Chapter 1
General Introduction

1. Botanical characteristics of Asparagus

Asparagus belongs to the family Liliaceae and is a monocotyledonous, rhizomatous, dioecious, herbaceous, and perennial plant (Tutin *et al*., 1980). Although there are several important ornamental species, such as *Asparagus asparagoides* (L.) W. F. Wight, *Asparagus densiflorus* (Kunth) Jessop, *Asparagus myriocladus* Baker, *Asparagus plumosus* Baker and *Asparagus virgatus* Baker, *Asparagus officinalis* L. is only used as an edible plant (Drost, 1997; Riccardi et al., 2012).

Edible asparagus is an ancient vegetable native to Europe (Tutin *et al*., 1980), where it has been cultivated since the times of the Greeks and Romans (Hexamer, 1914). As asparagus has cold tolerance (Yakuwa, 2004), drought tolerance (Wilcox-Lee, 1997) and salinity tolerance (Francois, 1987), it is currently cultivated under various climates all over the world and has become one of the most important perennial vegetables (Drost, 1997).

The edible portion of asparagus plant we need is the young spear just after sprouting in spring. Generally, spears 20–30 cm in length are harvested on ordinary farms in Japan. If spears are exposed to sunlight during their elongation, they become green. On the other hand, if sunlight to spears is blocked, they become white (Minagawa, 2004; Motoki, 2003; Yakuwa, 2004).

Under good environmental conditions, new spears continuously emerge from each bud cluster on the crown and grow into ferns for flowering. Otherwise, as environmental conditions become hostile, for example, lowering of air temperature, asparagus plants accumulate carbohydrates in the storage roots attached to the crown (Hikasa, 2000; Pressman et al., 1993; Shelton and Lucy, 1980; Taga et al., 1980). These carbohydrates are usually utilized for the spear and fern growth in the following spring and early summer (Hikasa, 2000; Pressman et al., 1993; Shelton and Lucy, 1980; Taga et al., 1980).

2. History of white asparagus production in Japan

Although asparagus has been cultivated in Europe for more than 2000 years (Hexamer, 1914), the history of asparagus cultivation in Japan is very short. Dutch traders introduced asparagus to Japan as an ornamental plant in the early 1780s (Kohmura, 2002; Sato and Motoki, 2002; Yakuwa, 2004). Some cultivation trials of asparagus as an edible plant were conducted in the 1870s, but the trials did not become widespread (Kohmura, 2002; Yakuwa, 2004). The first full-scale production of asparagus started in the early 1920s in Hokkaido (Kohmura, 2002; Sato and Motoki, 2002; Yakuwa, 2004). White asparagus spears were produced for canning at that time. Japanese canned white asparagus were widely consumed domestically and also exported overseas due to their high quality. White asparagus production then spread all over Japan. White asparagus became a major cash crop in Hokkaido (Sato and Motoki, 2002), and its production area in Hokkaido had increased to 5,210 ha till 1968 (Yakuwa, 2004).

However, the amount of cheaper canned products made in Taiwan and China started to increase rapidly since 1963, and domestic canning factories gradually reduced their production because of cost competition (Yakuwa, 2004). As a result, white asparagus production in Hokkaido decreased dramatically. Furthermore, as dietary habits of the Japanese diversified after 1960, green asparagus became popular among Japanese consumers for its healthy characteristics (Sato and Motoki, 2002; Yakuwa, 2004). The demand for green asparagus in domestic markets expanded greatly, leading the majority of Japanese farmers to convert their white asparagus fields to green ones, and the white asparagus growing area in Japan suddenly declined. Minagawa (2004) reported its area was only 158 ha in 1997 in Japan. Although there has been no record related to white asparagus production area in Japan since 1997, it is presumed to have decreased to less than 100 ha. Moreover, Benson (2012) reported that 99% of domestic asparagus production in Japan is green asparagus. Indeed, green asparagus is dominantly produced in Japan at present.

Fresh white asparagus is a common vegetable in European countries where white asparagus is dominantly cultivated and consumed (Motoki, 2003; Benson, 2012). This fact began to affect Japanese consumers around the year 2000 (Motoki, 2003). And, recently, the demand for fresh white asparagus in domestic markets is increasing because its distinctive taste and flavor have been

Figure 1-1. White asparagus production by the soil-mound method. (A) and (B) Soil mounds over asparagus rows in open field. (C) and (D) A farmer harvesting a white spear.

revaluated in Japan (Minagawa, 2004; Motoki, 2003).

Therefore, it is important to develop a new technique (a new cropping system) for white asparagus in order to produce a sufficient amount to meet the present and future demand in domestic markets. Since fresh asparagus is treated as one of the expensive vegetables, the introduction of white asparagus production by the new technique is expected to increase farmers' income.

3. Studies related to development of new cropping system of white asparagus

1) Shade control (Blanching method)

With respect to the blanching method in white asparagus cultivation, the soil-mound method has long been utilized as the sole means of white asparagus cultivation in open field (Minagawa, 2004; Motoki, 2003). To blanch the spears, a soil mound (ridged soil) of about 50 cm in height is set on asparagus rows before the start of spear growth (Figure 1-1A, B). Spears elongate in the soil mound, and are harvested with a special knife just before they reach the soil surface (Figure 1-1C, D). Hence, the white spear harvesting operation using this method is much more troublesome than that of green asparagus harvest, because farmers can not observe the spear elongating in the soil mound. The farmers usually can harvest green spears only once a day throughout the harvest period. Whereas white spears must be harvested two or three times a day because they become discolored when spears reach the soil mound surface and are exposed to sunlight. It takes much labor to harvest white spears with high quality in the soil-mound method. Alternative shading method with labor-saving should be developed.

Unique shading techniques are applied into welsh onion (*Allium fistulosum* L.) production. The blanched leaf sheath more than 30 cm in length is required in Japanese markets of welsh onion and farmers must mound soil around leaf sheathes of densely planted welsh onion five or six times during the growing period in open field (Wako et al., 2010). Whereas, in the greenhouse production of welsh onion, methods using rice hulls or rice straws (Kasai et al., 1993), using plastic shading films (Murayama et al., 1998) and using both flower nets and plastic shading films (Tokiwa et al., 2004) have already been established.

No report on a blanching method for white asparagus production in Japan has been published. But Makus and

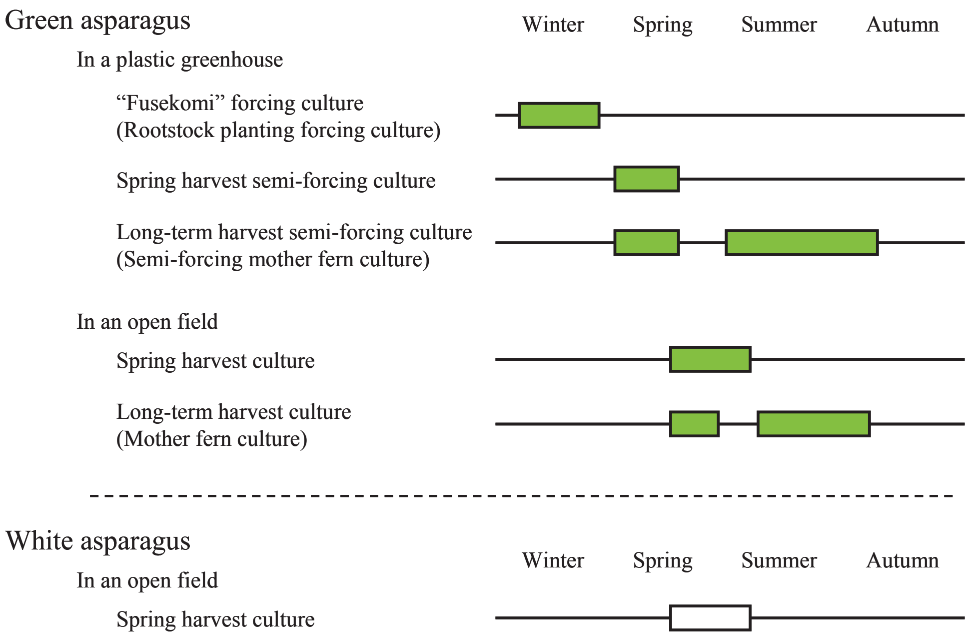


Figure 1-2. Comparison of cropping types between white and green asparagus production in Hokkaido. □ and ■ indicate harvest duration.

Gonzales (1991) indicated that white asparagus may be produced by only using a shading film in open field. Thus, in the present study, the new blanching method, based on the Makus and Gonzales (1991) method, was examined. Since white asparagus with no discoloration tends to be preferable in domestic markets, the spear color is considered to be one of the important characteristics. However, Makus and Gonzales (1991) did not describe the effect of their method on spear discoloration. Hence, the author focused on spear color in this study.

2) Greenhouse production to extend harvest period

The white asparagus production period is limited to spring-harvesting in open fields in Japan (Figure 1-2). In contrast, as the demand for green asparagus increased after 1960s, new cultivation techniques using greenhouses, particularly, plastic greenhouses, have been developed to extend the harvest period. Nowadays, five kinds of cropping types are found in Hokkaido (Figure 1-2) and 3 key techniques, "Fusekomi" forcing culture, spring semi-forcing culture and mother fern culture, are included. (1) "Fusekomi" forcing culture ("Fusekomi" means rootstock planting in Japanese) was established as a type of winter cropping in the early 1960s in Gunma Prefecture in Japan. In this type of cropping, one or two-year-old rootstocks grown in open fields were dug up in autumn and then transplanted in cultural beds in a plastic greenhouse. Green spears were produced in winter with warming cultural beds (Haruyama et al., 1985; Koizumi et al., 2002, 2003, 2013). (2) Spring semi-forcing culture in a plastic greenhouse was developed and led to early spring production in the early 1980s (Kobayashi and Shinsu, 1990). (3) Then, the mother fern cultivation system was established for long-term harvest in the southwestern region of Japan in the late 1980s (Ito et al., 1994; Abe et al., 1999). In this method, some qualified spears are carefully selected after spring harvest and grown into ferns, and all of the other spears are completely removed. Next, spears emerging at the base of mature ferns are harvested from summer to autumn, making it possible to establish the long-term harvest for green asparagus production. Therefore, currently, green asparagus production from December to October is feasible by the combination of these cultivation techniques in Japan (Figure 1-2). In the present study, the application of these established cropping types to white asparagus production is examined to extend the white asparagus harvest period.

In the application of greenhouse techniques (spring harvest semi-forcing and mother fern culture techniques) to white asparagus production, early spring harvest of white spears, from March to April, will be possible. And "Fusekomi" forcing culture will be useful for the production of white spears in winter. One-year-old rootstocks are ordinarily used in main production areas of this forcing culture instead of two-year-old rootstocks at present because of their easy handling size (Goto et al., 1994; Koizumi et al., 2003, 2013). Their compact rootstock size is very effective for dense replanting in artificial beds (compact cultural beds).

The extend of harvest period is succeeded in strawberry because of its compact crown size. The harvest period of June-bearing strawberry production in Japan has been advanced to Christmas, when a large amount of fruits are consumed for cakes, by the strict control of day length, temperature and nutrient management practice to compact crowns for the flower bud differentiation and the subsequent flowering, despite the fact that the June-bearing strawberry is essentially a spring harvest crop (Kakumu et al., 1991; Kumakura and Shishido, 1993; Matsumoto et al., 1981, 1987; Matsumura, 1991; Minegishi et al., 1988; Shishido et al., 1990; Ueki et al., 1993; Yoshida et al., 2012).

3) Breaking of dormancy

In the conventional spring production in asparagus, bud dormancy of rootstock is completely broken and new spears can emerge. On the other hand, the break of bud dormancy is important in the spear production in “Fusekomi” forcing culture in winter. Previous research (Haruyama et al., 1985; Koizumi et al., 2002, 2003) reported that break of bud dormancy of rootstocks was one of the most important problems for achieving high yield in “Fuseokmi” forcing culture for green asparagus production, and temperature below 5ºC was effective for breaking bud dormancy. But, there is little information about varietal differences in bud dormancy in autumn (Koizumi et al., 2002; Ku et al., 2007). Therefore, in the present study, the differences in the bud dormancy among three cultivars were also examined for the stable production of white spears in winter.

4) Spear quality harvested in new cropping method

In recent years, various vegetable consumptions have been increasing due to the rapid diversification of diet. This development includes not only fresh goods and processing materials but also medicated usages focused on functional components. Environmental conditions during crop growth influence the crop quality including the content of functional compound and Maeda et al. (2010) reported that light condition influenced the rutin and polyphenol contents of green asparagus spear in mother fern cultivation. The eating quality of a white asparagus spear is mainly determined by tenderness, sweetness and bitterness (Brueckner et al., 2010; Hoberg et al., 2008; Siomos et al., 1994). Especially, the bitterness of white spears has been attributed to the presence of saponin compounds, protodioscin (Kawano et al., 1975, 1977). It is essential to comprehend the spear qualities including the functional component and cooking characteristics of white asparagus obtained by this new cultivation method.

5) Long-term storage of rootstocks for summer harvest

There is no efficient cropping system for summer production of white spears. If asparagus rootstocks were stored every year until summer, white spears could be harvested in summer by applying the “Fusekomi” forcing technique mentioned above. Asparagus is one of the most widely traded vegetables in the world. Since it is highly perishable, many kinds of reports have been concerned with the postharvest treatment of both white and green asparagus spears for long-distance transportation (long-term storage), including precooling methods, storage temperature, and controlled atmosphere packaging (An et al., 2006; Gariepy et al., 1991; Lallu and Elgar, 2000; Lill and Corrigan, 1996; Lipton, 1965; Siomos et al., 2000; Villanueva et al., 2005). In contrast, there is still too little information available about the storage conditions for asparagus rootstocks.

Hokkaido is located in northern Japan and is well-known for its heavy snow in winter. The idea of storing snow and ice in winter to use as a cold energy resource for space cooling in summer is an old one (Harrington, 1933). Since snow and ice have considerable cold energy, they have a potential for contributing to energy conservation and reduction of CO_2 emissions if they can be utilized for space cooling and the storage of agricultural products in summer (Hamada et al., 2007, 2010). After snow and ice energy were acknowledged as a new type of energy in 2002 by the revision of the Japanese government ordinance under the Law Concerning Special Measures to Promote the Use of New Energy (Hamada et al., 2012), the heavy snow in Hokkaido started to be highly esteemed as one of the natural renewable energy resources. Snow cooling is often used to preserve agricultural products, such as rice grains and chinese yams, etc. (Fujikawa et al., 2010; Kobiyama and Yamagami, 2002; Nakamura and Osada, 2002).

According to Kim et al. (1989b), asparagus spears can elongate at an air temperature of more than 4ºC. Thus, in the present study, long-term storage of one-year-old rootstocks under low temperature (below 4ºC) using snow was examined to develop a white asparagus production system in summer.

4. Scope of this study

Long-term production of white asparagus is very important because the demand for fresh white asparagus in domestic markets has been steadily expanding.

Film-cover, greenhouse, "Fusekomi" forcing culture and storage of rootstocks will be important techniques for the establishment of long-term production of white asparagus and these techniques will be mixed in new cropping system. For example, "Fusekomi" forcing culture will be performed in the place covered with a shading film in a greenhouse for winter production. Following scientific problems are pointed from the related researches.

1) Effectiveness of film-cover as alternative blanching method for white spear production

Light and temperature conditions in tunnel covered with a plastic shading film were evaluated and white spear yield under the dark condition was compared with green spear one. And discoloration of white spear was observed and the suitable size of shaded tunnel was determined.

2) Quality of white spear harvested in film-cover method

Eating and processing qualities, such as sugar content and hardness, in white spear produced by the film-cover method were compared with conventional one produced by the soil-mound method. Besides, content of a functional component, protodiocin, in white spear was also compared between the film-cover and soil-mould methods.

3) Factors affecting spear yield in "Fusekomi" forcing culture with film-cover method

The effects of fresh weight of rootstock and soluble solid content of storage roots on white spear yield were evaluated. In addition, varietal difference in white spear yield was investigated and bud dormancy break was discussed.

4) Effectiveness of long-term storage of rootstocks with snow

Temperature inside the snow mound was recorded during the storage and storage ability of snow mount was valued. Physiological change of rootstocks, sugar contents etc, during the long-term storage with snow was examined and the spear yield after long-term storage was economically evaluated.

In the present paper, some examinations on these problems were carried out and finally new cropping system will be provided.

Chapter 2
Development of forcing production of white asparagus by environmental control with plastic shading film

Introduction

Asparagus (*Asparagus officinalis* L.) is becoming increasingly popular as a health-promoting vegetable (Chin et al., 2002; Maeda et al., 2005, 2010; Shao et al., 1996; Tsushida et al., 1994), and is available in most supermarkets throughout the year. Although green asparagus is the most commonly cultivated form, the consumption of fresh white asparagus has increased in Japan because of its attractive taste, and some farmers begin to produce white spears for the fresh market (Minagawa, 2004; Motoki, 2003). So, it is very valuable to improve the cultivation techniques of white asparagus in order to facilitate its production and response to its domestic demand.

As mentioned in Chapter 1, white asparagus has long been produced with the soil-mound method in Japan (Minagawa, 2004; Motoki, 2003; Yakuwa, 2004) as well as many European countries, such as Germany (Heißer et al., 2006), Greece (Siomos, 1996), Holland (Poll and van den Berg, 1999), and Poland (Knaflewski and Zurawicz, 2003), etc. In this method, for the purpose of blanching the spears, a soil mound (ridged soil) of about 50 cm in height is set on the asparagus row before the start of spear growth. Spears elongate in the soil mound, and are harvested just before they reach the soil surface because they become discolored if they reach the soil mound surface and are exposed to sunlight. Hence, the white spear harvesting operation using this method is more difficult than green asparagus harvest. What is worse, the spears must be harvested two or three times a day throughout the harvest period.

With regard to asparagus spear blanching method, Makus and Gonzales (1991) reported that white asparagus may be produced under opaque row covers in open field. Moreover, Pill et al. (1993) harvested white asparagus spears from one-year-old rootstocks covered by 0.15-mm-thick black plastic film in a cellar, though spears harvested in their study were very small because used rootstocks were not enough size (fresh weight: 100–300 g) to produce ordinary spears. In the present study, the new blanching method (described as ‘the film-cover method’ in latter part) for asparagus spears using only a plastic shading film without soil mounding, based on the Makus and Gonzales (1991) method, was examined.

On the other hand, the white asparagus production period is limited to spring-harvesting in open fields in Japan. Thus, the new technique to extend the harvest period of white asparagus is also needed to promote domestic consumption of fresh white asparagus. Green asparagus production in winter is performed by “Fusekomi” forcing culture explained in Chapter 1. And it is easier to control environmental condition for plant growth in this cropping type than the other cropping types of asparagus. If spears are blanched in this forcing culture, we can produce white spears in winter. Additionally, as white asparagus had never been produced in winter in Japanese asparagus history, this experiment was carried out using the forcing technique. In the present study, the effects of the film-cover method on the spear yield and the anthocyanin appearance on spears in three cultivars were examined.

