Assessment of Damage Caused by Two-Striped Leaf Beetle (*Medythia nigrobilineata* Motschulsky) Larval Feeding of Root Nodules in Soybean and Its Control during Furrow Cultivation at Seeding Time

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Abstract: Two-striped leaf beetle (TSLB) larvae are known to damage soybean root nodules but the effect they have on yield has not been quantitatively assessed. This study was aimed to evaluate the impact of TSLB and to establish a technique to control the larvae. We surveyed 106 farmers’ fields in Aichi prefecture for two years to quantify the changes in TSLB adult population, nodule damage by larvae, and nitrogen (N) content of seeds and stems, growth and yields of soybean plants treated with two different pesticides that were applied to furrow at the time of seeding. The results showed that damage caused by TSLB larval feeding of root nodules was observed particularly in soybeans grown in N-poor soil, and that ureide N content was decreased in plants with damage caused by larval feeding of root nodule, and such damage caused reduction of yield consistently in both years. Furthermore, we also showed the effectiveness of applying insecticides to the sowing furrow at the time of seeding. Specifically, the application of disulfoton to the sowing furrow at a rate of 4 g m⁻² as a simple method of pest control limited insect occurrences until the beginning of the blooming stage, curbed the feeding damage to root nodules caused by larvae, and increased the yield of soybean by 20%. This method has been adopted widely in Japan because it can be readily implemented using the fertilization application equipment installed on seeding machines and the insecticide is relatively inexpensive.

Key words: Nodule, Pest control, Soybean, Two-Striped leaf beetle.

Soybean (*Glycine max* (L.) Merr.) requires a large amount of nitrogen (N) for its growth and seed production. N in soybean is mainly supplied by its root nodules. N fixed by root nodules is transported to aboveground organs and tissues in the form of ureides (McClure and Israel, 1979; Schubert, 1986; Todd et al., 2006). Dependence on the root nodule for N fixation is the major feature of N nutrition in soybean. Damage to these organs by nodule-feeding pests may therefore reduce yield and seed quality.

The two-striped leaf beetle (TSLB) *Medythia nigrobilineata* Motschulsky is common in Japan and has been found in Yamagata (Saito et al., 1989), Ibaraki (Kikuchi and Mochida, 1992), Toyama (Sugimoto et al., 1994; Nitta, 2002), and Okayama (Nagai and Tsuboi, 1989) prefectures. The adult causes damage to soybeans by feeding on pods, leaves, and stems. The damage to pods as a result of adult feeding causes black spot on seeds (Sato and Fuse, 1983; Sato et al., 1989; Nitta, 2002), leading to economic loss owing to a decrease in seed quality. Feeding damage to leaves and stems inhibits vegetative growth and reduces yield (Yoshimeki and Kokayashi, 1955).

On the other hand, the larvae cause damage by feeding on the root nodules (Nawa, 1933; Koyama, 1938; Kawada, 1975; Saito et al., 1989), and the loss of soybean leaf color due to larval feeding damage to root nodules has been suggested to be a symptom of N deficiency (Kikuchi and Mochida, 1990). However, despite the reports of nodule
damage caused by TSLB larval feeding, its impact on yield and seed quality has not been determined. Therefore, the larvae has yet to be recognized as a major pest feeding on root nodules, possibly leading to a reduction in yield while adult TSLB has already been widely recognized as a pest causing damage to soybean by feeding on leaves and seeds among farmers, extension technicians and researchers.

Then, as a part of the research project aiming to identify major constraints for soybean production, we started large-scale field surveys on TSLB larval feeding in 2000 and found substantial regional variations with regard to the failure of soybean plants to grow properly owing to the presence of these larvae. Reduction in yield was more prominent in areas with yellow soil, which has relatively low fertility and N levels (Hamada et al., 2001). These findings suggested that inhibited root nodule function by TSLB larval feeding may be at least one of the major causes of low yield in this region, and we thus continued our study for another year.

