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Factors Affecting Germination of *Cuscuta japonica* Choisy

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Abstract

Dormancy of seeds of *Cuscuta japonica* Choisy was broken by mechanical scarification and by submersion in conc. sulfuric acid in the laboratory. In the field, dormancy could be broken as follows: 1) by the combined effect of soil factors and winter conditions. In the soil, the seed coat was removed gradually preparing the seeds for winter conditions where the fluctuation of temperatures near the freezing point could soften the seed coat and accelerate the germination; 2) burning and the high temperatures caused by burning could also break the dormancy of buried seeds. Moreover, the soil conditions after burning could be more suitable for emergence and early establishment of dodder than in unburned areas.

Germination of the non-dormant seeds could begin at temperatures lower than 5°C, that is intiation of germination. The rise in temperatures from 10 to 30°C caused most of the seeds which lost dormancy to germinate. Dodder seedlings could emerge from depths of up to 8 cm. As a result, the phenology of *C. japonica* in Hokkaido, and the behavior of dodder seeds buried in the soil were clarified in this study.

Key Words: Dormancy, burning, germination, phenology.

1. Introduction

As most of the seeds of dodder are hard coated, they are impermeable to water and oxygen. A pretreatment is frequently required to accelerate the germination. For example the submersion of seeds in conc. sulfuric acid solution or grinding them with sand paper and sometimes cold storage accelerate the germination (Gaertner 1950, Allred and Tingey 1964, Ashton and Santana 1976, Hutchison and Ashton 1980).

There is a few information on factors responsible for the break of dormancy of dodder seed in natural conditions. Ingestion of seeds by domestic animals (Khun 1877, according to Gaertner 1950), and overwintering of the seeds at different soil depths break dormancy (Hutchison and Ashton 1980).

In *Cuscuta japonica* choisy dormancy of seeds seems to be broken by the effect of fire (Ito unpublished). Ito observed an exciting flush of dodder on *Rosa rugosa* Thunb. community at Yambetsu in early spring of 1980, when fire burned some areas of the community. The Phenomenon is very comparable to prosperity of *Lespedeza bicolor* Turcz. in eastern Hokkaido, or of *Epilobium angustifolium* L. after frequent fires in Alaska. Heat pretreatment had been found to break the dormancy in *Lespedeza homoloba* Nakai (Iwara 1964), *Rhus javanica* L. (Iwata 1964;

Washitani 1986) and other plant species. A particular interest in the effect of heat pretreatment on the germination in dodder as shown already is the flush of dodder following grassland fire at Koshimizu, NE Hokkaido.

Recently, Solarization (heating the soil by using plastic mulch) proved to be effective in controlling several weeds including parasaitic plants. Horowitz *et al.* (1983) reported that temperature of soils covered with plastic mulch reached 60°C and caused thermal death of hydrated organisms. Burning crop residues and weeds will also help to kill or to stimulate buried seed populations depending on temperatures and the position of seeds in the soil. Therefore, it is important to investigate effect of burial and related factors on the germination. Because germination studies can yield basic ecological information on requirements by the seeds and the subsequent development. Knowledge of temperatures and the pretreatment of seeds for germination assesses the potential of seeds. This information provides insight of the establishment potential.

This study aims at the following objectives:

1) to clarify the life history of *C. japonica* (dodder);
2) to investigate temperature required for germination, of particularly pretreated seeds; and
3) to investigate factors responsible for the break of formancy and their subsequent effects on viability of seed,
   a. effect of burial depth,
   b. effect of burning.

2. Materials and Methods

2.1 *Seed collection*

Seeds of *C. japonica* were collected from dodder plants on *Rosa rugosa* Thunb. and *Artemisia montana* (Nakai) Pampan. of the general coastal meadow community dominated by *Rosa rugosa* at Koshimizu Genseikaen, northeastern Hokkaido, in the spring of 1985 (Figure 1). These seeds had overwintered under natural conditions before the collection. The seeds were stored in glass jars at room temperature (20°C) in the laboratory and used in the following experiments throughout this work.

2.2 *Pretreatment of seeds for the removal of the seed coat*

In this experiment the effect of acid pretreatment on viability and germinability of *C. japonica* was investigated and compared with the mechanical scarification as described previously (Zaroug and Ito 1987). Seeds of *C. japonica* were submerged in conc. (98%) sulfuric acid solution 1 : 2 v/v (one part seeds and two parts acid) for 5, 10, 15, 20, 25, and 30 min.