Materials and Methods

All experiments were conducted at Hokkaido Ornamental Plants and Vegetables Research Center (Takikawa, Hokkaido, Japan).

Experiment 1: Effect of shading condition on spear yield from one-year-old rootstock

Plant materials

One-year-old rootstocks of the cultivar ‘Gijnlim’ were used. Seedlings raised in 9 cm plastic pots for 92 days and seedlings raised in paper pots for 62 days were prepared for this experiment to make a wide range of variations in fresh weight of one-year-old rootstocks. Before planting these seedlings, compound fertilizer at the rate of 150 $kg \cdot ha^{-1}$ of N, 300 $kg \cdot ha^{-1}$ of P_2O_5, 150 $kg \cdot ha^{-1}$ of K_2O and 50 $t \cdot ha^{-1}$ of manure were applied to the experimental open field. The culture beds were ridged up to 30 cm and covered with green plastic films. Then 36 plastic pot and 36 paper pot seedlings were planted in rows 1.5 m apart, plant spacing of 30 cm, and plant depth of 5 cm on June 8, 2005 and grown for 5 months in the field. All rootstocks were dug from the ridged rows on November 8, 2005 before the field was covered with snow. These rootstocks were placed on the ground and covered with a plastic sheet to prevent from drying until replanting them in a greenhouse. All rootstocks were washed with water for removing soil and thier fresh weights were measured on November 31, 2005. And then 36 rootstocks with different fresh weight ranging from 369 to 1,398 g were carefully selected and randomly divided into two groups (18 rootstocks per group).

Four to six storage roots just below a crown, approximately 5 cm in length, were collected per rootstock. Sampled storage roots from each rootstock were squeezed by a hand extractor to obtain a moderate amount of sap and soluble solid content of each sap was measured by a refractometer (PR-101α; ATAGO Co., Ltd., Tokyo, Japan).

Shading condition in greenhouse

Forcing culture was performed in a heated glasshouse. Four culture beds, 0.3 m in depth, 0.6 m in width and 3.0 m in length, were prepared for the replanting rootstocks and electric wires were set to warm the soil at the bottom of the culture beds on December 1, 2005. Nine rootstocks from each group were planted in one bed on December 2, 2005. Plant spacing was 30 cm, and planting depth was about 3 cm below the ground level. And then a small tunnel 50 cm in height and 60 cm in width was set up over each bed. In two beds (green asparagus plots), a transparent plastic film was covered to produce green asparagus spears (Figure 2-1A). In the other beds (white asparagus plots), a shading plastic film with more than 99.99% of shading rate (White-Silver; Tokankosan Co., Ltd., Tokyo, Japan) was set over a small tunnel in order to produce white asparagus spears by eliminating sunlight (Figure 2-1B).

The lighting intensity was measured in daytime on January 14, 17, 18 and 21, 2006 with an illuminance meter (T-10; Konica Minolta Optics, Inc., Tokyo, Japan) to check light conditions in each experimental plot. A temperature data logger (Ondotori Jr.TR-52S; T&D Corporation, Nagano, Japan) was set in each plot to record the air temperature 15 cm above the ground hourly from December 9 to 22 in 2005. Throughout the experimental period, the soil temperature 15 cm below the ground surface was kept at 20ºC by electric wires. Water was supplied during the harvest duration as needed.

Spear Yield

Spears were harvested once a day with opening-closing operation of plastic films from December 15, 2005 to January 20, 2006 (for 37 days) (Figure 2-1A, B). Spears were cut off at the ground level when they had reached more than 24 cm in length. After trimmed to 24 cm, the number and weight of marketable spears obtained from individual rootstock were investigated. Remarkably irregular spears were removed even if less than 24 cm at every harvest. Spears less than 8 g, damaged, bent and with open tips were defined as unmarketable, and the number and weight of those were also investigated.

Experiment 2: Difference in white spear yield among three cultivars in forcing culture with the film-cover method and effect of shading tunnel size on anthocyanin appearance in spears of three cultivars

Most of the cultural practices were the same as the experiment 1.

Plant materials

Three cultivars, ‘UC157’, ‘Grande’ and ‘Gijnlim’, were used in 2005 and 2006. In 2005, 36 seedlings raised in 9cm plastic pots for 92 days and 36 seedlings raised in paper pots for 62 days of each cultivar were planted in the field on June 8, 2005. All rootstocks were dug on November 8, 2005. In 2006, 36 seedlings raised in 9 cm plastic pots for 97 days and 36 seedlings raised in paper pots for 64 days of each cultivar were planted on June 8, 2006 and dug on November 9, 2006. Randomized block design with three replications (plots), containing 12 plants (per plot), was adopted for the field plot arrangement in both years.

After measuring the fresh weight of all the rootstocks collected from each plot, rootstocks from the same cultivar plots were pooled. And then 13–18 rootstocks with different fresh weight of each cultivar were carefully selected in both years. The fresh weight of the rootstock of ‘UC157’, ‘Grande’ and ‘Gijnlim’ ranged from 279 to 1,135 g, from 373 to 1,178 g and from 344 to 1,398 g in 2005, and from 339 to 1,199 g, from 437 to 1,317 g and from 371 to 1,189 g in 2006, respectively. Soluble solid content of storage roots sampled from each rootstock was measured by the same method as experiment 1.

Shading condition in greenhouse

Forcing culture started from December 2, 2005 and from November 30, 2006, respectively. After replanting one-year-old rootstocks in the culture beds, small tunnels 50 cm in height and 60 cm in width, were set up over them in 2005, whereas a large tunnel 200 cm in height and 215 cm in width, was utilized in 2006 (Figure 2-2A, B). All tunnels were covered with the plastic shading film described above for the production of white spears. The lighting intensity was measured in daytime on January 5, 10, 15 and 26, 2007 as mentioned above to compare a dark condition between two different tunnel sizes.

<u>Spear Yield</u>

In 2005, spears were harvested once a day with opening-closing operation of plastic films from December 15, 2005 to January 20, 2006 (for 37 days) (Figure 2-2C). In contrast, in 2006, they were done once a day by persons wearing a flash light on their forehead inside the large shaded tunnel from December 18, 2006 to January 23, 2007 (for 37 days) (Figure 2-2D). Spears were investigated in the same manner of the experiment 1.

<u>Anthocyanin appearance in spear</u>

A pink discoloration in asparagus spear attributes to anthocyanin pigmentation (Wann and Thompson, 1965). Marketable spears with 13–20 g were used to clarify the difference in this discoloration among three cultivars and between two shaded tunnel sizes, small in 2005 and large in 2006. The extent of a pink discoloration in both the tip and base portions of those spears was evaluated by 4 subjective color scores (Score 1: no pink discoloration, 2: slight, 3: little, 4: deep) (Figure 2-3A, B).

Experiment 3: Winter productivity of white spear from one-year-old rootstock of 'UC157' in forcing culture with large shaded tunnel

<u>Plant materials and forcing culture with large shaded tunnel</u>

In 2007 and 2008, the productivity of white spears in winter was examined in detail by using only 'UC157'. Seedlings raised in 9 cm plastic pots for 110 days in 2007 and for 117 days in 2008 were prepared, and then planted in the field on June 4, 2007 and June 11, 2008, respectively. All rootstocks were dug on October 31 in both years. Forcing culture started from November 7, 2007 and November 26, 2008, respectively. These experiments were carried out with three replications (plots), containing 12 plants (per plot). Cultural management was the same as the experiment 1 and 2 except for plant spacing, 30cm in 2007 and 40 cm in 2008. A large shaded tunnel 200 cm in height was set up over the culture bed, and the harvest duration of white spears was 40 days in each year. Spears were investigated in the same manner of the experiment 1.

Figure 2-1. Asparagus spears at harvest in the experiment 1. (A) Green asparagus plot covered with a transparent plastic film. (B) White asparagus plot covered with a shading plastic film.

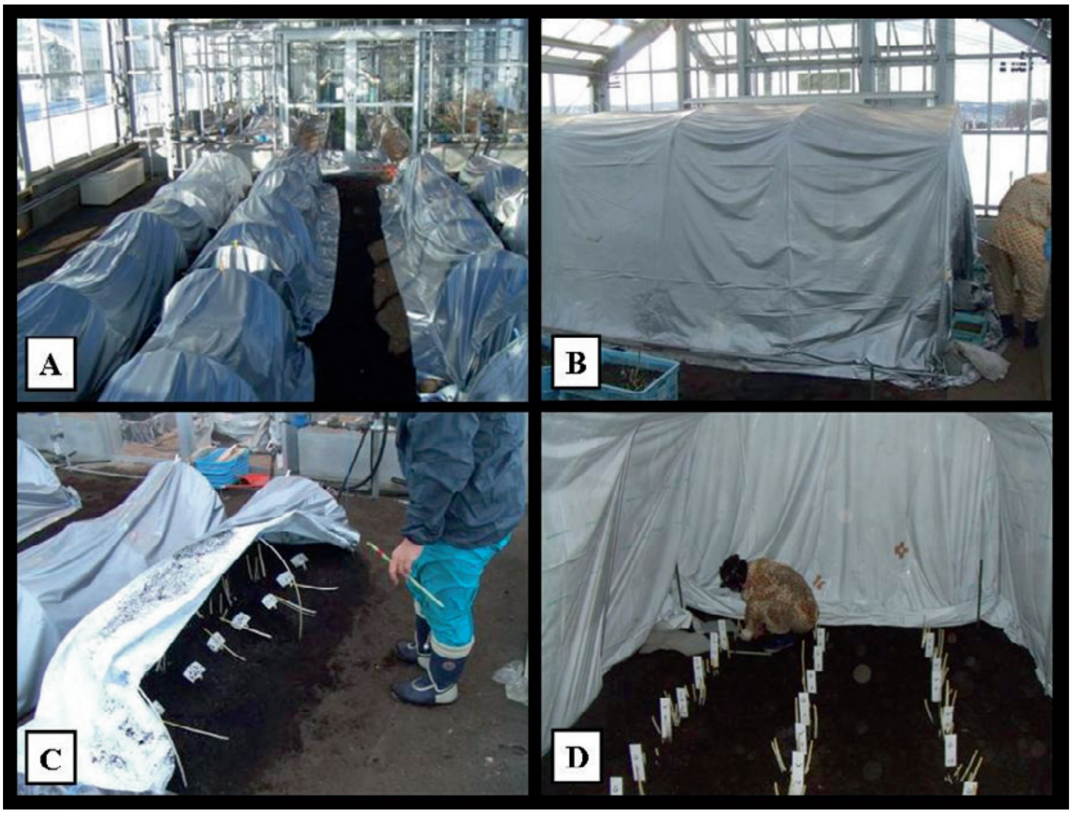


Figure 2-2. Shading treatment for asparagus spears in the experiment 2. External appearance of small shaded tunnel (A) in 2005 and large shaded tunnel (B) in 2006. Harvest work in small shaded tunnel (C) and large shaded tunnel (D).

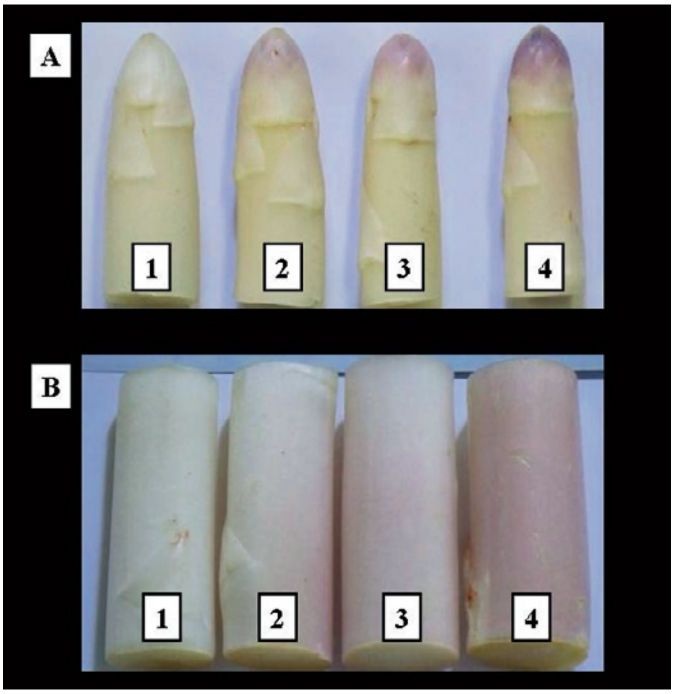


Figure 2-3. Color score for anthocyanin appearance in spear. (A) Four subjective color scores for tip portion of spear. (B) Four subjective color scores for bottom portion of spear. Score 1: no pink, Score 2: slight, Score 3: little, Score 4: deep.

Results

Experiment 1: Effect of shading condition on spear yield from one-year-old rootstock

The value of lighting intensity showed the range from 8.26 to 10, 400 lx in green asparagus plots and from 0.00 to 0.35 lx in white asparagus plots, respectively. The mean air temperature 15 cm above the ground in the tunnel in green plots from December 9 to 22 in 2006 was 17.9ºC (the air temperature ranged from 13.8 to 36.3ºC) and that in white plots was 16.6ºC (the air temperature ranged from 13.0 to 29.0ºC).

The soluble solid content of storage roots from one-year-old rootstocks prepared for green plots ranged from 11.1 to 24.5% (the mean±SD was 17.8±3.5), that for white plots did from 12.2 to 24.7% (the mean±SD was 18.4±3.6), respectively.

Since there was no significant correlation between the fresh weight of one-year-old rootstocks and the soluble solid content of storage roots, multiple regression analysis was performed. The yield characters (total weight of all spears, total weight of marketable spears, number of marketable spears, mean weight of marketable spears) were used as outcome variable, the fresh weight of one-year-old rootstock and the soluble solid content of storage roots as predictor variable. When the total weight of all spears was used as outcome variable, standard partial regression coefficient of the fresh weight of one-year-old rootstock and of the soluble solid content of storage roots in white plots was 0.6182 (significant at 1%) and –0.0632 (not significant), those in green plots was 0.8312 (significant at 1%) and 0.0165 (not significant), respectively (Table 2-1). Therefore the contribution rate of the soluble solid content of storage roots to the total weight of all spears was extremely small. As the similar results were obtained when the other yield characters except for mean weight of marketable spears were used as outcome variable, regression analysis was done by simple regression on the fresh weight of one-year-old rootstock to the yield characters.

A strong positive correlation between the fresh weight of one-year-old rootstock and the total weight of all spears, or the total weight of marketable spears, or the number of marketable spears, was found in both green and white asparagus plots (Figure 2-4). As the fresh weight of one-year-old rootstock was heavier, these yield characters tended to increase in both green and white plots. On the other hand, there was no correlation between the fresh weight of one-year-old rootstock and the mean weight of marketable spears. Hence, simple regression were used to assess the relations between the fresh weight of one-year-old rootstock and the total weight of all spears, or the total weight of marketable spears, or the number of marketable spears. Although no definite difference in the total weight of all spears, or the total weight of marketable spears, or the number of marketable spears was observed between green and white plots, the total weight of marketable spears in white plots tended to be larger than that in green plots within the range of the fresh weight of rootstock used in this experiment.

Table 2-1. Standard partial regression coefficient of predictor variable obtained from multiple regression analysis.

Outcome variable	Plot	Predictor variable	
		Fresh weihgt of rootstock	Soluble solid content of storage roots
Total weight of all spears	White	0.6182 **[z]	–0.0632
	Green	0.8312 **	0.0165
Total weight of marketable spears	White	0.5821 *	0.0505
	Green	0.7167 **	–0.1261
Number of marketable spears	White	0.6072 **	–0.0307
	Green	0.6630 **	–0.1061
Mean weight of marketable spears	White	–0.2322	0.1058
	Green	0.3628	–0.0399

[z] **, * indicate significant at $P < 0.01$ and $P < 0.05$, respectively.

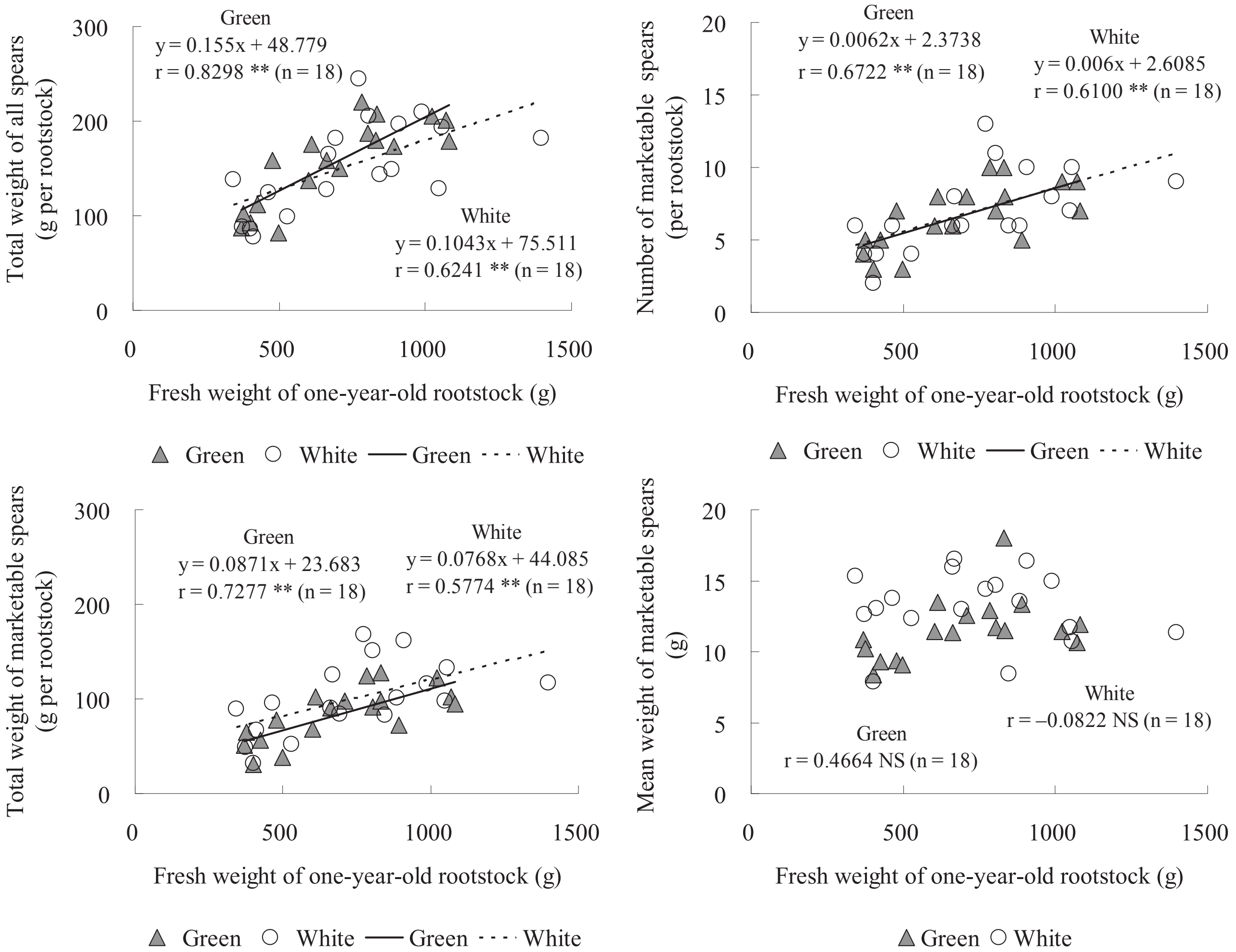


Figure 2-4. Relationship between fresh weight of one-year-old rootstock of Gijnlim and its yield characters in green and white plots. NS, ** indicate not significant, significant at $P< 0.01$, respectively.