We first analyzed the results for an initial 1-year experiment conducted in 2001 and reported that TSLB occurred in some farmers’ paddy fields for 3 generations, and soybean plant growth was inhibited by larval root nodule feeding damage, resulting in reduced yield, and that control of larval occurrence by applying a pesticide (disulfoton) during furrow cultivation at seeding time was effective, which decreased adult and larval occurrence density and root nodule damage, resulting in increased yield (Takei et al., 2002).

In the present study, we further analyzed the results for two years including the 2nd year experiment with additional farmers’ fields and an additional pesticide treatment, and examined the relationship between nodule damage by TSLB larval feeding, and the total N absorption and ureide N content of the plant, which derives from N fixed by root nodules. Overall objectives of this study were to verify damage caused by TSLB larval feeding of root nodules as a primary factor in the reduction of soybean yield in farmers’ fields, and to evaluate a countermeasure technique of insecticide application to control such damage.

**Materials and Methods**

The sites and pest control methods for the experiments, which were conducted in farmers’ fields in Anjo and Toyota cities, Aichi Prefecture, Japan in 2001 and 2002 are shown in Table 1. Paddy rice was previously cultivated on these fields which were recently converted for soybean cultivation. Their areas ranged from 0.1 to 0.3 ha, and soil type was mostly yellow. Fukuyutaka, the leading soybean cultivar in the region, was used in the present study. Seeds were sown at the rate of 3 to 5 g m$^{-2}$ with inter-row spacing of 70 to 80 cm from 20 June to 20 July, the optimal period for seeding. Conventional field cultivation management practices, except for insecticide application, were performed by the farm owners of the respective experimental fields. No fertilizer was applied because it was the standard cultivation in this region. R8 stage (full maturity) occurred between 10 and 20 November of each year.

1. **Pest control methods**

For pest control, disulfoton (Bayer, hereinafter referred to as disulfoton) and diazinon granules (Nippon Kayaku, hereinafter referred to as diazinon) were used as insecticides. Insecticide treatments were as follows: a) disulfoton for protecting the crop from field-invading adults at the beginning of soybean growth, b) diazinon for protecting the crop after the invasion of adults was confirmed, and c) no application of insecticide to serve as control. Disulfoton was applied to rows at a rate of 4 g m$^{-2}$ in the sowing furrow simultaneously with seed sowing in both 2001 and 2002. For simple and low-cost pest control, the insecticide was poured into the fertilizing equipment mounted on the seed sowing machine and applied to the sowing furrow. In contrast, diazinon was evenly spread over the soil surface of fields at a rate of 6 g m$^{-2}$ on 19 July 2001. In 2002, diazinon was mixed into the soil so that it would diffuse over the soil surface. It was applied at a rate of 6 g

| Parameters evaluated | Treatment | Number of experimental field | Anjo city | Toyota city | year |
|----------------------|-----------|------------------------------|-----------|-------------|------|
| **Adult populations** |           |                              |           |             |      |
| Disulfoton           | 3         | –                            | –         |             | 2001 |
| Diazinon             | 3         | –                            | –         |             | 2001 |
| Control              | 3         | –                            | –         |             |      |
| **Nodule damage**    |           |                              |           |             |      |
| Disulfoton           | 3         | –                            | –         |             | 2001 |
| Diazinon             | 3         | –                            | –         |             | 2001 |
| Control              | 3         | –                            | –         |             |      |
| Disulfoton           | 6         | 1                            | 1         |             | 2002 |
| Diazinon             | 3         | –                            | –         |             | 2002 |
| Control              | 6         | 1                            | 1         |             |      |
| **Growth, yield and N analysis** | |                              |           |             |      |
| Disulfoton           | 11        | 3                            | 3         |             | 2001 |
| Diazinon             | 3         | 1                            | 1         |             | 2001 |
| Control              | 11        | 3                            | 3         |             |      |
| Disulfoton           | 9         | 8                            | 8         |             |      |
| Diazinon             | 4         | 1                            | 1         |             |      |
| Control              | 9         | 8                            | 8         |             |      |
m² when the field was plowed with a tractor before the seeds were sown. Each insecticide and the application rates were handled carefully according to their instruction manuals.

