The Resistances to Rice Stripe Virus and Small Brown Planthopper in Rice Variety, IR 50

Hiroshi Nemoto¹⁾, Kouichi Ishikawa and Eiji Shimura

National Agriculture Research Center, Kannondai, Tsukuba, Ibaraki, 305

¹⁾ Present address : Ibaraki Agricultural Center, Plant Biotechnology Institute, Kamikunii, Mito, Ibaraki 311-42

Summary

Mechanism and inheritance of resistance to rice stripe virus (RSV) in several varieties were studied. It was apparent that Musashikogane and Minamihatamochi were highly resistant and IR 50 and TN 1 were moderately resistant to virus infection. IR 50 was found to be resistant to the small brown planthopper (SBPH) which was the vector of RSV. The inheritance of resistances to RSV and SBPH were studied by using the F₃ and F₆ generations of a cross of IR 50 with a susceptible variety, Reimei. The distribution of RSV and SBPH resistances at F₃ and F₆ were continuous and suggested a plural-genes control of resistance. The F₃ and F₆ lines were classified into groups of resistant (R), moderately resistant (M) and susceptible (S) to RSV infection and the SBPH resistance level of each group was evaluated. No significant difference was observed between the R and M+S groups of F₃, however, the R group of F₆ was significantly more resistant than the M and S groups. Thus an influence of SBPH resistance on the RSV infection of F₆ was suspected. It was estimated that SBPH resistance was effective in reducing RSV infection, and the RSV resistance in IR 50 based on the resistances to SBPH and virus infection and they were controlled by plural-genes.

Key Words: *Oryza sativa*, rice stripe virus, small brown planthopper, resistance.

Introduction

Rice stripe virus (abbr. RSV) is one of the most serious disease of rice in the temperate regions of East Asia. An estimated 500,000 to 620,000 hectares of paddy field in Japan was damaged by RSV every year during the period of 1963 to 1967. In 1986 alone, RSV attacked an estimated 170,000 hectares of paddy field (Syumiya and Nemoto 1990). This virus is transmitted in a persistent manner by some species of planthopper, predominantly, by the small brown planthopper (*Laodelphax striatellus* Fallén, abbr. SBPH). The typical symptoms of this disease are chlorotic stripes and mottling of the leaves. In a severe infection, some leaves emerge folded and twisted. Finally, the growth terminates and the plants die. These symptoms are called "Yuurei" in

Japanese which means "ghost".

Yamaguchi *et al.* (1965) reported that the genetic source for RSV resistance was abundant among the *indica* and *javanica* varieties, while all of the lowland *japonica* varieties were susceptible. Washio *et al.* (1967, 1968) reported that the RSV resistance in Japanese upland varieties is controlled by two pairs of dominant complementary genes *Stv-a* and *Stv-b*, and resistance in the *indica* varieties, including a Pakistani variety Modan, is controlled by one pair of incomplete dominant gene *Stv-bⁱ*. Two *japonica* resistant lines, St. No1 and Chugoku 31, were bred by Toriyama *et al.* (1966) and their resistance originated from Modan. Thereafter the breeding for RSV resistance was continued using these two lines as resistant donors. Resistant varieties such as Mineyutaka, Musashikogane, Hoshinohikari, *etc.* were bred and released to farmer. Hence RSV resistance in Japanese lowland varieties originated from Modan. If a virus strain can overcome the *Stv-bⁱ* of Modan, all of Japanese resistant varieties would become susceptible. Thus, RSV resistance sources need to be diversified.

One way to slow down the breakdown of a resistant variety is to produce a variety with diversified resistance functions. The accumulation of resistance genes with similar function is considered to be useful method to make stable resistant variety, however, the accumulation of several different resistance factors is also expected to be effective to control break-down. Resistance to virus transmitted by an insect can be due to resistances to the virus, vector or both. Furthermore, resistance to virus can be divided into resistance to virus infection and tolerance to virus (Fig. 1). In a successful breeding program to accumulate many resistance factors, it is necessary to identify resistant sources with the required resistance factors. The mode of inheritance of these factors also need to be understood. In this report, the resistance mechanism of some rice varieties was analysed. The inheritance of resistance factors in IR 50, an *indica* variety resistant to RSV, was also studied.

Fig. 1. A concept of resistance to rice stripe virus.