Experiment 2: Difference in white spear yield among three cultivars in forcing culture with the film-cover method and effect of shading tunnel size on anthocyanin appearance in spears of three cultivars.

In 2005, the small values of lighting intensity, ranging from 0.00 to 0.35 lx, were found in the small shaded tunnels. Additionally spears elongating in small shaded tunnels were inevitably exposed to sunlight for a moment when they were harvested. In contrast, a completely dark condition, constantly less than 0.01 lx, was maintained in the large shaded tunnel in 2006. As a result, a pink discoloration caused by anthocyanin pigmentation was observed at the bottom portion of spears in all cultivars harvested in 2005 (Table 2-2). In contrast, anthocyanin did not appear in all spears of each cultivar in 2006.

The soluble solid content of storage roots from one-year-old rootstocks of ‘UC157’, ‘Grande’ and ‘Gijnlim’ ranged from 9.8 to 17.7% (the mean±SD was 13.6±2.0), from 8.4 to 24.0% (the mean±SD was 19.2±4.1) and from 12.2 to 24.7% (the mean±SD was 18.4±3.6) in 2005, and from 22.8 to 27.9% (the mean±SD was 25.1±1.4), from 23.8 to 29.3% (the mean±SD was 26.0±1.6) and from 22.9 to 26.4% (the mean±SD was 24.1±1.0) in 2006, respectively.

At first, multiple regression analysis was performed likewise the experiment 1. The yield characters (total weight of all spears, total weight of marketable spears, number of marketable spears, mean weight of marketable spears) were used as outcome variable, the fresh weight of one-year-old rootstock and the soluble solid content of storage roots as predictor variable. But, the contribution rate of the soluble solid content of storage roots to each yield character tended to be extremely small like the results obtained in the experiment 1 (Data not shown), regression analysis was done by simple regression on the fresh weight of one-year-old rootstock to the yield characters.

A strong correlation was recognized between the fresh weight of one-year-old rootstocks and the total weight of marketable spears in three cultivars in both 2005 and 2006 (Figure 2-5). The total weight of marketable spears of ‘UC157’ tended to be larger than that of the others within the range of the fresh weight of rootstock used in the two years and ‘Gijnlim’ tended to be smaller than the others. Same tendencies were shown in the relationship between the fresh weight of one-year-old rootstocks and the total weight of all spears, or the number of marketable spears in all cultivars (Data not shown). But, no correlation was observed between the fresh weight of one-year-rootstocks and the mean weight of marketable spears except for ‘UC157’ in 2006 (Figure 2-5).

Table 2-3 represents the fresh weight of one-year-old rootstocks of three cultivars grown from 9 cm plastic pot seedlings in the field. These values were used to estimate the marketable yield of three cultivars using the simple regression formulas obtained in Figure 2-5. As a result, the marketable yield of ‘Gijnlim’ was significantly lower than the others in the two years (Table 2-3).

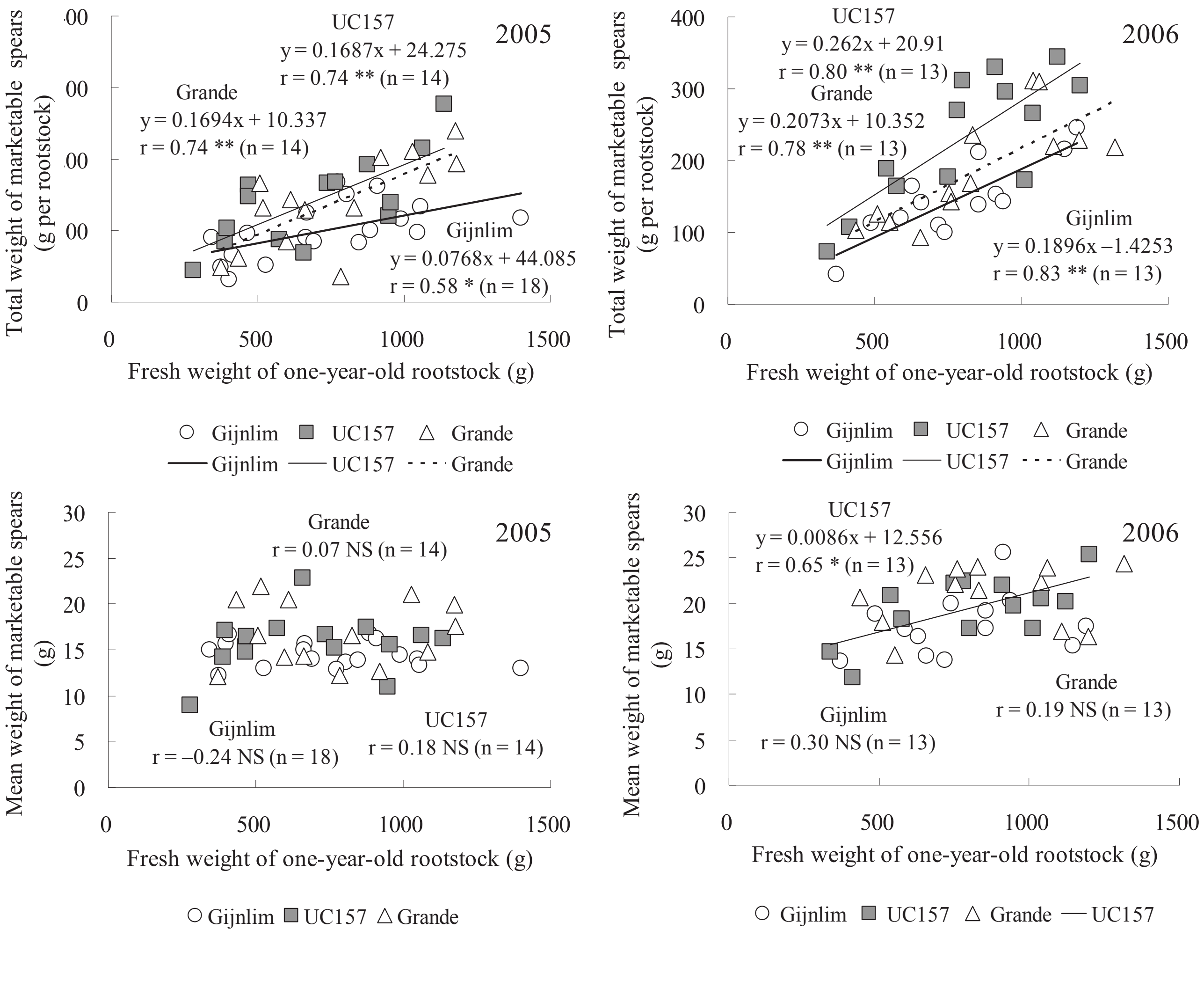


Figure 2-5. Relationship between fresh weight of one-year-old rootstock and total weight of marketable spears, or mean weight of marketable spears in three cultivars in forcing culture with shaded tunnels. NS, *, ** indicate not significant, significant at *P*<0.05 and at *P*<0.01, respectively.

Table 2-2. Effect of shaded tunnel size over cultural beds in forcing culture on anthocyanin appearance in white asparagus spears of three cultivars.

Cultivar	Portion	Small shaded tunnel (2005)					Large shaded tunnel (2006)				
		No. of	Color score ^{z}				No. of	Color score ^{z}			
		spears	1	2	3	4	spears	1	2	3	4
Gijnlim	Tip	24	24	0	0	0	10	10	0	0	0
	Base	24	1	4	16	3	10	10	0	0	0
UC157	Tip	10	10	0	0	0	13	13	0	0	0
	Base	10	7	3	0	0	13	13	0	0	0
Grande	Tip	17	17	0	0	0	18	18	0	0	0
	Base	17	8	8	1	0	18	18	0	0	0

^{z}The extent of pink discoloration (anthocyanin pigmentation) of each cultivar spear was evaluated by 4 subjective scores (Score 1: no pink discoloration, Score 2: slight, Score 3: little, Score 4:deep).

Table 2-3. Fresh weight of one-year-old rootstock and its marketable yield caluculated with simple regression formula in three cultivars.

Year	Cultivar	Fresh weight of one-year-old rootstock^z (g)	Marketable yield^y (kg•ha^{-1})
2005	Gijnlim	819 ± 43 b^x	2,377 ± 73 c
	UC157	832 ± 16 b	3,658 ± 59 b
	Grande	981 ± 26 a	3,922 ± 96 a
2006	Gijnlim	845 ± 18 a	3,527 ± 78 c
	UC157	983 ± 44 a	6,186 ± 258 a
	Grande	956 ± 34 a	4,634 ± 159 b

^{z}The weight of rootstocks grown from 9 cm plastic pot seedlings for one season in the open field.

^{y}The total weight of marketable spears caluculated with simple regression foumulas in Figure 2-5. The plant density was 22,222 plants•ha^{-1} in both years.

^{x}Values show the mean±SE (n = 3). Different letters in the column indicate significant at P<0.05 by Tukey's test.

Table 2-4. White asparagus productivity obtained from one-year-old rootstock of UC157 in forcing culture with large shaded tunnel.

Year	Marketable spears			Marketable yield^y
	Number^z (per rootstock)	Total weight^z (g per rootstock)	Mean weight^z (g)	(kg•ha^{-1})
2007	12.5 ± 0.5	205 ± 11.2	16.3 ± 0.4	4,553
2008	13.7 ± 0.7	308 ± 9.5	22.6 ± 0.4	5,138

^{z}Values indicate the maen±SE (n = 3).

^{y}Total weight of marketable spears obtained from all rootstocks grown for one saeson in the open field. The plant density was 22,222 plants•ha^{-1} in 2007, 16,667 plants•ha^{-1} in 2008, respectively.

Experiment 3: Winter productivity of white spear from one-year-old rootstock of 'UC157' in forcing culture with large shaded tunnel

White spears with no pink discoloration were harvested inside the large shaded tunnel in 2007 and 2008. The total weight of marketable spears per rootstock was 205 g in 2007 and 308 g in 2008, respectively (Tabla 2-4), resulting in the marketable yield of 4,553 kg·ha^{-1} in 2007 and 5,138 kg·ha^{-1} in 2008.

Discussion

In general, the productivity of asparagus depends on the vigor of rootstock grown in the previous season. Major components are thought to be the size of rootstock and soluble solid content of storage roots (Hikasa, 2000; Taga et al., 1980). Thus, multiple regression analysis was performed using these components. The yield characters (total weight of all spears, total weight of marketable spears, mean weight of marketable spears, number of marketable spears) were used as outcome variable, the fresh weight of rootstock and the soluble solid content of storage roots as predictor variable. The contribution rate of the soluble solid content of storage roots to each yield character except for mean weight of marketable spears tended to be much smaller than that of the fresh weight of rootstocks in both years. The 37 day-harvest period, not so long, and the small variation of the soluble solid content of storage roots from rootstocks used in this experiment may be the reasons for the small contribution rate of the soluble solid content of storage roots.

The result of simple regression analysis showed a strong positive correlation between the fresh weight of one-year-old rootstock and the total weight of all spears, or the total weight of marketable spears, or the number of marketable spears, respectively. As the fresh weight of rootstock increased, the total weight of all spears and the total weight of marketable spears became larger, the number of marketable spears also became greater. These results are consistent with the report of Haruyama et al. (1985), reconfirming that the fresh weight of rootstock just before replanting in a culture bed is one of the important yield components in “Fusekomi” forcing culture.

The elongation rate of asparagus spear is strongly affected by an air temperature in its growing period (Culpepper and Moon, 1939; Kim et al., 1989b). Thus, the mean air temperature 15 cm above the ground in both green and white plots of the experiment 1 was recorded. But the difference in the mean air temperature between green and white plots was very small (about 1ºC). Therefore, effect of the shading treatment on the spear yield was discussed as below.

There was no definite difference in the total weight of all spears, the total weight of marketable spears and the number of marketable spears between white and green asparagus plots. Kim and Sakiyama (1989a) reported that light conditions did not affect spear elongation and discussed that photosynthesis of spears did not have an influence on spear growth and carbohydorates needed for its growth were supplied from storage roots of a rootstock. Moreover, Ledgard et al. (1994) reported that the nitrogen accumulated in ferns in summer was transported to the crown and storage roots in autumn, and then preferentially utilized for spear growth in the following spring. Since it is speculated that the spear yield in forcing culture is strongly dependent on the fresh weight of rootstock dug up and is not affected by a light condition in a culture bed, the same yield in green asparagus forcing culture is considered to be obtained in white asparagus forcing culture by the film-cover method with large tunnel.

In white asparagus production by the soil-mound method, when spear tips reach the soil mound surface, and are exposed to sunlight due to the delay of harvest, a pink discoloration caused by anthocyanin pigmentation occurs in spear, leading to the grade down of products (Yakuwa, 2004). An obvious difference in this pink discoloration in spears harvested between two different shaded tunnel sizes was observed in the present study. In 2005, as the small values of lighting intensity, ranging from 0.00 to 0.35 lx, were found in the small shaded tunnels and spears growing in the small shaded tunnels were inevitably exposed to sunlight at harvest time because of the opening-closing operations of plastic shading films over the tunnels, a pink discoloration was observed at the bottom portion of spears in all cultivars. In contrast, no pink discoloration was found in all spears of each cultivar thanks to a complete dark in the large shaded tunnel in 2006.

These results suggest that the production of white asparagus spears without any pink discolorations derived from anthocyanin pigmentation is possible in “Fusekomi” forcing culture with large shaded tunnel.

A significant difference in white spear yield among three cultivars was recognized in forcing culture with the film-cover method in this study. It is well-known that asparagus shows the period of bud dormancy in autumn and the dormancy is broken by chilling treatment (Drost, 1997; Haruyama et al., 1985; Hayashi and Hiraoka, 1983; Kobayashi and Shinsu, 1990; Ku et al., 2007). And it was also reported that if asparagus rootstocks of which the dormancy are not completely broken are planted in a culture bed in “Fusekomi” forcing culture, they could not show their potential yields (Haruyama et al., 1985;

Koizumi et al., 2002, 2003). Moreover, it was revealed that there was an obvious difference in the chilling treatment including temperature, period, needed for breaking bud dormancy among cultivars (Koizumi et al., 2002; Ku et al., 2007), Furthermore, Koizumi et al. (2002) used the cumulative hours of chilling exposure to the air temperature below 5ºC as the indicator of breaking bud dormancy, and then concluded that two-year-old rootstocks of ‘UC157’ and ‘Grande’ required 350 hours and more than 500 hours for breaking their bud dormancy, respectively. So, this indicator was applied in the present study, 607 hours in 2005 and 600 hours in 2006 were calculated, respectively. Because Koizumi et al. (2002) also pointed out that the bud dormancy of one-year-old rootstocks was broken by shorter period of chilling than that of two-year-old rootstocks, the bud dormancy of one-year-old rootstocks of ‘UC157’ and ‘Grande’ used in the two years was considered to be completely broken by the sufficient chilling hours (more than 600 hours in both years). Such a result indicates that both ‘UC157’ and ‘Grande’ could show their potential spear yields at the culture beds in both years because of the release of bud dormancy.

On the other hand, the spear yield of ‘Gijnlim’ was significantly smaller than that of the other cultivars. Since it was reported that ‘Gijnlim’ showed higher productivity than ‘UC157’ in spring harvest open field culture in Hokkaido (Doi and Dohi, 2002; Uragami et al., 1993), this result implies that one of the factors for low productivity of ‘Gijnlim’ in this study attributed to the lack of chilling period needed for breaking bud dormancy, and ‘Gijnlim’ might need much longer chilling treatment for breaking its bud dormancy than ‘UC157’ and ‘Grande’. Further research on the difference in bud dormancy among cultivars in autumn is necessary for better understanding the precise timing of digging up rootstocks of which their bud dormancy are completely broken. These results demonstrate that ‘UC157’ and ‘Grande’ are suitable for white asparagus production in winter by “Fusekomi” forcing culture with the film-cover method from the standpoint of productivity. The experiment 3 indicated that marketable yield of 4,553 kg·ha^{-1} in 2007 and 5,138 kg·ha^{-1} in 2008 was obtained in white asparagus forcing culture with one-year-old rootstocks of ‘UC157’. These yields in both years are thought to be sufficient for famers considering a current yield, about 3,000 kg·ha^{-1}, obtained by green asparagus production in “Fusekomi” forcing culture in Japan (Koizumi et al., 2013).

Chapter 3
Application of the film-cover method with large tunnel to semi-forcing production in a plastic greenhouse

Introduction

The possibility of white asparagus production in winter by a large shaded tunnel was discussed in Chapter 2. Since this film-cover method is a very easy technique, it has potential for an application to the other asparagus cropping types. This means further extension of the harvest period of white asparagus in Japanese domestic production.

Therefore, the feasibility for the application of the film-cover method to an established plantation in spring harvest semi-forcing culture in a plastic greenhouse, where spears are harvested from April to May, was examined.

Materials and Methods

Plant materials and shading treatment

The yield of white spears produced by the film-cover method (white spear harvest plot) was compared to that of green spears by an ordinary method (green spear harvest plot) in spring harvest semi-forcing culture in a plastic greenhouse. Eight-year-old rootstocks of ‘UC157’ grown in a plastic greenhouse at Hokkaido Ornamental Plants and Vegetables Research Center (Takikawa, Hokkaido, Japan) were prepared for this experiment. The same experimental treatment was sequentially done in each plot from 2006 to 2007. Randomized block design with two replications (plots), containing 34 plants per plot (16.2 m^2 per plot), was adopted. According to the examination of spear yield during summer season in green asparagus long-term harvest culture (mother fern culture) in 2005, all plots used in this experiment showed an uniformity of spear productivity (the mean±SE (n = 2) of marketable spear yield in green plots was 11,310±495 $kg·ha^{-1}$ and that in white plots was 11,278±591 $kg·ha^{-1}$).