2. Monitoring of adult populations
To check the effectiveness of insecticides from the R1 (beginning of blooming) to R3 (beginning of pod development) reproductive stages, we monitored the numbers of adults on 5 plants from 3 randomly selected rows in each plot at 7-day intervals from 9 August to 20 September 2001. A drop cloth (diameter, 50 cm) was used to catch and count adults (Rudd and Jensen, 1977; Loughran and Ragsdale, 1986; Kikuchi and Mochida, 1992). Soybean maturity was estimated according to the method of Fehr et al (1971).

3. Assessment of damage to root nodule by larval feeding
Nodule damage by TSLB larvae was recorded at R1 (16 August 2001 and 2002) and R3 stages (13 September 2001 and 16 September 2002). Root systems of 5 randomly selected plants were carefully extracted using a shovel to minimize damage. Following this, the roots were carefully washed with water in a net to extract all root nodules. The root systems were microscopically examined for signs of larval feeding injury. The nodules were then detached and counted, and nodules with signs of insect feeding were also counted. The proportion of damaged nodules was expressed as the ratio of damaged to total nodules per plant.

4. Impact of pest control on soybean growth and yield
   (1) Impact of TSLB at the R1 stage
To evaluate the impact of TSLB damage to the root nodule at the R1 stage, we measured the main stem length and leaf color (SPAD value) using a chlorophyll meter (SPAD502, MINOLTA Co. Ltd.), were measured on 16 August in both years. Ten consecutive standing plants in a row were measured in 2 randomly chosen sections of row per plot.

   (2) Yield and seed quality
Plants were harvested from 2-m stretches of 2 ridges in 2 randomly chosen sections per plot at the R8 stage. After the plant samples were dried in a greenhouse under ambient conditions, all the pods were carefully detached and weighed. The yield and 100-seed weight were measured for filled seeds adjusted to a moisture content of 15% w/w.

   In 2001, 300 seeds were randomly sampled after the yields were recorded, and the occurrence ratio of black spot on seed was evaluated.

   (3) N analysis
At the R8 stage (early November in both years), 3 plants per row with moderate growth were harvested from 2 randomly chosen sections in each plot. Each plant was separated into the stem and seeds, which were then oven-dried at 70°C for 3 days and powdered separately. The N concentrations were measured using a dry combustion method (N.C-ANALYZER, Sumika Chemical Analysis Service, Co. Ltd.).

   At the R1 stage, ureide N content of stem and damage to root nodules were determined from 5 random plants. Ureide N was extracted from harvested stem tissues. Dried tissues were weighed, finely ground, and 0.2 g of the powder was extracted with 25 mL boiling water for 2 min (Herridge, 1982; Matsunaga and Matsumoto, 1986). The extract was filtered, made to a final volume of 50 mL, and analyzed according to the method of Christman et al. (1944).

Results

1. Effect of pest control with insecticides on TSLB damage
The adult TSLB populations are described in Table 2. Compared with the control, the seasonal average and peak of TSLB populations were significantly lowered by insecticide application, disulfoton being more effective than diazinon.

Table 2. Peak and average populations of adult TSLB per plant treated with insecticides (2001).

| Treatment        | Peak populations (no. plant⁻¹) | Average populations (no. plant⁻¹) |
|------------------|---------------------------------|-----------------------------------|
| Disulfoton       | 3.9                             | 1.8                               |
| Diazinon         | 5.1                             | 3.1                               |
| Control          | 12.6                            | 7.3                               |
| Treatment        | **                              | **                                |
| Location         | **                              | ns                                |
| Treatment × location | **                          | ns                                |

In the disulfoton plot, the adult population peaked on 20 September. In the diazinon and control plots, it peaked on 30 August. The analysis of variance (ANOVA) was conducted using data across locations. **: significant at \( p < 0.01 \); ns: not significant.
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than in the diazinon-treated plot at the R3 stage.

