Effects of Low Temperature and Shading during Flowering on the Yield Components in Soybeans

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Abstract: Northern Japan, Hokkaido has cold weather damage in agriculture almost every four years. Cold weather damage to soybeans [Glycine max (L.) Merr.] during flowering is especially severe and is caused by both low temperature and insufficient sunlight. Therefore, the damage should be analyzed from both aspects. We analyzed the effects of low temperature and shading during the flowering season on seed yield and yield components in two varieties of soybeans: cv. Hayahikari, an excellent cold weather tolerant variety, and cv. Toyomusume, a cold weather sensitive variety.

The soybean plants were exposed to a low temperature of 18°C day/13°C night, shaded (50%) without low temperature treatment, or shaded at a low temperature, during the four-week flowering season. The control plants were kept under normal conditions. The results indicated that cold weather damage is mainly caused by the low temperature, which severely reduced the number of pods per plant, in Toyomusume. However, shading also reduced the number of pods per plant in both varieties. All of the yield components examined were reduced by cold weather more severely in Toyomusume than in Hayahikari. Furthermore, shading combined with low temperature treatment caused greater damage in both Hayahikari and Toyomusume than either a low temperature or shading treatment alone.

Key words: Cold weather damage, Flowering stage, Low temperature, Pod, Seed yield, Shading, Soybean, Varietal difference.

Hokkaido, which is the largest soybean production area in Japan, is located in northern Japan. The Tokachi district is the main soybean producing region in Hokkaido, but its climate is not always appropriate for soybean production.

Soybeans currently grown in Hokkaido often suffer from low-temperature damage. There have been 11 years of cold weather damage in the past 44 years in the Tokachi district, and thus cold weather damage occurs at an average of once every four years (Tanaka, 1997). Cold weather damage is caused mainly by three factors (Yamamoto and Narikawa, 1966): 1) Poor growth due to low temperatures in the early stages of growth, 2) flower abscission and pod setting failure due to low temperatures before and during the flowering stage, 3) insufficient grain filling due to low temperatures in the pod filling stage. In only three of the 11 years with cold weather damage, soybean was damaged by one of these factors alone. In the other eight years of damage, the damage was caused by a combination of two or three of the factors mentioned above. The frequency of the years with damage by poor growth was the highest, but the damage caused by flower abscission and pod-setting failure was severe. In 1993, when the mean temperature during flowering stage was around 15°C and soybean yield in the Tokachi district was 87% below the average yield, flower abscission and pod-setting failure were remarkable (Matsukawa, 1994).

The cold weather damage in the Tokachi district was caused by both low temperature and insufficient sunlight. Numerous publications documented that the growth of soybean is sensitive to temperature and shading. Low temperature during the reproductive stage induces cleistogamy (Erickson, 1975; Thomas et al, 1981), which results in the development of many small seedless pods developing at the top of the plant, and the presence of multi-carpelate and deformed pods (Hume and Jackson, 1981; Thomas et al, 1981). Several studies have been conducted on pod setting as influenced by low temperature in the flowering stage (Saito et al, 1970; Duke et al, 1979; Marowitch, et al 1986; Purcell et al, 1987; Wang et al, 1997), carbohydrate metabolism (Thomas et al, 1981; Marowitch et al, 1986; Purcell et al, 1987), nitrogen fixation (Duke et al, 1979; Takahashi and Asanuma, 1996), and pollination (Goto and Yamamoto, 1972).

Shading reduces the number of pods (Mann and Jaworski, 1970; Tanaka et al, 1980; Ishikawa et al, 1984; Jiang and Egli, 1993) and the amount of nitrogen accumulation because of a reduction in nodule and root growth in the soybean plant (Tanaka et al, 1980).
While, Allen (1975) reported that reducing exposure to sunlight by 30% may be favorable during the excessively hot mid-day because it reduces soil and air temperature, wind speed, and water use. However, few attempts have been made on the effects of both low temperature and shading.

This study was conducted to determine the effects of low temperature, shading at a normal temperature, and shading at a low temperature using soybean cultivars with two different tolerance levels.

Materials and Methods

1. Plant materials

Two determinate cultivars, which have similar maturity, plant type and seed yield in normal years, were chosen for the experiment. Both varieties were developed at the Hokkaido Prefectural Tokachi Agricultural Experiment Station. One was Toyomusume with a yellow seed coat and hilum, gray pubescence and purple flowers (T/T, i/i, r/r, O/O, W/W; genotypes were summarized by Palmer and Kilen, 1987), which is a standard low-temperature sensitive variety. This variety was released in 1986 (Sasaki et al, 1986) and has been a main one grown in Hokkaido due to superior seed quality.

