ワルナスビ(Solanum carolinense L.)の種子による牧草地への侵入過程に関する研究

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Studies on the Invasion Processes of Horsenettle (*Solanum carolinense* L.) via Seeds in Pastures

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Abstract

Horsenettle (*Solanum carolinense* L.) is native to the Gulf States in the United States. It is a perennial weed that propagates by its seeds, roots and root cuttings. The plant has conspicuous spine-like prickles on the stems and leaves. It is also considered to be poisonous due to its solanine content. It is a troublesome weed in various types of crops such as corn, small grains and vegetables, as well as pastures in North America. Problems associates with this weed in pastures in Japan became noticeable in the 1970's, but occurrences of the weed were considered to have been restricted to small areas. However, it has become apparent that the area in which horsenettle can be found is expanding.

It is difficult to control the weed with ordinary pasture management practices such as grazing and cutting. Some of herbicides are effective in controlling the weed, but they are not practical in terms of the cost or efficiency. Therefore, the most efficient means of avoiding the negative effect of horsenettle is to prevent the weed from invading and being established in new areas.

The present study was conducted in order to elucidate the ways in which horsenettle invades pastures via seeds mixed into imported fodder crops and examine factors associated with seedling establishment.

The presence of horsenettle in pastures in central Japan was assessed by surveying 24 pastures from 1993 - 1998. Twenty-five percent of the surveyed pastures were found to be infested with the weed. Horsenettle is considered to be a species of alien weeds whose source of invasion is seeds mixed into imported fodder crops. The supply of concentrate applied during the grazing season, however, was not significantly related to the presence of horsenettle. The annual mean temperature in infested pastures was significantly higher than that in non-infested pastures. The probability of the weed being present was significantly lower in pastures where the average monthly air temperature over the three of the coldest months of the year was below the freezing point.

The effects of cattle digestion and of composting heat on the weed seed viability were investigated for the purposes of this study. Although the viability of horsenettle seeds ingested by cattle was significantly lower than that of non-ingested seeds, approximately 60% of the seeds that passed through the digestive tract of cattle remained viable. Furthermore, among the seeds estimated to be contained in excreta placed outdoors, 15% germinated and grew to come out. Composting heat was considered to be effective in reducing the viability of alien weed seeds, including horsenettle, if the manure was appropriately fermented and a temperature of 55℃ was maintained for 42 to 58 hours, or if a temperature of 60℃ was maintained for 10 to 17 hours.

The temperature range for germination, accumulated effective temperature and the effects of temperature fluctuations on horsenettle seeds were also investigated. Base temperature (T_b) and accumulated effective temperature (θ) to germinate seeds treated with gibberellin were calculated by Garcia-Huidobro's method. T_b was estimated to be approximately 15℃.

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Gompertz function adequately described the relationship between θ and germination fraction (%). The germination percentage of seeds exposed to diurnal temperature fluctuation with an upper limit of 15 – 30℃ and amplitudes of 5 – 15℃ for 10 or 20 cycles was significantly higher than that of seeds incubated at a constant temperature of 30℃. The seeds that were incubated at lower temperatures needed more cycles to increase their germination percentage. Horsenettle seeds appeared to respond to temperature fluctuation even below T_b.

The effect of sowing dates and competition with orchardgrass (*Dactylis glomerata* L.) on emergence and growth of horsenettle seedlings was investigated. The sowings were conducted at approximately monthly intervals from April through August in 1996 under conditions with and without orchardgrass sown in April 1996 (Hereafter, the former treatment will be referred as 'OG' and the latter as 'BARE'.). With the exception of the July sowing, the cumulative percentages of horsenettle emergence approximately two months after sowing in BARE were 80 % or higher for all sowings. In OG, the emergence percentages for the April and May sowings were higher than 45 %, while the percentages for the remaining sowings were nearly zero. Most of the seedlings for April through June sowings in BARE re-sprouted following the winter in May 1997. In OG, almost none of the shoots from any of the sowings were observed to have re-sprouted. The trend of horsenettle growth in September agreed well with that of the re-sprouted shoot number in May of the next year.

Experiments were also conducted to elucidate the possibility of seedling establishment for horsenettle during pasture renovation. Horsenettle seeds were sown on September 4 (SEP4), September 26 (SEP26) and October 15 (OCT15) in 2001 under conditions with and without orchardgrass sown on the same dates as the weed seeds except for OCT15 (Hereafter, the former treatment will be referred as 'OG' and the latter as 'BARE'.). For OCT15, the grass seeds were sown on September 26. The percentage of horsenettle that survived the winter was high for SEP4-BARE at 73%, but the percentages were below 10% for all other treatments. The freezing tolerance of horsenettle was investigated using seedlings sown on August 13 (AUG) and September 4 (SEP). The seedlings sown on each of these days were exposed to a temperature of -4℃ for a period of 3, 6, 9 and 12 hours, respectively. The viability of the seedlings for AUG was not reduced even after 12 hours of exposure, whereas the viability of those for SEP decreased as the exposure time increased. A 95% confidence interval for the exposure time at which viability was reduced to 10% was estimated to be 12 to 20 hours for the seedlings in SEP. Based on these findings, it was believed that the possibility of seedling establishment for horsenettle during pasture renovation would be low in the northern Kanto region and colder areas if the sown grasses became well established.

In order to prevent horsenettle invasion and establishment, the following were suggested based on the results of the study.

1) Manure should be treated adequately to raise its maximum temperature above approximately 60℃.
2) When pastures are renovated, sowing should be timed to enable the sown grasses to be well established. To avoid horsenettle establishment, late sowing is considered to be better for pastures where autumn sowing is appropriate.
3) Empty gaps in pastures should be minimized with adequate pasture management practices, such as over-sowing.
4) Dissemination of information on horsenettle to relevant people is important to prevent horsenettle invasion and allows them to take appropriate countermeasures before the weed becomes fully established.

Key words: *Solanum carolinense* L., Horsenettle, seed, invasion, cultural control

Chapter I INTRODUCTION

1. Review of horsenettle

(1) Taxonomy

Horsenettle belongs to Solanaceae, as known by the genus name. The chromosome number of n=12, 2n=24 has been determined for horsenettle plants in Canada [11] and in the US [20]. Zutshi and Kaul [156] reported that horsenettle in India is tetraploid, with 2n=48.

The weed is divided into three varieties: var. *carolinense*, var. *floridanum* (Shuttlw. Ex Dunal) Chapman, and var. *hirsutum* (Nutt.) Gray. The distribution areas of var. *floridanum* and var. *hirsutum* in the US are restricted to Florida and Georgia, and Alabama and Georgia, respectively

[141]. In Japan, there is no information available on the distribution of horsenettle at variety level.

(2) Geographical distribution

Horsenettle is native to the Gulf States in the US, and now it has spread across almost all of the continent US and into southern Ontario, in Canada [11, 141]. It is recorded in Haiti and Brazil [21]. In Asia, the weed is recorded in Bangladesh [42], Republic of Georgia [137], India [156], Japan [e.g. 100] and Nepal [42]. The weed is considered to have been introduced into Japan about 100 years ago [138], and has spread over all of the islands [e.g. 1, 37, 100, 113, 130]. The weed is recorded Croatia [30] and Norway [102] in Europe. The appearances of horsenettle were recorded in New Zealand [38] but may by now have been eradicated [139]. According to Parsons and Cuthbertson [104], the weed is considered to occur in Australia but Auld [7] reported that it has not been recorded as being naturalized in Australia.

(3) Biology and ecology

Horsenettle is a perennial plant that propagates by its seeds, roots and root cuttings. The germinable seed percentage is very high in general, and seeds are considered to play an important role in the plant's dissemination [46]. Freshly harvested seeds are highly dormant, but alternating temperatures from 20 to 30℃ stimulate germination. Ilnicki *et al.* [46] reported that light was not necessary for seed germination, while light promoted germination under some conditions in an experiment conducted by Suzuki [124]. NO_3 [46] or gibberellin [124] treatment can increase the germination percentage, under certain conditions. The seedlings can emerge from depths of 10 cm [46], and seeds retain their viability for at least four years when buried at depths of 8-12 cm [16].

Horsenettle grows well in sunny environments [130]. The weed appears to thrive on sandy or gravelly soils, but will grow in any type of soil [15]. It has an extensive root system, and is thought to be drought-tolerant [15].

The weed blooms from early summer through autumn [3, 11, 77, 81]. It is pollinated by bumble bees and carpenter bees [36]. The berries and seeds begin to mature by September [11].

The weed has an extensive root system with taproots and creeping, horizontal roots. Kiltz [54] showed that vertical taproots would grow to depths of 2.4 m and that horizontal root found in the upper 45 cm of soil. Both the vertical and horizontal roots have adventious buds and very small root cuttings are capable of producing shoots [121]. Ilnicki *et al.* [46] reported that 10-cm root cuttings produced shoots at a planting depth of 45.7 cm, whereas 5-cm root cuttings produced shoots only at shallow planting depths (5 and 10 cm).

Root cuttings appear to be susceptible to freezing temperatures [11, 146].

Ilnicki *et al.* [46] found that no shoots were produced from root cuttings exposed on the soil surface for three days.

Solomon [122] studied autoallelopathy in horsenettle seed germination. Germination of the weed seeds was inhibited by stem, root and leaf material incorporated into soil. The potency of the inhibition decreased with time and could be reversed by washing inhibited seeds and replanting them in fresh soil. This seems to demonstrate that autoallelopathy in this species can result in density-dependant regulation of population size.

Takahashi *et al.* [128] reported that aqueous extract of the plant considerably reduced the growth of lettuce seedlings. Wiepke and Glenn [150] reported that horsenettle extract in ethanol negatively affected the germination of turnip, and growth of corn and soybean radicle.

(4) Means of movement and dispersal

Horsenettle is disseminated by means of seeds, roots and root cuttings. Natural dispersal on a small scale could occur via seed dissemination. Horizontal roots can extend several meters from the taproot [54], and contribute to small-scale dissemination.

The seeds can maintain their viability after passing through the digestive tract of cattle, horse, pig, or sheep [67]. Ilinicki *et al.* [46] mentioned that berries may be eaten by farm animals and the seeds subsequently scattered over large areas by means of the animals' droppings.

Tillage in fields infested with horsenettle promotes the dissemination of the weed by cutting roots and dragging them elsewhere [140], provided that other favorable conditions obtain (*e.g.* good weather conditions, less competitive crop plants, ineffective weed-control). Additionally, harvesting operations may transport mature berries to other places, also encouraging dissemination.

Large-scale dissemination can occur by the contamination of crop or commercial seeds with horsenettle seeds. It has been surmised that horsenettle was introduced into Japan from the US via contamination of pasture-plant seeds [97, 138] and in fodder crop [90], although there is no definite evidence to support this theory.

(5) Impact on agriculture

Horsenettle is a troublesome weed in pastures [e.g. 77] and in field crops such as corn [107] and peanuts [34] in the US, Canada and Japan. It is also a problem in vegetable fields [11], orchards and tree nursery stock [26, 148]. It is also found on roadside, in waste areas, riverbanks and, occasionally, in gardens.

Horsenettle is listed as a noxious weed under the Seeds Act and Regulation administered by Agriculture Canada [11]. It is also listed in the Noxious Seeds Act of Manitoba.

This species is a declared noxious weed and/or noxious-weed seed in 38 states of the US (USDA, ARS, NGRP,
http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?100938).

The weed is classed as a member of the ten most troublesome pasture weeds in the southeastern United States [121].

The quarantine status of the weed in Australia is 'Prohibited Q' [7].

Horsenettle is considered to cause yield losses due to its competition with crops. Additionally, the presence of the weed fruits in the peanut harvests, as foreign material, affects the grade or quality assigned to the peanuts [151].

Frank [25] reported that horsenettle grown for three years and one year prior to planting snapbeans, reduced yield 48-65 % and 18-20 %, respectively. Hackett *et al.* [34] observed that maintenance of a weed-free environment for two or more weeks in a field previously infested with the weed permitted an increase in the yield of runner-type peanuts.

Horsenettle contains solanine and is poisonous to cattle, horse, and sheep when ingested [55]. Carlisle *et al.* [18] reported that the weed contained a potentially toxic level of nitrate.

The weed is also an important alternate host for insects of crop plants, such as the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] [64], the pepper maggot [*Zonosemata electa* (Say)] [24]. It is also host for the Potato Psyllid [*Paratrioza cockernelli* (Sulc.)] (which transmit Psyllid Yellow disease to potatoes and tomatoes) [143], tomato leafspot fungus [*Septoria lycopersici*] [106] and several viruses [108, 147].

(6) Control

1) Cultural control

Ilnicki *et al.* [46] reported that clipping the top growths, at least in July and August prevented this plant from producing viable seeds.

Because horsenettle has an extensive root system and small root cuttings can produce shoots, tillage is believed to enhance the spread of the weed [46, 121, 131]. However, the weed has been reported to become prevalent in conservation tillage systems [17, 22]. Therefore, young horsenettle which sprouts from root cuttings may be more susceptible to suppression by crop plants, and chemical and cultural control than well established plants. Muenscher [67] mentioned that a rotation that includes a clean cultivated crop every few years may reduce the presence of the weed, if the scattered plants that appear after the prior cultivation are hoed or pulled out.

Regehr and Janssen [109] observed that horsenettle population significantly declined in ridge-till systems of a soybean and sorghum rotation with herbicide treatments.

2) Biological control

Izhevskii *et al.* [49] studied the integrated control of horsenettle using tabacco mosaic virus str. Alke (TMV) and herbicides. Application of TMV to the weed prior to or during early bud formation allowed for satisfactory control of the weed in tea plantations. A prior herbicide treatment is recommended, to reduce the leaf surface-area of other weeds, and to ensure that the virus spray is deposited on horsenettle leaves only.

3) Chemical control

Horsenettle is reported to be susceptible to a wide range of herbicides. Some are effective for a short term, while others demonstrate long-term effectiveness.

Albert [2] concluded that in a pasture of bermudagrass, summertime application of 2, 4-D over a course of several years would be practical, although the Ontario Weed Committee lists the weed as resistant to 2, 4-D [11]. Foliar application of picloram in summer is very effective in controlling the root systems as well as the shoots of horsenettle. However, picloram poses a greater problem in its persistence and potential dispersion in the environment. Thus, triclopyr, which is as effective as picloram when used at higher rates than picloram may be appropriate for use in controlling this species in pastures [33].

Glyphosate is most effective when applied during the fruit-bearing period [8, 72]. Whitwell *et al.* [149] reported that a high temperature (32 ℃) resulted in more effective injury to the shoot but that a low temperature (13 ℃) during glyphosate treatment resulted in much less re-growth.

Smith and Calvert [121] reported that post-emergence applications of 2,4,5-T in water diluent would effectively control the weed .

In corn, post-emergence application of dicamba may be advisable [107].

Talbert *et al.* [134] reported that spot applications of acifluorfen were effective in controlling this weed species and minimized potential damage to developing strawberry buds, although repeated applications at two to three week intervals were required.

In peanuts, subsurface layered dinitramine or post-emergence 2,4-DB application is considered to provide acceptable levels of control [9, 34].

Horsenettle is also reported to be suceptible to amitorole [2], silvex, dinoseb [8], terbacil [103] and maleic hydrazide [131]. The seedlings are suceptible to atrazine, cloransulam, and metribuzin [142].

In Japan, the use of these herbicides except for dicamba, glyphosate and maleic hydrazide, is not acceptable in pastures [51]. The use of dicamba is restricted to at the end of grazing. The latter two can be use only at renovation. In Japanese pastures, prevention of establishment for horsenettle is the most efficient means of control.

2. Objectives and composition of the study

At the end of the Edo period in Japan, only 20 species were recorded as being naturalized. Following the Meiji Revolution in 1868, the number of naturalized plants began to increase, and approximately 1,200 species were recorded as being naturalized in 1995 [23]. It can therefore be seen that the rate of increase has risen remarkably since the end of World War Ⅱ.

Under these circumstances, the problem of alien weeds has increased on arable land. In 1977, Nakayama and Takabayashi [70] used questionnaires distributed to extension stations throughout Japan to study the problem of alien weeds in upland fields. They reported 10 new alien weeds that were said to have emerged and three other weeds that had the potential for invasion. By 1980, Saito [111] had recorded *Senecio jacobaea* L., which Nakayama and Takabayashi [70] had only mentioned as a weed with the potential for invasion, in Japan. Morita [66] argued that the accurate identification of rapidly spreading alien weeds and the establishment of a system to cope with these weeds were of utmost importance. However, alien weeds did not become a major problem among people involved in agriculture with the exception of those involved in pasture production.

Troublesome alien weeds such as broadleaf dock (*Rumex obtusifolius* L.) have been always associated with temperate grass pastures since use of temperate grasses was begun in Japan, because almost all of the grass seeds have been imported from overseas, and seed purification was not satisfactory in the past. However, alien weeds in pastures did not cause problems on arable land, including forage crop fields, and the more recently the grass seed was produced the fewer the number of weed seeds that were contaminated [39, 63, 68] after Japan became affiliated with the OECD Seed Scheme in 1967 [57]. Thus, alien weeds remained a problem mainly in terms of pasture production. In the late 1980s, alien weed problems became noticeable in forage crop fields. The weeds spread rapidly and the damage they caused was serious. Therefore, the Development of Technologies to Curb the Spread of Harmful Alien Plants research project was coordinated by the Ministry of Agriculture, Forestry and Fisheries from 1993 to 1996. The majority of the study reported here in this paper was conducted as part of this project.