2. Effect of insecticide application on soybean growth and yield

Table 4 shows the plant growth at the R1 stage in 2001 and 2002. In both years, the main stem length was similar in all plots. Disulfoton-treated plots showed significantly greener leaves than the control. Fig. 1 shows the yield and yield components in 2001 and 2002. Compared with the control, the application of disulfoton in the sowing furrow resulted in a higher number of pods m⁻². Consequently, the yield was higher than that of the control by approximately...
The amount of N in the seeds was significantly increased by the application of disulfoton, but not by diazinon. In contrast, the amount of N accumulated in the stem was much smaller than that in the seeds, and it did not differ significantly with the treatment. The relationship between the damaged nodule ratio at the R1 stage and the amount of N accumulated in the seeds and stems at the R8 stage was negative and significant (Fig. 3). Furthermore, ureide N content of the stem tissue showed a negative correlation with the damaged nodule ratio at the R1 stage (Fig. 4).

**Discussion**

We first conducted a study to quantitatively evaluate the influence of damage caused by TSLB larval feeding on root nodules on yield and seed quality of soybean plants that were grown in farmers’ paddy fields in 2001 (Takei et al., 2001). The yield of diazinon-treated plants was approximately 10% higher than that of the control. The effect of diazinon was weaker than that of disulfoton in terms of both diffusion and mixture in the soil. As a result, other yield components, including 100-seed weight and main stem length, did not differ significantly with the type of insecticide (Fig. 1).

The incidence of black spot on seeds did not differ significantly with the treatment: 1.0% for disulfoton, 1.1% for diazinon, and 1.4% for the control.

**3. Effects of insecticide application on N absorption**

Fig. 2 shows the amount of N accumulated in the seeds and stem of the plant grown under the 2 insecticide treatments in 2001 and 2002. The amount of N in the seeds was significantly increased by insecticide applications. In 2001, disulfoton was significantly more effective than diazinon, whereas in 2002, the amount of N in the seeds was significantly increased by the application of disulfoton, but not by diazinon. In contrast, the amount of N accumulated in the stem was much smaller than that in the seeds, and it did not differ significantly with the treatment.

The relationship between the damaged nodule ratio at the R1 stage and the amount of N accumulated in the seeds and stems at the R8 stage was negative and significant (Fig. 3). Furthermore, ureide N content of the stem tissue showed a negative correlation with the damaged nodule ratio at the R1 stage (Fig. 4).
al., 2002). Then, we further conducted another experiment in the following year with additional farmers’ fields and pesticide treatment. Through these experiments, we aimed to verify damage caused by TSLB larval feeding on root nodules as a primary factor in the reduction of soybean yield in farmers’ fields, and to evaluate a countermeasure technique of insecticide application to control such damage. Prior to our study, it was thought that the substantial damage caused by TSLB was primarily because of adult feeding on the leaf or seed. In addition, pest control methods are usually applied during later stages of growth, particularly after the pod-filling stage.

The results of this study showed that in farmers’ fields, more than 90% of root nodules in a plant were damaged by TSLB at the R1 stage (Table 3), with evidently less green and overall plant growth being suppressed (Table 4). This observation is in agreement with the report of Kikuchi and Mochida (1990) that leaf color deterioration was a result of inadequate N nutrition owing to damage caused by TSLB larval feeding on root nodules.

The soybean plant requires a large amount of N for growth and seed development. Approximately 40% – 70% of this N is assimilated and supplied by root nodules (Hoshi, 1982; Akao, 1991). In the fields used in this study, cultivating soybeans without fertilization required a high degree of dependence on root nodule N fixation. When the proportion of damage caused by larval feeding on root nodules is high at the R1 stage, larval feeding of root nodule impedes growth. Then ureide N content of the stem at the R1 stage became lower (Fig. 4), and the amount of N in the stem at the R8 stage was reduced (Fig. 3). Consequently, the yield of soybean was reduced (Fig. 1).

We showed that pest control is necessary during the early stages of growth because TSLB larvae cause feeding damage when soybean root nodules begin to form (Takei et al., 2002). We devised a method to apply the insecticide in labor-saving mechanized cultivation systems, rendering more efficient application of insecticides to the sowing furrow at the time of seeding (Takei et al., 2002).