Materials and Methods

1) Analysis of resistance mechanism in some varieties

The resistance to RSV in six varieties namely *indica* varieties IR 50 and TN 1, Japanese lowland varieties Musashikogane, Nipponbare and Reimei and Japanese upland variety Minamihatamochi were analysed. The RSV resistance to virus infection were tested by four methods, i.e. the field test, the net house test, the individual and mass inoculation tests. The SBPH resistance was evaluated by the non-preference and antibiosis tests. The test methods were described below.

Field test

The field test was done in 1984 at the Kounosu Branch of National Agriculture Research Center in Saitama, during a period of serious RSV epidemic in the prefecture. The lines were transplanted to the field in May which was the most susceptible period to the natural infection. The rate of viruliferous SBPH was estimated to be about 30 % by the random sampling test of hemagglutination method. Each test line was represented by one row of 25 plants, with a 18×30-cm spacing. The RSV incidence was symptomly evaluated at about 60 days after transplanting.

The net house test

The germinated seeds were sown in a 60×30-cm seedling case which had 12 rows and 13 hills per row. One row represented a line and each line was replicated twice. When seedlings were about at 1.5 leaf stage, case was transferred into a mosquito net (1.8×3.0×1.5-m) containing about 500 SBPH. The frequency of viruliferous SBPH of this population was randomly tested by the Enzyme-linked immunosorbent assay (abbr. ELISA) method and estimated that about a half of SBPH carried virus. The seedlings were exposed for inoculation access for seven days. After inoculation, the seedlings were applied with insecticides and transferred into a green house. The frequency of plants showing RSV symptoms was assessed at 30 days after inoculation.

The individual inoculation test

In this method, every line was inoculated isolately from the other lines. Twenty seeds of a line were sown in a petri dish filled with soil. A petri dish was covered by a plastic cylinder and fixed together by using of vinyl tape. The upper end of the cylinder was covered with tetron gossamer. Two hundred nymphs of SBPH at second to third instar were released into each cylinder when the seedlings were at 1.5 leaf stage. The rate of viruliferous SBPH was estimated to be about 80 % by using ELISA method. The inoculation access period was three days and the experiments were replicated three times.

The inoculated seedlings were maintained under the artificial light for seven days after inoculation. The

RSV infection on each seedling was tested by using the simplified ELISA method as described by Ishikawa *et al.* (1987). Samples were crushed in the well of ELISA plate which was precoated with γ -globulin. After adding the conjugate, the plates were kept at 37 °C for four hours and then washed. Substrate was added and the reaction were evaluated at 405-nm by using a Microplate reader MR 600 (Dynatech). The γ -globulin and conjugate used in the experiments were produced in the Virus Control Laboratory of National Agriculture Research Center, Tsukuba, Japan.

The mass inoculation test

In this method, test lines were inoculated together in a cage, and the SBPH could choose any individual lines. The germinated seeds were sown in rows in a 15.0×26.0-cm plastic tray. Each row of 10 seeds represented a line and each line was replicated twice. The tray was placed in a cage of 32×32×43 (height)-cm. Second to third instar nymphs of SBPH were released into the cage when the seedlings were at 1.5 leaf stage. The inoculation were continued for three days and at the rate of about 10 SBPH per seedling. The percentage of viruliferous SBPH was estimated to be about 80 % by using ELISA method. The percentage of infected plant was determined by the simplified ELISA method.

The non-preference test

The germinated seeds were sown in 24 rows in a 15.0×26.0-cm plastic tray. Each row of 10 seeds represented a line and each line was replicated four times. The two trays were placed in a cage of 32×32×43-cm. Second to third instar nymphs of SBPH were released into the cage when seedlings were at 1.5 leaf stage. The number of SBPH that settled on each row was counted every day. Total number of settled SBPH for three days was utilized as the index of the non-preference of each line.

The antibiosis test

Seedlings of a line at 2.0 leaf stage were placed in a 2.5×20.0-cm test tube with some water. Five SBPH nymphs at second to third instar were introduced into each test tube. The upper top of the test tube was covered by the tetron gossamer. The survival rate of the introduced SBPH was determined at five days after the introduction and was used as the index of the antibiosis of each line. The experiments were done in five replications.