Shimizu *et al.* [119] revealed that velvetleaf (*Abutilon theophrasti* Medic.) was found to be the most abundant and widespread alien weed on arable land throughout Japan using questionnaires distributed to extension stations throughout Japan in 1993. Other widespread species included horsenettle, spiny amaranth (*Amaranthus spinosus* L.), burcucumber (*Sicyos angulatus* L.), oriental cocklebur (*Xanthuium occidentale* Bertoloni) and common pokeweed (*Phytolacca americana* L.). These alien weeds occurred not only in forage crop fields but also in pastures, upland fields for crops and vegetables and orchards. Although horsenettle had already been recognized as a weed in pastures and orchards [e.g. 70], it also began to appear in forage crop fields.

According to a survey on alien weeds in pastures conducted using questionnaires in 1994 [90], broadleaf dock was found to be present on 70 farms out of the 101 farms that responded to the questionnaires. The survey revealed that bull thistle (*Cirsium vulgare* (Savi) Tenore) and horsenettle, both of which had been considered to be limited to small areas [35, 59], were more widespread than anyone had imagined. Horsenettle was reported to be present on only 0.4% of the farms that participated in a survey carried out in 1981, but the percentage of farms on which the weed was found increased to approximately 25% in the most recent survey.

Increase in consumption of imported fodder crops could involved in increase in appearance of alien weeds in forage crop fields and pastures, because it was reported that many weed seeds were mingled in imported fodder crops [118]. Each year, 17 million tons of fodder crops were imported during the period between 1989 and 1998 [60, 61]. For weed seeds mixed into imported fodder crops, however, there are several possible barriers before they are carried to pastures. Some of imported fodder crops are initially fumigated with methyl bromide in Japan [115]. In this treatment, however, weed seeds mixed into fodder crop are not affected [116]. Secondly, a large amount of fodder crops is crushed into pieces which pass through a 2 mm sieve used with a rotary crusher in factories. Weed seeds, especially small ones, possibly pass through this process and maintain their viability. Third, animal digestion is considered to reduce weed seed viability. However, it is reported that some weed seeds remained viable after being digested by cattle [6, 67, 126]. Thus, viable weed seeds would be carried to fields in the manure when the manure was not sufficiently fermented [6, 126].

Horsenettle has conspicuous spine-like prickles on its stems and leaves. The weed is also considered to be poisonous due to its solanine content [11]. Thus, cattle avoid eating this weed and it forms thick stands in pastures. Horsenettle has an extensive root system [46, 48, 54]. Once the weed becomes established, it is very difficult to eradicate with mechanical weed control methods such as cutting [46] or chemical control methods [98]. Moreover, these methods are sometimes not very practical for use in pastures in terms of cost, topography and safety for cattle and the environment. Therefore, the prevention of weed establishment is the most important and efficient means of control in pastures. Once the weed becomes established, it appears to spread primarily by means of vegetative reproduction. In long-distance dispersal, however, the role of seeds is considered to be important. The recent increase in the appearance of the weed in pastures is presumably due to seed contamination in imported fodder crops as mentioned above. Furthermore, because horsenettle is less prevalent than broadleaf dock, the areas of pastures in which weed invasion via seeds may occur are large. Therefore, it is important to determine the routes of invasion in pastures, and the conditions under which seedling emergence and establishment of horsenettle occur in order to develop countermeasures.

The objectives of the present study were to elucidate the routes of horsenettle invasion of pastures via cattle and examine factors associated with seedling establishment. In Chapter Ⅱ, the author discuss presence of horsenettle in pastures of central Japan, as well as the conditions under which it is likely to be invasive. In Chapter Ⅲ, the effects of cattle digestion and of composting heat on weed seed viability were investigated to prove the possibility of invasion of the weed via cattle. Furthermore, the temperatures and durations of time necessary to kill the weed seeds in manure are determined. In Chapter Ⅳ, the effect of temperature on germination of horsenettle seeds was investigated and the base temperature for the germination, the relationship between the cumulative germination percentage and accumulated effective temperature as well as the effect of temperature fluctuation on the germination were clarified. In Chapter Ⅴ, the author investigated the effect of sowing date and competition with orchardgrass (*Dactylis glomerata* L.) on seedling emergence and overwintering for the weed. In Chapter Ⅵ, the author studied seedling emergence and overwintering of horsenettle sown in autumn and the freezing tolerance of horsenettle seedlings to prove the possibility of establishment of the weed seedlings during pasture renovation.

Chapter Ⅱ PRESENCE OF HORSENETTLE IN PASTURES OF CENTRAL JAPAN

The authors' previous study [90] using a questionnaire has revealed that the percentage of pastures where the weed is present has increased in comparison with that in a study conducted in 1981 [35], and that infested pastures have spread all over Japan from Hokkaido to Okinawa. According to the literature, the weed is now recorded in Hokkaido [113], Honshu [e.g. 13, 101, 124, 130] and Okinawa [37]. Therefore, the climatic condition does not appear to limit the distribution of horsenettle in Japan, in a general sense. However, further studies are needed to elucidate the detailed distribution and spread of the weed. In this chapter, presence of horsenettle in pastures of central Japan is discussed based on an investigation in Niigata, Nagano and Tochigi Prefectures, as well as the conditions under which it is likely to be invasive.

1. Presence of horsenettle in pastures of central Japan

(1) Materials and methods

The presence of horsenettle was investigated in 24 pastures between 1993 and 1998: 19 public pastures, one private meadow, and two pastures and two meadows in agricultural research institutes in the three prefectures mentioned. Presence of the weed was recorded in three sown pastures, while in 20 pastures the presence of other plant species was also noted. In the three pastures, information on the herbage species sown was available from records. For the pasture in the Nagano Institute of Animal Industry, data on the presence of the weed, sown herbage species and management of the pasture were obtained from a scientist who worked in the institute.

The management of each pasture (the harvesting system, type of cattle, supply of concentrate applied during grazing season and manure spreading) was determined by interviews with the managers or a questionnaire sent to these individuals. The monthly and annual mean air temperatures of each pasture were estimated from grid data supplied by the Meteorological Agency of the Ministry of Transport. The estimation was made using a computer program [155] developed in the Grassland Resources Evaluation Laboratory of the NILGS.

The relationship between the management or air temperature and the presence of horsenettle was analyzed using Fisher's exact tests. Data on the type of cattle or air temperature in the private meadow were not available, and that meadow was thus not included in the analysis of these data. There were three types of harvesting systems used in the pastures, *i.e.*, mowing, grazing and their combination. In analyzing the relationship between the harvesting system and the weed presence, the combined harvesting system was grouped with grazing since cattle were also allowed in the pastures under this system. Each occurrence of a harvesting system (mowing or grazing) was regarded as a sample. If both were used in one pasture that would give two samples; therefore, the number of samples was 29 in this case. There were three types of cattle, *i.e.*, dairy, beef and their combination. The combination type was grouped with dairy cattle in the analysis since pastures for dairy cattle are generally grazed more intensively than pastures for beef, and if there are dairy cattle in a pasture, the pasture is probably being managed intensively. In analyzing the relationship between the supply of concentrate used during grazing season and horsenettle presence, the pastures where mowing was the only harvesting system used were excepted, so that the number of samples was 20.

(2) Results and discussion

The total area of each of the 19 public pastures ranged from 28 to 587 ha. Temperate grasses were sown in all including the pasture in which the vegetation was determined from the records. The dominant species in the pastures differed with *Zoysia japonica* Steud. being dominant in a few. The planted area in the private meadow was 1.5 ha in 1998, and *Phalaris arundinacea* L. was dominant.

Horsenettle was present in six of the 24 pastures (Fig. 1). In one public pasture horsenettle was present in the 1980s, but was eliminated before our investigation by topsoil removal after the spraying glyphosate. In the analysis of the relationship between management or air temperature and the presence of horsenettle, this pasture was grouped into those where horsenettle was present.

There were no significant relationships between the system of harvesting pastures or the type of cattle and the presence of horsenettle ($p>0.05$) (Tables 1 a and b). It has been reported that imported concentrate was contaminated with many weed seeds [117, 118], and that horsenettle seeds passing through the cattle digestive remained viable [67]. Thus,

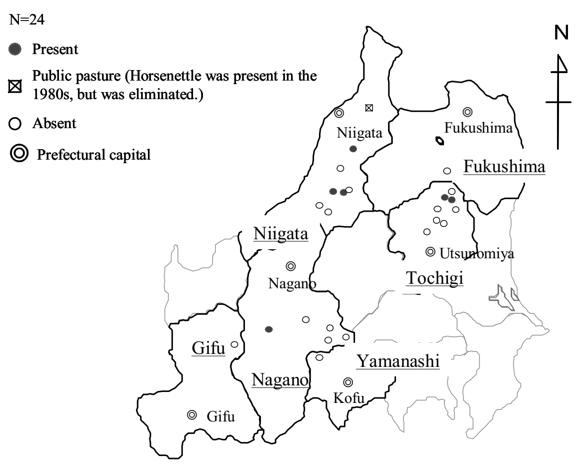

Fig. 1. Presence of horsenettle (*Solanum carolinense* L.) in pastures in several prefectures of central Japan.

it was presumed that the supply of concentrate during grazing season and the spreading of manure would influence the presence of horsenettle. That presumption does not hold, however, as these factors were not significantly related to the presence of horsenettle in this study ($p>0.05$) (Tables 1 c and d).

The relationship between the annual mean temperature in the pastures and the presence of horsenettle was of interest because horsenettle seeds require temperatures of above approximately 15 ℃ to germinate as will be mentioned in Chapter Ⅳ. The annual mean temperature in the infested pastures was significantly higher than that in non-infested pastures ($p<0.01$) (Table 2). The roots of horsenettle are reportedly susceptible to freezing temperatures [11]. Data on soil temperature in the investigated pastures was not available in this experiment, but the relationship between the average monthly air temperature of three of the coldest months and the presence of horsenettle was determined; the probability of the weed presence was significantly ($p<0.01$) lower in pastures where the average air temperature was below the freezing point (Table 3). Therefore, it was evident that a horsenettle invasion was more likely in the warmer areas of central Japan than in the cooler areas. Horsenettle is native to the Gulf States [11] and the weed may favor the warmer temperature. Although horsenettle was present in a pasture where the average temperature was below zero (Table 3) and was recorded in Sapporo [113] where the average temperature was −3.3℃ [56], soil freezing and its effect on the roots of horsenettle [11] may be one factor preventing the weed from invading the cooler areas of central Japan. The possibility of horsenettle invasion may not be completely ruled out by the freezing temperatures but may vary

Table 1. Relationship between pasture management and the presence of horsenettle.

a) Harvesting system (n=29)

	Mowing	Grazing
Present	4	3
Absent	5	17

b) Type of cattle (n=23)

	Dairy	Beef
Present	6	0
Absent	13	4

c) Supply of concentrate during grazing season (n=20)

	Supply	No
Present	3	0
Absent	12	5

d) Manure spreading (n=24)

	Spread	No
Present	5	2
Absent	10	7

Note:
There were no significant relationships between the harvesting system, type of cattle, supply of concentrate or manure spreading and the presence of horsenettle ($p>0.05$). The relationship was tested using Fisher's exact test.

a) The harvesting system using both mowing and grazing was grouped with grazing. Each harvesting occurrence was regarded as a sample. Thus if two occurred in a pasture that was viewed as two samples.

b) When both dairy and beef cattle were in a pasture, they were grouped with the type of dairy cattle.

c) Pastures where mowing was the only harvesting system were not included.

Table 2. Annual mean temperature in the pastures where horsenettle was present and absent.

	Annual mean temperature (℃)
Present	11.98 (0.69) [a]
Absent	8.62 (2.13)

The annual mean temperatures were tested using the separate variance t-test.
There is a significant difference ($p<0.01$).
a) Values in parentheses represent S.D.

Table 3. Relationship between the average monthly temperature of three of the coldest months and the presence of horsenettle. (n=23)

	Temperature <0℃	Temperature >0℃
Present	1	5
Absent	14	3

There is a significant relationship between temperature and the presence of horsenettle ($p<0.01$). The relationship was tested using Fisher's exact test.

depending on the depth of freezing and the extent of the roots when they are exposed to freezing temperature. Roots of well-established horsenettle penetrate very deeply [e.g. 46, 124] and the root cuttings can sprout from soil as deep as 45.7 cm [46]. The growth of horsenettle roots may be influenced by other species in which the weed occurs and air temperatures affect the competitive relationship between the weed and other species. Further studies are required to ascertain the effect of temperatures focusing on both the competitive relationship and the growth of the weed itself.

Horsenettle was recorded in Niigata and Nagano in the 1940s [112*, 130] and in Tochigi in the 1960s [13], both in the relatively distant past. The percentage of pastures infested with horsenettle in this study was 25% (Fig. 1, excluding one public pasture), almost the same as that (27%) in the authors' previous study in which the presence of alien weeds in pastures throughout Japan was surveyed by questionnaire [90]. These percentages are lower than the 69% of *Rumex obtusifolius* L. although both weeds were introduced into Japan at approximately the same period [69, 101, 138]. Although the temperature is believed to be involved in limiting the range of horsenettle expansion in the cooler areas, the information currently available about spread of the weed is not enough to discuss this issue. Further studies are needed regarding the expansion of horsenettle range.

2. Summary

The presence of horsenettle in pastures was surveyed in 24 pastures in central Japan. The percentage of infested pastures was found to be 25%. None of the management practices in the pastures (the harvesting system, type of cattle, supply of concentrate applied during grazing season and manure spreading) was significantly related to the presence of horsenettle. The annual mean temperature in infested pastures was significantly higher than that in non-infested pastures. The probability of the weed's presence was significantly lower in pastures where the average monthly air temperature for three of the coldest months was below the freezing point. It was considered that horsenettle invasion was more likely in warmer areas of central Japan than in the cooler areas.

* In this paper, horsenettle (*Solanum carolinense* L.) was misidentified as *S. aculeatissimum* Jacq. (Imai, pers. Commun.).

Chapter Ⅲ HORSENETTLE INVASION VIA CATTLE FED ON IMPORTED FODDER CROPS

The results in Chapter Ⅱ indicated that 25% of pastures investigated was infested with horsenettle, and that the percentage was almost the same as that (27%) in our previous study in which the presence of alien weeds in pastures throughout Japan was surveyed by questionnaire [90]. The percentage of pastures infested with the weed was lower than that of those infested with broadleaf dock, which was 69% in the previous study [90]. The most efficient means of avoiding weed impacts is to prevent their invasion. Thus, prevention of horsenettle invasion is very important before it becomes prevalent.

Horsenettle could be one of these species whose source of invasion is seeds mixed into imported fodder crop. Therefore, the effect of cattle digestion and of composting heat on horsenettle seeds was investigated. Further, the duration of heat exposure sufficient to kill the weed seeds was determined.

1. Effect of cattle digestion on horsenettle seeds

In this section, 1) the percentages of recovery, germination and viability of horsenettle seeds passing through the digestive tract of cattle, 2) seedling emergence from feces placed outdoors were investigated.

(1) Materials and methods

Experiment was conducted at the NILGS, Tochigi, Japan, from October to November 1995.

1) Horsenettle seed

Horsenettle seeds were collected at the NILGS in the spring of 1995 and stored under room conditions until use in this experiment. To determine the percentage of germination and viable seeds, 200 seeds were placed on moistened filter paper in two Petri dishes in a growth chamber (each Petri dish contained 100 seeds). The seeds were kept under alternate temperature and light conditions, *i.e.* 35℃ and light for 12 hours, and 25℃ and dark for 12 hours. Germination count was performed over a 25-day period. Seeds that did not germinate were subsequently tested for viability using the tetrazolium test [114].

2) Cattle and diets

Two diets with different concentrates were fed to two dry Holstein cows at maintenance level, respectively. Diet barley

Table 4. Composition and nutritive value of the diets.

	Diet	
	Barley	Corn
Composition (DM%)		
Alfalfa silage	17.0	17.0
Grass silage	31.0	34.0
Flaked barley	40.2	-
Flaked corn	-	33.2
Soybean meal	11.0	15.0
Mineral mix	0.5	70.5
Vitamin mix	0.3	0.3
Nutritive value (DM%)		
CP	16.90	16.98
OCW	41.12	41.38
TDN	70.80	71.70

contained 40% flaked barley (on a DM basis) and 48% silage. Diet corn contained 15% flaked corn and 51% silage. The nutritive value of the two diets was identical (Table 4). Cows were kept in digestion trial stall [65] separately, and fed the diet in the morning and evening. After the diets were offered for 14 days, 10,000 horsenettle seeds (18.5 g) mixed in the concentrates were fed to the cows once. All the feces were collected during a period of six days after feeding of the seeds. The trial was repeated three times, every three weeks. Mean weights (± SD) of the three cows fed the diet barley and diet corn were 660 ± 57 kg and 687 ± 16 kg, respectively.

3) Feces collection, and recovery of seeds and seedling emergence from feces

Total feces were collected twice a day for the 1st day (18 and 24 hours after feeding of the seeds), four times a day for the 2nd day (30, 36, 42 and 48 hours after feeding of the seeds), twice a day for the 3rd, 4th and 5th days (60, 72, 84, 96, 108 and 120 hours after feeding of the seeds) and once a day for the 6th day (144 hours after feeding the seeds). Thirteen fecal collections were weighed and a subsample (0.25 – 4 FW kg, mean 1.2 FW kg) was taken from each fecal collection to determine the seed recovery, percentage of germination and viability. Subsamples were put in nylon bags (300 mesh), washed and then air-dried at room temperature. Sound horsenettle seeds were collected from dried subsamples and then their number was counted. Seed recovery, expressed as a percentage of seed input, was calculated by multiplying the number of seeds recovered per gram of subsample by the total fecal output per collection, and adding the number of seed recovered from 13 collections and then dividing by the number of seeds fed. The seeds recovered from feces were tested, as described in 1), for the determination of the percentages of germination and viability.