Disulfoton applied to the sowing furrow at the seeding stage as a simple method of pest control remains effective for approximately 2 months. Disulfoton limits the number of insects at the R1 stage and damage caused by larvae feeding on the root nodules. Because a large amount of N absorbed during the R1 stage is translocated to the seeds (Kato et al., 1983; Yamagata and Kanamori, 1990), it is extremely important to avoid root nodule damage at the R1 stage to attain higher yields. In addition, it is well established that if a large amount of N is taken up from the R1 to R3 stages, the growth of pods is improved (Aragaki and Fujii, 1991; Takahashi et al., 1994). At the R5 stage, this rate is the most important factor for achieving high yields in soybeans (Shiraiwa et al., 2004). Thus, when the damage to root nodule caused by TSLB larval feeding was controlled by pest management measures, including the efficient use of insecticides, the amount of N in stem was increased (Fig. 2), resulting in a larger number of pods m$^{-2}$ and high yield (Fig. 1). This result was due to the sufficient amount of N supplied from the root nodule to the aboveground parts at the R1 stage.

In contrast, diazinon was less effective than disulfoton (Table 2). This suggests that when a field is sprayed with insecticide after insect incidence has occurred, there is a possibility that eggs have already been laid in the soil; therefore, efficacy of decreasing larval density is low. The benefit of pesticide application to the soil surface before seeding is lower than that of application in the sowing furrow at the seeding time.

Several reports on TSLB infections (Sato et al., 1983; Sato et al., 1989; Nitta, 2002) have emphasized the occurrence of black spot on seed. These authors found that the most effective stage for controlling TSLB using insecticides is at the peak of the second generation of TSLB, which coincides with the R3 stage in the present study (Table 2). However, using a conventional pest control at the R3 stage when the number of adults has already increased, is too late to prevent the larval feeding damage to the root nodule that has occurred during the R1 to R3 stage.

Saito et al. (1989) reported that because the degree of damage differed according to seed type, the prevention standard for seed damage was set above 5%. Although marked insect damage was observed in the control plots, an extremely low percentage of seeds (1.4%) were affected by black spots. In the disulfoton-treated plants, only 1.0% of seeds were affected by black spots, which was not significantly different from the percentage in the control plants. However, the adult population was controlled by disulfoton application even after the end of August (Table 2). This result suggests that the use of disulfoton in areas where black spot outbreaks are expected could be effective for their prevention, even when other management options are used to reduce their incidence.

The application of disulfoton to the sowing furrow increased soybean yield. This method can be easily applied to the use of fertilizing equipment mounted on the seeding machine. This pest control method does not increase labor cost. The economic benefit of using disulfoton is high because of its affordable price and increased yield. We believe that this pest control method is a key method for maximizing soybean yield with the labor-saving feature demanded by large-scale farming.

In Aichi prefecture, Japan, where this study was conducted, the soybean growing area was approximately 4,260 ha as of 2012 (MAFF, 2013). To meet the high demand for drastic improvement of cultivation technology, we conducted a series of large-scale surveys of soybean production from 1999 to 2000 (Hamada et al., 2001). We
identified several factors that contributed to low yield (1.4 t ha⁻¹), including excessive soil moisture, drought, and deterioration of soil physics (Hamada et al., 2001). In the present study, we have shown that the major cause of low yield was damage caused by TSLB to root nodules. The pest control method suggested in the present study increased yield by approximately 20%. This method is now being adopted on approximately 2,000 ha, i.e., 45% of the total soybean cultivation area in Aichi prefecture, and some other parts of Japan such as Hokuriku region.

Although TSLB damage to soybean has not yet been reported in other countries, in US, the bean leaf beetle Cerotoma trifurcata is reported to be a pest whose larvae cause similar damage to root nodules of soybeans (Smelser and Pedigo, 1992; Riedell et al., 2005; Lundgren and Riedell, 2008). Thus, understanding of occurrence pattern and characteristics of feeding on root nodule of such pest may help establish the control method for increased soybean yield.