The other was Hayahikari, which has a yellow seed coat and brown hilum, tawny pubescence and white flowers (i/i, i/i, T/T, r/r, O/O, W/W). Hayahikari showed the lowest seed yield reduction among soybean varieties and lines in 1993, where soybean was most severely damaged by cold weather in the past 50 years. Field experiments in cooler locations and the experiments in a phytotron kept at a low temperature also showed that Hayahikari is highly tolerant to cold weather. As a result, Hayahikari was released in 1998 as one of the most cold weather tolerant varieties (Yumoto et al, 2000).

2. Plant culture

Experiments were conducted from May to October in 1997 at the Hokkaido Prefectural Tokachi Agricultural Experiment Station (42°55’ N, 143°03’ E, 95 m above sea level). On 27 May, twelve seeds per pot were planted in pots (1/2000 a) filled with dry andosol soil supplemented with a synthetic fertilizer (0.6 N· 5.3 P₂O₅· 2.7 K₂O kg⁻¹). The plants were thinned to two per pot, two weeks after emergence.

The plants were grown in an experimental facility under a plastic roof without walls. The average temperature during soybean growth was 14.5°C and that in the flowering season (20 July to 30 August) was 18.3°C (Fig 1), 0.7°C and 1.2°C lower than the average of the last ten years, respectively. The sunlight duration of the flowering season was 140.7 hours, 5.8 hours less than the average. During the half of the flowering season, the weather was clear. The growing conditions were those of a normal year, not a cold year.
pods, ovules and grains per pod, the fertilization rate, 100 seed weight (seed size) and the seed yield were subjected to the t-test.

Results

1. Effects of cooling and shading treatments

In the control conditions, Toyomusume matured on 5 October, and Hayahikari 4 days earlier on 1 October. The shading treatment did not greatly affect the maturity date in either variety; it delayed maturity by 3 days in Toyomusume and hastened by 2 days in Hayahikari. The cooling treatment and the shading + cooling treatment delayed the maturity date by almost 20 days in both varieties. Effects of cooling and shading treatments on the vegetative factors were not large in either variety. Neither cooling nor shading treatment significantly affected the number of main-stem nodes per plant, the number of branches per plant and the total number of nodes per plant in either variety (Fig 2, Table I).

Although the seed yield was approximately the same, about 20 g per plant, in both varieties under the control conditions, it was higher in Hayahikari than in Toyomusume after the cooling treatment. The seed yield after the shading treatment at a normal temperature was nearly the same in the two varieties (Fig 3).

The seed yield in Toyomusume was reduced to 2.6 g (13% of the control) by the cooling treatment, but that in Hayahikari only to 15.6 g (77% of the control). Thus, the results confirmed that Hayahikari is more tolerant to low temperatures than Toyomusume. In Toyomusume, all yield components, the number of pods per plant, the number of ovules and grains per pod, the fertilization rate and 100 seed weight, were reduced significantly (p < 0.01) by the cooling treatment. In Hayahikari, however, only the number of grains per pod and the fertilization rate were reduced significantly (p < 0.01). The largest difference between the two varieties in the response to the cooling treatment was the number of pods per plant, which was reduced from 35.2 to 12.8 (36% of the control) in Toyomusume, and from 23.6 to 22.3 g (95% of the control) in Hayahikari. The number of ovules, the number of grains per pod and the fertilization rate in Toyomusume were reduced by the cooling treatment to 90, 64 and 72% of the control, respectively, while those in Hayahikari were reduced only to 96, 87 and 91% of the control, respectively.

The number of pods per plant was significantly reduced by the shading treatment (to about 80% of the control) in both varieties (p < 0.05 in Toyomusume and

![Fig. 2. Effects of cooling and shading treatments on vegetative organs of soybeans. Numbers in parentheses are percent of the control.](image)

Table 1. Effects of cooling and shading treatments: t-test results.