2) Genetic analysis of resistance to RSV and SBPH in IR 50

IR 50 is a semi-dwarf *indica* variety bred at International Rice Research Institute (IRRI) in the Philippines. Reimei is a *japonica* type variety. The cross of Reimei and IR 50 was made in 1981, and its hybrid population had been grown in field and maintained at every generation by the two-seeds-descent method. In 1987, populations of F₂ and F₅ were grown in the field. About 50

plants of each generation were assessed for their major agronomic characteristics, such as heading date, culm length and panicle length, seed fertility and shattering. These plants were harvested individually and used as the material lines to study the mode of inheritance of RSV and SBPH resistances. RSV and SBPH resistances were assessed by using the mass inoculation test and the non-preference test respectively.

The virus source and the SBPH used in these experiments were collected at the Kounosu Branch of National Agriculture Research Center which was located at Saitama, Japan. After collection, the SBPH was selected for their ability to carry virus in the Paddy Insect Laboratory in National Agriculture Research Center. These selected SBPH were used in the experiments except for the field test.

Results

1) Analysis of resistance mechanism in some varieties

Table 1 shows the reactions of tested varieties to RSV infection under four test methods. Ninety-six percent of Nipponbare and 76 % of Reimei were infected in the field test. IR 50, TN 1, Musashikogane and Minamihata-mochi did not show any symptoms. In the net house test, the incidences of RSV infection on Nipponbare and Reimei were 77 and 85 % respectively, and these were significantly higher than those of the other four varieties. IR 50, TN 1, Musashikogane and Minamihata-mochi showed an obviously low infection rate by these two test methods. Also no significant difference was observed between these varieties. However, results of the individual inoculation test showed that RSV incidences on Musashikogane and Minamihata-mochi were 27 and 25 %, whereas on IR 50 and TN 1, they were 60 and 58 % respectively. There was a significant difference between the two groups. The results of the individual inoculation test indicated that Musashikogane and Minamihata-mochi showed a low infection rate consistently similar to those obtained in the field and the net house tests. However, RSV incidences on IR 50 and TN 1 were higher than those of the first two tests. The mass inoculation test gave RSV incidence on TN 1 as

Table 1. Rice stripe virus incidence of test varieties under four test methods

Varieties	Field test ¹⁾	Net house test ¹⁾	Inoculation test	
			Individual ²⁾	Mass ²⁾
IR50	0 ^a	19 ^a	60 ^b	20 ^a
TN1	0 ^a	15 ^a	58 ^b	55 ^b
Musashikogane	0 ^a	8 ^a	27 ^a	21 ^a
Minamihata-mochi	0 ^a	19 ^a	25 ^a	20 ^a
Nipponbare	96 ^b	77 ^b	90 ^c	70 ^b
Reimei	76 ^b	85 ^b	82 ^c	60 ^b

¹⁾ Based on symptom observation.

²⁾ Based on symplified ELISA method.

³⁾ Means with the same letter are not significantly different at 0.05 by DMRT.

55 % which was almost comparable to the level obtained by the individual inoculation test. However, the RSV incidence on IR 50 was 20 % which was lower than that in the individual inoculation test and comparable to the level of infection on Musashikogane and Minamihata-mochi.

Table 2 shows the reaction of tested varieties to SBPH. The non-preference test showed the total number of settled SBPH on TN 1, Musashikogane and Minamihata-mochi were comparable to those on Nipponbare and Reimei which were infected by RSV with high percentage. However, the total number of settled SBPH on IR 50 was significantly lower than those on the other five varieties. In the antibiosis test, the average survival rate of SBPH on IR 50 was also lower than those of the other five varieties.

2) Genetic analysis of resistance to RSV and SBPH in IR 50

Fig. 2 shows the reactions to SBPH resistance of the F₁, F₃ and F₆ generations from the cross of Reimei and IR 50. The total number of settled SBPH on F₁ plants located between those of IR 50 and Reimei. The total number of settled SBPH of F₃ and F₆ lines distributed continuously. Fig. 3 shows the segregation patterns to RSV infection in the F₁, F₃ and F₆ generations. The percentage of RSV infection of F₁ generation was located between the parents. The frequency of the F₃ and F₆ lines on RTV infection were continuously distributed between parents.