References
Akao, S. 1991. Nitrogen fixation and metabolism in soybean plants. JARQ 25: 83-87.
Aragaki, K. and Fujii, H. 1991. Effect of coated urea top-dressing at hilling time on growth and yield of soybean plant. Jpn. J. Soil Sci. Plant Nutr. 62: 75-78***.
Christman, A.A., Foster, P.W. and Esterer, M.B. 1944. The allantoin content of blood. J. Biol. Chem. 155: 161-171.
Fehr, W.R., Caviness, C.E., Burmood, D.T. and Pennington, J.S. 1971. Stage of development descriptions for Soybeans, Glycine max (L.) Merrill. Crop Sci. 11: 929-931.
Hamada, Y., Tani, T., Yoshida, T., Nakajima, Y., Shirota, M. and Shaku, I. 2001. Investigation into the soybean culture in Nishi-Mikawa region in Aichi prefecture. Res. Bull. Aichi Agric. Res. Cent. 33: 87-92**.
Herridge, D.F. 1982. Relative abundance of Ureides and nitrate in plant tissues of soybean as a quantitative assay of nitrogen fixation. Plant Physiol. 70: 1-6.
Hoshi, S. 1982. Nitrogen fixation, growth and yield of soybean. In Japanese Society of Soil Science and Plant Nutrition eds., Nitrogen Fixation in Root Nodules. Hakuyusha Publishers, Japan. 5: 33***.
Kato, T., Yamagata, M. and Tsukahara, S. 1983. Absorption and utilization of NH₄⁺-N (¹⁵N) and NO₃⁻-N(¹⁵N) in soybean plants. Jpn. J. Soil Sci. Plant Nutr. 54: 25-29***.
Kawahara, A. 1975. Encyclopedia of Crop Disease and Insect Damage. Yokendo, Tokyo. 1045-1046****.
Kikuchi, A. and Mochida, O. 1990. Estimation of damaged amounts of soybean root nodules by larvae of Medythia nigrobilineata (Coleoptera: Chrysomelidae) with measurement of leaf color by a chlorophyll meter. Proc. Kanto-Tosan Plant Prot. Soc. 37: 183-184*.
Kikuchi, A. and Mochida, O. 1992. Seasonal abundance of Medythia nigrobilineata Coleoptera Chrysomelidae on soybean in Tsukuba, Ibaraki, Japan. Proc. Kanto-Tosan Plant Prot. Soc. 39: 193-195*.
Koyama, T. 1938. Morphology and ecology of two-striped leaf beetle. Appl. Entomology. 1: 169-176****.
Loughran, J.C. and Ragdole, D.W. 1986. Life cycle of the bean leaf beetle, Cerotoma trifurcata (Coleoptera: Chrysomelidae), in southern Minnesota. Ann. Entomol. Soc. Am. 79: 34-38.
Lundgren, J.G. and Riedell, W.E. 2008. Soybean nitrogen relations and root characteristics after Cerotoma trifurcata (Coleoptera: Chrysomelidae) larval feeding injury. J. Entomol. Sci. 43: 107-116.
MAFF (Ministry of Agriculture, Forestry and Fisheries Government of Japan) 2013. [Online]. Available at www.maff.go.jp/j/tokei/kouyou/sakumotu/sakkyou_kome/index.html#r (accessed 19 May 2013). Japan Statistics on Crops.
Matsunaga, R. and Matsumoto, S. 1986. N fixation (C₄H₄ reduction) and ureide concentration in several soybean cultivars. Jpn. J. Crop. Sci. 55: 223-228.
McClure, P.R. and Israel, D.W. 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiol. 64: 411-416.
Nagai, K. and Tsuibo, A. 1989. Life cycle and seasonal changes in population density of the two-striped leaf beetle Medythia nigrobilineata (Motschulsky) (Coleoptera: Chrysomelidae) in southwestern Japan. Kinki Chugoku Agric. Res. 77: 10-20***.
Nawa, U. 1933. Regarding two-striped leaf beetle (Monolepta nigrobilineata Motsch.). Insect World 37: 293-296****.