Other subsamples were taken from feces collected during the 2nd day and 3rd day after feeding of the seeds. Subsamples collected at 30, 36, 42 and 48 hours after feeding of the seeds and subsamples collected at 60 and 72 hours were grouped into days 2 and 3, respectively. A subsample for each day weighed about 2 kg. Subsamples were put on cheesecloth in plastic plates containing small holes and then placed outdoors. Seedling numbers were counted in May 1996. Percentage of emergence from feces was calculated by dividing the number of seedlings by the number of seeds estimated to be in the subsample.

4) Statistical analyses

Data were arcsin-transformed prior to analyses [52].

The effects of the diet on the percentages of seed recovery and germination and viability of total seeds recovered were analyzed in a randomized block design, where the diets were treatments and the trial periods were blocks.

The difference between the percentages of germination and viability of total seeds recovered and of non-ingested seeds was tested in relation to that of non-digested seeds as population mean with population variance unknown.

The effect of the number of days after ingestion on germination and viability was analyzed in a randomized block design, where the number of days after ingestion were treatments and the trial periods were blocks, for each diet.

(2) Results and discussion

The diet difference had no effect on the percentage of seed recovery, percentages of germination or viability of total seeds recovered, or on the percentage of seedling emergence from feces (Table 5). Seeds fed to the cows were recovered at a percentage of 83% (mean of the two diets). The germination and viability were 61% and 76% (mean of the two diets), respectively. The germination and viability of non-ingested seeds were 63% and 83%, respectively. The germination was not affected by cattle digestion, while the viability of the seeds recovered decreased ($P < 0.01$). Among the seeds contained in the subsamples placed outdoors, 15% (mean of the two diets) emerged, and it was revealed that horsenettle seeds ingested by cattle could emerge from feces, outdoors.

The percentages of germination and viability of the seeds recovered each day after ingestion are presented in Table 6. Only the results from days 2-5 were included in the analysis of variance, because more than 100 seeds were collected and

Table 5. Effect of diets on percentages of seed recovery[a)], germination and viability of total seeds recovered, and seedling emergence[b)] from feces.

Diet [c)]	Recovery	Germination	Viability	Emergence
		(%)		
Barley	84	58	73	18
Corn	81	65	78 [d)]	12

a) Total recovery six days after ingestion was calculated as percentage of seeds ingested.
b) Subsamples collected during the second and third days were used for the determination.
c) The composition and nutritive value of the two diets were almost the same, except for concentrates.
d)Viability of a subsample collected 48 h after ingestion in block 1 could not be measured, and it was not included.
No significant difference between diets at P = 0.05.

Table 6. Effect of number of days after ingestion on the percentages of germination and viability of seeds recovered.

Diet		Days after ingestion 1	2	3	4	5	6
		%					
Barley	G	58	59	59	58	49	58
	V	70	75	71	73	68	75
Corn	G	58	59	70	64	61	62
	V	71	78	79	79	73	77

G: germination, V: viability
No significant difference in the percentages of germination or viability in each diet at P = 0.05 between days after ingestion (the second - the fifth day).

tested for germination and viability, except on the 5th day in block 3 in the diet barley (only 37 seeds were collected). Difference between the diets was not compared. The duration of the period during which seeds remained in the digestive tract had no effect on the percentages of germination or viability which did not change over the period, regardless of the diets. There were no major differences in the percentages of germination or viability between the number of days after ingestion.

Total recovery of horsenettle seeds from feces was not affected by the diets and the mean of the two diets was 83%. This percentage was much higher than that reported in similar experiments using pasture species [120, 152)]. The viability of all the seeds recovered was high (76%, mean of the two diets), though it was lower than that of non ingested seeds ($P<0.01$). The viability of other weed species recovered from cattle feces [6)] and exposed to rumen digestion by cattle [14)] was much lower than the values obtained in this experiment, and it was considered that the tolerance of horsenettle seeds to cattle digestion was relatively higher than that of other species.

The width, length and thickness of horsenettle seeds which were collected in Tochigi in 1993 were 1.87 ± 0.16, 2.36 ± 0.17 and 0.68 ± 0.09 mm, respectively (Nishida, unpublished data). Particles which pass through a 2.36 mm sieve are considered to flow out from the rumen of cattle [105)]. Since many sound horsenettle seeds were recovered in this experiment, it was considered that they could avoid physical destruction caused by mastication and rumination. Hence the size of horsenettle seed is considered to contribute to the tolerance. Besides, as the viability of the seeds recovered did not change over the period during which they remained in the digestive tract, horsenettle seeds may not be very susceptible to the chemical effect of digestion.

The germination percentage of all the seeds recovered was 61% (mean in the two diets), and there was no difference between that of ingested and non-ingested seeds. Soft seeds of legumes are readily digested, and the percentage of hardseedness among the recovered seeds increased with the duration of the retention time [152)]. The percentage of dormancy of horsenettle seeds recovered did not change with the duration of the retention time.

Among the seeds which were estimated to be contained in the subsamples placed outdoors, 15% (mean of the two diets) emerged, a lower value than the percentage of emergence expected from the viability of seeds recovered. Emergence might have continued after seedling counts were performed in May 1996. However, 59 seedlings emerged from a subsample on an average and the possibility for horsenettle to become established in the fields via cattle feces was confirmed. Yamada *et al.* [153)] reported that there was a close correlation between the number of seedlings from feces and the number of sound seeds in the feces. The recovery and viability of horsenettle seeds were very high and seedlings emerged from feces placed outdoors in this experiment. Therefore, cattle feces are a possible source of weed infestation in forage crop fields and pastures if weed seeds are mixed in feed.

It is considered that flaked barley disintegrates faster than flake corn in the rumen and induces a lower ruminal pH. However, there was no difference between the recovery of horsenettle seeds in diet barley and in diet corn in this experiment. Ishida and Kayo measured the ruminal pH (every

2 hours from 2 to 8 hours after ingestion) using fistulated cows which were fed the same diets as in this experiment (2 cows for each diet). The lowest pH values in diet barley and diet corn were 5.69 and 5.86, respectively, and there was no significant difference (at P=0.05) between the two diets (unpublished data). This was considered to be one of the reasons why the difference in diets did not affect the recovery of horsenettle seeds in this experiment.

2. Effect of composting heat on weed seeds

In section Ⅲ-1, it was revealed that approximately 60% of horsenettle seeds ingested by cattle was excreted and remained viable. It means that manure could be one of the sources of horsenettle infestation if composting heat was not able to kill the seeds.

The germination percentage of 15 weed species including horsenettle exposed to composting heat was determined in this section. It is believed that the data are useful to determine whether alien weed seeds mingled in imported fodder crops could become established in forage crop fields and pastures via cattle excreta, and develop strategies to reduce the spread of alien weed seeds.

(1) Materials and methods

The experiment was conducted at the NILGS from December 1993 to November 1994.

1) Composting tubes and materials

Composting tubes were made of chloridized vinyl. The diameter and height of the outside part of a tube were 46 cm and 46 cm, respectively. The capacity was 52.1 L. To obtain various composting heat temperatures, tubes were packed with cattle feces and sawdust by varying the ratios, densities or sizes of materials, and ventilation conditions. The temperature was measured at the center of the tube every hour.

2) Weed seeds

Fifteen weed species including horsenettle were selected for the experiment (Table 7). These are alien species which had become common [119] or common native species in forage crop fields in Japan. Weed seeds were collected in Tochigi Prefecture in the autumn of 1993 and stored under room conditions until use in this experiment. Weed seeds were placed in small cheesecloth bags, with one species per bag which contained 50 seeds each. The 15 cheesecloth bags were placed in larger nylon mesh bags. The large bags were placed in the center of a composting tube. Seeds were exposed to composting incubation for 7-25 days and then removed to test for germination. The seeds were placed on a moistened filter paper in Petri dishes in a growth chamber. The seeds were kept under alternate temperature and light conditions, *i.e.* 35℃ and light for 12 hours, and 20℃ and dark for 12 hours. Germination count was performed over a 7-25-day period. Eight trials were conducted over the course of 1 year, using a total of 39 tubes. Control seeds were tested for germination in January, April and October 1994, using 100 seeds for each species for each test.

(2) Results and discussion

Moisture content of the compost at the beginning of the exposure of seeds was 72 ± 4% (mean of 39 tubes). The maximum temperatures of 39 tubes ranged from 18.5 to 75.3℃.

Table 8. Germination of control seeds.

	Test time		
Species	Jan.	Apr.	Oct.
		%	
A. trifida	8	9	8
B. frondosa	42	37	6
C. vulgare	23	4	14
S. americanum	77	55	58
S. carolinense	9	45	46
A.thophrasti	53	69	100
P. americana	51	18	34
A. patulus	65	94	96
A. spinosus	87	94	100
A. viridis	3	77	98
P. lapathifolia	45	70	0
P. longiseta	46	73	0
D.ciliaris	51	72	88
E. crus-galli	15	100	96
P. dichotoomiflorum	3	1	84

Table 7. Tested weed species.

Family	Species
Asteraceae	*Ambrosia trifida* L., *Bidens frondosa* L., *Cirsium vulgare* Tenore
Solanaceae	*Solanum americanum* Mill, *S. carolinense* L.
Malvaceae	*Abutilon theophrasti* Medic.
Phytolaccaceae	*Phytolacca americana* L.
Amaranthaceae	*Amaranthus patulus* Bertoloni, *A. spinosus* L., *A. viridis* L.
Polygonaceae	*Persicaria lapathifolia* S. F. Gray, *P. longiseta* Kitag.
Poaceae	*Digitaria ciliaris* Koeler, *Echinochloa crus-galli* Beauv., *Panicum dichotomiflorum* Michx

Control seeds of *P. lapathifolia* or *P. longiseta* did not germinate in October 1994 (Table 8), but the maximum germination percentage of the two species which were exposed in October was 16%. Therefore, it was considered that 15 weed species remained viable during the experimental period. Since the germination of control seeds of *S. carolinense*, *A. theophrasti*, *A. patulus*, *A. viridis*, *D. ciliaris*, *E. crus-galli* and *P. dichotomiflorum* increased with time, the dormancy of these seeds was considered to be broken gradually during the experimental period.

The relationship between the maximum temperature to which weed seeds were exposed and the percentage of species which retained their germinability is depicted in Fig. 2. A set of models of straight lines with two intersecting points [99] was calculated by letting the maximum temperature be an independent variable and the percentage of the species which retained their germinability a dependent variable. The effects were not obvious until the maximum temperature reached 46℃. Thereafter there was a rapid decline in germinability, and all the species lost their germinability when the temperature rose above 57℃.

The percentage of the species which retained their germinability, the maximum temperature and duration of the period during which temperature was higher than 45℃ are presented in Table 9. The duration was 142 hours in the 23 tubes in which the maximum temperatures were higher than 55.1℃ and all the species lost their germinability.

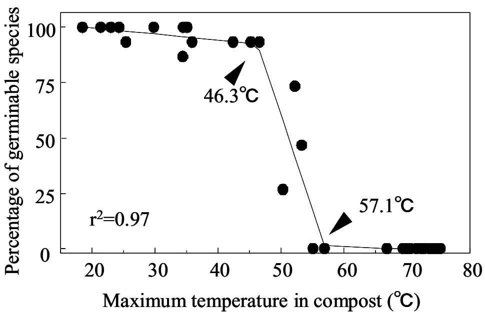

Fig. 2. Percentage of weed species which retained their germinability after being exposed to varying temperatures in compost.

The intersecting points of the set of models fitted between the maximum temperature and the percentage of species which retained their germinability corresponded to 46 and 57℃. These values indicate that some species lost their germinability when temperatures rose above 46℃ and all the species lost their germinability when temperatures rose above 57℃ in this experiment. It is considered that the germinability of seeds is affected not only by the maximum temperature but also by the duration of the period during which seeds are exposed to temperatures higher than a certain degree. Duration of the period during which the temperature was higher than 45℃ was 142 hours in tubes where the maximum temperatures were higher than 55.1℃ and all the species lost their germinability. Therefore, raising temperature above 60 ℃ and keeping the duration longer than 142 hours enable to kill non-dormant seeds in compost. However, it is considered that the higher the maximum temperature, the shorter the duration required to kill seeds. Hence, further studies about the maximum temperature and the duration of the period required to kill seeds are needed.

Weed seeds were considered to imbibe enough water in this experiment because the water content of the compost was 72% which is considered to be relatively high. Lethal temperature and duration of the period during which seeds are exposed to temperatures vary with the water content of seeds [43, 94, 126]. Hence, the water content of compost for which seeds imbibe enough water is important to kill weed seeds in the composting process.

Lethal temperature for non-dormant weed seeds in compost was determined in this experiment. However, it is considered that dormant seeds could be more tolerant to heat [44]. Dormancy is not broken in a short time after seed setting. Hence, if there are many young seeds in the compost, higher temperature and longer duration are required to kill them. Further studies on lethal temperature for dormant seeds should be carried out to control weed infestation in fields via compost.

Table 9. Percentage of species which retained germinability, maximum temperature and duration of the period during which temperature was higher than 45℃.

Maximum temperature in compost (℃)	≦42.4	45.2	46.6	50.3	52.2	53.3	≧55.1
Tube number	11	1	1	1	1	1	23
Gemination of species (%)	97(5)	93	93	27	73	47	0
Duration (h)	0	9	27	76	40	109	142(40)

Values in () represent s.d.

3. Effect of duration of heat exposure on weed seeds

In Section Ⅲ-2, it was revealed that raising temperature above 60 ℃ and keeping the duration longer than 142 hours enable to kill non-dormant weed seeds in compost. However, the relationship between the percentage of heat death of weed seeds and shorter terms of heat exposure remains unclear. Additional experiments are required to reveal this relationship because the process of composting cannot always provide the conditions necessary to kill seeds, especially during the wintertime [91].

The heat tolerance of seeds varies with their water uptake, and water-imbibing seeds have lower heat tolerance than air-dried seeds [43, 94, 126]. The water content of manure is usually about 65 to 70% when composting begins [19], and it is assumed that seeds embedded in manure absorb water from manure before being exposed to composting heat. Hence, the death percentage of weed seeds which absorbed water at 15℃ for 24 hours before heat treatment are examines in this section, although the water uptake of seeds in manure, before being exposed to heat, may vary depending on several factors such as the water content and temperature of manure, the length of time that the seeds are embedded in the manure and the degree of their dormancy, *etc.*

Although it is reported that the temperature of 45℃ adversely affected the germination of *Solanum nigrum* [31] and *Abutilon theophrasti* seeds [43], in our previous study some seeds of the tested weed species retained their viability after exposure to a 45℃ temperature over the course of an entire week [89]. The maximum temperature in manure tends to rise above 45℃ when the manure temperature remains above 45℃ for more than one week [5, 91, 126]. Weed seeds lose germinating abilities following exposure in compost to temperatures above approximately 60℃ as shown in the previous section. Therefore, the author investigated the effect of duration of heat exposure at 55 and 60℃, respectively, on the viability of seeds of horsenettle and of other common weeds in this section.

(1) Materials and methods

The experiments were conducted from March to September in 1997.

1) Weed seeds

Ten weed species that commonly occur in forage-crop fields and pastures in Japan were selected: *Solanum carolinense* L. (horsenettle), *S. americanum* Mill., *Abutilon theophrasti* Medic., *Phytolacca americana* L., *Amaranthus spinosus* L., *A. patulus* Bertoloni, *Persicaria lapathifolia* (L.) S. F. Gray, *Panicum dichotomiflorum* Michx., *Echinochloa crus-galli* (L.) Beauv. var. *crus-galli* and *Digitaria ciliaris* (Retz.) Koeler. Among these, *Persicaria lapathifolia*, *E. crus-galli* and *D. ciliaris* are native to Japan; the other weeds were introduced from overseas after 1854 when Japan was opened to foreign intercourse. The seeds for the experiment were collected in Tochigi Prefecture during 1994 and 1996, air-dried and stored under room conditions.

2) Water uptake

Samples consisted of 150 air-dried seeds for *Amaranthus* species, 90 for *S. americanum*, *Panicum dichotomiflorum* and *D. ciliaris*, 60 for *E. crus-galli* and 30 for horsenettle and others. A sample of each species was weighed, placed on filter paper and absorbent cotton in a glass Petri dish (d. 6-cm), and supplied with 20 ml of deionized water. The seeds were incubated at 15℃ for 24 hours in darkness and re-weighed for water uptake after removal of external moisture by blotting.

3) Heat treatment

Thirty seeds of each species which absorbed water under the conditions given above were exposed to a temperature of 55℃ for a period of 24 hours, and to a temperature of 60℃ for 3 hours. After exposure to heat stress, they were incubated for a germination test at alternate temperature and light conditions of 30℃ / 12 hours light and 20℃ / 12 hours dark. A germination count was made over a 20-day period. Sound seeds that did not germinate were subsequently tested for viability by tetrazolium (Tz) test [74]. Seeds that germinated or were stained with Tz were classified as 'viable seeds'. Those that collapsed when they were pinched with a pair of tweezers or were not stained were classified as 'dead seeds'. For the species of which seeds remained viable after this heat exposure, the duration of exposure at each temperature was extended until all seeds were killed. The germination and viability percentages of the seeds were recorded at each duration and temperature.