There was no correlation between RSV and SBPH resistances in the F₃ and F₆ generations of the cross (correlation coefficient at F₃: 0.1435, F₆: -0.0821). The RSV resistance of F₃ and F₆ lines were classified into groups of resistant (R) and moderately resistant to susceptible (M + S), and groups resistant (R), moderately resistant (M) and susceptible (S) respectively. The groups' antibiosis and non-preference to SBPH were compared in Table 3. No significant difference to SBPH resistance was observed between R and M+S groups of the F₃ generation. However, significant difference occurred in the SBPH resistance in groups of the F₆. The antibiosis and non-preference values of R group of

Table 2. The resistance to small brown planthopper of rice varieties

Varieties	Non-preference ¹⁾	Antibiosis ²⁾
IR50	49 ^a	64 ^a
TN1	95 ^{bc}	92 ^b
Musashikogane	88 ^b	100 ^b
Minamihata-mochi	118 ^c	88 ^b
Nipponbare	110 ^{bc}	96 ^b
Reimei	82 ^b	92 ^b

¹⁾ Non-preference was indicated by the number of total SBPH settled on test line for three days.

²⁾ Antibiosis was indicated by the survival rate of SBPH at five days after introduction.

³⁾ Means with the same letter are not significantly different at 0.05 by DMRT.

Fig. 2. The segregation for the non-preference to small brown planthopper (SBPH) in F_1 , F_3 and F_6 generations of the cross between IR 50 and Reimei.

Fig. 3. The segregation for the rice stripe virus (RSV) incidence in F_1 , F_3 and F_6 generations of the cross between IR 50 and Reimei.

Table 3. Comparison of resistance to small brown planthopper (SBPH) in the reaction groups to rice stripe virus (RSV) in F_3 and F_6 generations of the cross between IR50 and Reimei

Generation Varieties	Reaction group to RSV	SBPH resistance	
		Non-preference ¹⁾	Antibiosis ²⁾
F_3 (Reimei/IR50)	R group ³⁾	47bc	36bc
	M+S group	51c	38c
F_6 (Reimei/IR50)	R group	42a	17a
	M group	53c	34b
	S group	48c	24ab
IR50		38a	16a
Reimei		62d	53c

¹⁾ Non-preference was indicated by the total number of settled SBPH on plant for three days.

²⁾ Antibiosis was indicated by the survival rate of SBPH at five days after introduction.

³⁾ Test lines were classified by their RSV incidence.

F_3 generation R: 0-20 %, M+S: 21-100 %

F_6 generation R: 0-20 %, M: 21-50 %, S: 51-100 %

⁴⁾ Means with the same letter are not significantly different at 0.05 by DMRT.

F_6 were comparable to that of IR 50, while the values of M and S groups were significantly higher than those of the R group and IR 50. Therefore, lines within the R group of F_6 generation were similar to that of IR 50, while lines of M and S groups took a middle position between Reimei and IR 50 in terms of their SBPH resistance.

Table 4 shows the correlation coefficient of RSV and SBPH resistances to other agronomic characteristics. Heading time, culm and panicle lengths, seed fertility and shattering were independently inherited with RSV and SBPH resistances.

Discussion

In general, it is not easy to study the mechanism and inheritance of resistance to viral diseases transmitted by insect. Their virus resistance is sometimes correlated to the vector resistance. The study of rice viruses is relatively more advanced in rice tungro virus (abbr. RTV) which is the most serious viral disease in the tropics. Kobayashi *et al.* (1983) and Takita and Habibuddin (1986) reported that RTV resistance in IR 42 was based on its resistance to the vector and its resistance was controlled by two pairs of dominant genes. Daquioag *et al.* (1984) and Dahal and Hibino (1985) reported that rice varieties Habiganj DW 8, Utri Merah, Utri Rajapan were resistant to RTV but susceptible to the vector, and their resistance were based on resistance to rice tungro spherical virus (RTSV). Hibino (1988) identified some IR lines with RTSV resistance that originated from TKM 6. Daquioag *et al.* (1986) found that the tolerant variety Balimau Putih that was susceptible to RTV infection but showed only light symptoms.