| Variety               | Main stem nodes per plant | Branches per plant | Total nodes per plant | Pods per pod | Ovules per pod | Grains per pod | Fertilization rate | 100 seed weight | Seed yields per plant |
|-----------------------|---------------------------|--------------------|-----------------------|--------------|---------------|---------------|-------------------|-------------------|---------------------|
| Control vs Cooling    | ns                        | ns                 | ns                    | *            | ns            | ns            | **                | *                 | ns                  |
| Toyomusume Control vs Shading | ns                 | ns                 | ns                    | ns           | ns            | ns            | ns                | *                 | *                   |
| Cooling vs Shading + Cooling | ns               | ns                 | ns                    | *            | ns            | ns            | ns                | **                | ns                  |

| Variety               | Main stem nodes per plant | Branches per plant | Total nodes per plant | Pods per pod | Ovules per pod | Grains per pod | Fertilization rate | 100 seed weight | Seed yields per plant |
|-----------------------|---------------------------|--------------------|-----------------------|--------------|---------------|---------------|-------------------|-------------------|---------------------|
| Control vs Cooling    | ns                        | ns                 | ns                    | *            | ns            | ns            | ns                | *                 | *                   |
| Toyomusume Control vs Shading | ns                 | ns                 | ns                    | ns           | ns            | ns            | ns                | ns                | ns                  |
| Cooling vs Shading + Cooling | ns               | ns                 | ns                    | *            | ns            | ns            | ns                | ns                | ns                  |

* and ** indicate significant at p = 0.05, p = 0.001 and not significant difference, respectively.
Fig. 3. Effects of cooling and shading treatments on yield components of soybeans.

Fertilization rate = Grains per pod/Ovules per pod × 100. Numbers in parentheses are percent of the control.

2. Leaf temperature

In the control, the leaf temperature of Hayahikari was the same as the outside temperature during cloudy weather (Table 2), but it was markedly higher (29.5°C) than the outside temperature (24.5°C) during clear weather.

In the phytotron, during cloudy weather, the leaf temperature was almost the same as the controlled air temperature (18°C) irrespective of shading. However, during clear weather, although the leaf temperature of the cooling treatment was about 6°C higher (24.6°C) than the air temperature, that of the shading + cooling treatment (18.6°C) was nearly the same as the air temperature. Thus, the leaf temperature during the cooling treatment was markedly raised by sunlight but not under shading.

Discussion

The reproductive growth was greatly affected by low temperature, but the vegetative growth was not in either variety. The number of main stem nodes per plant, the number of branches per plant and the total number of nodes per plant after the cooling treatment were approximately the same those in the control conditions in both varieties. It is assumed that the differentiation of vegetative growth is almost completed at the beginning of the flowering period, and low temperature increases the partitioning of total biomass to vegetative organs, while it decreases its partitioning to the reproductive organs (Seddigh and Jolliff, 1984; Wang et al, 1997). Thus, the
effects of low temperature during the flowering period on vegetative organs are small.

Although low temperature significantly damaged seed yields in both varieties, Hayahikari showed higher tolerance than Toyomusume. This varietal difference was due to different reduction rates in the number of pods per plant, the number of grains per pod, the fertilization rate and 100 seed weight. This means that the difference between Hayahikari and Toyomusume in the decrease of seed yield due to cold weather is derived from the tolerance to low temperature of several components, not just one component, related to yield in Hayahikari.

The most drastic difference between Toyomusume and Hayahikari in the response to the cooling treatment was the reduction in the number of pods per plant. It was significantly reduced in Toyomusume, but not in Hayahikari. This indicates that pod number is the most important component responsible for cold weather tolerance. It can be considered that Hayahikari has a higher pod setting ability in cold weather than Toyomusume. However, this result does not mean that Hayahikari has a superior pod setting ability during low temperature period. In other words, there is a possibility that most of Hayahikari’s pods were set after the low-temperature period. Further investigation about how Hayahikari’s pods are set in cold weather is necessary.

The other reproductive growth components, the numbers of ovules and grains per pod and the fertilization rate, also varied with the cultivars. The number of grains per pod and the fertilization rate were significantly reduced by the cooling treatment in both varieties, although the degree of the reduction in Toyomusume was greater than that in Hayahikari. More generally, our results showed that low temperature affects the process of fertilization: the number of unfertilized ovules was increased, therefore the number of grains per pod and the fertilization rate were decreased by the cooling treatment. This finding is also supported by the study of Goto and Yamamoto (1972), who found that cooling treatment before flowering decreased pollen activity and pollen density per stigma, and thus increased the number of unfertilized flowers.

Low temperature decreased the seed size more in Toyomusume than in Hayahikari. The 100 seed weight of Toyomusume in the control was 30.2 g, which was about 7 g heavier than that of Hayahikari. However, the 100 seed weight of Toyomusume was about 6 g lighter than that of Hayahikari after the cooling treatment, showing a drastic reduction of seed weight by low temperature. In this experiment, the pod size and seed size were approximately equal in the two varieties at the end of the cooling treatment (data not shown), and the period from the end of the cooling treatment to maturity was the same in the two varieties. Therefore, it is assumed that the difference in the reduction of the seed weight by the cooling treatment in the two varieties was caused by the difference in pod filling after the cooling treatment.