At the beginning of the experiment, untreated seeds were also tested for germination and viability, as were the treated seeds. Dormancy percentages were calculated by subtracting the germination percentage from the viable seed percentage for untreated seeds. As the seed dormancy percentage for *Abutilon theophrasti* was quite high at the beginning of the experiment, this percentage with untreated seeds was re-examined at the end to determine if there were any changes in the percentage of dormancy over the duration of the experiment using the germination test.

4) Statistical analyses

In all cases, the experimental design was Completely Randomized Design with three replications. Germination and viability percentages were arcsin transformed prior to analyses of variance [52]. Means of germination and viability within each species were tested between treatments by the l. s. d. method [40].

For *A. theophrasti*, which required the longest period of heat exposure to kill all seeds at each temperature, confidence intervals of LD_{90} values were calculated using the probit method [154]. The probit method assumes a normal distribution for the dose that induces response. The response percentage at each dose is converted into 'surveyed probit' using a normal distribution table and a transformation equation. 'Estimated probits' are calculated from surveyed probits, and then modified in order to obtain 'practical probits'. A weighted regression equation between the doses and practical probits is calculated. Then the confidence interval of LD_{90} value is calculated using the equation.

(2) Results and discussion

1) Water uptake

Following water absorption, the water uptake of the various species of seeds ranged from 6 to 60% (Table 10). The water uptake of horsenettle seeds was 44%, which was relatively high among those of the tested species. Water uptake was lowest for *Phytolacca americana* and highest for *E. crus-galli*.

2) Dormancy

The dormancy percentages for horsenettle, *Persicaria lapathifolia* and *Panicum dichotomiflorum* were quite low in this experiment, although it has been reported that their seeds do demonstrate dormancy [46, 47, 129] (Table 10). It appears that the dormancy dissipated during the storage period of one to two years before the beginning of this experiment.

Dormancy percentages for *D. ciliaris* and *E. crus-galli* and seeds were 14 and 34%, respectively.

The highest dormancy percentage of 80% was recorded for *A. theophrasti* seeds. The germination percentage for *A. theophrasti* seeds at the beginning and at the end of the experiment was 13 and 11%, respectively. The dormancy percentage for *A. theophrasti* appeared to remain unchanged during the experiment.

Table 10. Percentages of water uptake and dormancy of untreated seeds.

Species	Water uptake [a)]	Dormancy percentage [b)]
	%	
Solanum carolinense	44 (2) [c)]	2 (2)
S. americanum	37 (2)	0 (0)
Abutilon theophrasti	12 (5)	80 (6)
Phytolacca americana	6 (1)	2 (4)
Amaranthus spinosus	33 (3)	0 (0)
A. patulus	29 (1)	1 (2)
Persicaria lapathifolia	24 (3)	0 (0)
Panicum dichotomiflorum	20 (2)	6 (2)
Echinochloa crus-galli	60 (3)	34 (13)
Digitaria ciliaris	54 (3)	14 (4)

Note:

a) Weed seeds were supplied 20 ml of deionized water, and the water uptake was measured after incubation at 15 ℃ for 24 hr.

$$\text{Water uptake (\%)} = \frac{\text{WISW} - \text{ADSW}}{\text{ADSW}} \times 100$$

WISW: water-imbibing seed weight

ADSW: air-dried seed weight

b) The dormancy percentage was calculated by subtracting the germination percentage from the viable seed percentage after absorption of water and incubation for 20 days at 20/30 ℃.

Dormancy percentage (%)= Viability (%) - Germination percentage (%)

c) Values in parentheses represent SD.

3) Heat treatment

i) Effect of heat treatment

Seeds of horsenettle and of other species except for *A. theophrasti* were killed by exposure to temperatures of 55 and 60℃ for the respective durations of 72 and 24 hours (Table 11).

The germination percentage for *A. theophrasti* was 0% following 24 hours of heat treatment at both 55 and 60℃ but the seeds remained viable. The time required to kill all *A. theophrasti* seeds at temperatures of 55 and 60℃ was 120 and 30 hours, respectively.

The briefest heat treatment, 24 hours at 55℃ and 3 hours at 60℃ significantly reduced viability of horsenettle seeds, although the viability was 72 and 67 % at 55 and 60℃, respectively, which appeared to be relatively high. The viability of horsenettle seeds, however, was almost 0% at both temperatures, when the duration of treatment became longer. The pattern of germination for horsenettle seeds was similar to that of the viability. The patterns of percentages of germination and viability for *S. americanum* and *A. patulus*

Table 11. Percentages of germination and viability of weed seeds exposed to heat for different periods at 55 and 60℃.

		Un-treated	55 ℃ hours					60 ℃ hours			
			24	48	72	96	120	3	6	24	30
							%				
Solanum carolinense	G [a]	97 a	19 bc	3 cd	0 d			32 b	0 d	0 d	
	V [b]	99 a	72 b	7 c	0 c			67 b	9 c	0 c	
S. americanum	G	97 a	28 c	0 d				78 b	0 d	0 d	
	V	97 a	79 a	0 b				84 a	6 b	0 b	
Abutilon theophrasti	G	13 a	0 c	0 c	0 c	0 c	0 c	9 ab	3 b	0 c	0 c
	V	93 a	23 bc	12 cd	9 cde	2 ef	0 f	39 b	23 bc	7 de	0 f
Phytolacca americana	G	92 a	0 c					49 b	0 c	0 c	
	V	94 a	0 c					57 b	3 c	0 c	
Amaranthus spinosus	G	94 a	2 b	0 b				20 b	0 b	0 b	
	V	94 a	2 c	0 c				24 b	1 c	0 c	
A. patulus	G	96 a	38 b	0 c				70 b	0 c		
	V	97 a	38 c	0 d				74 b	0 d		
Persicaria lapathifolia	G	83 a	0 b					0 b			
	V	83 a	0 b					0 b			
Panicum dichotomiflorum	G	90 a	0 c					26 b	0 c		
	V	96 a	0 c					46 b	0 c		
Echinochloa crus-galli	G	41 a	0 b	0 b	0 b			3 b	0 b		
	V	76 a	6 b	6 b	0 b			6 b	0 b		
Digitaria ciliaris	G	52 a	0 b					7 b	0 b		
	V	67 a	0 b					10 b	0 b		

Note:
Values in the same row followed by the same letter are not different at 1% level of significance.
a) germination percentage b) viability

seeds were similar to those for horsenettle seeds. Some seeds of *Phytolacca americana*, *A. spinosus* and *Panicum dichotomoiflorum* remained viable after 3 hour treatment at 60℃, but almost all seeds for these species were killed after 24 hour treatment at 55℃. The briefest fatal exposures were for *Persicaria lapathifolia*, *E. crus-galli* and *D. ciliaris*, with minimum durations of 24 hours at 55℃ and 3 hours at 60℃. Thus, heat tolerance was considered to be highest for *A. theophrasti*, and second highest for horsenettle, *S. americanum* and *A. patulus* among the species tested.

ii) Fatal exposure duration for *A. theophrasti*

Because the duration of exposure to heat needed to kill all the seeds was longest for *A. theophrasti*, the viability of the seeds can be considered as an index of fatal exposure for the tested species. In order to quantify the relationship between death percentage and duration for which *A. theophrasti* seeds were exposed, a detailed analysis was carried out. Confidence intervals of LD_{90} values were calculated using the probit method. These were 1.62 to 1.76 or 42 to 58 hours at 55℃ and 1.01 to 1.24 or 10 to 17 hours at 60℃ (Fig. 3).

iii) Possibility for composting process to kill weed seeds in manure

Reports indicate the process of composting can provide temperatures above 55℃ for 96 to 144 hours [5, 91, 126] and temperatures above 60℃ for 72 to120 hours. Thus, given normal composting conditions, both temperature and length of heat exposure are sufficient to kill horsenettle and other weed seeds, including dormant *A. theophrasti* ones. The experiment design does not rule out the possibility that other factors, including ammonia [58, 95] and microorganisms, may have significant effects on the viability of weed seed in manure.

4. Summary

Experiments were conducted to determine whether horsenettle seeds mixed into imported fodder crops could become established in pastures via cattle excreta. Horsenettle seeds were fed to cows which received 2 diets with different concentrates, *i.e.* flaked barley and corn, and the seeds recovered from excreta were tested for germination and viability. The 2 diets had no effect on the seed recovery, or on the germination or viability of recovered seeds. The mean percentages of seed recovery, germination and viability of recovered seeds were 83, 61 and 76%, respectively. Among the seeds estimated to be contained in excreta placed outdoors, 15% emerged. Likewise, the germination

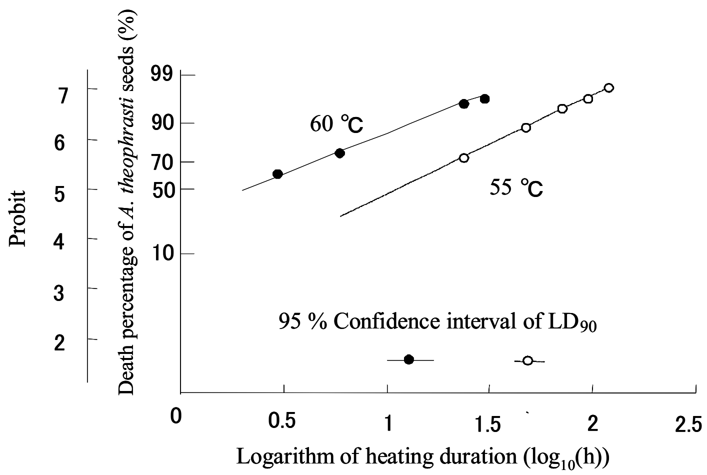

Fig. 3. Relationship between logarithms of heating duration and death percentage of *Abutilon theophrasti* seeds.

Circles show 'estimated probits' at each temperature (○ 55 ℃, ● 60 ℃).
Weighted regression equations between logarithms of heating duration and 'practical probits' that were modified 'estimated probits', were calculated.
55 ℃ $y = 2.884 + 2.009\,x$ $\chi^2 = 4.794 < \chi^2_{(3,\,0.05)}$
60 ℃ $y = 4.469 + 1.648\,x$ $\chi^2 = 4.115 < \chi^2_{(2,\,0.05)}$
According to χ^2 of the equations, their linearity was undeniable.

percentage of horsenettle and 14 other weed species exposed to compost incubation was determined. Weed seeds were placed in 39 composting tubes (52.1L) for 7-25 days, and the regression equation was calculated between the maximum compost temperature and the percentage of species which retained their germinability. The effects were not conspicuous until the maximum temperature reached 46℃. Thereafter there was a rapid decline in the percentage of germinable species, and none of the species germinated when the temperature was above 57℃.

Water-imbibing seeds of horsenettle and of 9 other weed species were exposed to temperatures of 55 and 60℃, respectively, to determine the duration of heat exposure sufficient to kill weed seeds in manure. The durations of heat exposure tested were 24, 48, 72, 96 and 120 hours at 55℃, and 3, 6, 24 and 30 hours at 60℃. All seeds of each species were killed by exposure to heat at 55℃ for 72 hours and at 60℃ for 24 hours except for those of *Abutilon theophrasti* (dormant seed percentage 80%) which were killed by exposure for 120 and 30 hours at the respective temperatures.

For *Abutilon theophrasti*, which required the longest duration of heat exposure to kill all seeds at each temperature, confidence intervals of LD_{90} values were calculated using the probit method, and were 42 to 58 and 10 to 17 hours at 55 and 60℃, respectively. According to the literature, the process of composting was considered to be capable of providing sufficient length of heat exposure to kill all weed seeds in manure at both temperatures.

The results showed that imported fodder crops containing horsenettle seeds could become a source of weed infestation unless animal excreta were treated adequately.

Chapter Ⅳ EFFECT OF TEMPERATURE ON GERMINATION OF HORSENETTLE SEEDS

Because horsenettle grows in corn and soybean fields in the US [11, 46, 121, 132], which exports a large amount of fodder crops to Japan, the rapid increase in incidence of the weed is believed to be due to contamination by the weed seeds along with other alien weed seeds in imported fodder crops [117, 118]. Horsenettle seeds partially survive after passing through the cattle digestive tract. They also retain their viability in manure if cattle excrement is not adequately fermented as shown in Chapter Ⅲ. Thus, live horsenettle seeds would be spread over the field when such manure is used. The seeds thus appear to play an important role in large-scale infestations. Several studies have been conducted on germination of horsenettle seeds, however, further studies are still needed to elucidate the relationship between temperature and germination of the weed seeds.

The accumulated effective temperature for horsenettle seeds to germinate was calculated by Garcia-Huidobro's [28, 29] method in this chapter. The accumulated effective temperature is applicable even if the period has a decimal fraction, and the base, optimum and maximum temperatures for germination are obtained by calculations using this method. In these points, accumulated effective temperature is different from total effective temperature described as $\Sigma(T - T_b)$. In addition to obtaining the accumulated effective temperature and temperature range for horsenettle seeds to germinate, the effect of temperature fluctuation on the germination of the weed seeds was investigated.

1. Range of germination temperature and accumulated effective temperature

(1) Materials and methods

The experiment was conducted from June to July in 1993.

1) Seeds

Horsenettle berries were collected from a pasture in the NILGS in November 1992. The seeds were separated from the berries, washed and stored under room conditions until the experiment.

2) Germination test

Forty seeds were placed on a filter paper in a 9 cm-diameter Petri dish. Due to low germination percentages of the seeds in a preliminary experiment in which deionized water was used, 5 ml of 100 ppm gibberellin (Wako, GA_3:85 – 93%, GA_1+GA_4:7 – 15%) was added to the Petri dish. Then the seeds were incubated at constant temperatures of 15, 20, 25, 30, 35 or 40℃ (light / dark, 10 / 14 hours). The treatments were replicated five times. The number of seeds that germinated was recorded daily for 40 days.

3) Application of Garcia-Huidobro's method

The range of germination temperature and accumulated effective temperature (θ) for the germination of different fractions of the single seed lot were calculated by Garcia-Huidobro's method [28, 29]. This method is based on an idea of accumulated effective temperature (θ), expressed by the following equation:

$$\theta = (T - T_b) \times t$$

where θ = accumulated effective temperature (℃-day), T = temperature (℃), T_b = base temperature (℃), t = period (day)

In this method, T_b and θ are obtained by regressing between temperature and 1/t for different germination fractions. The idea of accumulated effective temperature is easy to understand and regression parameters are relatively easy to calculate. Thus, this method was used in this experiment.

The germination fraction, G_x, was defined as the germination percentage calculated of the final percentage obtained at each temperature. The reciprocal of period at a given germination fraction (1/t) was defined as reciprocal of time (t) taken for G_x to germinate. Each 1/t was plotted against the temperature for G_x. Optimum temperature, T_o, was the temperature at the maximal 1/t. The value of 1/t increased linearly with temperatures up to T_o and decreased thereafter. The accumulated effective temperatures (θ) and the base temperatures (T_b) were calculated as described in Garcia-Huidobro's [28, 29] methods since a linear relationship was clearly observed between 1/t and temperature in each G_x below T_o. θ is the reciprocal of the slope of the regression line fitted for each G_x and T_b is the temperature-axis intercept. For the seeds that were incubated at temperatures above T_o, the same procedure was undertaken to calculate θ and a maximum temperature, T_m. A Gompertz function was fitted to describe the relation between θ and G_x of seeds incubated at below T_o [12]:

$$G_x = 100 \times \exp\{-a \times \exp(-b \times \theta)\}$$

$$\theta = (T - T_b) \times t$$

where G_x = germination fraction (%); θ = accumulated effective temperature (℃-day); T = incubation temperature (℃); T_b = base temperature (℃); t = period of incubation (day); a, b = parameters

(2) Results and discussion

Figure 4 shows the trend of germination of horsenettle seeds treated with gibberellin. Germination was highest at 30 – 40℃ with 77 – 88%. The respective final germination percentages on the 40th day were above 20% at each temperature regime except at 15℃. Therefore, the results of this experiment, except for 15℃, were used to calculate the temperature range of germination and θ.

If the seeds are incubated above T_b over an adequately long period of time, the length of time period for them to attain 100% germination can be calculated at any temperature above T_b. However, from Fig. 4, it did not appear that the germination of horsenettle seeds could ever reach 100%, even if the incubation periods were extended. Therefore, the final

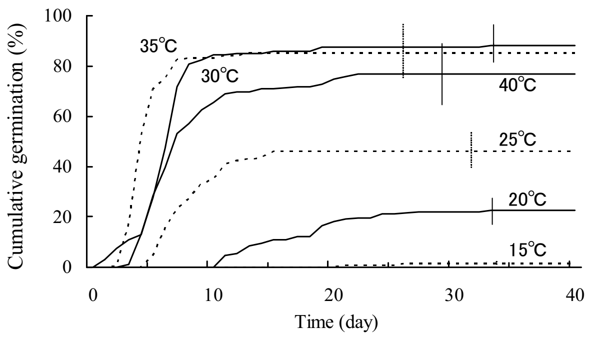

Fig. 4. Course of cumulative germination for horsenettle seeds treated with gibberellin.

Vertical bars represent common standard deviation at each temperatre.

Table 12. Estimated base temperatures (T_b), accumulated effective temperatures (θ) and regression coefficient of temperature against reciprocal of period at a given germination fraction(r^2) for different values of germination fraction (G_x).