In the case of RSV resistance, Washio *et al.* (1967, 1968) studied the inheritance of resistance to virus infection with the artificial inoculation method which was similar to our individual inoculation test to eliminate effect of SBPH resistance. They reported that RSV resistance was mainly controlled by virus resistance genes, *Stv-a*, *Stv-b* or *Stv-bⁱ*. According to Washio's results, it was guessed that Minamihatamochi had *Stv-a* and *Stv-b*, while Musashikogane, IR 50 and TN 1 had *Stv-bⁱ*. However, our study indicated that Musashikogane and Minamihatamochi were highly resistant to RSV infection, while TN 1 and IR 50 were moderately resistant and their resistant reaction was different from that

Table 4. The correlation coefficient of resistances to rice stripe virus (RSV) and small brown planthopper (SBPH) with other agronomic characteristics of the F_6 lines of IR50 and Reimei

Resistance	Heading time	Culm length	Panicle length	Seed fertility	Seed shattering
RSV ¹⁾	-0.003	0.189	-0.024	0.066	-0.145
SBPH ²⁾	0.022	0.217	0.246	0.246	-0.122

¹⁾ RSV resistance was indicated by RSV incidence in the mass inoculation test.

²⁾ SBPH resistance was indicated by the antibiosis test.

of Musashikogane (Table 1). Okamoto and Inoue (1967) reported that SBPH resistance source was abundant among the *indica* varieties which could control the survival and multiplication of SBPH. However, their results showed that SBPH resistance was not stable and complete control was difficult to be achieved. In our studies, it was indicated that TN 1 was susceptible to SBPH, while IR 50 was resistant to SBPH. RSV infection on TN 1 was almost similar under both mass and individual inoculation tests. On the other hand, RSV infection on IR 50 was lower under the mass inoculation test. In the individual inoculation test, the released SBPH were forced to suck the test seedlings, hence the vector preference was eliminated and the resistance to virus was fully tested. On the other hand, the preference existed under the mass inoculation test. SBPH resistant seedlings had fewer SBPH on them, hence their RSV incidence became relatively lower than the SBPH susceptible seedlings. The low level of RSV incidence on IR 50 under the mass inoculation test (Table 1) was believed to be due to its resistance to SBPH (Table 2). Based on our present results, the mechanism of resistance among the tested varieties were;

IR 50 : Moderately resistant to virus infection,
Resistant to vector.

TN 1 : Moderately resistant to virus infection,
Susceptible to vector.

Musashikogane : Highly resistant to virus infection.
Susceptible to vector.

Minamihatamochi : Highly resistant to virus infection.
Susceptible to vector.

Nipponbare : Susceptible to virus and vector.

Reimei : Susceptible to virus and vector.

The evaluation of SBPH resistance was tried by the non-preference test and antibiosis test in this study. Tested varieties showed similar results under both methods (Table 2). The non-preference test is more convenient than the antibiosis test. However, 12 lines are evaluated in a cage and it is possible that the number of settled insect is influenced by the composition of test materials. For example, when a moderately resistant line is tested with other resistant or susceptible lines, the evaluation of moderately resistant line will be fluctuated by the partners. The refining is necessary to non-preference for SBPH resistance.

Ishikawa *et al.* (1987) reported that RSV resistance could be classified to the Musashikogane-type or the

TN1-type depending on their RSV incidence and symptoms. Varieties belonging to the Musashikogane-type showed a lower percentage of RSV incidence but the infected plants showed "Yuurei" symptoms which was serious symptoms. On the other hand, the TN 1-type showed moderate values of percentage of RSV incidence but the infected plants rarely showed "Yuurei" symptoms. The difference between both types was thought to be based on the degree of resistance to virus infection and tolerance. It was considered that the Musashikogane-type had a high resistance to infection but without tolerance. However, the TN 1-type varieties had a moderate resistance to infection but with some degree of tolerance. According to Ishikawa's classification, Musashikogane and Minamihatamochi were included in the Musashikogane-type, while TN 1 and IR 50 were included in the TN1-type, and they had tolerance to virus.

The progeny populations of the cross between IR 50 and Reimei was maintained by the two-seed-descent method. The monogenic segregation was observed for seed shattering and phenol reaction at F_2 and F_5 generations (Table 5). These results showed that the tested F_6 lines had been randomly maintained. RSV and SBPH resistances of IR 50 was estimated to be controlled by the plural-genes when based on the results of F_3 and F_6 generations. However, the relationship of RSV resistance with SBPH resistance was different between F_3 and F_6 generations. In the F_3 generation, the R and M+S groups of RSV infection had the similar resistance level to SBPH (Table 3). The ratio of SBPH resistance was so heterogenous that it was not detected in the F_3 generation. However, the SBPH resistance in the F_6 generation was genetically fixed and there was a difference in the SBPH resistance between the R and M groups (Table 3). The SBPH resistance in the F_6 generation had influenced the distribution of RSV resistance among the F_6 lines. Hence it could be concluded that SBPH resistance was effective in reducing RSV infection, and the resistances to RSV infection and SBPH in IR 50 were controlled by plural-genes.