Nitta, A. 2002. Studies of seasonal occurrence and control of two-striped leaf beetle, Medythia nigrobilineata Motschulsky, on soybeans in Hokuriku District. Proc. Assoc. Plant Prot. Hokuriku 50: 229-232***.
Riedell, W.E., Lundgren, J.G., Osborne, S.L. and Pikul Jr., J.L. 2005. Effects of Soil nitrogen Management on Soybean Nitrogen Relations and Bean Leaf Beetle (Coleoptera: Chrysomelidae) Biology. J. Agric. Urban Entomol. 22: 181-190.
Rudd, W.G. and Jensen, R.L. 1977. Sweep net and ground cloth sampling for insects in soybeans. J. Econ. Entomol. 70: 301-304.
Saito, T., Sato, M. and Fuse, H. 1989. Ecology and control of two-striped leaf beetle, Medythia nigrobilineata Motschulsky, on soybean in Yamagata prefecture. 2. Seasonal prevalence and control threshold of the adults. Bull. Yamagata Agric. Exp. Stud. 24: 53-61*.
Sato, M. and Fuse, H. 1983. Damage of soybean by the two-striped leaf beetle, Medythia nigrobilineata Motschulsky, and its control by insecticides. Ann. Rep. Plant Prot. North Jpn. 34: 37-39***.
Sato, M., Saito, T., Fuse, H. and Takeda, T. 1989. Ecology and control of two-striped leaf beetle, Medythia nigrobilineata Motschulsky, on soybean in Yamagata prefecture. 1. Infestation and chemical control of the adults in Shonai district. Bull. Yamagata Agric. Exp. Stud. 24: 37-51**.
Schubert, K.R. 1986. Products of biological nitrogen fixation in higher plants: Synthesis, transport, and metabolism. Ann. Rev. Plant Physiol. 37: 539-574.
Shiraiwa, T., Ueno, N., Shimada, S. and Horie, T. 2004. Correlation between seed filling stage in various soybean genotypes. Plant Prod. Sci. 7: 138-142.
Smelser, R.B. and Pedigo, L.P. 1992. Soybean seed yield and quality reduction by bean leaf beetle (Coleoptera: Chrysomelidae) pod injury. J. Econ. Entomol. 85: 2390-2403.
Sugimoto, N., Noda, T. and Nitta, A. 1994. Seasonal prevalence of the two-striped leaf beetle, Medythia nigrobilineata Motschulsky (Coleoptera: Chrysomelidae) and the proper timing of insecticide application. Proc. Assoc. Plant Prot. Hokuriku 42: 94-99***.
Takahashi, Y., Chushini, T., Nagumo, Y., Nakano, T. and Ohyama, T.
1994. Yield components of soybean plant with deep placement of N fertilizer, related to high productivity. *J. Niigata Agric. Exp. Stn.* 40: 7-15.

Takei, M., Nakamura, M. and Hamada, Y. 2002. The studies on the eating damage for soybean root nodule by two-striped leaf beetle, *Melythia nigrolineata*, and the development of chemical control method. *Res. Bull. Aichi Agric. Res. Cent.* 34: 31-36*.

Todd, C.D., Tipton, P.A., Blevins, D.G., Piedras, P., Pineda, M. and Polacco, J.C. 2006. Update on ureide degradation in legumes. *J. Exp. Bot.* 57: 5-12.

Yamagata, M. and Kanamori, T. 1990. Distribution of the top-dressing nitrogen (15N) in soybean plant on paddy field rotated to upland as affected by irrigation. *Jpn. J. Soil Sci. Plant Nutr.* 61: 61-67*.

Yoshimeki, M. and Kobayashi, S. 1955. Analytical study of injury by the two-striped leaf beetle, *Paraluperodes suturalis nigrolineata* Motschulsky, in soybean plant. *Bull. Inst. Agric. Res. Tohoku Univ.* 7: 19-22**.

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