G_x (%)	T_b (℃)	θ (℃-day)	r^2
G_{20}	14.03	64.05	0.989**
G_{30}	14.08	70.50	0.956*
G_{40}	14.15	75.15	0.968*
G_{50}	15.03	77.33	0.969*
G_{60}	15.62	80.57	0.975*
G_{70}	15.06	92.51	0.982**
G_{80}	15.61	94.91	0.991**
G_{90}	14.10	127.45	0.992**
average	14.71		

** : P<0.01 * : p<0.05

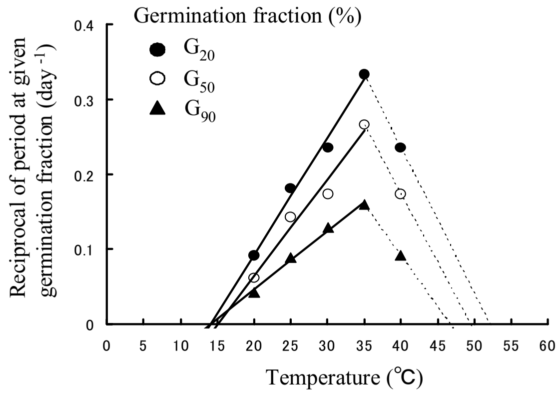

Fig. 5. Relationship between temperature and reciprocal of period at a given germination fraction (1/t).

Solid lines are the regression line of the relationship between temperature and 1/t below T_0. The regression line above T_0 are represented by dotted lines since only two points were obtained in this study.

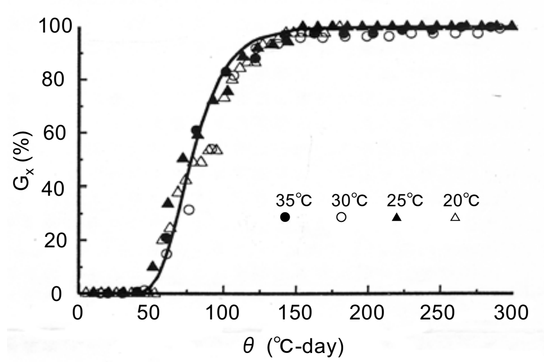

Fig. 6. Germination fraction (G_x) at accumulated effective temperature (θ) for horsenettle seeds treated with gibberellin and incubated at 20, 25, 30 and 35℃.
Solid line is the estimated value from equation (1).

germination percentage obtained at each temperature regime was considered as the highest possible percentage of germinable seeds and this value was taken as 100% in this study.

Each reciprocal of period at a given germination fraction (1/t) was plotted against the incubation temperatures for G_x. G_{20}, G_{50} and G_{90} are shown in Figure 5. Below 35℃, the regression lines between the temperature and 1/t were significant ($P<0.05$) for each germination fraction of G_{20}–G_{90} (Table 12). Thus, base temperatures (T_b) and accumulated effective temperatures (θ) for different G_x to germinate were calculated. The results are shown in Table 12. The T_b was estimated to be between 14.03 and 15.62℃. The base temperatures estimated from the literature were about 5℃ for *Persicaria lapathifolia* (L.) S. F. Gray, *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Beauv. var. *praticola* Ohwi [145] and 13℃ for *Digitaria ciliaris* (Retz.) Koeler and *Cyperus microiria* Steud. [93]. Compared with these weeds, horsenettle has a higher base temperature. This may be one of the factors that impede occurrence of horsenettle in the field. Although a maximum temperature (T_m) could not be calculated in this study because only two points were obtained above T_o, it was surmised to be between 45 – 50℃ from the graph (Fig. 5).

The Gompertz function has been shown to be suitable for describing cumulative germination [136]. The following equation

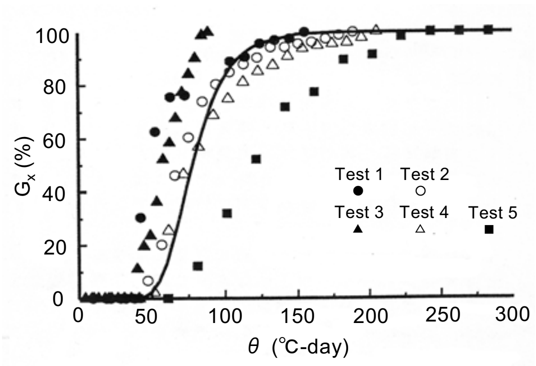

Fig. 7. Germination fraction (G_x) at accumulated effective temperature (θ) for horsenettle seeds in different tests.
Solid line is the estimated value from equation (1).

Table 13. Conditions and final germination percentages of tests whose data were used to validate equation (1).

	Condition of test	Final germination (%)
test 1	Gibberellin + constant 15 ℃ followed by constant 25 ℃	85
test 2	30/20℃ (10/14 hr)	79
test 3	25/15 ℃ (10/14 hr)	38
test 4	Gibberellin + constant 25 ℃	88
test 5	Constant 35 ℃	29

In all the tests, deionized water was properly supplied during the incubation.
In test 1 and 4, five ml of 100 ppm gibberellin was supplied at the beginning of the incubation.

was obtained by fitting the data of this study into the Gompertz function:

$$G_x = 100 \times \exp\{-74.85 \times \exp(-0.056 \times \theta)\}$$

$$r^2 = 0.994 \qquad (1)$$

$$\theta = (T - 14.7) \times t \qquad (2)$$

In Equation (2), T_b was set to be 14.7, which is the mean of T_b for G_{20}–G_{90} (Table 12). Figure 6 shows the pattern of G_x values with increasing accumulated effective temperature. It is evident that equation (1) adequately described the relationship between θ and G_x. Equation (1) was validated using data obtained from five separate tests (Fig. 7). The experimental conditions of the tests used in Fig. 7 are shown in Table 13. Equation (1) adequately described data from tests 2 and 4. Data from tests 3 and 5 with relatively low final germination percentages and those from test 1 in which the temperature regime was changed during the course of incubation were not as adequately described as data from tests 2 and 4. Further studies are needed to clarify the reasons for this and to improve the estimation.

The range of germination temperature and accumulated effective temperature to germination were calculated using gibberellin treated horsenettle seeds in this experiment. There is a possibility that temperature response of gibberellin-treated seeds is different from that of non-treated seeds. However, gibberellin treatment was considered necessary in order to obtain adequate germination percentages to calculate because of low germination percentages in the preliminary experiment. Because Equation (1) adequately described the germination course of non-treated seeds (Fig. 7, test 2), it was not considered that the resultant findings were limited to the case of gibberellin-treated horsenettle seeds.

2. Effect of temperature fluctuation on germination

(1) Materials and methods

The experiment was conducted from January to February in 1994.

1) Seeds

Berries of the weed were collected from a pasture in the NILGS in November 1992. The seeds were separated from the berries, washed and stored under room conditions until the experiment.

2) Germination test

Forty seeds were placed on a filter paper and supplied with 5 ml of deionized water in a 9 cm-diameter Petri dish. The seeds were exposed to the following temperature conditions:30/20, 25/20, 25/15, 25/10, 20/15, 20/10 and 15/10℃ for 10 hours in the light /14 hours in the dark, respectively, for four different cycles (1, 5, 10 and 20 cycles). The seeds in each treatment were then incubated at a constant 30℃ for 30 – 40 days in total. The control was seeds exposed to a constant 30℃ for 30 days. Germination during the period was recorded. The experiment was set up in a completely randomized design with five replicates. Germination percentages were arcsine-transformed [52] prior to analysis of variance. Means were tested by Scheffe's method and differences between the germination percentages of each treatment and the control were tested by Dunnett's method [127]. The significant level was 0.05 for all cases.

(2) Results and discussion

The germination percentages obtained at various alternating temperatures were significantly higher than that at control (constant 30℃) when the number of cycles was 10 or more, except for 20/15 and 15/10℃ (Table 14). At these temperatures, 20 cycles were needed to raise the germination percentages to levels significantly higher than that of the control. In general, with regard to the alternating temperature regimes, the more cycles the seeds were exposed to, the higher was the germination percentage obtained. More cycles were required to raise the germination percentage when seeds were incubated at lower temperature regimes. However, the maximum germination percentages attained at the different alternating temperature regimes were similar with values of 60 – 69%.

It appeared that temperature fluctuation cumulatively promoted the germination of horsenettle seeds with increase in the number of cycles and that its effect varied among the temperature and thermal amplitudes. Benech Arnold *et al.* [12] classified viable seeds of *Sorghum halepense* (L.) into three states, namely, State Ⅰ: seeds that have been broken from dormancy and are responding to temperature; State Ⅱ: seeds that have not yet satisfied their fluctuating temperature requirements for dormancy breaking; State Ⅲ: seeds that have no evident response to temperature. Applying this classification to horsenettle seeds, all the seeds in State Ⅱ appeared to change to State I under all the alternating temperature regimes tested at 10 – 20 cycles, since the maximum germination percentages were similar in all cases.

Table 14. Effect of number of cycles of temperature fluctuation on the germination percentage of horsenettle seeds.

Thermal regime(℃)	Number of cycles of temperature fluctuation			
	1	5	10	20
	germination (%)			
Const. 30	39 (control)			
30/20	41 b	46 ab	63 a	**65** a
25/20	41 b	51 ab	**60** a	58 a
25/15	40 b	45 b	**61** a	61 a
25/10	45 c	47 bc	60 ab	**67** a
20/15	32 c	50 b	48 b	**64** a
20/10	38 c	42 bc	57 ab	**65** a
15/10	37 b	39 b	44 b	**69** a

Underlined values are significantly different from that of control (constant 30 ℃)($p<0.05$).
The maximum germination percentage at each thermal regime is shown in bold. They were not significantly different ($P>0.05$).
Values in the same row followed by the same letter are also not significantly different ($p>0.05$).

The results of SectionⅣ-1 showed that T_b of horsenettle seeds was 14 – 16℃. Even temperature fluctuation below T_b was effective in promoting the germination of horsenettle seeds in State Ⅱ since the seeds responded to 15/10℃ in this experiment. It was suggested that horsenettle seeds which require temperature fluctuation to germinate may respond to temperature fluctuation below T_b, and then germination may begin with the temperature above 14 – 16℃ under field conditions. Ilnicki *et al.* [46] reported that horsenettle seeds scattered on the soil surface began to emerge in the middle of May. The average air temperature of May in the district is surmised to be 13 – 19℃ [73] although data on soil temperature is not shown in their paper. The suggestion mentioned above was thought to be roughly in line with the emergence of the seeds under field conditions.

3. Summary

The temperature range of germination, accumulated effective temperature and the effects of temperature fluctuation on horsenettle seeds were investigated. Base temperature (T_b) and accumulated effective temperature (θ) to germination for seeds treated with gibberellin were calculated by Garcia-Huidobro's method. T_b was estimated to be between 14 – 16℃. Maximum temperature was surmised to be between 45 – 50℃ from a graph. Gompertz function adequately described the relationship between θ and germination fraction (%), but a modification of the equation was needed in some cases.

The germination percentage of seeds exposed to diurnal temperature fluctuation with an upper limit of 15 – 30℃ and amplitudes of 5 – 15℃ for 10 or 20 cycles was significantly higher than that of seeds incubated at a constant 30℃. Seeds incubated at lower temperatures needed more cycles to increase the germination percentage, but the maximum percentage obtained with each temperature regime was similar. Horsenettle seeds appeared to respond to temperature fluctuation even below T_b because exposure to 15/10℃ for 20 cycles increased the germination percentage.

It was suggested that horsenettle seeds which require temperature fluctuation to germinate may respond to temperature fluctuation below T_b, and then germination may begin with the temperature above 14 – 16℃ under field conditions.

Chapter V EMERGENCE CHARACTERISTICS OF HORSENETTLE FROM SEEDS

The base temperature for germination of horsenettle seeds was approximately 15 ℃, and that alternating temperature regimes, including the 10 to 15 ℃ range, promoted the germination of horsenettle seeds, according to the results in Chapter IV. Ilnicki *et al.* [46] reported that 50% of the seedlings that had emerged by the middle of May survived the summer under the field conditions in the northeastern US. However, further knowledge about seedling emergence and establishment of horsenettle is required to develop effective weed-control practices in Japan.

The effect of sowing date and competition with orchardgrass on the emergence and growth of horsenettle plants was investigated in this chapter. The results should provide a basis for developing weed-control practices to prevent further establishment of the species.

1. Emergence characteristics of horsenettle from seeds

(1) Materials and methods

The experiment was conducted at the NILGS from 1996 to 1997.

1) Seeds

Horsenettle berries were collected from a field in the NILGS in April 1996. Seeds separated from the berries were washed under running water and then air-dried. The seeds were stored under room conditions until the experiment was conducted.

2) Sowing experiment

Thirty plastic containers (45 (L) × 31 (W) × 26 (D) cm^3) were filled with soil in which fertilizer (200 g / m^2 ground dolomitic limestone and 10, 20 and 10 g / m^2 of N, P_2O_5 and K_2O, respectively) had been incorporated. Each container had nine holes, about one cm in diameter, at the bottom. These containers were placed outdoors. On April 8, 1996, 3 g / m^2 of orchardgrass (*Dactylis glomerata* L. cv. Akimidori) seed was sown in each container. Forty horsenettle seeds per container were sown one cm deep in the containers at approximately monthly intervals from April through August 1996. The sowing dates were April 24, May 24, July 2, July 26 and August 26 (hereafter abbreviated to April, May, June, July, and August, respectively). Horsenettle seeds were sown under two conditions, namely BARE and OG, at each sowing. In BARE, orchardgrass was removed before horsenettle seeds were sown. The weed seeds were sown between orchardgrass in OG. In OG, orchardgrass length was measured at 10 points per container before weed seed sowing in April through June. The plant length was higher than 10 cm in June, and orchardgrass was cut at 10 cm above the soil surface before weed seed sowing thereafter. Each round of sowing under each condition was replicated three times, and the containers were watered as needed in 1996.

Newly emerged horsenettle seedlings were counted at weekly intervals, as a rule, for about two months after sowing (The counting period was about one month for August sowing). The number of living seedlings was also counted on July 2, July 26, August 26 and October 16. All horsenettle seedlings in one container for each sowing under each condition were dug up, and lengths and dry weights of shoots and roots were measured on September 25, 1996. When weighing was conducted, all seedlings in a container were gathered and measured at once. The plant length for orchardgrass was also measured at 20 points per container for all containers in OG. The relative light intensity in OG was measured on the ground surface with a portable solarimeter developed by Fukuyama *et al.* [27] for containers that were undisturbed. In these measurements, four out of five light detectors were used.

The number of emerged shoots and seedlings for the weed in undisturbed containers was counted separately on May 30, 1997. The shoot length for all of the plants was measured and then they were dug up. The root length and dry weights of shoots and roots were measured in the same manner as the previous measurement in September 1996. For orchardgrass, the coverage, plant length (15 points per container) and dry matter weight of above ground parts (cut at five cm above the ground) were measured on May 1 and 30, respectively.

All containers received 5, 5 and 5 g / m^2 of N, P_2O_5 and K_2O in August 1996 and April 1997, respectively.

3) Data analysis

The experiment was set up in a randomized block design. One- or two-way ANOVA [45] was applied depending on the case and then means were separated using the Studentized range [40]. Percentages of emergence were arcsine-transformed [52] prior to the analysis. The significant level was 0.05 for all cases.

(2) Results and discussion

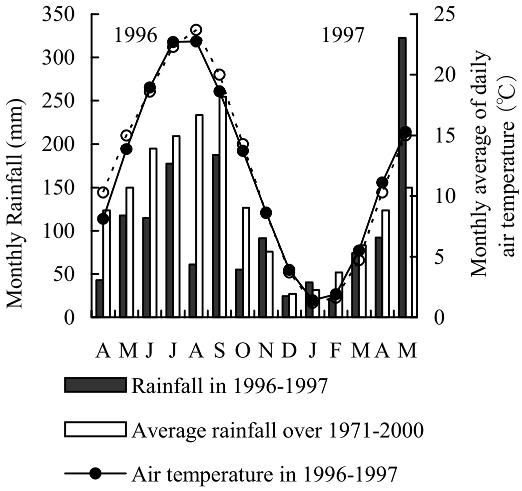

Fig. 8. Weather conditions during the experimental period[75].

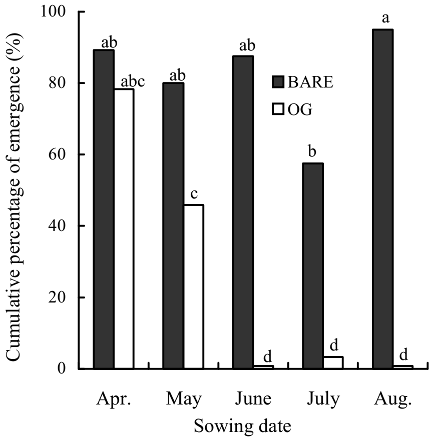

Fig. 9. Effect of sowing dates and conditions on cumulative percentage of seedling emergence for horsenettle during the two months after sowing.

Colums with the same letter(s) do not differ significantly.

1) Weather conditions and the state of orchardgrass during the investigation

Monthly air temperatures for the year in which sowings were conducted (1996) were slightly lower than average, and those in 1997 were slightly higher (Fig. 8). Monthly rainfall in 1996 was less than average, and that in August was about one fourth of the average. Monthly rainfall in May 1997 was approximately twice the average.