A large tunnel 200 cm in height and 215 cm in width was set up and covered with a plastic shading film (White-Silver; Tokankosan Co., Ltd., Tokyo, Japan) as described in Chapter 2 on April 17, 2006 and April 19, 2007 just before sprouting in each white plot. Although this plastic film has white and silver color sides, white side was used as outside in this experiment because an air temperature inside the tunnel became more than 45 ºC when its silver side was used as outside in the preliminary experiment. The lighting intensity inside each shaded tunnel was measured in the morning from April 25 to 27 and from May 15 to 17, 2006, and from April 26 to May 1 in 2007 with an illuminance meter (T-10; Konica Minolta Optics, Inc., Tokyo, Japan) to check light conditions in each experimental plot. A temperature data logger (Ondotori Jr.TR-52; T&D Corporation, Nagano, Japan) was set in all plots to record the air temperature 15 cm above the ground and the soil temperature 15 cm below the ground hourly from April 28 to June 6 in 2006 and from April 20 to June 1 in 2007. The humidity 15cm above the ground in all plots was measured with a hygrometer in the morning from May 22 to 27 in 2006 and from May 4 to 12 in 2007. Spears were harvested once a day for 35 days in both years, from May 4 to June 7 in 2006 and from April 29 to June 2 in 2007 (Figure 3-1). The shaded tunnels were removed just after 35 day-harvest in white plots.

Compound fertilizer at the rate of 200 $kg·ha^{-1}$ of N, 197 $kg·ha^{-1}$ of P_2O_5, 191 $kg·ha^{-1}$ of K_2O and 40 $t·ha^{-1}$ of manure were applied in each year. The other cultural managements were based on the conventional methods in Hokkaido.

Spear yield

Spears were cut off at the ground level when they had reached more than 24 cm in length. After trimmed to 24 cm, the number and weight of marketable spears were investigated. And every marketable spear was classified into 4 grades (8 g≦S＜13 g, 13 g≦M＜20 g, 20 g≦L＜33 g, 33 g≦2L). The weight of unmarketable spears including less than 8 g, damaged, bent and with open tips was also measured.

Spear thickness and weight

This measurement was performed from May 8 to 14 in 2007. Spears with 13.0–16.0 mm in maximum diameter at the basal portion (24.0 cm from tip) were carefully collected from each plot. Spear length at harvest, maximum diameter in two spear portions (upper: 5.0 cm from tip; middle: 12.0 cm from tip) and spear weight after trimmed to 24 cm in length were measured.

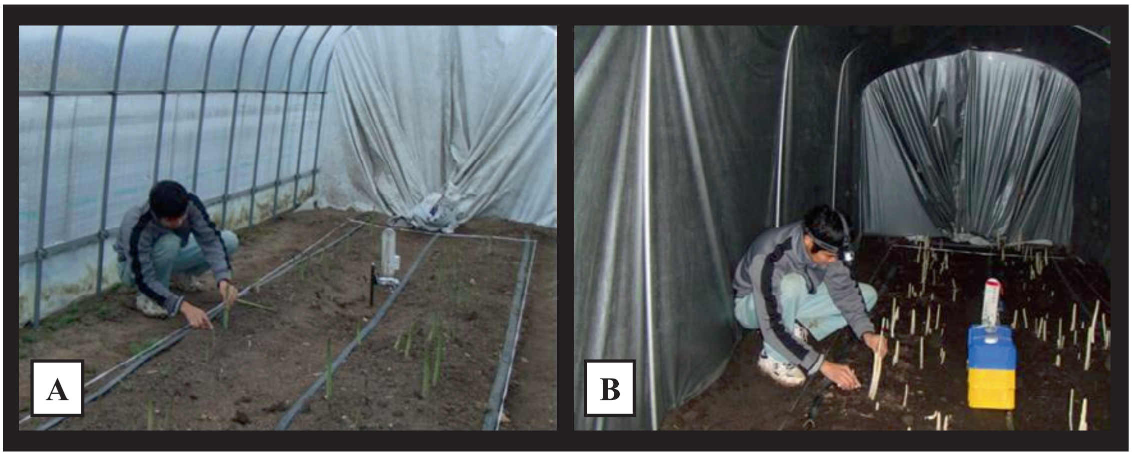


Figure 3-1. Experimental plots in a plastic greenhouse. (A) Green spear harvest plot and (B) white spear harvest plot.

Figure 3-2. Asparagus spears obtained from experimental plots. Left, Spears from green plots. Right, Spears from white plots.

Results

The value of lighting intensity in green plots showed the range from 50,120 to 75, 250 lx in 2006 and from 3,110 to 69,000 lx in 2007. In contrast, that in white plots was invariably kept less than 0.01 lx in both years thanks to a large shaded tunnel.

The mean air temperature 15 cm above the ground in white plots was 16.5ºC in 2006, 15.3ºC in 2007, and that in green plots was 15.3ºC in 2006, 15.3ºC in 2007. The mean air temperature in white plots tended to be approximately 1ºC higher than that in green plots in 2006, and no difference was found in 2007. On the other hand, the mean soil temperature 15 cm below the ground in white plots was 13.6ºC in 2006, 13.0ºC in 2007, and that in green plots was 16.2ºC in 2006, 14.6ºC in 2007. The mean soil temperature in white plots tended to be lower than that in green plots in both years.

The humidity in green plots greatly varied with a wide range from 5 to 70% because it was strongly dependent on weather condition, meanwhile, that in white plots was comparatively stable (from 69 to 75%) in the two years.

White spears with no pink discoloration were harvested inside the large shaded tunnel throughout this examination (Figure 3-2). The marketable spear yield in white plots was obviously smaller than that in green plots at the first week of harvest, but it gradually became larger than in green plots as the harvest progressed (Table 3-1). As a result, there was no difference in the total marketable spear yield harvested for 35 days between white and green plots. Furthermore, no difference in the total spear yield including unmarketable spears was also found between them. The number of marketable spears in white plots was significantly smaller compared to that in green plots (Table 3-2). On the other hand, since the number of 2L grade spear was large and the other grades were small in white plots compared with green plots, the mean weight of marketable spears in white plots was significantly larger than that in green plots.

In white plots, the spear length was 2.4 cm shorter, and the maximum spear diameter in upper and middle portions was 2.8 mm and 2.7 mm longer, the spear weight was 8.2 g larger compared with green plots (Table 3-3).

Table 3-1. Comparison of spear yields between white and green plots in spring harvest semi-forcing culture in a plastic green house.

Year	Cultivation[z]	Marketable spear yield ($kg{\bullet}ha^{-1}$)						Total spear yield[xy] ($kg{\bullet}ha^{-1}$)
		1st week	2nd week	3rd week	4th week	5th week	Total[y]	
2006	White	187	2,296	3,300	2,330	1,847	9,960 ± 187	14,411 ± 144
	Green	1,674	2,620	2,044	1,649	1,626	9,613 ± 60	14,584 ± 259
2007	White	232	1,581	1,609	1,420	1,419	6,261 ± 1,854	8,580 ± 2,499
	Green	1,137	1,529	1,141	1,281	1,045	6,133 ± 1,060	10,120 ± 1,688
Two-way ANOVA[w]	Cultivation (C)						NS	NS
	Year (Y)						*	*
	C × Y						NS	NS

[z]White spears were producd by the film-cover method in white plots.
[y]Values indicate the mean±SE (n = 2).
[x]Total spear yield includes unmarketable yield.
[w]NS, * indicate not significant, significant at P<0.05, respectively.

Table 3-2. Comparison of the number and mean weight of marketable spears between white and green plots in spring harvest semi-forcing culture in a plastic green house.

Year	Cultivation[z]	Number of marketable spears（per rootstock）					Mean weight of marketable spears[y](g)
		2L	L	M	S	Total[y]	
2006	White	6.8	1.6	0.4	0.2	9.0 ± 0.2	44.7 ± 1.7
	Green	4.6	5.2	2.7	1.0	13.5 ± 0.2	28.9 ± 0.2
2007	White	3.3	2.7	1.5	1.0	8.5 ± 1.1	29.3 ± 4.9
	Green	1.8	4.1	3.0	1.8	10.7 ± 1.1	23.1 ± 1.6
Two-way ANOVA[x]	Cultivation (C)					*	*
	Year (Y)					NS	*
	C × Y					NS	NS

[z]White spears were producd by the film-cover method in white plots.
[y]Values indicate the mean±SE (n = 2).
[x]NS, * indicate not significant, significant at P<0.05, respectively.

Table 3-3. Comparison of spear lenght at harvest, spear diameter and weight after trimmed of marketable spears between white and green plots in spring harvest semi-forcing culture in a plastic green house.

Cultivation[z]	Number of spears inevestigated	Spear length at harvest[y] (cm)	Spear diameter[y] (mm) Upper (5.0 cm from tip)	Middle (12.0 cm from tip)	Basal (24.0 cm from tip)	Spear weight after trimmed[y] (g)
White	37	25.6 ± 0.19	13.0 ± 0.17	15.1 ± 0.18	14.4 ± 0.15	33.6 ± 0.74
Green	52	28.0 ± 0.32	10.2 ± 0.12	12.4 ± 0.12	14.4 ± 0.14	25.4 ± 0.47

[z]White spears were producd by the film-cover method in white plots.

[y]Values indicate the mean±SE. Harvested spears were trimmed to 24 cm in length and then spear diameter and weight were investigated.

Discussion

In the present study, setting up a large shaded tunnel 200 cm in height just before spears emergence above the ground surface made it possible to maintain a complete dark condition (lighting intensity: less than 0.01 lx) in white spear harvest plots. Although the humidity in the large tunnels constantly showed high range (from 69 to 75%), an occurrence of both physiological disorder and disease damage derived from this humidity was not observed. It is known that a pink discoloration in asparagus spear attributes to anthocyanin pigmentation (Wann and Thompson, 1965). White spears with no pink discoloration were harvested inside the large shaded tunnel in both years. These results demonstrate that white spears with no pink discoloration can be also produced in spring harvest semi-forcing culture when a large shaded tunnel in which a complete dark condition is consistently maintained is set up on asparagus rows.

As the result of this study indicated that the mean soil temperature in white plots tended to be lower than that in green plots in both years, a large shaded tunnel was considered to suppress the increase of a soil temperature in white plots. Hayashi and Hiraoka (1978) reported that the higher a soil temperature became within its range from 12 to 26 ºC, the earlier asparagus spears sprouted. Therefore it was suggested that this effect of shading treatment on a soil temperature caused the lower yield of marketable spears in white plots than in green plots at the first week of harvest in both years. But there was no difference in the total marketable spear yield harvested for 35 days between white and green plots, although the number of marketable spears in white plots was less than in green plots, as the mean weight of marketable spears in white plots was larger than in green plots. These results suggest that if white asparagus spears are produced by the film-cover method in spring harvest semi-forcing culture in a plastic greenhouse, the same yield as that of green asparagus production can be obtained.

Since the mean weight of marketable spears and the number of 2L grade spears in white plots was obviously larger and greater than in green plots in 2006, the measurements of spear length at harvest, spear thickness and weight were performed in 2007. When spears with the same diameter at the basal portion were compared between white and green plots, the spear length in white plot was shorter, the maximum spear diameter in the upper and middle portions was larger, and the spear weight was heavier than in green plots. Thus, these results suggest that in white plots longitudinal elongation of spears is suppressed, lateral growth of spears is stimulated and those spear weight becomes larger, resulting in the increase of the number of 2L grade spears.

Makus and Gonzales (1991) indicated that white spears with the larger weight than green spears were produced by setting a shaded tunnel 35 cm in height on asparagus rows in an open field. This is consistent with the present result. Moreover, they reported that the yield of white spears was also larger than that of green ones because the same number of marketable spears as in green plots was harvested in white plots. Therefore, it is expected that when the large shaded tunnel is set up in a plastic greenhouse, if some kinds of cultural techniques to warm a soil temperature in the tunnel are conducted at the same time, much larger spear yield in white asparagus production by the film-cover method can be obtained than in green asparagus production in spring harvest semi-forcing culture.

The results in this chapter will serve to facilitate white asparagus production in spring harvest semi-forcing culture, leading to further extension of the harvest period of white asparagus in Japanese domestic production.

Chapter 4
Comparison of quality of white asparagus spears produced by two different blanching methods

Introduction

In Japan, two blanching methods start to be used to produce white asparagus spears. In the film-cover method, blanched spears grow above the ground surface even though they are in the complete dark in the shaded tunnels, and harvesting is much easier than in the soil-mound method. Therefore, this film-cover method is rapidly spreading for white asparagus production among Japanese growers at present.

However, some growers point out that spears produced by the film-cover method may be different from those from the traditional method in external qualities such as shape and color, and several restaurant cooks and can processing manufacturers also comment that the shape of spears produced by the film-cover method tends to be lost because of their tenderness if they are cooked or processed like spears produced by the soil-mound method. Additionally, some growers and consumers claim that taste and flavor differ between white asparagus spears blanched using the film-cover method and the soil-mound method. The major factors that determine the eating quality of a white asparagus spear are tenderness, sweetness and bitterness (Brueckner et al., 2010; Hoberg et al., 2008; Siomos et al., 1994). The bitterness of white spears has been attributed to the presence of saponin compounds (Kawano et al., 1975, 1977). Protodioscin, a furostanol saponin, was confirmed as the principal saponin compound in white asparagus spears (Brueckner et al., 2010; Chin et al., 2002; Schwarzbach et al., 2006; Shao et al., 1996; Wang et al., 2003).

As there is little information on the difference in the quality of spears produced by the two blanching methods mentioned above, those spears are treated as the same product in many markets. It is necessary to provide precise information on the differences in spear characteristics between the two different blanching methods to make effective use of spears obtained from either blanching method.

The objective of this study was to compare the qualities of white asparagus spears produced by two different blanching methods, the soil-mound method and the film-cover method, in detail, focusing on tip tightness, color, thickness, weight, hardness, and components contribute to taste, such as sweetness and bitterness; sugar (glucose, fructose and sucrose) and protodioscin contents, and finally to clarify the differences between them.

Materials and Methods

Plants and experimental treatments

All experiments except for the sugar and protodioscin analyses were conducted at Hokkaido Ornamental Plants and Vegetables Research Center (Takikawa, Hokkaido, Japan) from 2006 to 2008. Two-year-old rootstocks of 'UC157' were used over two years. Seeds were germinated in paper pots on April 5, 2006 and on April 6, 2007, respectively. Seedlings were raised for about 2 months and transplanted to the experimental open field on June 8, 2006 and June 4, 2007. These seedlings were grown under the conventional method. Two-year-old rootstocks were collected on October 31, 2007 and on October 31, 2008, respectively. It is known that two-year-old rootstocks of 'UC157' are apparently in a dormant stage in late autumn, and their dormancy is broken by chilling treatment (Koizumi et al., 2002). Thus, all rootstocks were put into plastic bags and placed in a refrigerator at 2–4ºC for 15 days in 2007 and 20 days in 2008 in order to be exposed to sufficient chilling to break dormancy. Forcing culture was performed in a heated glasshouse during winter. Soil in this glasshouse consisted of ash soil and peat moss, and its bulk density was 0.86 $g{\cdot}cm^{-3}$. The culture bed for rootstocks was 0.3 m in depth, 2.3 m in width and 4.4 m in length. It was prepared in early November in both years and electric wires were set to warm the soil at the bottom of the bed. After chilling treatment, 68 rootstocks in 2007 and 108 rootstocks in 2008 were randomly distributed into four groups, 17 plants per group in 2007 and 27 plants per group in 2008.

Two groups were planted in soil-mound sites and the others in film-cover sites on November 15, 2007 and on November 20, 2008. Planting depth was 5 cm below the ground level. Soil was mounded approximately 22 cm in height above the ground in the soil-mound plots (Figure 4-1A). Adequate water was supplied after planting and no watering was performed until the end of the experiment.

A 200 cm-high tunnel covered with a plastic shading film (White-Silver; Tokankosan Co., Ltd., Tokyo, Japan) was set over the culture bed in order to keep all the experimental plots under the same environmental conditions except for soil mounding treatments. Throughout the experimental periods, the soil temperature 15 cm below the ground surface was kept at 20ºC by electric wires and the air temperature 15 cm above the ground surface in the tunnel was controlled within a range from 10 to 25ºC by the air conditioners and ventilation systems in the glasshouse. Emerged spears were harvested for 21 days, December 9 to 29 in 2007, and 19 days, December 9 to 27 in 2008, respectively (Figure 4-1B, C). Harvesting was performed once a day and spears from the same blanching plots were pooled. Straight, undamaged and not flattened spears ranging from 21.0 to 25.0 cm in length were carefully selected and trimmed to 21.0 cm in spear length. The maximum diameter was measured at the bottom of each spear. Spears 12.0–15.0 mm in diameter at the bottom were exclusively used for this study. All of the investigations except for the analysis of sugar and protodioscin contents were carried out on the harvest day.