Hitherto, high resistance to virus infection have been used in the breeding of RSV resistant variety, and SBPH resistance was believed to be not effective to control SBPH and RSV. However, the moderate RSV resistant varieties such as IR 50 and TN 1 are found to diversify the sources of RSV resistance, and SBPH resistance was shown to reduce RSV infection significantly in the moderately resistant varieties. SBPH resist-

Table 5. The segregation of seed shattering and phenol reaction in F_2 and F_5 generations of the cross between IR50 and Reimei

Generation	Shattering		Expected ratio	P	Phenol		Expected ratio	P
	Hard	Easy			Pos.	Neg.		
F_2 (Reimei/IR50)	36	13	3 : 1	0.8-0.9	37	12	3 : 1	0.8-0.9
F_5 (Reimei/IR50)	20	22	1 : 1	0.8-0.9	20	22	1 : 1	0.8-0.9

ance alone is not enough to control RSV infection. However, an accumulation of SBPH resistance in virus resistant varieties is useful in reducing the RSV infection.

Literature Cited

- Dahal, G. and H. Hibino (1985) Varieties with different resistance to tungro (RTV) and green leafhopper (GLH). *Int. Rice Res. Newsl.* 10 (1) : 5-6.
- Daquioag, R. D., E. R. Tiongeo and H. Hibino (1984) Reaction of several rice varieties to rice tungro virus (RTV) complex. *Int. Rice Res. Newsl.* 9 (2) : 5-6.
- , P. Q. Cabauatan and H. Hibino (1986) Balimau Putih, cultivar tolerance of rice tungro-associated viruses. *Int. Rice Res. Newsl.* 11 (6) : 8.
- Hibino, H., R. D. Daquioag, P. Q. Cabauatan and G. Dahal (1988) Resistance to rice tungro spherical virus in rice. *Plant Disease* 72 : 843-847.
- Ishikawa, K., H. Nemoto, T. Omura and T. Tsuchizaki (1987) Improvement of test method for rice stripe virus with simplified ELISA. *Ann. Phytopath. Soc. Japan.* 54 : 123.
- Kobayashi, A., M. A. Supaad and O. Othman (1983) Inheritance of resistance of rice tungro and biotype selection of green leafhopper in Malaysia. *JARQ* 16 (4) : 306-311.
- Okamoto, D. and H. Inoue (1967) Studies in the smaller brown planthopper, *Laodelphax striatellus* Fallén as vector of rice stripe virus, II. Varietal resistance of rice to the smaller brown planthopper. *Chugoku Agr. Exp. Sta., Bull. E.* 1 : 115-136.
- Syumiya, A. and H. Nemoto (1990) Rice breeding for virus resistance. *Nogyo Gijyutsu* 45 (5) : 36-44.
- Takita, T. and H. Habibuddin (1985) Relationship between laboratory-developed biotypes of green leafhopper and resistant varieties of rice in Malaysia. *JARQ* 19 (3) : 219-223.
- Toriyama, K., Y. Sakurai, A. Ezuka, and O. Washio (1966) The breeding of resistant varieties for rice stripe virus. *Nogyo Gijyutsu* 21 : 16-19.
- Washio, O., K. Toriyama, A. Ezuka and Y. Sakurai (1967) Studies on the breeding of rice varieties resistant to stripe disease, II. Genetic study on resistance to stripe disease in Japanese upland rice. *Japan. J. Breed.* 18 : 96-101.
- , —, — and — (1968) Studies on the breeding of rice varieties resistant to stripe disease, III. Genetic study on resistance to stripe in foreign varieties. *Japan. J. Breed.* 18 : 167-172.
- Yamaguchi, T., S. Yasuo and M. Ishi (1965) Studies on rice stripe disease, III. Study on varietal resistance to stripe disease of rice plant. *J. Vent. Agr. Exp. Sta.* 8 : 109-160.