The plant length for orchardgrass and relative light intensity in OG in September 1996 were 28 ± 3 cm (mean ± SD) and 22 ± 6%, respectively. The coverage, plant length and dry weight of above ground parts in May of the next year were 92 ± 3%, 39 ± 9 cm and 180 ± 35 g / m^2, respectively. The relative light intensity and dry matter weight in the present experiment were both within the range reported in previous studies [50, 82].

2) Emergence and overwintering of seedlings

With the exception of July sowing, cumulative percentages of emergence for horsenettle approximately two months after sowing in BARE were 80% or higher for all sowings (Fig. 9). In OG, the emergence percentage for April was comparable with that in BARE. The percentages in OG, however, decreased as sowing dates were delayed and almost no seedlings emerged after May. The percentage of emergence for July sowing in BARE was lowest among the sowings in BARE, although the difference was only significant between the percentage for July sowing and that for August sowing. A similar pattern was observed in previous studies [4, 53, 144], in which emergence patterns for summer annual species were investigated. Among the species, *Solanum nigrum* was reported to become dormant or conditional dormant [10] in August [110] when the percentage of emergence was lowest under the field conditions [53].

The relative cumulative percentage of emergence (RCPE) was calculated for each container by assuming that the cumulative percentage of emergence approximately two months after sowing (Fig. 9) in the container was 100%. Accumulated effective temperature (AET) was calculated according to Chapter Ⅳ as follows:

$$AET = \sum T$$

$$T = \sum_{i=1}^{24} (t_i - 14.7)/24$$

where t_i is the mean air temperature from hour i−1to i (℃). Air temperatures recorded at the NILGS were used.

The relationship between AET and RCPE was similar between BARE and OG when the comparison was made for the month (Fig. 10). From April through July sowings in BARE, the later the seeds were sown, the slower the emergence rate was. The rate of emergence for August sowing was faster than that for July sowings. The AETs at which RCPE reached 50% for each sowing under each condition (AET_{50}) are shown in Table 15. AET_{50}s for April and May sowings were significantly lower than those for June and July sowings in BARE. In OG, the value was comparable

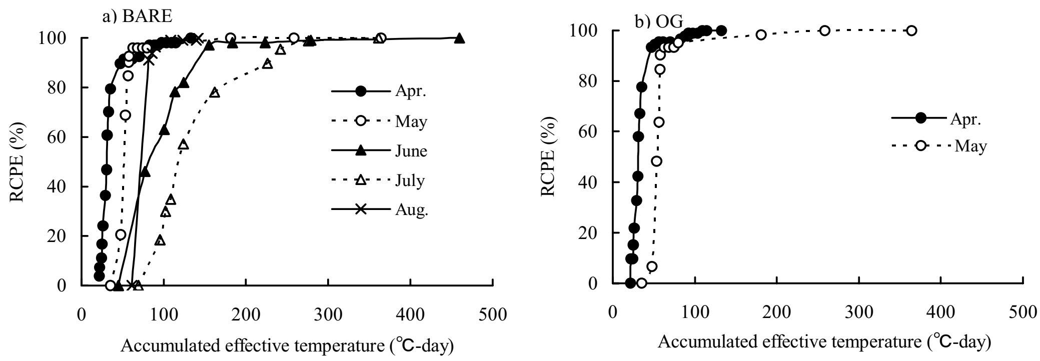

Fig. 10. Relationship between accumulated effective temperature and RCPE for horsenettle in BARE and OG.
RCPE: Relative cumulative percentage of emergence (see text for details)
Values for sowings after May in OG were omitted because almost no seedlings emerged.

Table 15. Effect of sowing dates on the accumulated effective temperature at which RCPE for horsenettle reached 50 % in BARE and OG.

Condition	Sowing date				
	Apr.	May	June	July	Aug.
	℃-day				
BARE	30 d	51 cd	84 b	122 a	72 bc
OG	31 b	54 a	—	—	—

RCPE: Relative cumulative percentage of emergence (see text for details)

Values for sowings after May in OG were omitted because almost no seedlings emerged.

Values within rows followed by the same letter do not differ significantly.

with that in BARE when the comparison was made for each month.

According to Baskin and Baskin [10], seeds of some species annually cycle between dormancy and nondormancy and those of others cycle between conditional dormancy and nondormancy. They also mentioned that temperature ranges, percentages and rates of germination are dependent on dormancy states. The cumulative percentage of emergence for July sowing was the lowest and the emergence rate for the month was the slowest in this experiment. Dormancy may be involved in the relatively hindered emergence in July. Because horsenettle seeds used in this experiment were stored under room conditions, further studies using horsenettle seeds stored under natural conditions are necessary to elucidate seasonal change in dormancy of horsenettle seeds.

Water stress during the hot summer was possibly one of the other reasons for the reduction in the percentage of emergence for July sowing. However, the water stress was not considered to have occurred in this experiment since the containers were watered as needed.

It was shown that the germination percentage for horsenettle incubated at a 40℃ constant temperature regime was as high as those incubated at lower constant temperature regimes of 30 and 35℃ in Chapter Ⅳ. Therefore, even considering that the soil temperatures at a depth of one cm might be higher than air temperatures [123], the emergence of horsenettle seeds in July sowing were not likely to have been hindered by high temperatures (Fig. 8).

Although the cumulative percentage of emergence in OG was comparable with that in BARE in April, the percentages in OG were always significantly lower than those in BARE in May and after for each month. Therefore, it was considered that the growth of orchardgrass prevented the weed seeds from emerging. Sunlight filtered through orchardgrass leaves may inhibit the weed seed germination [10]. The effect of filtered sunlight on the germination of horsenettle seeds, however, has not been studied although it was reported that light was not necessary for the weed seed germination [46]. Thus, further studies are needed. As orchardgrass roots grow, the physical conditions of the soil may become unfavorable for the emergence of horsenettle seeds. Although the present experiment was not designed to analyze the adverse effects of orchardgrass on horsenettle seeds, it was revealed that competition with orchardgrass severely reduced the percentage of emergence for horsenettle seeds.

The percentage of emergence in the next spring was not affected by sowing dates. Sowing condition, however, did affect the percentage, and the percentage in BARE was significantly higher than that in OG (Table 16).

The cumulative percentage of emergence through the experimental period for all sowings in BARE and April and May sowings in OG was significantly higher than those in June through August sowings in OG (Table 17). Almost no emergence occurred in the latter. The cumulative percentage of emergence though the experimental period in BARE reached nearly 100%, and it was suggested that the ability to emerge under the field conditions for horsenettle seeds was potentially very high.

Most of the seedlings for April through June sowings in BARE overwintered and re-sprouted in May of the next year (Fig. 11). For July sowing in BARE, the number of seedlings emerged was similar to that of shoots re-sprouted in May of the next year, but the standard errors were large. The number of re-sprouted shoots for August sowing in BARE was fewer than that of seedlings emerged. Thus, it was considered that late sowings led to fewer seedlings capable of overwintering. In OG, almost no re-sprouted shoots were observed for any of the sowings in May 1997.

3) Growth

In the growth investigation for horsenettle in September 1996, only one container out of three was sampled for each sowing date under each sowing condition. For this reason, only the effects of the main factors, namely sowing condition and sowing date were evaluated [127] (Table 18). The effect of

Table 16. Effect of sowing conditions on the percentage of seedling emergence for horsenettle in the next spring after sowing.

Sowing condition	Emergence percentage
	%
BARE	63 [a]
OG	0 [b]

Emergence percentage in the next spring after sowing = Number of seedlings emerged in May 1997 / (Number of seeds sown - Number of seedlings emerged during 1996) ×100

The investigation was conducted on May 30, 1997.

The percentage in BARE is signifficantly different from that in OG.

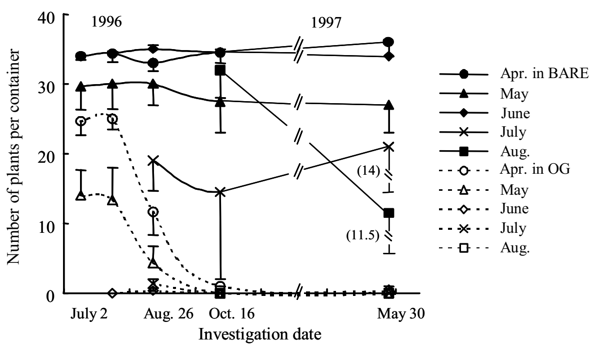

Fig. 11. Course of changes in the number of living plants in BARE (——) and OG (----).

The number of living seedlings and re-sprouted shoots is presented for 1996 and 1997, respectively.
Bars attached to the symbols represent standard error. Values in parentheses represent standard error for July and August sowings in BARE on May 30, 1997, respectively.

Table 17. Effect of sowing dates and conditions on the cumulative percentage of seedling emergence for horsenettle through the experimental period.

Condition	Sowing date				
	Apr.	May	June	July	Aug.
			%		
BARE	96 [a]	94 [a]	94 [a]	75 [a]	99 [a]
OG	83 [a]	55 [a]	1 [b]	3 [b]	0 [b]

Values followed by the same letter do not differ significantly.

Table 18. Effect of sowing conditions on growth of horsenettle in September in the year of sowing.

Condition	Sowing date	Length		Dry weight	
		Shoot	Root	Shoot	Root
		cm		g / plant	
BARE	Apr.	22.79	72.11	1.1432	2.3413
	May	19.88	57.85	0.5156	1.7488
	June	9.81	44.53	0.2672	0.8056
	July	11.00	24.36	0.1933	0.2633
	Aug.	2.15	7.37	0.0040	0.0014
OG	Apr.	4.18	8.79	0.0036	0.0073
	May	2.03	3.68	0.0003	0.0003
	June	0.00	0.00	0.0000	0.0000
	July	3.10	10.00	0.0050	0.0005
	Aug.	1.60	3.90	0.0010	0.0010
Mean of the sowing condition					
BARE		13.13 [a]	41.25 [a]	0.4247	1.0321
OG		2.18 [b]	5.27 [b]	0.0020	0.0018

The lengths were measured for each individual plant, respectively, and then averaged for each container. In weighing, all seedlings in a container were gathered and measured at once.
The shoot and root lengths in BARE are significantly different from those in OG, respectively.

sowing condition was significant for shoot and root lengths, and those in BARE were longer than those in OG. The dry weights of shoots and roots in BARE were greater than those in OG, although the differences were insignificant. Results suggested that competition with orchardgrass suppressed the growth of horsenettle seedlings. The effect of the sowing date was not significant in the ANOVA. Thus, the relationship between sowing date and growth for horsenettle in BARE was evaluated using regression analysis. The sowing date was found to be negatively related to lengths (Fig. 12 a) and dry weights (Fig. 12 b) of shoots and roots in BARE.

The trend in seedling growth agreed well with that of the number of re-sprouted shoots in May of the next year (Fig. 11).

In BARE in May 1997, the earlier the seeds were sown, the greater the plant growth was (Table 19). The length and dry weight of roots in BARE were similar to those in September of the previous year (Table 18). According to previous

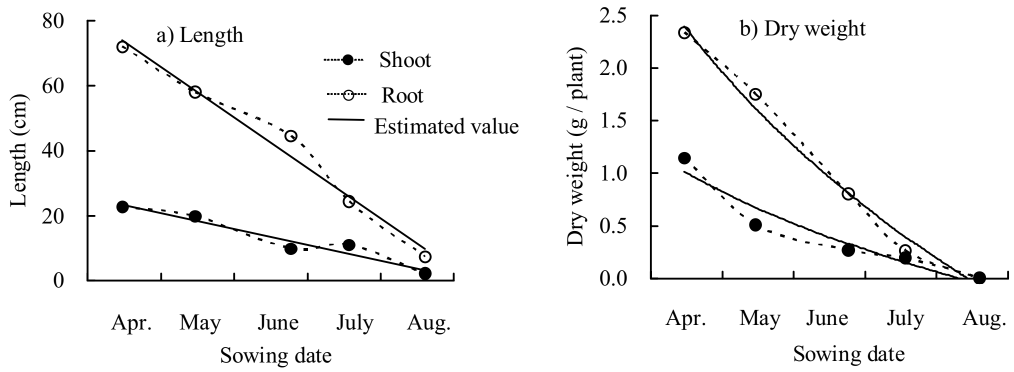

Fig. 12. Relationship between sowing date and length and dry weight of shoots and roots.

The investigation was conducted in September 1996.
Shoot length = -0.16 × day + 42.03, $R^2 = 0.94$
Root length = -0.52 × day + 133.28, $R^2 = 0.98$
Shoot dry weight = -1.45 × ln(day) + 7.91, $R^2 = 0.93$
Root dry weight = -3.36 × ln(day) + 18.32, $R^2 = 0.99$
day = days from January 1, 1996 until each sowing date
All equations are significant at P = 0.05.

Table 19. Effect of sowing dates and conditions on growth of horsenettle in May of the next year after sowing.

Condition	Sowing date	Length: Shoot	Length: Root	Dry weight: Shoot	Dry weight: Root
		cm	cm	g / plant	g / plant
BARE	Apr.	15.91 a	77.69 a	0.5189 a	2.2029 a
	May	9.99 ab	68.58 ab	0.2629 ab	1.6797 ab
	June	9.25 abc	53.02 ab	0.2924 ab	0.9278 bc
	July	3.74 bcd	29.69 bc	0.1099 b	0.3177 cd
	Aug.	1.33 bcd	6.08 c	0.0133 b	0.0093 d
OG	Apr.	0.00 d	0.00 c	0.0000 b	0.0000 d
	May	0.00 d	0.00 c	0.0000 b	0.0000 d
	June	0.75 cd	2.00 c	0.0005 b	0.0005 d
	July	0.00 d	0.00 c	0.0000 b	0.0000 d
	Aug.	0.00 d	0.00 c	0.0000 b	0.0000 d

The lengths and dry weights were measured as mentioned in Table 18.

Values within columns followed by the same letter(s) do not differ significantly.

studies [46, 77], starch content in horsenettle roots decreased between the beginning of May and the middle of June, and the content began to increase thereafter, although the trend may not be consistent between years. Thus, the similarity in the length and dry weight of roots in September and in May of the next year in this experiment might be due to the fact that the measurement was conducted before matter accumulation began in horsenettle roots. However, the changes in starch content and possibly in the root dry weight during September and May were not ruled out.

With the exception of June sowing, no re-sprouted shoots were observed in OG (Fig. 11), and horsenettle growth for June sowing was considerably low in May 1997 (Table 19). The adverse effect of orchardgrass on horsenettle growth was clearly shown.

It was reported that the adverse effect of cutting on the growth of horsenettle was furthered by competition between the weed and orchardgrass [82]. In the previous study, the effect of over-sown orchardgrass on the growth of horsenettle which sprouted from roots was investigated. In the present experiment, the outcome of competition between the two species at earlier stage was determined. It was suggested that the adverse effect of orchardgrass on horsenettle was more serious in the present study than that in the previous study.

4) Possibility for horsenettle to become established from seeds in pastures

This experiment revealed that the percentage of emergence for horsenettle seeds was very high if they were sown under conditions in which there was no competition with other species and no water stress. Results also indicated that horsenettle seeds that encountered favorable conditions from April through August could emerge during the year and that the seedlings were capable of overwintering.

In pastures, cattle grazing and environmental stress often lead to disappearance of grasses, thus causing bare patches to appear. Therefore, it was considered that horsenettle seeds could emerge and become established in pastures if they encountered such empty patches. In thick pastures, however, horsenettle seeds would rarely emerge and become established because the percentages of emergence and growth for the weed under the condition with orchardgrass were considerably low in this experiment.

2. Summary

The effect of sowing dates and competition with orchardgrass (*Dactylis glomerata* L.) on emergence and growth of horsenettle seedlings was investigated. Horsenettle seeds were sown in plastic containers that were filled with soil and placed outdoors. The sowings were conducted at approximately monthly intervals from April through August in 1996 under conditions with (OG) and without (BARE) orchardgrass sown in April 1996. With the exception of July sowing, cumulative percentages of emergence for horsenettle approximately two months after sowing in BARE were 80 % or higher for all sowings. In OG, the emergence percentages for April and May sowings were higher than 45 %, while the percentages for the remainder were nearly zero. Most of the seedlings for April through June sowings in BARE overwintered and re-sprouted in May 1997. In OG, almost no re-sprouted shoots were observed in any of the sowings. It was found that the earlier the seeds were sown, the greater the plant growth was in September 1996 and May 1997. This trend was prominent in BARE. The horsenettle growth in OG was much less than that in BARE. The trend of horsenettle growth in September agreed well with that of the re-sprouted shoot number in May of the next year. From these findings, it was suggested that horsenettle seedlings rarely become established in pastures thick with orchardgrass.

Chapter Ⅵ OVERWINTERING AND FREEZING TOLERANCE OF HORSENETTLE SEEDLINGS

It was shown that the base temperature for germination of horsenettle seed was approximately 15 ℃, and that alternating temperature regimes, including the 10 to 15℃ range, promoted germination of horsenettle seeds in Chapter Ⅳ. Sowing experiments revealed that the percentage of overwintering for horsenettle sown in bare ground was high, while that of the weed sown among orchardgrass was nearly zero when the weed seeds were sown between April and July in Chapter Ⅴ. The possibility of seedling establishment for horsenettle during pasture renovation, however, has not been clarified.

The results in Chapter Ⅱ indicated that occurrence of the weed was more likely in warmer areas than in colder areas based on the investigation on presence of the weed in pastures of central Japan. A few studies do exist on freezing tolerance of the weed [11, 146]. However, further studies are necessary to clarify the effects of low temperatures on the weed.

The emergence and overwintering of horsenettle sown in autumn and freezing tolerance of the seedlings were investigated.