Other researchers also studied the low-temperature tolerance of soybean varieties. Some researchers reported that the pubescence color is related to cold weather tolerance. Morrison et al (1994) reported that the yield of tawny pubescence soybeans is higher than that of gray pubescence soybeans in cooler regions. Takahashi and Asanuma (1996) concluded that the tawny gene T is associated with cold tolerance during the flowering stage. Actually, the pubescence color of Hayahikari is tawny (gene T), and that of Toyomusume is gray (t). Thus, the varietal difference in low-temperature tolerance in this experiment might be related to T/t.

Note that the number of pods per plant was reduced by the shading treatment similarly in the two varieties. In both Toyomusume and Hayahikari, it was decreased by about 20 % compared with the control. Mann and Jaworski (1970) and Jiang and Egli (1993) also reported that shading decreased the number of pods per plant. It is supposed that insufficient sunlight decreases photosynthetic activity and reduces assimilates, which results in pod abscission as Schou et al (1978), Egli and Zhen-wen (1991) described. Both varieties demonstrated a parallel reduction of the number of pods per plant by shading, and no major difference was found in the other yield components. Therefore the seed yield reduction was almost parallel to the reduction of the number of pods per plant in both varieties. This result shows that there is no difference between the two cultivars in the susceptibility to shading at normal temperatures.

Hayahikari showed a high tolerance to the cooling treatment, but less tolerance to the shading + cooling treatment. Furthermore, the seed yield after the shading + cooling treatment was lower than that after the cooling treatment in both Toyomusume and Hayahikari. The damage caused by the shading + cooling treatment

| Outside Temperature (°C) | Amount of sunlight (kcal h⁻¹ m⁻²) | Leaf Temperature (°C) |
|--------------------------|-----------------------------------|----------------------|
| Clear | Cloudy | Clear | Cloudy | Control | Shading + Cooling treatment | Cooling treatment |
| 24.5±0.9 | 21.3±0.3 | 1062±24.6 | 350±172.6 | 29.5±1.7 | 21.8±0.5 | 18.6±0.2 | 18.0±0.0 | 24.6±0.9 | 18.3±0.8 |

Date are means±standard errors.
appeared to be a combination of that caused by shading and cooling. In both varieties, the reduction of the numbers of ovules and grains per pod, and the fertilization rate by the shading+cooling treatment paralleled the reduction by the cooling treatment. However, pod number was decreased more strongly by the shading+cooling treatment than the cooling treatment. Shading at a normal temperature significantly decreased the number of pods per plant but did not seriously affect other yield components in either variety. A greater reduction in the number of pods per plant by the shading+cooling treatment than by the cooling treatment shows the combined effects of cooling and shading.

This phenomenon can be partially explained by leaf temperature. According to Marowitch et al (1986), the lower the plant temperature, the lower the rate of photosynthesis and translocation of assimilates in soybeans during cold weather. In cloudy weather, the leaf temperature during the cooling treatment was unaffected by shading. In clear weather, however, the leaf temperature during the cooling treatment (24.6°C) was lowered to 18.6°C by shading. In other words, the leaf temperature during the cooling treatment was 6°C higher than that during the shading+cooling treatment in clear weather. Therefore, the cause of the severe decrease in the number of pods and seed yield by the shading+cooling treatment can be considered as follows. When the low temperature is combined with sufficient sunlight during the flowering period, which is an essential stage for pod setting, the leaf temperature may be high enough for photosynthesis and translocation of assimilates. However, when the low temperature is combined with insufficient sunlight, the temperature may be too low to support photosynthesis and translocation of assimilates, cause severe damage to plants.

We found that the total pod setting ability of Hayahikari for low temperature was higher than that of Toyomusume, but both varieties showed the same susceptibility to shading. Cold weather tolerant varieties such as Hayahikari have shown good performance in most years with cold weather damage. However, in the years with extremely severe damage, they also sometimes received some damage. Therefore, it is important to develop new types of tolerance. Our studies indicate that to breed a variety which has a superior pod setting ability not only at a low temperature but also at a low temperature under insufficient sunlight would be an effective way to improve cold weather tolerance.

In conclusion, a low temperature during the flowering season markedly reduced seed yield and shading during the same period reduces pod number. Shading combined with low temperature treatment causes greater damage than either low temperatures or shading treatment alone. A varietal difference in the seed yield after the low temperature treatment is attributed to the difference in the response of yield components, especially the number of pods per plant, to the low temperature. We are now investigating the pod setting ability, pollination and fertilization at low temperatures, since the number of pod is the most important factor in cold weather tolerance.

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