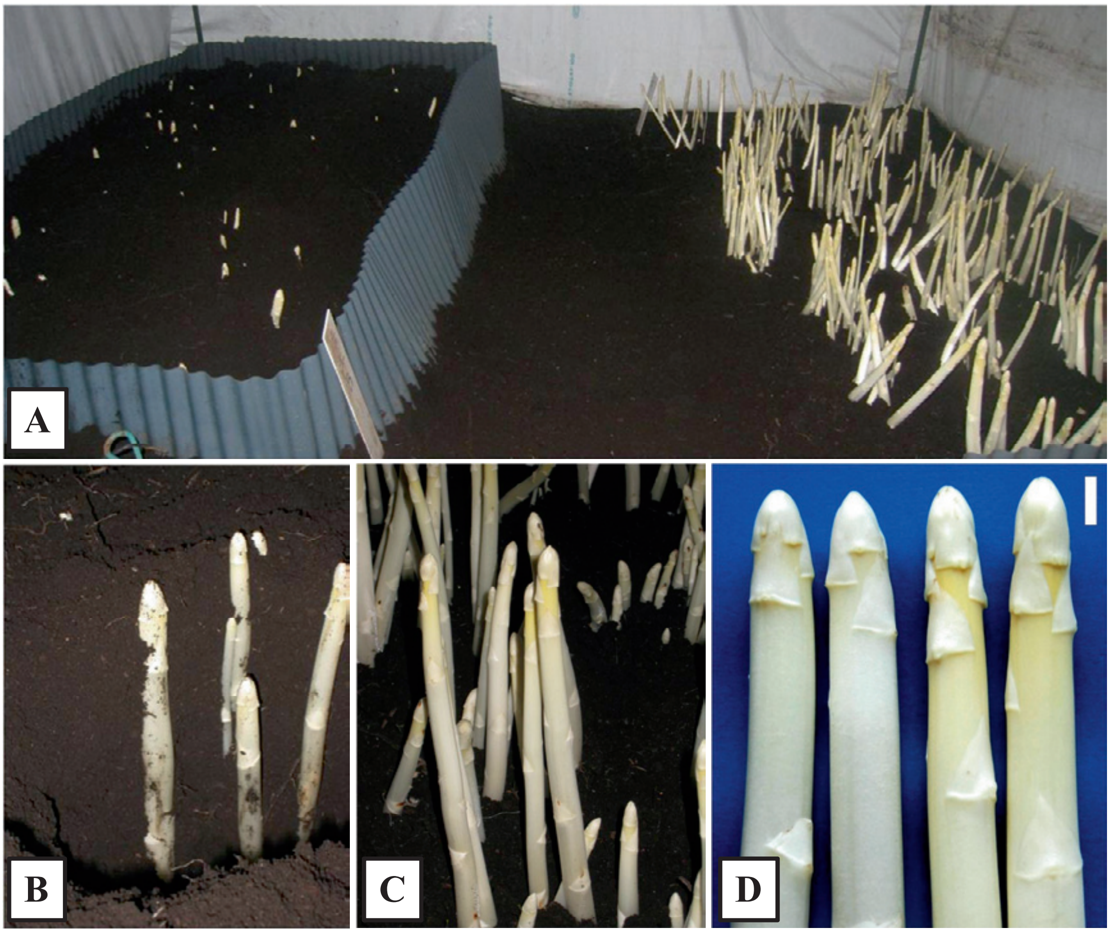


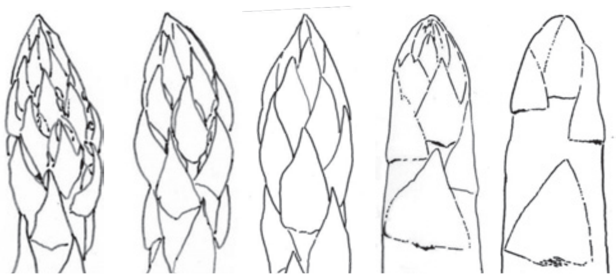
Figure 4-1. Two blanching methods for asparagus spears. (A) Experimental treatments on a heated bed (left, soil-mound method; right, film-cover method). (B) White asparagus spears growing in soil-mound plot. (C) White asparagus spears growing in film-cover plot. (D) Tip portion of harvested spears (left, soil-mound method; right, film-cover method). Bar indicates 10 mm.

<u>Spear color</u>

Spears harvested from December 12 to 16 in 2007 and from December 20 to 22 in 2008 were used for color investigation. To determine the external color of the spear, color measurements were performed in three portions (upper: 2.0 cm from tip, middle: 10.5 cm from tip, lower: 19.0 cm from tip) of each spear using a spectrophotometer (NR-3000; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). The color was quantified using the values of L*, a*, b* color space.

<u>Spear tip tightness</u>

Spear tip tightness was evaluated by the 5 objective scores shown in Figure 4-2. Spears harvested during the two periods in 2007 (December 9 to 15, 23 to 29) and 2008 (December 18 to 20, 24 to 26) were utilized for this observation.



Score 1 (Open)　2　3　4　5 (Tight)

Figure 4-2. Scores for spear tip tightness.

<u>Spear thickness and weight</u>

The same spears evaluated for spear tip tightness were also used for the measurements of diameter and weight. Maximum diameter in three spear portions (upper: 4.0 cm from tip; middle: 10.5 cm from tip; basal: 21.0 cm from tip) and spear weight were measured.

<u>Spear hardness</u>

Spears obtained from December 9 to 10 in 2007 and from December 16 to 19 in 2008 were used for the examination of spear hardness. These spears were boiled for 10 min and immediately cooled in an ice water bath. Hardness of the spear was determined in three portions (upper: 4.0 cm from tip, middle: 10.5 cm from tip, lower: 19.0 cm from tip) using a texture analyzer (TA-XT2i; Stable Micro Systems, Godalming, UK) set up with a stainless cylinder, 2 mm in diameter, and the value (N) of maximum load during probe penetration at speed of 2 $mm \cdot s^{-1}$ was defined as the index of spear hardness.

<u>Sugar and protodioscin contents in spear</u>

Spears harvested between December 13 and 16 and between December 24 and 29 in 2007, and from December 21, 24 and 27 in 2008, were used for the sugar and protodioscin analyses, and these analyses were conducted at Graduate School of Agriculture, Hokkaido University (Sapporo, Hokkaido, Japan) and at Faculty of Agriculture and Life Science, Hirosaki University (Hirosaki, Aomori, Japan). Frozen spears were cut into 3 sections of equal length, and then the bottom sections from 3 to 5 spears were used for analysis. In the preliminary study, it was observed that both sugars and protodioscin were accumulated the most from the bottom section of a spear (data not shown). Therefore, the bottom section was used for the analyses. Spears were lyophilized and milled into a fine powder.

Sugars (fructose, glucose and sucrose) were extracted from 20 mg of the freeze-dried powder with 70% ethanol for 1 h at room temperature. The extracted solution was centrifuged, and the supernatant was used for sugar analysis. Sugar content was determined using a high-performance liquid chromatography (HPLC) system equipped with a Shodex Asahipak NH2P-50 4E (4.6 × 250 mm) column. The mobile phase consisted of 80% acetonitrile. Analysis was performed by running each sample for 25 min at a column temperature of 35°C, with a flow rate of 1.0 $mL \cdot min^{-1}$. Each run was monitored by a refractive index detector. Lactose was used as the internal standard.

Protodioscin and other saponin compounds were extracted from 50 mg of freeze-dried powder with 1 mL of 70% ethanol. The extracted solution was centrifuged (10,000 rpm, 10 min), and the supernatant was collected in a test tube. The extraction and centrifugation procedures were repeated. The solution was loaded on a C18 solid phase cartridge (Bond Elut C18, 500 mg, 3 mL; Agilent Technologies, Tokyo, Japan), and the cartridge was washed twice with 3 mL of pure water. A sample fraction containing the saponin compounds was eluted with 99.5% ethanol, and then evaporated to dryness at 40°C with a CVE-200D centrifugal evaporator (Tokyo Rikakikai Co., Tokyo, Japan) and dissolved with 500 μL of 70% ethanol. The extraction procedures were repeated 3 times.

In 2007, protodioscin content in the sample solution was determined by high-performance thin-layer chromatography (HPTLC) using a modified method described by Schwarzbach et al. (2006). A silica gel 60 F_{254S} HPTLC plate (200 × 100 mm) (Merck, Darmstadt, Germany) was used for the analysis. Each sample solution (1 μL) was applied on the baseline (18 mm above the bottom edge of the plate) at a width of 6 mm. The external standard solution consisted of 500 and 1000 ppm (in 70% ethanol) of purchased protodioscin (ChromaDex Inc., Irvine, USA). After drying, the bottom of the plate was dipped in a developing solvent (ethanol : acetonitrile : chloroform : water = 4 : 2 : 4 : 0.5, v/v). After development, the plate was air-dried, dipped for 1 s in Ehrlich's reagent (2 g of p-dimethylaminobenzaldehyde dissolved in 20 mL concentrated hydrochloric acid and 180 mL methanol), and heated on a plate heater (EC-1200N; As One Co., Osaka, Japan) at 110°C for 5 min. Protodioscin was identified as a pinkish-red spot with the same Rf value as the external standard. The plate was scanned into a personal computer, and the density of the protodioscin spot on the image file was analyzed by densitometry using Image J gel analysis software (http://rsbweb.nih.gov/ij/).

In 2008, protodioscin content in the sample solution was determined using HPLC equipped with an ELSD, which was developed for the detail protodioscin analysis by Maeda et al. (2012). HPLC analysis was conducted using a Shimadzu LC9A system (Shimadzu Co., Kyoto, Japan) equipped with a Model 300S ELSD (M&S Instruments Inc., Osaka, Japan) and a Waters Sunfire C18 (4.6 × 250 mm) column (Nihon Waters K. K., Tokyo, Japan). The mobile phases consisted of 0.1% trifluoroacetic acid (A) and acetonitrile (B). Analysis was performed by running each sample for 30 min at a column temperature of 40 °C and using a linear gradient system at a flow rate of 1.0 $mL{\cdot}min^{-1}$. The gradients were as follows: 0 min, 84% solvent A and 16% solvent B; 20 min, 60% solvent A and 40% solvent B; and 30 min, 40% solvent A and 60% solvent B; the post-running time was 10 min. The electrical signal from the ELSD fed into a personal computer was monitored and analyzed using an HPLC data integrator system (Chromato-PRO; Run Time Instruments Co., Kanagawa, Japan). The chromatogram peak for protodioscin was confirmed at the retention time of 19.9 min by the injection of commercial protodioscin (ChromaDex Inc., CA, USA) standard solution. Protodioscin content of the sample solution was calculated from the peak areas with reference to a calibration curve prepared using 0, 25, 50 and 125 ppm of external standard solution.

Results

External quality of spears

Comparing the two different blanched spears, there was a significant difference in both a* and b* scores in the upper portion in the two years (Table 4-1). Although negative a* indicates a hue of bluish-green and positive b* indicates yellow (McGuire, 1992), differences in b* scores at the upper portion were also easily recognized by visual observation, which showed that spear color in the portion 2 cm below the tip was more yellowish in the film-cover plots than in the soil-mound plots (Figure 4-1D). There was no difference in spear color in the middle portion in the two years and the results of a* and b* scores in the lower portion were not constant between the two years (Table 4-1).

In the film-cover plots, spear tightness ranged from a score of 2 to 5, an especially high frequency was observed in score 4, and less than 30% of spears showed a score of 5,

Table 4-1. Effect of different blanching methods on spear color.

Year	Blanching method	Number of spears	Spear color in each section determined by spectorophotometer[z]								
			Upper (2.0 cm from tip)			Middle (10.5 cm from tip)			Lower (19.0 cm from tip)		
			L*	a*	b*	L*	a*	b*	L*	a*	b*
2007	Film-cover	19	70.7	–6.6	18.9	72.4	–16.7	2.3	69.8	–20.8	4.4
	Soil-mound	10	71.3	–4.0	9.5	72.7	–24.6	2.1	69.6	–12.3	3.2
	t-test[y]		NS	**	***	NS	NS	NS	NS	*	NS
2008	Film-cover	32	72.9	–7.7	18.7	73.6	–19.1	2.2	69.5	–18.4	4.9
	Soil-mound	28	73.5	–4.4	7.3	71.7	–23.1	2.1	68.5	–17.0	2.3
	t-test[y]		NS	***	***	NS	NS	NS	NS	NS	***

[z]Values show the mean.

[y]NS, ***, **, * indicate not significant, significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

Table 4-2. Effect of different blanching methods on spear tip tightness.

Year	Date	Blanching method	Number of spears	% of spear tip tightness				
				Score 1 (Open)	Score 2	Score 3	Score 4	Score 5 (Tight)
2007	December 9–15th	Film-cover	38	0	0	5.3	73.7	21.1
		Soil-mound	33	0	0	0	6.1	93.9
	December 23–29th	Film-cover	25	0	0	8.0	88.0	4.0
		Soil-mound	29	0	0	0	6.9	93.1
2008	December 18–20th	Film-cover	37	0	2.7	32.4	56.8	8.1
		Soil-mound	37	0	0	2.7	18.9	78.4
	December 24–26th	Film-cover	34	0	0	11.8	58.8	29.4
		Soil-mound	18	0	0	0	11.1	88.9

Table 4-3. Effect of different blanching methods on spear diameter in each section and its weight.

Year	Date	Blanching method	Number of spears	Spear diameter^z (cm)			Spear weight^z (g)
				Upper (4.0 cm from tip)	Middle (10.5 cm from tip)	Basal (21.0 cm from tip)	
2007	December 9–15th	Film-cover	38	10.87	12.86	13.38	21.84
		Soil-mound	33	11.26	13.34	13.53	23.49
		t-test^y		NS	*	NS	*
	December 23–29th	Film-cover	25	11.00	12.88	13.18	21.74
		Soil-mound	29	11.27	13.21	13.50	23.15
		t-test^y		NS	NS	NS	NS
2008	December 18–20th	Film-cover	37	10.46	12.61	13.57	22.80
		Soil-mound	37	11.63	13.83	13.74	25.28
		t-test^y		***	***	NS	**
	December 24–26th	Film-cover	34	10.57	12.60	13.54	22.71
		Soil-mound	18	11.74	13.73	13.80	25.62
		t-test^y		***	**	NS	**

^{z}Values show the mean.

^{y}NS, ***, **, * indicate not significant, significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

Table 4-4. Effect of different blanching methods on spear hardness estimated by texture analyzer.

Year	Blanching method	Number of spears	Hardness in each spear section^z (N)		
			Upper (4.0 cm from tip)	Middle (10.5 cm from tip)	Lower (19.0 cm from tip)
2007	Film-cover	16	0.29	0.89	6.14
	Soil-mound	22	0.50	2.64	7.04
t-test^y			***	***	NS
2008	Film-cover	34	0.36	0.81	7.06
	Soil-mound	41	0.48	2.19	5.93
t-test^y			***	***	**

^{z}Values show the mean.

^{y}NS, ***, ** indicate not significant, significant at $P < 0.001$ and $P < 0.01$, respectively.

which was defined as the tightest throughout this experiment (Table 4-2). On the other hand, more than 78% of spears grown in the soil-mound plots were classified as a score of 5 in all experimental periods.

In 2007, spear diameter in the upper and middle portions and spear weight tended to be greater and heavier in the soil-mound plots than the film-cover plots (Table 4-3), although significant differences were recognized only in spear diameter in the middle portion and spear weight during the period from December 9 to 15. In 2008, spear diameters in the upper and middle portions were significantly greater and spear weight was significantly heavier in the soil-mound method than the film-cover method in both periods.

Hardness of spears

In both blanching methods, the values for hardness of harvested spears increased basipetally (Table 4-4). Comparing the two blanching treatments, the values in both upper and middle portions were significantly larger in the soil-mound plots than the film-cover plots in the two years. Hardness in the lower portion was changeable; there was no difference between the two blanching methods in 2007, but the value in the film-cover method was larger than in the soil-mound method in 2008.

Sugar and protodioscin contents in spears

In 2007, significant differences in glucose and sucrose contents between two blanching methods were recognized, but they did not affect the total sugar content in both blanching methods at all (Table 4-5). Sugar components remained stable throughout the harvest period for both the film-cover and soil-mound methods, consisting of 47–50% fructose, 46–48% glucose and 3–5% sucrose. In 2008, although all the sugar contents varied among the harvest periods, no difference in the total sugar content between two blanching methods was observed (Table 4-6). Irrespective of the blanching method, sugar components in spears invariably consisted of 42–47% fructose, 42–43% glucose and 10–16% sucrose.

A clear calibration curve was obtained when HPTLC analyses were conducted more than 3 times per sample solution, but variations in the spot density of the protodioscin were observed on the HPTLC plates. Thus, quantitation of protodioscin consisted of 3 HPTLC analyses for each sample. In 2007, protodioscin contents in white spears produced by the film-cover method varied slightly during the harvest period (from 1.0 to 1.7 mg·g DW^{-1}), and no definite variation was observed (Figure 4-3). Protodioscin contents in spears produced by the soil-mound method were more varied (from 1.8 to 3.6 mg·g DW^{-1}), however protodioscin contents in samples harvested on the same day tended to be higher in the soil-mound plots than in the film-cover plots. A two-way ANOVA test revealed a significant difference at the 1% level between the two blanching methods. Irrespective of the blanching method, all values of protodioscin contents in 2008 tended to be higher than those in 2007 because of the improvement of analysis accuracy for protodioscin (Figure 4-4). Protodioscin contents in white spears produced by the film-cover method varied from 4.5 to 6.2 mg·g DW^{-1}, those in spears produced by the soil-mound method did from 4.8 to 10.6 mg·g DW^{-1}. However, the same tendency was also recognized in 2008, a significant difference at the 1% level between the two blanching methods was found by a two-way ANOVA test. Protodioscin contents in the soil-mound method were higher than that in the other method, especially on December 24 and 27 (Figure 4-4).

Table 4-5. Effect of different blanching methods on sugar content in white asparagus spears in 2007 experiment.

Date of Harvest	Blanching Method	Sugar contents^z (mmol・g DW^{-1})			
		Fructose	Glucose	Sucrose	Total
Dec 13–14	Film-cover	1.11	1.15	0.11	2.38
	Soil-mound	1.19	1.15	0.09	2.43
Dec 15–16	Film-cover	1.16	1.12	0.10	2.38
	Soil-mound	1.21	1.14	0.08	2.44
Dec 24–25	Film-cover	1.15	1.11	0.11	2.36
	Soil-mound	1.25	1.18	0.10	2.53
Dec 26–27	Film-cover	1.19	1.11	0.12	2.43
	Soil-mound	1.26	1.20	0.09	2.55
Dec 28–29	Film-cover	1.12	1.09	0.11	2.32
	Soil-mound	1.20	1.16	0.09	2.45
Two-way ANOVA^y	Blanching method (B)	NS	*	**	NS
	Date of Harvest (H)	NS	NS	NS	NS
	B × H	NS	NS	NS	NS

^{z}Values show the mean (n = 3).

^{y}NS, **, * indicate not significant, significant at P<0.01and P<0.05, respectively.

Table 4-6. Effect of different blanching methods on sugar content in white asparagus spears in 2008 experiment

Date of Harvest	Blanching Method	Sugar contents[z] (mmol•g DW^{-1})			
		Fructose	Glucose	Sucrose	Total
Dec 21	Film-cover	1.26	1.15	0.29	2.70
	Soil-mound	1.34	1.19	0.29	2.83
Dec 24	Film-cover	1.08	1.00	0.29	2.37
	Soil-mound	1.01	0.96	0.28	2.25
Dec 27	Film-cover	0.74	0.74	0.27	1.76
	Soil-mound	1.09	1.02	0.28	2.39
	Blanching method (B)	NS	NS	NS	NS
Two-way ANOVA[y]	Date of Harvest (H)	**	**	**	**
	B × H	NS	NS	NS	NS

[z]Values show the mean (n = 3).
[y]NS, ** indicate not significant, significant at P <0.01, respectively.