1. Effect of sowing date and competition with orchardgrass on overwintering

(1) Materials and methods

The experiment was conducted at the NILGS from 2001 to 2002.

1) Seeds

Horsenettle berries were collected from an NILGS field in April 2001. Seeds separated from the berries were washed under running water and then air-dried. The seeds were stored under room conditions until it was time to conduct the experiment.

2) Sowing experiment

Eighteen plastic containers (45 (L) × 31 (W) × 26 (D) cm^3) were filled with soil in which fertilizer (200 g / m^2 ground dolomitic limestone and 10, 20 and 10 g / m^2 of N, P_2O_5 and K_2O, respectively) had been incorporated. Each container had nine holes, measuring approximately one cm in diameter, at the bottom. These containers were placed outdoors. On September 4 (SEP4), September 26 (SEP26) and October 15 (OCT15), 2001, forty horsenettle seeds per container were sown at a depth of one cm in the containers under conditions with (OG) and without (BARE) orchardgrass (*Dactylis glomerata* L. cv. Akimidori). The grass seeds (3 g / m^2) were sown on the same dates as the weed seeds, except for OCT15. For OCT15, the grass seeds were sown on September 26. Each round of sowing under each condition was replicated three times, and the containers were watered as needed in 2001.

Newly emerged horsenettle seedlings were counted until November 21, 2001. The height of the seedlings was measured on November 9, 2001. All of the horsenettle seedlings in one container for each sowing under each condition were dug up, and the lengths and dry weights of roots were measured on November 21, 2001. When weighing was conducted, all of the seedlings in a container were gathered and measured at one time. The number of re-sprouted shoots and emerged seedlings for the weed in undisturbed containers was counted separately from May 2 through June 10, 2002. The shoot length was measured for all of the plants and then they were dug up. The root length and dry weights of shoots and roots were measured in the same manner as the previous measurements taken in November 2001. The dry weights of both parts for re-sprouted shoots and emerged seedlings were weighed separately.

For orchardgrass, plant length and density were measured in November 2001. The plant length and dry matter weight of above ground parts (cut at five cm above the ground) for the grass were measured in June 2002.

Tochigi Prefecture is zoned as being transitional in terms of forage grass production, and the optimum sowing period for temperate grasses is from the end of August through the middle of September [125].

The experiment was set up in a randomized block design. One or two-way ANOVA [45] was applied depending on the case and then means were separated using the studentized range [40]. Percentage of emergence and overwintering shoots was arcsine-transformed [52] prior to the analysis. The significant level was 0.05 for all cases.

(2) Result and discussion

1) Weather conditions and state of orchardgrass during the investigation

Monthly means of daily mean air temperatures from September through November 2001 were similar to the average, and that in December was slightly lower (Fig. 13). Those from January through April 2002 were higher than average and returned to normal thereafter. Monthly means of daily lowest air temperatures showed the same trend as monthly means of daily mean air temperatures. Monthly rainfall in October 2001 and January 2002 was higher than average, and that in February and April 2002 was lower than average.

In November 2001, plant lengths and densities for orchardgrass sown on September 4 and 26 were 11.30 ± 0.91 (mean ± SD) and 5.50 ± 0.13 cm, and 1,452 ± 293 and 1,599 ± 149 plants / m^2, respectively. Only plant length was found to be significantly different between the sowing dates. In June 2002, plant length and dry matter weight for the grass were not found to be significantly different between the sowing dates, and they were 69.56 ± 3.58 cm and 201.4 ± 17.1 g / m^2, respectively.

2) Emergence, overwintering and growth of seedlings

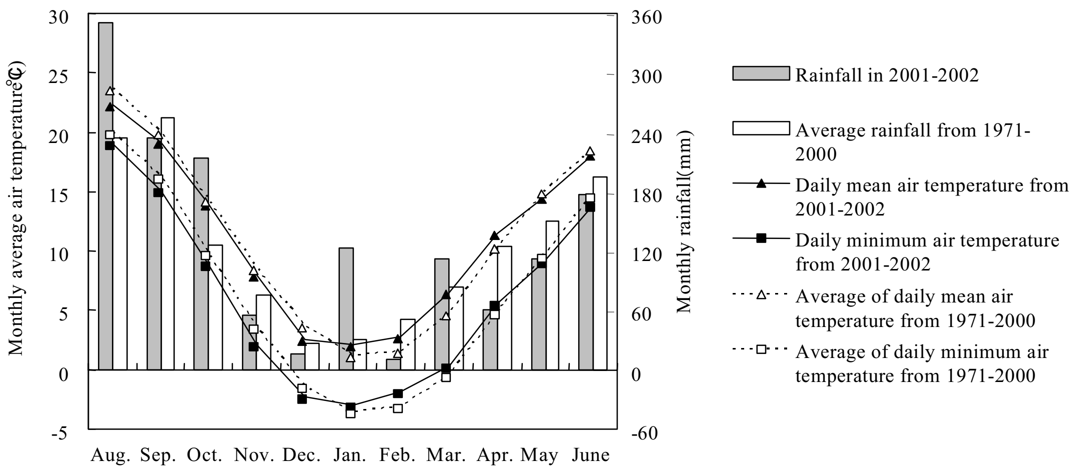

Fig. 13. Weather conditions during the experimental period[75].

emerged in the year of sowing

It was discovered that the earlier the weed seeds were sown, the higher the cumulative percentage of emergence for the weed was in the year of sowing (Table 20). The cumulative percentage of emergence for the weed was higher for BARE than that for OG when comparisons were made between the two conditions. For OCT15, however, the percentages were nearly zero for both conditions. Shoot length for horsenettle in November for SEP4 was significantly higher than that for SEP26 (Table 21). The difference between the sowing conditions, however, was not found to be significant. The root lengths of the weed for SEP4-BARE, SEP4-OG, SEP26-BARE and SEP26-OG were 16.1 ± 0.6 (mean ± SD, n (number of seedlings measured) = 22), 9.8 ± 1.0 (n = 22), 4.2 ± 0.6 (n = 16) and 3.8 ± 0.9 (n = 10) cm, respectively. The root dry weight for the weed under each treatment was, in the same order as above, 8.2, 3.1, 0.9 and 1.0mg/plant, respectively. Thus, horsenettle for SEP4-BARE was considered to be the largest among all of the treatments.

The percentage of overwintering shoots was calculated by dividing the number of re-sprouted shoots in 2002 by the number of seedlings that emerged in 2001. The percentage was high for SEP4-BARE at 73%, and the percentages were below 10% for all other treatments (Fig. 14).

The growth of re-sprouted horsenettle shoots in 2002 was measured in June 2002, and the results for SEP4 are shown in Table 22. It appeared that the growth of horsenettle for BARE was greater than that for OG, but the difference between the sowing conditions was not significant. This might be because seedlings that were smaller than a certain criterion could not survive the winter. Thus, the difference in growth for the re-sprouted shoots between the sowing conditions was not found to be significant in June 2002, despite the fact that the percentage of overwintering shoots was significantly higher for SEP4-BARE than that for SEP4-OG. The horsenettle growth for SEP26 in June 2002 was not compared between the sowing dates and conditions because only one plant was measured for each condition.

Makuchi and Sasaki [62] reported that the death of broadleaf dock (*Rumex obtusifolius* L.) seedlings in winter was due to the desiccation of roots by frost heaving, and that the mortality of the weed seedlings in winter was higher in bare ground than in a pasture and a meadow, based on the experiments they conducted in Miyagi Prefecture. In the present experiment, the percentage of overwintering shoots was higher for SEP4-BARE than for SEP4-OG, and the results were contrary to those from Makuchi and Sasaki [62]. One reason for this was thought to be that the density of orchardgrass was high in this experiment because the investigation was conducted between the year the grass was sown and the next. Thus, the adverse effects of the grass on horsenettle growth were severe and horsenettle seedlings could not grow well enough to survive the winter. On the other hand, Makuchi and Sasaki [62] carried out their investigations in the pasture and meadow that were established eleven and five years before, respectively, and empty patch size was bigger than that in this experiment. The grasses in their study might have actually served as a shelter that protected the weed seedlings from frost heaving.

Table 20. Effect of sowing dates and conditions on the cumulative percentage of seedling emergence for horsenettle in the year of sowing.

Condition	Sowing date			
	SEP4	SEP26	OCT15	Mean
	%			
BARE	68.3	40.8	0.8	36.7 a
OG	60.8	29.2	0.0	30.0 b
Mean	64.6 A	35.0 B	0.4 C	

Newly emerged seedlings were counted until November 21, 2001.
Values within the mean column and row followed by the same letter do not differ significantly.

Table 21. Effect of sowing dates on shoot length of horsenettle in November in the year of sowing.

Condition	Sowing date	
	SEP4	SEP26
	cm	
BARE	1.24	0.78
OG	1.22	0.73
Mean	1.23 a	0.76 b

The length is significantly different between the sowing dates.

Table 22. Effect of sowing conditions on the growth of re-sprouted horsenettle shoots for SEP4 in June in the next year of sowing.

Condition	Length		Dry weight	
	Shoot	Root	Shoot	Root
	cm		mg / plant	
BARE	0.97	14.22	3.87	4.98
OG	0.73	11.58	1.75	3.59

Values are only shown for SEP4 since there were few re-sprouted shoots for SEP 26.
There is no significant difference between the conditions for each item measured.

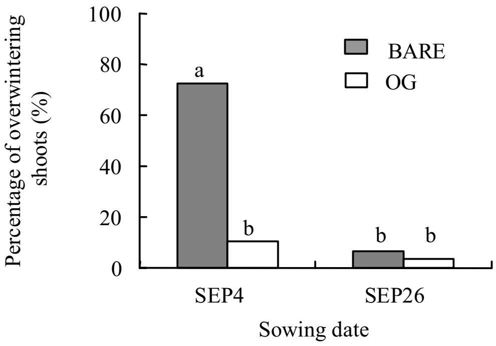

Fig. 14. Effect of sowing dates and conditions on the percentage of overwintering shoots for horsenettle.
Bars followed by the same letter do not differ significantly.

Table 23. Effect of sowing dates and conditions on the cumulative percentage of seedling emergence for horsenettle from May through June in the next year of sowing.

Condition	Sowing date			
	SEP4	SEP26	OCT15	Mean
	%			
BARE	1.3	10.0	26.7	14.6 b
OG	0.0	2.5	1.7	1.4 a
Mean	0.6 B	6.3 AB	14.2 A	

Values within the mean column and row followed by the same letter do not differ significantly.

Table 24. Effect of sowing conditions on the growth of horsenettle seedlings in June in the next year of sowing.

Condition	Length		Dry weight	
	Shoot	Root	Shoot	Root
	cm		mg / plant	
BARE	1.54 a	14.13 a	4.21 a	4.03 a
OG	0.50 b	4.38 b	0.53 b	0.36 b

The comparison between the conditions was made by pooling the data among the sowing dates.
Values are significantly different between the conditions for each item.

The present experiment was not designed to clarify the cause of death for horsenettle seedlings in winter, but it can be hypothesized that it may have been a result of frost heaving and/or coldness itself.

3) Emergence and growth of horsenettle in the next year of sowing

The percentage of emergence was higher for BARE than for OG, and the later the seeds were sown, the higher the percentage was in 2002 (Table 23). Seedling growth was compared between the sowing conditions by pooling the data among the sowing dates since the effect of sowing dates on growth was considered to be negligible in the next year of sowing. The growth of seedlings was significantly greater for BARE than for OG (Table 24), and the size of the seedlings for BARE was comparable to that of re-sprouted shoots (Table 22).

4) Possibility of seedling establishment for horsenettle during pasture renovation

The percentage of existing horsenettle (seedlings + re-sprouted shoots) in 2002 to seeds sown in 2001 was

Table 25. Effect of sowing dates and conditions on the percentage of existing horsenettle to seeds sown in June in the next year of sowing.

Condition	Sowing date			
	SEP4	SEP26	OCT15	Mean
		%		
BARE	55.0	12.5	26.7	30.7 a
OG	6.3	3.8	1.7	3.6 b
Mean	30.6 A	8.1 B	14.2 B	

Values within the mean column and row followed by the same letter do not differ significantly.

higher for BARE than for OG (Table 25). Among the sowing dates, the percentage for SEP4 was higher than those for other treatments. The percentage of existing horsenettle for OG was very low, and the sizes of seedlings and re-sprouted shoots for the weed (Tables 22 and 24) appeared to be very small in comparison with the size of orchardgrass (the plant length was approximately 70 cm). Therefore, the possibility of seedling establishment for horsenettle during pasture renovation would be low if sown grasses became established well. On the other hand, the mean percentage of existing horsenettle for BARE was 30.7% after pooling the data among the sowing dates. According to the findings in ChapterⅤ, horsenettle seedlings that emerged from seeds sown between April and August in the bare ground appeared to be capable of being established. Thus, it was considered that seedling establishment for horsenettle could occur in pastures in which the weed seed sources (*e.g.* cattle excreta and/or seed bank) existed when empty patches appeared.

Brown and Porter [16] reported that the germination percentage for horsenettle seeds that had been buried in the ground for four years was 98%, according to experiments conducted in Iowa. Solomon [122] mentioned that horsenettle seeds remained viable for at least seven years under laboratory conditions. There is no information available on the longevity of horsenettle seeds in Japan. Studies on horsenettle seed longevity under the field conditions of Japan are essential.

2. Effect of sowing date on freezing tolerance

(1) Materials and methods

The experiment was conducted at the NILGS from 2001 to 2002.

1) Seeds

Horsenettle berries were collected from an NILGS field in April 2001. Seeds separated from the berries were washed under running water and then air-dried. The seeds were stored under room conditions until it was time to conduct the experiments.

2) Freezing tolerance examination

Soil in which the same amount of fertilizer as the experiment 1 had been incorporated was filled in 1 / 5,000 Wagner pots. The pots were placed outdoors. Three horsenettle seeds per pot were sown on August 13 (AUG) and September 4 (SEP), 2001. For AUG, some of the seedlings sown on August 14 were transplanted in the pots on September 12. On December 11, 2001, when shoots of the weed had been killed by frost, the seedlings were dug up and washed. The length and diameter of roots were measured after blotting off the extra water. Five plastic bags containing ten roots per bag were prepared for AUG and SEP, respectively [96]. These bags were placed in an incubator set at 2℃ and kept there for approximately 18 hours. The bags were then exposed to a temperature of −4℃ for 3, 6, 9 and 12 hours, respectively. After being exposed for the designated number of hours, bags were replaced in the incubator offset at 2℃. A bag that was kept in the incubator set at 2℃ during the treatment was designated as the non-treated control for AUG and SEP, respectively. Approximately 27.5 hours after the commencement of exposure, the bags were taken out of the incubator, and the roots were planted in containers (47 (L) × 32 (W) × 8 (D) cm^3). The containers had been filled with a mixture of soil and vermiculite (2 : 1, v : v), were kept in a glasshouse where they were watered as needed and were fertilized with liquid fertilizer (1 ml Hyponex / 2000 ml water) every two weeks, as a rule, starting two weeks after planting. The roots were dug up on April 22, 2002. Roots with green leaves and/or white buds on them were counted as being viable. Roots without them were replanted in containers (48 (L) × 33 (W) × 7 (D) cm^3) filled with soil fertilized with the same amount of fertilizer as the soil in the Wagner pots. The containers were kept in the glasshouse until June 11, 2002, at which time the roots were dug up and viability was determined. Roots that could not be recovered were regarded as having died.

The means of root length and diameter were separated between the sowing dates using a t-test [45]. A logistic regressive equation [135] was calculated between exposure time and viability for horsenettle seedlings.

(2) Results and discussion

1) Relationship between viability of the seedlings and exposure time

The root length for AUG was significantly longer than that for SEP, while the diameter remained the same between the sowing dates (Table 26). The relationship between the viability for the seedlings and exposure time for each sowing date are depicted in Fig. 15, respectively. For AUG, viability was not reduced by increasing exposure time at the significant level of 0.05 since the P-value of Wald χ^2 for β (coefficient of exposure time) was 0.401 (Table 27). On the other hand, viability decreased as exposure time increased for SEP since the P-value of Wald χ^2 for β was 0.005. A 95% confidence interval for exposure time at which viability was reduced to 10% was estimated as being 12 to 20 hours for SEP.

Wehtje *et al.* [146] reported that horsenettle root fragments were killed by exposure to 12 hours of freezing conditions (−2 to −4℃). On the contrary, viability for AUG was not reduced even after 12 hours of exposure to a temperature of −4℃ in this experiment. The contrariety between the two experiments may be due to the difference in root fragment size and/or biotype. It is necessary to clarify the reasons for this.

2) Possibility of restricting horsenettle distribution by low temperatures in winter in Japan

Meteorological data from the National Agricultural Research Center for Hokkaido Region (NARCH) (Sapporo and Kawanishi) and the NILGS (Nasu and Miyota) was surveyed (Table 28). In Kawanishi, the daily mean temperatures at 10 cm below the soil surface sometimes dropped below −4℃. At 30 cm below the soil surface, temperatures did not drop below zero, although the observation period was short. In Sapporo, winters during which temperatures at 5 cm below the soil surface went below zero for approximately four months were found to occur occasionally. The minimum value of the temperatures, however, was −3.3℃ (January 5, 1980), and temperatures did not drop below −4℃. In Miyota, the minimum value of temperatures 5 cm below the soil surface was −0.8℃ (March 1, 2000). In Nasu, temperatures at 10 cm below the soil surface did not drop below zero. According to the data, there was a possibility that horsenettle seedlings could be killed as a result of low temperatures in the Tokachi area.

