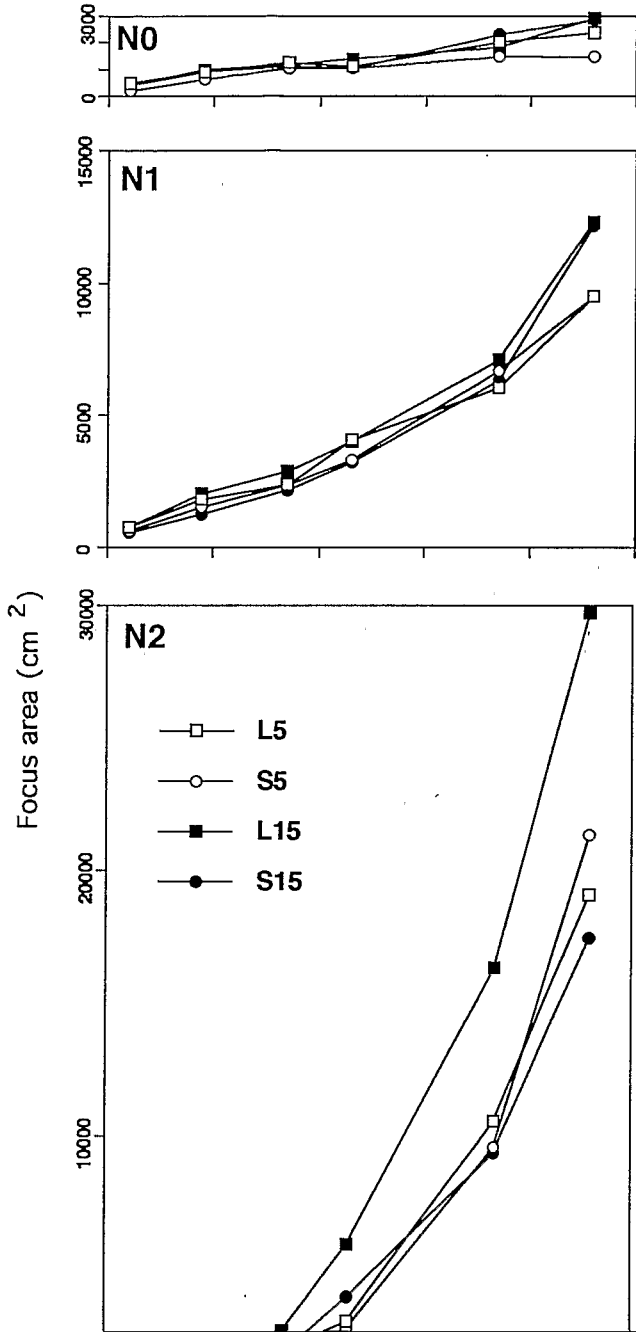
**Leaf wetness.** In both experiments, the nitrogen treatments resulted in significant differences in mean leaf wetness ratings at 6 a.m. ( $w$ ) averaged over 9 observations ( $N_0 = 1.38 \pm 0.12$ ,  $N_1 = 1.70 \pm 0.11$ ,  $N_2 = 1.78 \pm 0.10$ ,  $P < 0.05$ , in experiment 1;  $N_0 = 1.61 \pm 0.11$ ,  $N_1 = 2.11 \pm 0.14$ ,  $N_2 = 2.38 \pm 0.07$ ,  $P < 0.01$ , in experiment 2). There were strong differences between the two experimental seasons in accumulated rainfall during the observation periods (424.5 mm in 35 days in experiment 1; 19.9 mm in six days in experiment 2). These rainfall differences, combined with differences in  $w$ , resulted in contrasted values of leaf wetness indices ( $W$ ), ranging from 0.58 ( $N_0$ , experiment 2) to 13.27 ( $N_2$ , experiment 1).

**Leaf nitrogen.** Significant differences were observed in areas under leaf nitrogen content curves ( $N$ ) among the three nitrogen treatments ( $P < 0.01$  in both experiments). First experiment means:  $N_0 = 70.3 \pm 1.8$ ,  $N_1 = 77.2 \pm 1.8$ ,  $N_2 = 85.6 \pm 4.0\%$  per day; second experiment means:  $N_0 = 65.0 \pm 1.6$ ,  $N_1 = 65.0 \pm 2.0$ ,  $N_2 = 75.0 \pm 2.5\%$  per day.

**Focal expansion.** Focal expansion was strongly influenced by

In the second experiment, sheath blight foci had very low rates of expansion in treatment  $N_0$  (Fig. 3). In  $N_1$  and  $N_2$ , inoculation treatments  $L_5$  and  $L_{15}$  resulted in greater focal areas than  $S_5$  and  $S_{15}$ . In some cases ( $S_5$  and  $S_{15}$  in  $N_2$ , and  $L_{15}$  in  $N_1$ ), focal area

declined in the period just before the last assessment date. In general, however,  $L_{15}$  corresponded to the largest maximum focal size in  $N_1$  and  $N_2$ . The analyses of variance (Table 3) over successive observation dates showed a significant effect of nitrogen over the entire observation period, and a significant contrast between inoculated versus controls was indicated. The effect of inoculum position on the source hills on focal size was significant on all assessment dates, while the effect of inoculum amount was significant only early in the experiment.