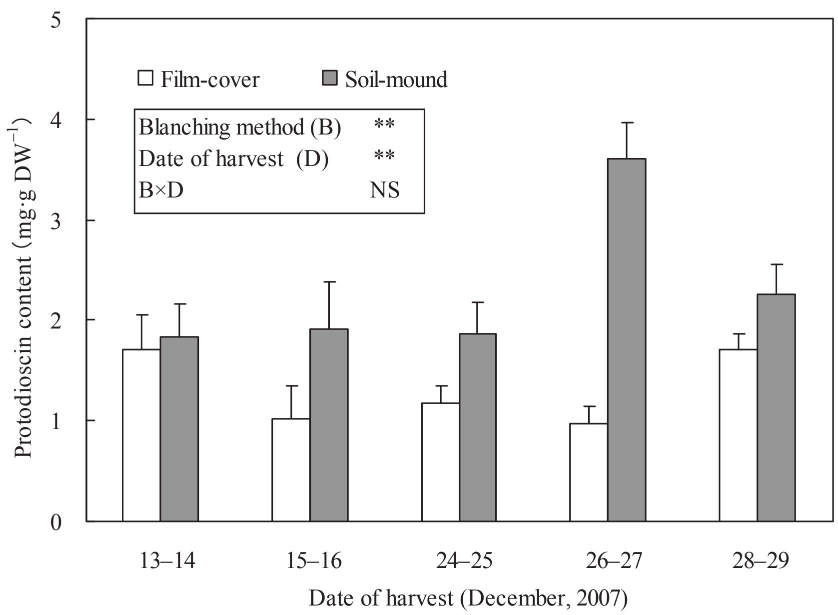


Figure 4-3. Effect of different blanching methods on protodioscin content in white asparagus spears in 2007 experiment. Bars indicate SE (n = 3). NS, ** in the box denote not significant, significant at P<0.01, respectively by the two-way ANOVA test.

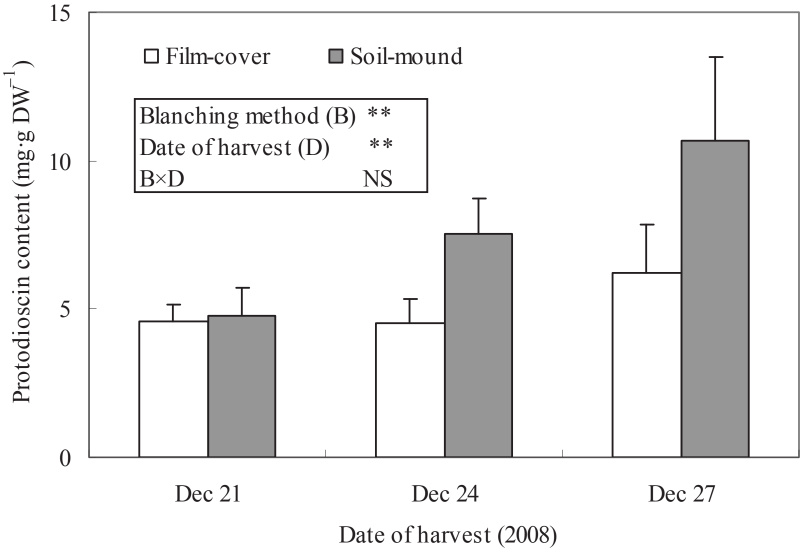


Figure 4-4. Effect of different blanching methods on protodioscin content in white asparagus spears in 2008 experiment. Bars indicate SE (n = 3). NS, ** in the box denote not significant, significant at *P*<0.01, respectively by the two-way ANOVA test.

Discussion

The two different blanching methods obviously influenced spear tip color and tightness in the present experiment. The color of the spear tip portion (2 cm from the tip) in the film-cover method was more yellowish than in the other. Throughout the experimental periods, yellow spots were frequently observed in spear tips in the film-cover plots. The author confirmed the same color on the spear tips when asparagus plants were grown in a completely dark room in several other experiments (data not shown). Therefore, soil mounding appears to be effective for the production of complete white spears. Since these yellow spots rapidly turned green when exposed to light, such responses might be attributed to the existence of chlorophyll and carotenoids. Further compositional research is needed on spear tip color, and its relationship with soil mounding should be elucidated. The results of this experiment also indicate that the spear tip tended to be tighter in the soil-mound method than the film-cover method. van Os and Simonse (1988) produced white asparagus spears without soil mounding in a dark climate-controlled chamber in hydroponic forcing culture using two-year-old rootstocks, and described the tip of the emerged white asparagus, grown without soil mounding, as less closed than that of field-grown asparagus with soil mounding, probably because of low pressure at the tip. Although they did not show any data on spear tip tightness in their reports, their description is consistent with the results obtained here. Therefore, soil pressure over crowns in the soil-mound method is thought to influence the shape of the spear tip. As differences in spear tip color and tightness between the two blanching methods were visually distinguishable, these characteristics might be striking visible criteria to help discriminate between the two kinds of white spears produced by different blanching methods.

Spear hardness is an important factor in determining eating quality (Scott and Kramer, 1949; Siomos et al., 2000). However, there is no information on the effect of blanching methods on spear hardness. The present study indicates that white asparagus spears grown by the traditional soil-mound method were significantly harder in the upper and middle portions than by the film-cover method, suggesting that soil pressure during spear elongation in the soil mound also makes the spears harder.

The author found that white spears grown by the film-cover method in a plastic greenhouse tended to be tougher than green spears in the other experiment (data not shown). Thus, spear hardness in the film-cover method is thought to be between that of green spears and white spears in the soil-mound method, which may mean that cooking time (heating time) for spears produced by the film-cover method should be longer than for green spears and shorter than for white spears by the soil-mound method. In the present examination, spear diameter in upper and middle portions and spear weight obtained from the soil-mound plots were greater and heavier than those from the film-cover plots. These differences may be closely associated with spear hardness, especially for spear fibrousness, as described below.

It is known that a well-lignified fiber sheath present in white asparagus is missing in green asparagus from microscopic observations (Chang, 1983), and white spears harvested using the soil-mound (Papadopoulou et al., 2003) and film-cover (Brovelli et al., 1998) methods were more fibrous than green spears. A correlation between fiber content and shear-press maximum peak value measured by a texture test system was found in green asparagus (Clore et al., 1976). These reports suggest that the hardness of asparagus spears was closely related to fiber content irrespective of the blanching method. Additionally, Haard et al. (1974) reported that ethylene induced isozyme (isoperoxidase) changes during fiber formation in postharvest asparagus. In order to better understand differences in spear hardness between the two blanching methods, further research on the relationships among spear hardness, fiber content, and metabolic changes, such as ethylene production, will be necessary.

Results of sugar analysis in both years showed no significant difference in the total sugar content between white spears produced by the film-cover and soil-mound methods, and sugar components in those spears were constantly stable. These results suggest that the blanching method had no effect on sugar content or components in white spears. In contrast, a significant difference in protodioscin content between the two blanching methods was observed in both years. Some previous studies indicated that saponin compounds act as a defensive substance against fungal and bacterial pathogens and probably some other pests or stressors (Hughes et al., 2004; Osbourn, 1996). Wu et al. (2005) reported that osmotic stress treatment activated saponin biosynthesis in cultured cells of *Panax ginseng*. Shimoyamada et al. (1990, 1996) reported that saponin compounds AS-1 and AS-2-I, found in asparagus, showed antifungal activity on some species of fungi. Therefore, saponin biosynthesis regulation in white asparagus spears may be associated with an antistress and/or defensive action against pathogen or insect attack. Spears produced by the soil-mound method may be exposed to a more stressful environment than spears produced by the film-cover method because they must penetrate the soil mound. Environmental stresses may include low temperature, higher physical soil load, scratches, and wounds caused by rubbing against soil grains, and possible attacks from fungi and/or other microorganisms. Results of this study suggest that biosynthesis of saponin compounds, including protodioscin, may be accelerated by some stresses derived from soil mounding in white spears. Further biochemical investigations are needed to determine the relationship between soil-borne stress and saponin biosynthesis in white asparagus spears to clarify the mechanism of saponin accumulation in white spears.

The results of this study suggest that the blanching method has no effect on sugar content or components and, therefore, no effect on sweetness. Protodioscin content, however, appears to be affected by the blanching method. Thus, the difference in flavor of white spears between the two blanching methods appears to be caused mainly by the bitterness associated with differences in the contents of saponin compounds, especially protodioscin (the dominant saponin in white asparagus spears) that may be influenced by soil-borne stresses.

The differences in external appearance, hardness and protodioscin content between white asparagus spears produced by the two blanching methods are discussed here. This basic information will improve cooking and processing of white asparagus, and contribute to furthermore increase of the domestic consumption for fresh white asparagus, soon.

Chapter 5
Feasibility of summer production of white asparagus from one-year-old rootstocks stored with snow for long-term period

Introduction

White asparagus production in summer is extremely small since the efficient cropping system for summer production has not yet been developed. Hojo et al. (2006) reported that white asparagus could be produced by covering a small shaded pipe 25 cm in length and 4 cm in diameter on individual spears during summer harvest in the mother fern culture which established in southwestern Japan in the late 1980s (Kobayashi and Shinsu, 1990; Ito et al., 1994). But, according to the method, growers must prepare many pipes for shading spears at every harvest time, resulting in the low efficiency of harvest work. Furthermore, since white asparagus spears with no pink discoloration caused by anthocyanin pigmentation tend to be preferable in Japanese domestic markets, growers must put the pipe only on spears just before they have emerged above ground, because pink discoloration in spears occurs if they are exposed to sunlight even in a short. This restricts production, so, another easier method to produce a large number of white asparagus spears with no pink discoloration in summer is required by Japanese growers. It is also important to establish the technique to extend the harvest period of white spear for further expansion of domestic demand and consumption of fresh white asparagus.

The possibility of white asparagus production by a large shaded tunnel, white asparagus forcing culture in winter, was discussed in Chapter 2. If asparagus rootstocks were stored every year until summer, white spears could be harvested in summer by applying the “Fusekomi” forcing culture technique mentioned in Chapter 2.

Hokkaido is located in northern Japan and is well-known for its heavy snow in winter. Thus, the author attempted to utilize this natural resource to store asparagus rootstocks for a long period, without refrigerated facilities requiring electric power.

It was reported that asparagus plants accumulated carbohydrates in storage roots in autumn and then consumed them for spear or fern growth the following spring. The amount of carbohydrates is closely related to the spear yield (Haynes, 1987; Pressman et al., 1993; Shelton and Lucy, 1980). Hence, in this study, the effects of long-term storage using snow on the sugar contents in storage roots of one-year-old rootstocks and the young spear yield were examined to establish a summer-harvest system for white asparagus.

Materials and Methods

The examinations were carried out twice, from 2008 to 2009 and from 2009 to 2010. One-year-old rootstocks of ‘UC157’ were used.

Plant material for 2008–2009 experiment

Approximately 200 rootstocks grown under the conventional practices in Hokkaido were dug up from a farmer’s field (Kuriyama, Hokkaido, Japan) on November 6, 2008, and transferred to the Experiment Farm of Field Science Center for Northern Biosphere, Hokkaido University (Sapporo, Hokkaido, Japan), a region above 15 m sea level with 1.0 m snow cover. These rootstocks were placed on the ground and covered with a large blue plastic sheet, which was not made of perfectly impermeable materials. This type of sheet is often used as covering material in agricultural practice to prevent rootstocks from drying until the start of long-term storage. On November 23, rootstocks too large, or too small, or damaged, were removed, and 150 medium-sized rootstocks without any damage were finally prepared for the 2008–2009 experiment. These rootstocks were randomly transferred to 30 plastic containers (H 30 cm, W 36 cm, L 52 cm), namely, five rootstocks were set in each plastic container with 20 L of the field soil. This setting of rootstocks into the containers was defined as the start of long-term storage in this study.

All containers were moved to Yoichi Orchard Farm, Hokkaido University (Yoichi, Hokkaido, Japan), a region above 5 m sea level with 1.2 m snow cover on December 18. They were set on the ground surface, covered with the large blue plastic sheet mentioned above, and left under natural conditions. On February 11, a large snow mound more than 3.5 m in height was made over the containers, and the rootstocks were stored under the snow mound for 5 different storage periods: 0, 2, 4, 6 and 6.5 months, including ordinary snow cover from November 23 to February 11.

Six containers were taken out from their storage places on November 26 (0 months storage), January 27 (2 months storage), March 26 (4 months storage), May 25 (6 months storage) and on June 8 (6.5 months storage), respectively (Figure 5-1). In each treatment, the container was recognized as a unit of replication. Three containers were immediately moved to a completely dark room adjusted at 20ºC air temperature to grow young spears. Then 2.5 L of water was supplied to each container weekly during the spear harvest. Rootstocks in the other three containers were utilized for physiological investigation. The fresh weight of all rootstocks was measured after they were washed by a high-pressure water sprayer to remove soil. Five storage roots just below a crown, 10 cm in length, were collected per rootstock, and all root samples from 5 rootstocks were pooled in each container. Next, 10 cm storage roots were cut into 2 parts; the base and head. The basal part, 0–5 cm from the crown, was used to measure soluble solid content, and the head part, 5–10 cm from the crown, was lyophilized and stored at –20ºC until the sugar analysis.

Plant material for 2009-2010 experiment

About 100 rootstocks (sown on February 6, 2009 and transplanted on June 13, 2009) were dug up from the Experiment Farm of Field Science Center for Northern Biosphere, Hokkaido University, on November 12, in 2009. All rootstocks were stored by the same method used in the previous season until the start of storage treatment. On December 5, 70 medium-sized rootstocks without any damage were carefully selected for the 2009–2010 experiment. The mean fresh weight of rootstocks in this experiment was estimated by averaging eight randomly selected plants (rootstocks). 48 rootstocks were randomly divided into 12 groups, and 12 plastic containers were prepared. Then four rootstocks were set in each plastic container with 20 L of the soil medium (Takii-ikubyoubaido; Takii Seed Co., Ltd., Kyoto, Japan). These containers were placed in the experimental field with the large blue plastic sheet over them and left under natural conditions.

On January 21, six containers were taken out from the storage place (after 1.5 months of storage treatment) (Figure 5-1). On March 11, the other six containers were transported to the experimental site of Yubari City (a region above 200 m sea level with 1.5 m snow cover, Hokkaido, Japan), and set inside a large snow mound more than 3.0 m in height. All the containers were completely wrapped with an impermeable plastic film so that they would not touch the snow directly. This year, the snow mound was covered with the large blue plastic sheet. Many mesh plastic bags filled with rice hulls, which functioned as a heat insulation material, were laid over all snow mound surfaces, and covered with a second large blue plastic sheet. These containers were taken on July 28 (after 7.5 months of storage treatment). After 1.5 and 7.5 months of storage, containers taken out from under ordinary snow cover or the snow mound were utilized for spear yield examinations, with the same procedures used for the 2008–2009 experiment. In addition, two containers in which seven rootstocks were planted with the same soil medium mentioned above were prepared for the sugar analysis of their storage roots. One container was provided for 1.5 months of storage treatment, and the other for 7.5 months of storage treatment, respectively. Five storage roots just below a crown, 10 cm in length, were collected from each rootstock, and each obtained sample was

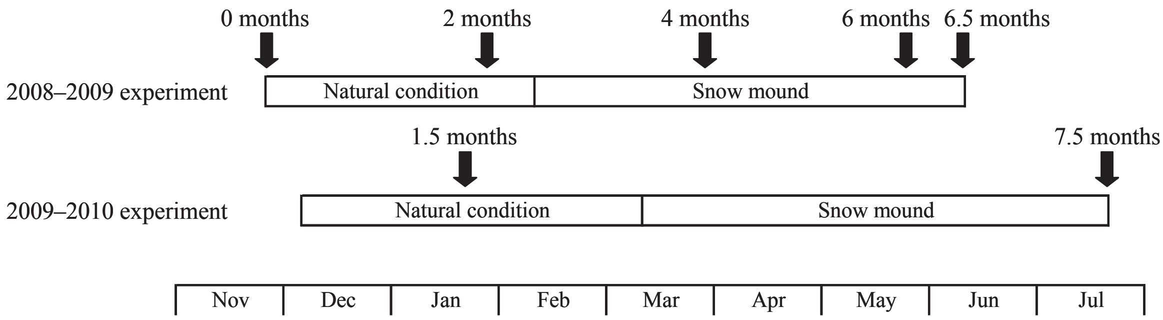


Figure 5-1. Schedule for long-term storage of asparagus rootstocks in 2008–2009 and 2009–2010 experiments. Black arrows show the transfer of containers to a temperature-controlled room at 20ºC.

lyophilized and then stored at –20ºC until the sugar analysis.

Storage temperature

A temperature data logger (Ondotori Jr.TR-52S; T&D Corporation, Nagano, Japan) was set in one of the containers stored for the longest period in both years to record the hourly soil temperature during storage under the natural condition and snow mound.

Spear yield

Spear yield was recorded for 45 days after transferring containers into the dark room controlled constantly at 20 ºC. Spears in each container were harvested daily and trimmed to 24 cm in length. Only straight, undamaged and not flattened spears were recognized as marketable. In this experiment, apparently unmarketable spears were also cut every day even if their spear length did not reach 24 cm. The number and fresh weight of marketable spears were recorded.

Soluble solid and sugar contents in storage roots

Sampled storage roots were squeezed by a hand extractor to obtain a moderate amount of sap, and soluble solid content of each sap was measured by a refractometer (PR-101α; ATAGO Co., Ltd., Tokyo, Japan). 2.0 g of lyophilized storage roots from each sample was broken up into small pieces and homogenized with 100 mL of 80% (v/v) ethanol using the homogenizer (Ultra-Turrax® T25; Rose Scientific, Inc., Cincinnati, USA) for two minutes. The solution was filtered and its volume was adjusted to 200 mL by adding 80% (v/v) ethanol. 100 mL of the extract was dispensed and evaporated, and finally its volume was concentrated up to 25 mL by adding distilled water. The total and reducing sugar contents in each extract were analyzed using a slightly modified Somogyi-Nelson method (Hodge and Hofreiter, 1962). In this study, the absorbances were measured at 520 nm by a spectrophotometer (UV-2500PC; Shimadzu Co., Kyoto, Japan).

Results and Discussion

Storage temperature of rootstock

The soil temperature in the containers covered with the plastic sheet varied within the range of 0–5ºC from the start of storage to middle December in both years, because there was not a sufficient amount of snow over the storage place (Figure 5-2). The containers started to be covered with snow from December 27, 2008 and January 4, 2010 in the 2008–2009 and 2009–2010 experiments, respectively. The highest snow cover was 100 cm in the former experiment and 80 cm in the latter. After all the containers were fully covered with snow, particularly after they were set under snow mounds, the soil temperature was kept stable at 0–1ºC until the end of long-term storage. This result also made us realize how snow becomes a precious natural resource for effective and stable cooling if snow-cover lasts for a long time.