Other than coldness itself, the death of seedlings can also

Table 26. Root length and diameter of horsenettle for each sowing date.

Sowing date	Root Length	Root Diameter
	cm	mm
AUG	34.84 [a]	0.49
SEP	17.10 [b]	0.48

The length is significantly different between the sowing dates.

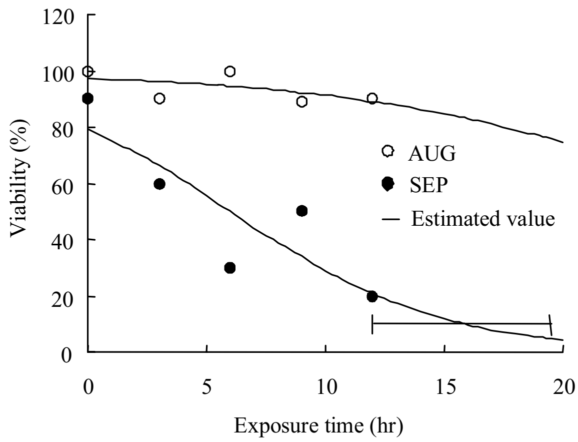

Fig. 15. Logistic regression of viability for horsenettle seedlings exposed to −4℃ in relation to exposure time.

The horizontal line shows a 95% confidence interval for exposure time at which seedling viability is reduced to 10% for SEP.

Table 27. Analysis of the relationship between the viability of horsenettle seedlings and exposure time.

Sowing date	Pearson Goodness-of-Fit Statistics			Analysis of Maximum Likelihood Estimate			
	DF	χ^2	Pr > χ^2	α	β	Wald χ^2 for β	Pr > χ^2
AUG	3	2.040	0.564	3.608	-0.127	0.706	0.401
SEP	3	3.645	0.303	1.348	-0.225	7.98	0.005

$\log\{p(x) / (1-p(x)\} = \alpha + \beta x$

p(x) = The number of living horsenettle / the number of horsenettle exposed to freezing treatment

x = exposure time

Table 28. Meteorological data surveyed.

Observation point		Item	Period
National Agricultural Research Center for Hokkaido Region	Sapporo	Temperatures at 5 and 10 cm below the soil surface	1976.1 - 2003.4
	Kawanishi	Temperature at 10 cm below the soil surface	1988.1 - 2003.3
		Temperature at 30 cm below the soil surface	1996.12 - 2003.4 [a)]
National Institute of Livestock and Grassland Science	Nasu	Temperature at 10 cm below the soil surface	1989.1 - 2000.12
	Miyota	Temperatures at 5 and 15 cm below the soil surface	1991.12 - 2000.3

Temperatures are the daily mean for all.

a) There were some missing values.

be caused by frost heaving [78]. It was reported that horsenettle could sprout from root fragments buried at 45.7 cm below the soil surface [46]. Thus, horsenettle with its extensive root system [48] was considered to be capable of surviving winter even in the Tokachi area if the root was cut by frost heaving.

Horsenettle was not mentioned in Takita [133], which covered approximately 2,200 species in Hokkaido primarily centering on eastern Hokkaido. The weed was therefore considered not to occur in eastern Hokkaido. The occurrence of the weed, however, was recorded in Sapporo [113] and Tobetsu (Ishikari gun), indicating that horsenettle could become established in southern Hokkaido. In Hokkaido, there are large areas of grassland and the damage could be severe if the weed were to begin to expand. It is therefore necessary to clarify the factors that restrict horsenettle distribution to prevent the weed from spreading to the colder areas of Japan.

The growth of the weed is known to be affected by many factors such as weather conditions during the growing season, soil fertility and competition with other plants under field conditions. Therefore, it is impossible to estimate precisely the potential distribution of the weed based only on the findings in this study and the meteorological data during winter. Results in this section, however, suggested that the potential distribution areas of the weed would be large even in Hokkaido.

3. Summary

Experiments were conducted to elucidate the possibility of seedling establishment for horsenettle during pasture renovation at the NILGS.

Horsenettle seeds were sown in plastic containers that were filled with soil and placed outdoors to investigate the effect of sowing date on overwintering of the weed seedlings. The sowings were conducted on September 4 (SEP4), September 26 (SEP26) and October 15 (OCT15) in 2001 under conditions with (OG) and without (BARE) orchardgrass (*Dactylis glomerata* L.) sown on the same dates as the weed seeds except for OCT15. For OCT15, the grass seeds were sown on September 26. It was found that the earlier the weed seeds were sown, the higher the cumulative percentage of emergence of the weed was in the year of sowing. The cumulative percentage of emergence of the weed was higher for BARE than that for OG when comparison was made between the two conditions. For OCT15, however, the cumulative percentages of emergence of the weed were nearly zero for both conditions. The percentage of horsenettle that survived the winter was high for SEP4-BARE at 73%, and percentages were below 10% for all other treatments.

The freezing tolerance of horsenettle was investigated using seedlings sown on August 13 (AUG) and September 4 (SEP). The seedlings sown on each day were exposed to a temperature of −4℃ for a period of 3, 6, 9 and 12 hours, respectively. Viability for AUG was not reduced even after 12 hours of exposure, whereas the viability for SEP decreased as exposure time increased. A 95% confidence interval for exposure time at which viability was reduced to 10% was estimated to be 12 to 20 hours for SEP.

From these findings, it was believed that the possibility of seedling establishment for horsenettle during pasture renovation would be low in the northern Kanto region and colder areas if sown grasses became established well. It was also suggested that the possibility of establishment of the weed seedlings was lower in colder areas than in warmer areas.

GENERAL DISCUSSION AND CONCLUSION

In recent years, the increase of alien weeds in pastures has become a serious problem. The percentage of farms in which horsenettle was found increased from 0.4% in 1981 [35] to approximately 25% in 1994 [90]. The recent increase in appearance of the weed in pastures is presumably due to seed contamination in imported fodder crops [90]. Therefore, this study was conducted to elucidate the routes of horsenettle invasion of pastures via cattle and examine factors associated with seedling establishment, with the intention of preventing further invasion and establishment of horsenettle in pastures.

Invasion of pastures via cattle

Routes of horsenettle invasion from seeds were assumed as follows: horsenettle seeds mixed into fodder crops were fed to cattle and then manure was spread over pastures: or cattle which were fed on such fodder excreted on pastures. In ChapterⅢ, the effect of cattle digestion and of composting heat was investigated. Although viability of horsenettle seeds ingested by cattle was significantly lower than that of non-ingested seeds, approximately 60% of the seeds passed through the digestive tract of cattle without losing viability. Further, among the seeds estimated to be contained in excreta placed outdoors, 15% emerged. The results indicated that feces on pastures could be a source of horsenettle infestation if fodder crops containing large numbers of the seeds were fed.

Composting heat was considered to be effective in reducing viability of horsenettle seeds if manure was appropriately fermented and provided a temperature of 55℃ was maintained for 42 to 58 hours, or if 60℃ was maintained for 10 to 17 hours. Otherwise, manure could be a source of infestation because the effect of composting heat on germination of horsenettle and 14 other weed species was not significant until the maximum temperature reached a threshold of 46℃. Hokuchi [41] reported that cattle excrement was merely piled up on 90% of cattle farms. With this method, the probability of killing weed seeds is low, especially during winter when the temperature of manure may not readily rise. Adequate treatments to promote fermentation of manure are necessary to minimize manure as a source of weed infestation.

Potential distribution in Japan

The results in ChapterⅡ, Ⅳ and Ⅵ suggest that seedling establishment for horsenettle is more likely in warmer areas than in the cooler areas of Japan. This, however, does not mean that horsenettle would not become established in the northern parts of Japan. Viability of horsenettle seedlings that emerged from seeds sown in August was not reduced even after 12 hours of exposure to a temperature of −4℃. For seedlings which emerged from seeds sown in September, 12 to 20 hours of exposure to −4℃ were estimated to be necessary to reduce the viability to 10%. A survey of meteorological data in ChapterⅥ revealed that the daily mean temperatures at 10 cm below the soil surface sometimes dropped to less than −4℃ in Kawanishi, Hokkaido, and that temperatures did not drop below zero at 30 cm below the soil surface. Horsenettle can sprout from root fragments buried at 45.7 cm below the soil surface [46]. Thus, the seedlings could be killed as a result of low temperatures in Kawanishi, but horsenettle could survive the winter there if roots grow sufficiently. Therefore, the potential distribution area of horsenettle was considered to be large, even in Hokkaido.

Seedling establishment in pastures

Seedling establishment of horsenettle seldom occurred in pastures thick with grasses, according to the results in ChapterⅤ and Ⅵ. The investigation in ChapterⅡ, however, revealed that 25% of pastures investigated were infested with the weed. The author observed that reed canarygrass (*Phalaris arundinacea* L.) meadows were heavily infested with horsenettle in the investigation, despite the fact that reed canarygrass has been reported to inhibit the growth of broadleaf dock [76]. Therefore, mechanisms certainly exist that allow horsenettle to thrive in pastures. The results of this study suggested that horsenettle might have persistent seed banks [32]. Cattle grazing and environmental stress often lead to the disappearance of grasses, thus causing bare patches to appear in pastures. Horsenettle seedlings may emerge and become established if they encounter these empty patches. The high percentage of the seedling emergence on bare ground throughout the growing season supports this hypothesis. Solomon [122] also suggested the existence of seed banks in his study on autoallelopathy in horsenettle. He reported that germination of horsenettle seeds was inhibited by weed leaf material incorporated into the soil, and that the potency of the inhibitor decayed after several weeks of moist storage. He also mentioned that autoallelopathy in this species functions to maintain a viable seed bank while reducing intraspecific competition between seedlings and adults. Seed longevity under natural conditions, however, has not been studied except for Brown and Porter [16], who reported that 98% of horsenettle seeds germinated after four years burial in soil. Studies are necessary to elucidate the dynamics of seed banks for the weeds in Japan.

Seriousness of horsenettle as a pasture weed

Broadleaf dock has been recognized as one of the most noxious and prevalent weeds in pastures [35, 59]. Recognition of horsenettle as a noxious weed occurred much more recently than for broadleaf dock, and the former is not as prevalent as the latter [35, 90]. According to a previous study conducted at the NILGS, the density of broadleaf dock seedlings in November was almost the same as that in April of the following year under competition with orchardgrass [71]. On the other hand, in this current study, it was shown that horsenettle rarely re-sprouted in spring of the year following sowing under competition with orchardgrass. Although the experimental conditions were not exactly the same between the two studies, horsenettle was considered to be weaker than broadleaf dock during seedling establishment. This may be one of the reasons that horsenettle is not as prevalent as broadleaf dock, although both weeds were introduced to Japan approximately 100 years ago [69, 138]. For broadleaf dock, however, there are a few effective herbicides available [79]. Further, a wide range of studies [80] have been done on the weed, and control practices have been established [79]. On the other hand, herbicides that are registered for use in pastures are only partly effective on horsenettle [98]. Control practices against fully established horsenettle in pastures without renovation are not available and there are few studies on renovation of pastures infested with the weed [72, 85]. Thus, once the weed has become established, it may actually be more of a nuisance than broadleaf dock. Prevention of horsenettle invasion and establishment is of utmost importance.

Proposal to prevent further invasion in pastures

As mentioned above, the most efficient means of avoiding horsenettle impacts is to prevent its invasion and establishment. Manure should be treated adequately to raise the maximum temperatures in manure above approximately 60℃. If the maximum temperatures reached above 60℃, the process of composting could provide both temperatures and duration sufficient to kill horsenettle seeds. When pastures are renovated, sowing should be timed to get sown grasses well established. To avoid horsenettle establishment, late sowing is considered to be better for pastures where autumn sowing is appropriate. Over-sowing is also effective to prevent horsenettle from becoming established. Empty gaps in pastures should be minimized. Horsenettle is not as prevalent as broadleaf dock in pastures, but it appears that control of fully established horsenettle is more difficult than that for broadleaf dock. Moreover, the potential distribution area of horsenettle is considered to be large even in Hokkaido. Thus, dissemination of information on horsenettle to relevant people is important to prevent horsenettle invasion and allow them to take countermeasures before the weed becomes fully established.

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ワルナスビ（*Solanum carolinense* L.）の種子による牧草地への侵入過程に関する研究

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摘　　要

ワルナスビは米国メキシコ湾岸州原産の多年生雑草で，茎葉に鋭いトゲを持ち家畜に有害なソラニンを含む。種子と根，根片で繁殖し，北米では，トウモロコシ，ダイズあるいは牧草地等の強害雑草である。日本では，1970 年代に牧草地の雑草として問題になったが，被害は一部地域に限定されると考えられていた。しかし，現在その分布範囲は広がっている。

ワルナスビは，一般的な草地管理では，防除が困難であり，安価で効率的な除草剤も現在のところ登録されていない。従って，新たな侵入・定着を阻止することが最も効率的な防除法である。

このような背景を踏まえ，ワルナスビの種子による侵入経路の解明と，実生の定着に関する要因を明らかにするために本研究を実施した。

1）　日本中央部の牧草地におけるワルナスビの分布

新潟県，長野県および栃木県を中心とした日本中央部の 24 の牧草地で，1993～1998 年にかけて，ワルナスビの有無を調査した。ワルナスビの発生が観察された調査地は約 1/4 であった。ワルナスビの侵入源は，輸入濃厚飼料に混入した種子と推測されるので，濃厚飼料給与の有無等の管理方法とワルナスビの発生との関係を調べたが，有意な関係は認められなかった。一方，ワルナスビが存在した調査地の年平均気温は，ワルナスビが存在しなかった調査地の年平均気温よりも有意に高く，また，最寒３ヶ月の月平均気温が氷点下になる調査地では，そうでない調査地に比べてワルナスビの発生する確率が有意に低かった。

2）　輸入濃厚飼料を採食した牛を経由したワルナスビの侵入

牛に採食されたワルナスビ種子は，糞中から 83％が回収され，その内 76％は生存していた。また，ワルナスビ種子を採食した牛の糞を戸外に置くと，中に含まれると推定される種子の約 15％が出芽した。堆厩肥中に埋設されたワルナスビを含む 15 種類の雑草種子は，堆厩肥の最高温度が約 60℃以上に上昇すると発芽力を失った。また，ワルナスビ種子よりも熱耐性のあるイチビ種子の死滅率が 90％になる時間の 95％信頼区間は，55℃では 42－58 時間，60℃では 10－17 時間と計算された。

3）　ワルナスビ種子の発芽に及ぼす温度の影響

ジベレリン処理をしたワルナスビ種子を供試し，発芽最低温度，および有効積算温度と発芽率との関係を調査した。発芽最低温度は約 15℃と推定され，有効積算温度と累積発芽率との関係はゴンペルツ曲線でよく表された。また，ワルナスビ種子を温度や変温幅および回数を変えた変温条件に曝し，発芽率を調査した結果，発芽最低温度よりも低い 15/10℃の変温条件でも暴露回数が多くなれば発芽率が向上した。

4）　ワルナスビ実生の出芽特性

4～8 月にワルナスビ種子を裸地条件とオーチャードグラス（OG）との競合がある条件で 1 ヶ月おきに播種した結果，播種後約 2 ヶ月間の累積出芽率は，裸地区では，7 月播種区を除き約 80％以上と非常に高かった。また，裸地条件の 4～6 月播種区では出芽した個体のほとんど全てが越冬した。しかし，OG との競合条件下では，OG の草冠が形成された 6 月播種区以降は，ほとんど出芽せず，また，4 および 5 月播種区も含めて，播種翌年に再萌芽した個

体はほとんど観察されなかった。また，播種翌年5月の萌芽数は，播種当年9月のワルナスビの生育量の傾向と良く一致した。

5) ワルナスビ実生の越冬性および耐凍性

9月上，下旬および10月中旬に，裸地およびOGとの競合がある条件で，ワルナスビ種子を播種した。裸地条件で，9月上旬に播種したワルナスビは約73%が越冬したが，それ以外の区の越冬率は10%以下であった。ワルナスビの播種数に対する播種翌年6月の生存個体割合は，9月上旬播種区が最も高かった。

8月および9月に播種したワルナスビ実生を当年12月に堀上げ，-4℃で3～12時間処理した結果，8月播種のワルナスビは，12時間処理でも無処理の場合とほぼ同程度の生存率を保った。一方9月播種のワルナスビは処理時間の経過とともに，生存率が低下し，回帰分析の結果，生存率が10%となる処理時間の95%信頼区間は12-20時間と計算された。

以上の結果から，ワルナスビの新たな侵入・定着を防止するために次のことを提言する。

1) 堆厩肥は最高温度が約60℃以上となるよう，適切に発酵させる。
2) 草地更新は，播種牧草の定着を確保するため，播種適期に行う。また，秋播きを行う地域では，ワルナスビ実生の定着を避けるためには，適期の後半に播種することが望ましい。
3) 牧草地内の裸地を最小限に保つよう，追播技術もワルナスビの定着を防ぐために有効である。
4) 牧草地の強害雑草として古くから認識されているエゾノギシギシに比較して，ワルナスビの分布域は現在のところ小さい。しかし，前者については防除技術が一応の完成をみているのに対して，ワルナスビが蔓延した場合には更新以外に有効な防除手段がない。また，日本における潜在的な分布可能域は北海道を含めかなり広いと考えられるため，蔓延を未然に防ぐための関係者への情報の伝達が重要である。

キーワード：*Solanum carolinense* L.，ワルナスビ，種子，侵入，耕種的防除