**Regression and path analysis.** Significant colinearity occurred among the variables *l*, *s*, *C*, *W*, and *N* (Table 4). A first regression equation (Table 5) in which all variables were entered into the model accounted for a very large fraction of the variation in *LR* ( $R^2 = 0.959$ ). The contribution of *N* to this first model was, however, not significant ( $P = 0.186$ ). Its removal led to another equation involving *W*, *C*, *s*, and *l*, which accounted for a similar variation in *LR* ( $R^2 = 0.957$ ). This second equation was also the outcome of a stepwise, upward-selection procedure involving initially all variables. A regression equation involving *W* and *C* only accounted for a large fraction of variation in *LR* ( $R^2 = 0.939$ ), while a regression equation involving *s* and *l* only accounted for a small fraction ( $R^2 = 0.378$ ). An analysis involving *N*, *W*, and *C* indicated that consideration of *N* in combination with *W* and *C* was superfluous in describing the data. Conversely, replacing *W* and *C* by *N* in the second equation (and involving *N*, *l*, and *s* simultaneously,  $R^2 = 0.870$ ) did not improve data description.

Path coefficient analysis partitioned correlation among variables into direct and indirect effects on sheath blight focal expansion (Fig. 4). This analysis identified two groups of variables affecting *LR* in the combined data set: environment-related and crop growth-related variables (*C* and *W*), which are affected to a large degree by nitrogen treatments and initial disease variables (*s* and *l*), reflecting the inoculation treatments. Strong direct effects of *W* and *C* on *LR* were indicated. The direct effect of *N* on *LR* was marginal (as shown in Table 5), but its effects on *W* and *C* were strong and significant ( $P < 0.0001$ ). The effect of *s* on *LR*

was weaker than that of *l*, but the two variables showed some degree of colinearity, indicating that *s* affected *LR* partly via *l* (and vice versa). *U* represents all undetermined influences on *LR*.

Path coefficient analyses using the same framework led to similar conclusions when experiments 1 and 2 were considered separately. The analyses yielded significant ( $P < 0.05$ ) path coefficients between *W* and *LR* (0.235 and 0.335 for experiments 1 and 2, respectively) and between *C* and *LR* (0.554 and 0.157), while path coefficients between *N* and *LR* were not significant. The path coefficients between *l* and *LR* (0.106 and 0.211) were larger than those between *s* and *LR* (0.012 and 0.039).

## DISCUSSION

Crop growth, as measured by frequency of contacts between plant organs, was greater in the second (dry season) than in the first (rainy season) experiment; this was likely due to differences in the amount of radiation received by each crop. Differences in crop growth among experiments, however, were small relative to within-experiment variation in both leaf-to-leaf (a threefold to fourfold increase) and leaf-to-sheath contacts (a twofold to threefold increase in both experiments) across the range of nitrogen treatments employed. The nitrogen treatments also resulted in small, but significant, differences in the nitrogen content of the leaves (an increase of 17.8 and 13.4% from  $N_0$  to  $N_2$  in experiments 1 and 2, respectively). Additionally, increased nitrogen supply to the crop resulted in an increased capacity of the canopy to retain moisture (*w*).

TABLE 3. Analyses of variance of area of rice sheath blight foci (*a*) measured at several observation dates (*t*) after inoculation of a source hill at the center of rice plots

Source of variation <sup>a</sup>	df	Significance of main effect or interaction term <sup>b</sup>											
		Experiment 1 <sup>c</sup>					Experiment 2 <sup>c</sup>						
		<i>t</i> = 12	<i>t</i> = 19	<i>t</i> = 27	<i>t</i> = 33	<i>t</i> = 47	<i>t</i> = 56	<i>t</i> = 13	<i>t</i> = 21	<i>t</i> = 27	<i>t</i> = 35	<i>t</i> = 49	<i>t</i> = 56
Replication (R)	7			*	**			**	**	*			
Nitrogen level (N)	2	**	**	*	**	**	**	**	**	**	**	**	*
Error (a)	14												
Inoculation (I)	4	**	**	**	**	**	**	**	**	**	**	**	**
a2: among inoculations	3	**	**	*				**	**	**	**	**	*
Height (H)	1	**	**	*	*			**	**	**	**	**	**
Amount (A)	1	*	*					*		*	**	**	
H × A	1												
Control vs a2	1	**	**	**	**	**	**	**	**	**	**	**	**
N × I	8	*	**		**			**	**	**	**	**	**
N × a2	6		*							**	**	**	**
N × H	2									**	**	**	**
N × A	2									*		*	
N × A × H	2												
N × (Control vs a2)	2	**	**	**	**	*	**	**	**	**	**	**	**
Error (b)	84												
Total	119												
cv (a) %		7.1	5.4	9.2	7.3	25.1	22.2	4.4	5.5	7.6	7.3	9.8	24.4
cv (b) %		6.2	4.6	6.9	7.8	17.8	16.6	5.3	5.2	5.2	7.4	7.2	21.4

<sup>a</sup> The analysis includes partitioning of effects of height of positioning and amount of inoculum in the source hills.

<sup>b</sup> \*\*:  $P < 0.01$ ; \*:  $P < 0.05$ .

<sup>c</sup> Experiment 1: rainy season; experiment 2: dry season.

The inoculation treatments resulted in the expected pattern of blight of soybean is favored by permanent wetness and correla-

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