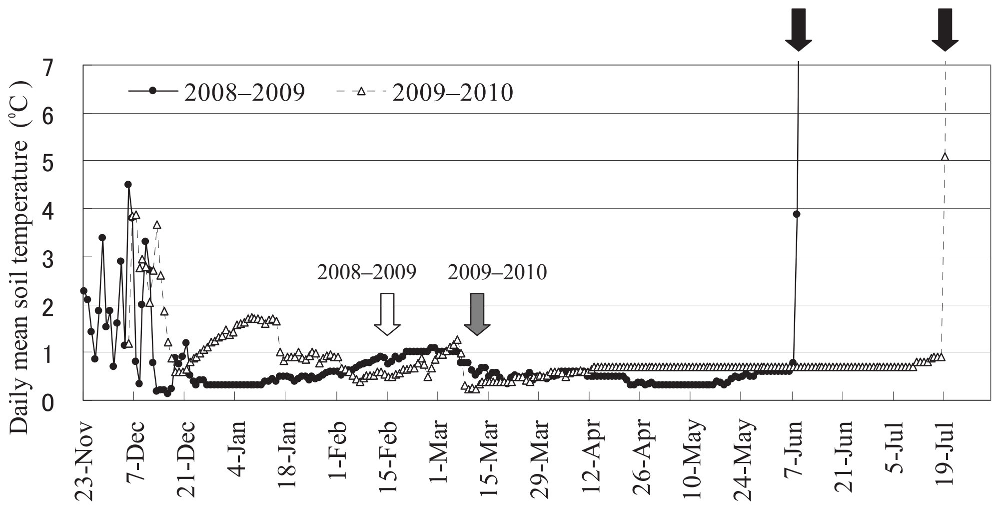


Figure 5-2. Schedule for long-term storage of asparagus rootstocks in 2008–2009 and 2009–2010 experiments. Black arrows show the transfer of containers to a temperature-controlled room at 20ºC.

Table 5-1. Effects of storage period on rootstock fresh weight, soluble solid contents and sugar contents of storage roots in the 2008–2009 experiment.

Storage period[z]	Rootstock fresh weight[y] (g)	Storage roots: Soluble solid content[y] (%)	Storage roots: Sugar contents[y] (mg•g DW^{-1}) Total	Reducing
0 months	929 ± 47 a	21.9 ± 0.4 ab	612 ± 25 a	44 ± 11 b
2 months	927 ± 68 a	23.7 ± 0.2 a	599 ± 14 a	83 ± 3 a
4 months	1,006 ± 116 a	21.5 ± 1.9 ab	563 ± 29 a	32 ± 11 b
6 months	1,068 ± 66 a	20.7 ± 0.7 ab	599 ± 13 a	29 ± 7 b
6.5 months	864 ± 15 a	19.2 ± 0.6 b	557 ± 8 a	21 ± 4 b

[z]The storage started on November 23, 2008.
[y]Values indicate the mean±SE (n = 3). Different letters in the column show significant differences at 5% by Tukey-Kramer HSD.

Table 5-2. Effects of storage period on spear yield of rootstock in the 2008–2009 experiment.

Storage period[z]	Marketable spear: Number[y]	Marketable spear: Yield[y] (g)	Marketable spear: Average spear weight[y] (g)	Total spear yield[y] (g)
0 months	16.5 ± 1.0 a	309 ± 37 a	18.7 ± 1.4 a	400 ± 42 a
2 months	15.6 ± 1.5 a	270 ± 21 a	17.4 ± 0.4 ab	346 ± 23 a
4 months	16.4 ± 0.4 a	263 ± 7 a	16.1 ± 0.2 ab	400 ± 4 a
6 months	16.1 ± 2.6 a	234 ± 31 a	14.7 ± 0.5 b	347 ± 30 a
6.5 months	12.1 ± 2.0 a	202 ± 34 a	16.6 ± 0.2 ab	308 ± 33 a

[z]The storage started on November 23, 2008.
[y]Values indicate the mean±SE (n = 3) per plant. Different letters in the column show significant differences at 5% by Tukey-Kramer HSD.

Table 5-3. Effects of storage period on sugar contents of storage roots in the 2009–2010 experiment.

Storage period[z]	Sugar contents[y] (mg•g DW^{-1}) Total	Reducing
1.5 months	626 ± 20	60 ± 8
7.5 months	531 ± 22	22 ± 5
t -test[x]	**	**

[z]The storage started on December 5, 2009.
[y]Values indicate the mean±SE (n = 7).
[x]** shows significant at 1%.

Table 5-4. Effects of storage period on spear yield of rootstock in the 2009–2010 experiment.

Storage period[z]	Marketable spear: Number[y]	Marketable spear: Yield[y] (g)	Marketable spear: Average spear weight[y] (g)	Total spear yield[y] (g)
1.5 months	15.8 ± 0.9	237 ± 13	15.1 ± 0.7	364 ± 15
7.5 months	14.3 ± 0.6	192 ± 13	13.5 ± 0.6	311 ± 15
t -test[x]	NS	*	NS	*

[z]The storage started on December 5, 2009.
[y]Values indicate the mean±SE (n = 6) per plant.
[x]* and NS show significant at 5% and non-significant, respectively.

<u>*Sugar contents of storage root and spear yield from stored rootstocks*</u>

In the 2008–2009 experiment, there was no significant difference in the fresh weight of the rootstocks among the storage periods (Table 5-1). Meanwhile, the values of soluble solid and reducing sugar contents in storage roots increased at 2 months storage and then gradually decreased until 6.5 months of storage. The total sugar content tended to decrease after the start of storage although no significant difference in its content was found. Similarly, there were no significant differences in the marketable and total spear yields harvested from the rootstocks among the storage periods (Table 5-2). However, the tendency of the yield to reduce was recognized as the storage period became longer. In particular, the marketable yield at 6.5 months storage was 202 g per plant, which was 34.6% lower than that at 0 months, and the total spear yield at 6.5 months storage was 308 g per plant, 23.0% lower than at 0 months.

White asparagus spears with no pink discoloration were harvested in all the experimental treatments like Chapter 2, 3 and 4. There was no problem related to spear quality (external appearance) throughout this experiment.

As mentioned above, the large blue plastic sheet was utilized as a covering tool for the rootstocks stored under the snow mound in 2008–2009 experiment. As this sheet was not made of perfectly impermeable materials, melting water from the snow mound was considered to constantly pour into the containers. Araki (2002) observed the serious damage to the roots and crowns in the asparagus rootstocks grown in the drained paddy where the reduced layer was well developed, and suggested that moisture stress and oxygen deficiency in the reduced layer may damage asparagus rootstocks. This melting water might have brought about similar damage to the stored rootstocks.

In the 2009–2010 experiment, an impermeable plastic film was prepared for rootstock storage treatment to prevent melting water of the snow mound from penetrating into the storage containers. The fresh weight of the rootstock population used in the 2009–2010 experiment showed 897±160 g (mean±SD, n = 8) just before the storage. A significant difference was found in the total and reducing sugar contents in storage roots between the two storage treatments (Table 5-3). The total sugar content after 7.5 months of storage was 531 mg·g DW^{-1}, which indicated a 15.1% reduction compared to the content at 1.5 months. Although white asparagus spears, which had the desirable external quality for fresh white asparagus, were also harvested in this experiment, the marketable yield obtained from the 7.5 months' storage treatment was 192 g per plant, significantly 19.0% lower than that from 1.5 months, and the total spear yield from after 7.5 months was 311 g per plant, also significantly 14.6% lower than at 1.5 months (Table 5-4).

All harvested marketable spears were completely white and were regarded as the commodity for fresh white asparagus; that was more than 190 g marketable spear yield per plant after the snow storage for 6.5 months and 7.5 months in both experimental years. Such yield will be sufficient because the economical standard yield of green spear is 200 g per plant in winter production by "Fusekomi" forcing cropping.

These results showed that long storage under snow mounds reduced rootstocks' spear yield, even if melting water of the snow mount was blocked off completely by impermeable sheets. van Os and Simonse (1988) described that spear production of two-year-old asparagus rootstocks dug up in autumn and stored at a temperature of 1ºC for 4 or 12 weeks, with a relative humidity of 95%, was up to 16% lower than that of the rootstocks stored for 0 weeks (forcing cropping just after digging up). Although the relative humidity was not recorded in the present study, it was presumed to be more than 95% under snow mounds (Muramatsu, 1987), and our results seemed to be consistent with their description.

It has been well-documented that the carbohydrates of storage roots accumulated in autumn are consumed when a spear or fern grows the following spring, and that the amount of carbohydrates is closely related to the spear yield of asparagus (Haynes, 1987; Pressman et al., 1993; Shelton and Lucy, 1980). Therefore, the decrease in the total sugar content observed during the long-term storage in both years was thought to greatly affect the spear yield in stored rootstocks. As this decrease is considered to be caused mainly by respiration of the rootstocks, a future study addressing the measurement of rootstock respiration under snow mounds should be performed.

In the present study, all the rootstocks were dug up in autumn and stored from autumn to summer in both years, resulting in a long-term storage period of more than 6.5 months. Moreover, as there was not a sufficient amount of snow over the storage place from November to December under natural conditions, asparagus rootstocks could not be stably stored at 0–1ºC during this period. But it is not always necessary to dig up rootstocks in autumn; there is also a method to dig up rootstocks in spring and store them only from spring to summer under snow mounds. In this method, farmers can easily prepare for the snow mound just before the start of storage and can also store the rootstocks just after they have been dug up. And the storage period under snow mound becomes 3–4 months, the decline in the total sugar content and the spear yield is also expected to be suppressed compared to the storage period examined in this study. Thus, this method must be examined for the improvement of spear yield in summer in our further research.

Year-round production of chicory (*Cichorium intybus* L. var. *folisum*) is found in many European countries by means of the long storage of the plant's roots. And for long-term storage such as 9 months, chicory roots are usually stored at –2 to –1ºC (Delele et al., 2009; Neefs et al., 2000; Schenk et al., 2003). In order to better understand the most efficient storage condition for the asparagus rootstock, the optimum temperature should be defined in our future research.

Disease problems in rootstocks should be overcome in a storage system with snow mound. When asparagus rootstocks were dug up from fields, many storage roots were inevitably excised to some extent. Occasionally, some decay caused by physical damage or/and by subsequent fungal infection was observed in the excised portions of rootstocks with longer storage duration. Therefore, it is necessary to establish a technique to overcome physical damage influences; for example, by drying treatment, or by spraying fungicides to the rootstocks just after digging them up.

Although the storage under snow mounds in this experiment could not suppress the reduction of sugar contents, white asparagus spears with no pink discoloration were harvested throughout all the storage treatments and a marketable spear yield of more than 190 g per plant was obtained from rootstocks of about 900 g stored after 6.5 months or 7.5 months in the two years. Because white asparagus production in summer is extremely small in the present domestic markets, white spears produced by this cultivation method can be regarded as high-value-added products with presumably higher prices than in other seasons. Thus, these results suggest that white asparagus spears can be commercially produced in summer from one-year-old rootstocks stored with a simple and low-cost system using snow energy. The present data will contribute to the summer demand of fresh white asparagus and promote the further expansion of domestic consumption for fresh white asparagus.

Chapter 6
General Discussion

Recently, white asparagus production is taken increasing attention in Japanese markets. For the year-round supply of white asparagus, new cropping system should be established because white asparagus is harvested only spring season in open field in traditional cropping. The author is thinking that 2 key systems should be developed for the year-round supply, one is 1) artificial complete dark circumstance (condition) when asparagus spears are coming up, and the other is 2) expanding harvest season.

The author pointed out following scientific problems in Chapter 1 to realize the 2 key systems mentioned above.

1) Effectiveness of film-cover as alternative blanching method for white spear production

2) Quality of white spear harvested in film cover method

3) Factors affecting spear yield in "Fusekomi" forcing culture with film-cover method

4) Effectiveness of long-term storage of rootstocks with snow

1. Artificial complete dark condition by film-cover

1) Effectiveness of film-cover method for white spear production

The film-cover method for asparagus spears using only a plastic shading film without soil mounding, based on Makus and Gonzales (1991) method, was examined in Chapter 2. The results indicated that white asparagus with no pink discoloration could be produced under a complete dark condition inside the shaded tunnel, and the yield of white spear production by this method was the same as that of green asparagus production. It was reported that pink discoloration in asparagus spears is derived from anthocyanin pigmentation (Wann and Thompson, 1965) and light induces the accumulation of anthocyanin in epidermal tissues of asparagus spears (Flores et al., 2005). Thus, maintaining a complete dark condition, keeping less than 0.01 lx, inside the shaded tunnel from spear emergence to harvest, is the most important point in white asparagus production by the film-cover method because white spears with no pink discoloration tend to be desired for fresh white asparagus markets in Japan. Although Makus and Gonzales (1991) did not describe pink discoloration in spears in their report, the results of the present study showed definite pink discoloration at the bottom portion of spears in all cultivars produced in the small shaded tunnel of 50 cm height. In the film-cover method with small tunnel, it is always necessary to remove the shading film over the tunnel at every harvest time, and consequently spears elongating inside the small tunnels are always exposed to sunlight at the moment. Furthermore, since it is quite difficult to check the light condition inside the tunnel after covering the shading film, sunlight may penetrate the gap between the film and the ground to inside the tunnel. Therefore, a small shaded tunnel is not considered suitable for the film-cover method because of the opening-closing operations of plastic films over the tunnels at every harvest time. In contrast, a large shaded tunnel can almost completely exclude sunlight, thereby producing white asparagus spears with no pink discoloration. It is also considered to be a labor-saving way compared to the small shaded tunnels because it is not always necessary to remove the shading film over the tunnel at harvest time.

Finally, the film-cover method with large tunnel is recommended as the new blanching method for asparagus spears for Japanese farmers since no pink discoloration is one of the most appealing features of fresh white asparagus in domestic markets.

In the present study, only one kind of plastic shading film with a very high shading rate, more than 99.99%, was utilized for the shading material. However, this material is an expensive means to guarantee a high shading rate. To reduce the initial cost of the shaded tunnel to increase farmer's income, the possibility of usage of other cheaper materials as a shading film for this cultivation should be examined. Further detailed research is also needed to elucidate the light condition which affects the accumulation of anthocyanin in epidermal tissues of asparagus spears, not only the lighting intensity but also the exposure time.

2) Quality of white spear harvested in film-cover method

With the spread of the film-cover method among asparagus farmers, the differences in some qualities between white asparagus spears produced by two different blanching methods are beginning to emerge. However, there is no information about them. The spear quality between the film-cover and traditional soil-mound methods was compared in Chapter 4. With regard to external quality, obvious differences in spear tip color and tightness were found between the two blanching methods. As these differences were visually distinguishable, they were considered to be remarkably visible indicators to help discriminate between white spears produced by these two different methods. Although it was assumed that the difference in spear tip tightness might be attributed to the existence or non-existence of soil pressure, the factor causing the difference in spear tip color was unclear. More detailed research including componential analysis is needed to reveal this difference.

The results of the present study showed a significant difference in spear hardness between the two blanching methods. The hardness of spears boiled for 10 minutes from the film-cover method was significantly tenderer in the upper (4 cm from tip) and middle (10.5 cm from tip) sections than that from the soil-mound method. Spear hardness is an important factor to determine the eating quality (Scott and Kramer, 1949; Siomos et al., 2000), and this feature appears to directly affect heating time when spears are cooked. Clore et al. (1976) reported that fiber content influenced hardness in green asparagus spear. Moreover, Chang (1983) found that a well-lignified fiber sheath present in white asparagus is missing in green asparagus. Histological observation and quantitative determination of spear fiber contents may show the difference in spear hardness between the two blanching methods more clearly.

The results of protodioscin analysis revealed that protodioscin content in spears produced by the soil-mound method was significantly higher than with the film-cover method. Protodioscin, a furostanol saponin, was confirmed as the principal saponin compound in white asparagus spears and it affected the bitterness of white spears (Brueckner et al., 2010; Chin et al., 2002; Kawano et al., 1975, 1977; Schwarzbach et al., 2006; Shao et al., 1996; Wang et al., 2003). Some previous studies indicated that saponin compounds act as a defensive substance against fungal and bacterial pathogens and probably some other pests or stressors (Hughes et al., 2004; Osbourn, 1996). Shimoyamada et al. (1990, 1996) reported that saponin compounds AS-1 and AS-2-I, found in asparagus, showed antifungal activity on some species of fungi. Therefore, biosynthesis of certain saponin compounds is likely to be regulated by soil-borne stresses and/or diseases. It is assumed that spears in the soil-mound method may grow in a more stressful environment due to soil pressure than in the film-cover method because soil-mounding causes many stresses, such as the higher physical soil load, scratches, fungi or insect attack etc. Thus, further investigations are needed to elucidate the relationship between the soil-borne stress and the saponin accumulation in white asparagus spears. Maeda et al. (2012) reported that application of methyl jasmonate, one of the plant hormones, to spears grown by the film-cover method enhanced the protodioscin biosynthesis in them. Their result may soon make it possible to artificially control saponin contents in white spears produced by the film-cover method in a commercial field.

As the results of the present study revealed obvious differences in spear color, tightness, hardness and bitterness, it is presumed that it is necessary to choose which spears cultivated by the film-cover method or the soil-mound method, are preferred in each case, according to the desired spear characteristics. The basic information about spear quality of two kinds of white asparagus spears is important for not only farmers but also sellers and users and will serve to further increase domestic consumption of fresh white asparagus.

2. Expanding of spear harvest period

1) White spear production in semi-forcing culture

In Chapter 3, the application of the film-cover method with large tunnel to spring harvest semi-forcing culture was examined. As a result, white spears with no pink discoloration could be produced in a plastic greenhouse in which eight-year-old rootstocks of asparagus were grown. There was no difference in the total marketable spear yield between white and green spear productions. Furthermore, no difference in the total spear yield including unmarketable spears was also found between them. These results indicate that the new cropping type of white asparagus for early-spring production is sufficiently developed, and the harvest period for fresh white asparagus can be extended further (Figure 6).

It is very easy for asparagus farmers to introduce this film-cover method to an asparagus planted field in a plastic greenhouse because they can start to produce white asparagus spears as soon as they have on hand the materials for the large shaded tunnel. Actually, farming households introducing such a blanching method to plastic green houses have been increasing every year in Hokkaido. As described in Chapter 1, the mother fern cultivation system, a long-term harvest semi-forcing culture in which spears are harvested from spring to summer, was established in the southwestern region of Japan in the late 1980s (Kobayashi and Shinsu, 1990; Ito et al., 1994; Abe et al., 1999) and currently used in the plastic greenhouses in Hokkaido (Jishi et al., 2006). Thus, the application of this film-cover method to mother fern cultivation system only during the spring harvest season is expected to make it possible to harvest white spears in spring and green ones in summer using the same plastic house (Figure 6). Some farmers have already tried this method in mother fern culture for several years without any serious problems to date.

2) Bud dormancy break in forcing production

The present study also revealed that white asparagus could be produced by "Fusekomi" forcing culture with the film-cover method in winter. This also means that the new cropping system for white asparagus can extend the harvest period in domestic production (Figure 6). In fact, however, it is necessary to carefully select the suitable cultivars as in green asparagus production. It is well-known that asparagus shows the bud dormancy in autumn and its dormancy is broken by chilling treatment (Drost, 1997; Haruyama et al., 1985; Hayashi and Hiraoka, 1983; Kobayashi and Shinsu, 1990; Ku et al., 2007). It was also reported that there was an obvious difference in the chilling treatment which is needed for breaking bud dormancy among asparagus cultivars in autumn (Koizumi et al., 2002; Ku et al., 2007). Hence, it is expected that the earlier the bud dormancy breaks, the earlier farmers can dig up the rootstock, resulting in earlier harvest of white spears.

'Gijnlim' showed higher productivity than 'UC157' in spring harvest open field culture in Hokkaido (Doi and Dohi, 2002; Uragami et al., 1993), but the yield of 'Gijnlim' was lower than those of 'UC157' and 'Grande' in "Fusekomi" forcing culture in the present study. This result implies that one of the factors for low productivity of 'Gijnlim' attributed to the lack of chilling period needed for breaking bud dormancy, and 'Gijnlim' might need much longer chilling treatment for breaking its bud dormancy than 'UC157' and 'Grande'. From the standpoint of yield stability, it is considered preferable to select the cultivars with shallower dormancy such as 'UC157' and 'Grande' for this white asparagus forcing culture.

Further research on the difference in bud dormancy among cultivars in autumn is needed for better understanding of the precise timing for digging up rootstocks in which their bud dormancy are completely broken. Although the cumulative hours of chilling exposure to the air temperature below 5ºC used by Koizumi et al. (2002) are the most widely known indicator for breaking dormancy in "Fusekomi" forcing culture, it is also necessary to develop simpler and more accurate methods to comprehend the state of bud dormancy of asparagus rootstocks.

Asparagus plants accumulate carbohydrates in the storage roots in autumn, and these carbohydrates are usually utilized for the spear and fern growth in the following spring (Hikasa, 2000; Pressman et al., 1993; Shelton and Lucy. 1980; Taga et al., 1980). As this accumulation of carbohydrates occurs around the same time when the dormancy is just being broken by chilling exposure, the relationship between these phenomena in autumn should be addressed.

There is little information about the mechanism of bud dormancy of asparagus found in autumn. Therefore, further study including not only dormancy breaking but also dormancy induction must be promoted in order to elucidate this mechanism at some future day.

3) Effectiveness of long-term storage of root stocks with snow

In Chapter 5, summer production of white asparagus was examined in order to extend the harvest period. Considering the limited oil resources and the problem of global warming, effective usage of renewable energies for agricultural production also has significant implications for our future. After snow and ice energy was acknowledged as a new type of energy in 2002 by the revision of the Japanese government ordinance under the Law Concerning Special Measures to Promote the Use of New Energy (Hamada et al., 2012), snow started to be highly valued as one of the natural renewable energy resources. Therefore, the present study focuses on the heavy snowfall in winter in Hokkaido.

Rootstocks were stably stored at 0–1ºC when fully cov-

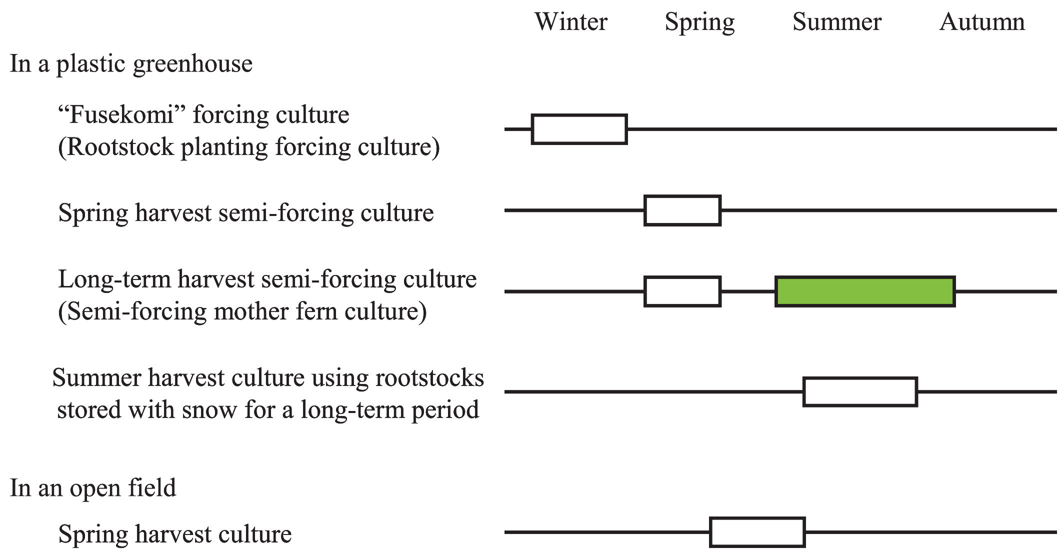


Figure 6. Cropping system of white asparagus in Hokkaido in future.
and indicates white and green harvest duration, respectively.

ered with an adequate amount of snow. The total sugar contents in storage roots of rootstocks tended to decrease as the storage duration was longer. However, white spears with no pink discoloration could be produced and the marketable spear yield per plant from the rootstock stored for more than 6.5 months with snow showed the commercial level, 190 g per plant. These results of this study suggest that commercial production of white asparagus in summer will be feasible from one-year-old rootstocks stored with snow, a simple and low-cost system.

Kim et al. (1989b) reported that asparagus spears showed high-temperature injury at temperatures of more than 35ºC. In this study, snow was used only for the storage of rootstocks. But, in Hokkaido, the air temperature sometimes reaches above 30ºC during the summer season. Thus, such cooling energy may be also used for the environmental control of spear growth, namely, the control of air temperature in cultural beds in which white spears are cultivated.

If the decline of spear yield from stored rootstocks is improved and rootstocks are stored with snow for longer period, autumn production of white asparagus may become possible. Future study is to improve the method to store rootstocks more efficiently and effectively.

4) Toward sustainable production of white asparagus in future

The author showed the new 2 cropping types, forcing production in winter, semi-forcing production in early spring, and the feasibility of summer production by mixing the film-cover method and long-term storage of rootstocks using snow. These results will enable farmers to produce white asparagus from winter to summer by such new cropping and conventional soil-mound cropping in a short time (Figure 6). And the year-round production system of white asparagus will further develop by many kinds of basic research in future.

In the forcing production in winter and summer production, rootstocks have to be prepared and transplanted in culture beds. Since spear yield had a strong relationship with the size of rootstocks, agricultural techniques for promoting rootstock growth should be improved, especially in Hokkaido, where the growth period is limited by the cool climate.

Besides, the heating system in winter and cooling system in summer using low-cost energy including natural renewable energy should be developed for the year-round production of white asparagus because energy costs for heating and cooling are very important for farm management. In the present research (Chapter 5), snow cool energy was used for long-term storage of rootstocks. And the result obtained also made us realize how snow becomes a precious natural resource for effective and stable cooling. From the viewpoint of energy-saving, the utilization of natural renewable energy is of significance to establish agriculture of environmental conservation type in future, and will lead to the low carbon society in which the input of energy is reduced. Agricultural commodity produced by using natural renewable energy is also expected to be the value-added one and finally contribute to regional development. The present attempt is a pioneer work and its data will serve to future agriculture with low carbon concept.

It is hoped that the results obtained in this study will provide helpful information for all persons engaged in handling fresh white asparagus in Japan, including asparagus farmers, breeders, buyers and consumers, and will also facilitate the utilization of natural renewable energy in agriculture.

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Summary

Asparagus (*Asparagus officinalis* L.) belongs to the family Liliaceae. This perennial plant is an ancient vegetable native to Europe where it has been cultivated since the times of the Greeks and Romans. The edible portion of asparagus plant we need is the young spear just after sprouting in spring. Generally, spears 20–30 cm in length are harvested on ordinary farms in Japan. If spears are exposed to sunlight during their elongation, they become green. On the other hand, if sunlight to spears is blocked, they become white. The first full-scale commercial production of white asparagus for canning started in the early 1920's in Japan, and canned white asparagus were exported overseas due to their high quality. Then, white asparagus became a major cash crop in Hokkaido, and its production area there had increased to 5,210 ha by 1968. However, due to the increase of cheaper canned products made in Taiwan and China in the 1960's and the domestic consumption of green asparagus, the white asparagus growing area in Hokkaido decreased dramatically to less than 100 ha in the late 1990's. But, recently, the demand for fresh white spear in domestic markets is increasing because its distinctive taste and flavor have been revaluated in Japan. Therefore, studies on the development of the new cultivation method by easier environmental control compared to the conventional method and the extension of harvest period for white asparagus were examined in order to facilitate further expansion of demand and consumption for fresh white asparagus.

In Chapter 2, to produce white spears in winter, the new blanching method for asparagus spears, the environmental control with a plastic shading film, was examined in "Fusekomi" forcing culture. A strong correlation was observed between the fresh weight of one-year-old rootstocks and their yields. Light conditions during the harvesting time had little influence on the yield, but they influenced anthocyanin pigmentation in the spear. When asparagus spears were harvested in complete darkness maintained by a plastic shading film, anthocyanin pigments in the spears disappeared. Differences among cultivar's yields were caused by the differences in the extent of chilling exposure necessary for each cultivar to break the bud dormancy of its rootstock. These results indicate that white asparagus spears can be produced with no anthocyanin pigment in forcing culture by using one-year-old rootstocks of cultivars with shallow dormancy cultivars which do not require long chilling period for bud dormancy, if spears are harvested in a completely dark condition maintained by a plastic shading film.

In Chapter 3, the author examined the application of the film-cover method described in Chapter 2 to an established plantation in spring harvest semi-forcing culture in a plastic greenhouse. When a large shaded tunnel 200 cm in height with a shading film was set up in the plastic greenhouses before the spears emerged above the ground surface and a complete dark condition was maintained throughout the harvest period, white asparagus spears with no anthocyanin pigment were produced in shaded tunnels. The number of marketable white spears was smaller than that of marketable green spears. However, the weight of white spears was greater than that of green spears. Consequently, there was no difference between white and green asparagus production with regard to marketable yield. These results indicate that the production of white spears in early spring is possible in established fields in plastic greenhouses using the film-cover method. .

In Chapter 4, the differences in the spear qualities between white asparagus spears produced by two different blanching methods, the soil-mound and film-cover methods, were examined in "Fusekomi" forcing culture with two-year-old rootstocks. The blanching method had an obvious influence on the color and tightness of the spear tip. Color in the portion 2 cm below the spear tip was more yellowish in the film-cover method than in the soil-mound method. The spear tips tended to be rougher in the film-cover method than in the soil-mound method. These results suggest that both the spear tip color and tightness might be striking visible features to help one discriminate between the two blanching methods. The hardness of spears boiled for 10 minutes from the film-cover method was significantly tenderer in the upper (4 cm from tip) and middle (10.5 cm from tip) sections than that from the soil-mound method. No significant differences were observed in sugar content or composition in spears produced by the two methods. With regard to

protodioscin related to bitterness, its content was significantly lower in spears produced by the film-cover method than in those by the soil-mound method. These results revealed that the blanching methods affect the qualities of white spear closely associated with eating and cooking quality.

In Chapter 5, the feasibility of summer harvest of white spear from asparagus rootstocks stored under snow was examined. One-year-old rootstocks were dug up in early November. Medium-sized rootstocks were put into the soil in containers and stored under snow for about 7 months. After snow storage, sugar content of storage root was measured and containers with rootstocks were transferred to a dark room at 20ºC for examination of white spear yield. Rootstocks were stably stored at 0–1ºC when fully covered with an adequate amount of snow. The total sugar contents in storage roots of rootstocks tended to decrease as the storage duration was longer. Spear yield in summer harvest after more than 6.5 months of storage under snow tended to be lower than those in winter harvest after 0 or 1.5 months of storage. The decrease of total sugar contents in storage roots during storage might cause the reduction of spear yield from the rootstocks. The marketable spear yield from one-year-old rootstocks with about 900 g fresh weight stored for more than 6.5 months under snow mound was more than 190 g per rootstock in both years. Such a spear yield suggests that white asparagus spears can be commercially produced in summer from one-year-old rootstocks stored under snow.

The present study demonstrated that the application of the film-cover method, the new blanching method using large tunnel covered with a plastic shading film, to “Fusekomi” forcing and spring harvest semi-forcing cultures made it possible to harvest white spears in winter and early spring. The study also provided fundamental information about the production of white spears in summer from rootstocks stored with snow for a long-term period. Thus, in response to the present year-round demand for white asparagus, the academic and fundamental information obtained in the present study will contribute to the establishment of a long-term white asparagus production system through the combination of the conventional and new cropping types in future. Besides, the present study also revealed the differences between the qualities of white spears produced by the two different blanching methods. It is sincerely hoped that the results obtained in the present study will provide precious information to all persons including asparagus farmers, breeders, buyers and consumers for further expansion of both production and consumption of fresh white asparagus in Japan.

Furthermore, the utilization of snow for rootstock storage shown in the present study is considered to be an environmental control technique in agriculture corresponding to the low carbon society and will contribute to the establishment of environmental friendly agriculture in future.

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根株貯蔵とフィルム遮光によるホワイトアスパラガスの長期収穫体系に関する研究

地　子　　立

要約

アスパラガス（*Asparagus officinalis* L.；英名：Asparagus）はユリ科に属する多年生の植物で，ギリシャ，ローマ時代からヨーロッパにおいて栽培されてきた野菜である．我々が食用とするのは萌芽直後の若茎で，太陽光のもとで育てるとグリーンアスパラガス，太陽光を遮って栽培するとホワイトアスパラガスとして収穫できる．

北海道では1920年代に缶詰加工用の原料としてホワイトアスパラガスの商業的生産が始まり，その品質の高さから，缶詰は海外へ輸出された．北海道でのホワイトアスパラガスの栽培面積は急速に増加して1968年には5,210haに到達し，重要な輸出品目としての地位を確立した．1960年代からより安価な台湾，中国産缶詰の生産と輸入増加，国内においてもグリーンアスパラガスへの需要転換等により，1990年代後半には道内の栽培面積が100ha未満にまで激減した．近年，その独特な風味と食感が再評価され，缶詰用原料ではなく，青果品としてのホワイトアスパラガスの需要が増加傾向となり，ホワイトアスパラガスの栽培が再度注目を浴びている．そこで，ホワイトアスパラガスの需要拡大に対応すべく，既存の栽培法よりも簡易な環境制御による栽培法開発と出荷期拡大へ向けた研究を実施した．

第２章では，ホワイトアスパラガスの冬季生産を目標に，伏せ込み促成栽培においてアスパラガス若茎の新たな軟白方法として遮光フィルムを用いたトンネル内生産について検討した．１年生株の根株生重とそれらから得られる若茎収量には強い正の相関が認められたが，軟白時の光条件は若茎収量に影響を与えなかった．一方，若茎のアントシアニン発色にはわずかな光照射も影響したが，遮光フィルム被覆により完全暗黒条件が形成されると，アントシアニン発色がない高品質なホワイト若茎が収穫できた．若茎収量には品種間差が認められ，根株の休眠打破に必要とされる低温遭遇量の品種間差に起因すると考えられた．これらより，遮光フィルム活用で，休眠性が浅い（低温遭遇要求の少ない）品種を用いた伏せ込み促成栽培において，冬季のホワイト若茎生産が可能となった．

第３章では，第２章において開発したフィルム被覆法のハウス半促成春どり栽培における適用について調査した．ビニールハウス内に定植されているアスパラガスの若茎萌芽前に高さ200cmのトンネルを設置し，その上に遮光フィルムを被覆して完全な暗黒条件を維持すると，アントシアニンの発生がないホワイト若茎が生産できた．フィルム被覆法により生産されたホワイトアスパラガスの規格内若茎数はグリーン栽培のそれよりも少なかったが，平均若茎重が大きく，グリーン栽培と同程度の規格内収量が得られた．これらより，ハウス半促成春どり栽培においてもフィルム被覆法によりホワイトアスパラガスが栽培可能であることが示された．

第４章では，ホワイトアスパラガス生産のための２種の軟白方法（慣行の培土法と新規のフィルム被覆法）により生産された若茎品質の差異を，２年生株の伏せ込み促成栽培で調査した．フィルム被覆法で生産された若茎の外観は黄色味が強く，先端のしまり程度がゆるく，これらは軟白方法を見分ける指標になると考えられた．10分間茹でた若茎の硬度は，フィルム被覆法により生産された若茎の方が培土法のそれより低かった．苦み成分のプロトディオシン含量もフィルム被覆法により生産された若茎が培土法のそれより少なかった．糖含量とその組成には軟白方法の差は認められなかった．これらの結果は軟白方法，すなわち若茎の生育環境が食味，調理特性および苦味成分等に影響を与えることを明らかにした．

第５章では，雪下で長期間貯蔵したアスパラガス根株からのホワイト若茎の夏季生産について検討し

た．11 月上旬に掘り取った 1 年生株をコンテナの土中に埋め込み，約 7 か月間雪下に貯蔵した．貯蔵後，貯蔵根の糖含量を測定し，20℃暗室で若茎収量を調査した．充分量の雪下では根株は安定的に 0〜1℃で貯蔵されたが，貯蔵期間が長くなるほど貯蔵根の糖含量は減少した．また，夏季収穫では冬季収穫と比較して，若茎収量は低くなり，貯蔵期間における貯蔵根の糖含量減少が影響したと思われた．しかしながら，6.5 月以上貯蔵した 1 年生根株（約 900 g）から 190g 以上の規格内若茎が得られ，これは商業的な夏季生産が可能なレベルと判断された．

本研究により大型遮光トンネルを用いて暗黒条件を維持する新たな軟白方法（フィルム被覆法）を，伏せ込み促成栽培やハウス半促成春どり栽培に適応することで，冬季や早春のホワイトアスパラガス生産が可能となった．また，雪を利用して長期間貯蔵した根株からの夏季生産の可能性についての基礎的な知見が得られ，今後の夏季生産の技術開発にも寄与できると考えられた．将来的には各種作型の組合せによりホワイトアスパラガスの長期収穫体系が確立されると考えられ，周年需要に応える学術的基礎知見を提示できた．加えて，本研究では二つの異なる軟白方法により得られた若茎の品質の違いを明らかにした．

本研究で得られた成果は，今後の青果用ホワイトアスパラガスの生産および消費拡大へ向けて，生産者，育種家，販売者，消費者を含む全ての関係者にとって貴重な情報となることが期待される．また，根株貯蔵における雪冷熱利用はエネルギー投入を低減させた低炭素社会を目指した環境制御技術であり，環境保全型農業に貢献できると考えられる．