2.3 *Determination of temperatures required for dodder seed germination*

The seeds pretreated in conc. sulfuric acid and non-treated seeds were placed on moistened filter paper in petri-dishes. These were replicated four times each containing 25 seeds and then placed in an incubator at six temperature regimes,

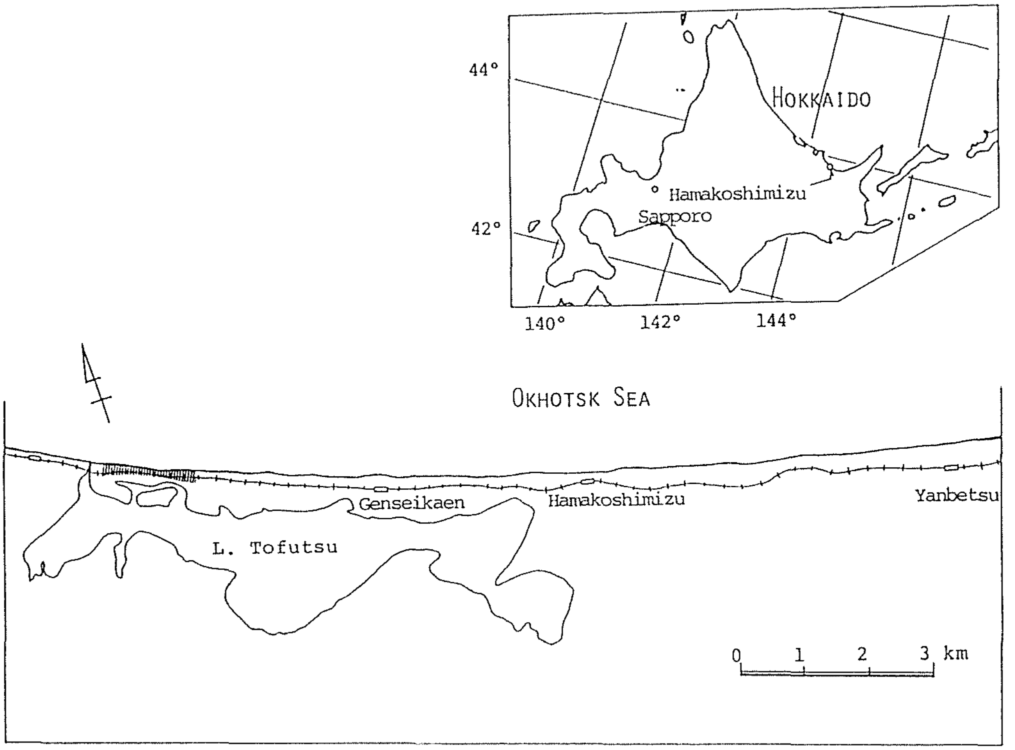

Figure 1. Study area. Shaded area in the detailed map shows the area burned in the spring of 1987.

namely 5, 10, 15, 20, 25 and 30°C. Germination was recorded over the first two weeks every two days. Then, the non-treated seeds were kept for 16 weeks at 25°C until no germination was recorded. These were then tested for viability.

2.4 *Effect of burial depth on dormancy and viability of dodder seeds*

The effect of burial depth and duration on the dormancy and viability of dodder seeds was investigated in the following: 97% to 100% dormant viable seeds were obtained from the field. On 17 June 1985 approximately 300 seeds (1.6 g) were placed in polyester cloth bags, which were permeable to water, and attached to a plastic coated wires to facilitate recovery. The wires were anchored to the soil surface. The seeds were then buried at 1, 5, 10 and 15 cm in depths. It was replicated four times in a randamized block design. Air temperatures and soil temperatures measured at one and five cm soil depth were obtained from the Nursery of the Experimental Forest, Department of Forestry, Faculty of Agriculture, Hokkaido University.

Since the first recovery in September of the same year did not show any change in the germination. The recovery were then made on 17 April, 17 May, and 17 June of 1986. The total buried seeds were analyzed immediately after recovery in order to determine the percent of the germination *in situ* and the remaining seeds were incubated at 25°C for a four weeks. The seeds germinated during this period were recorded and calculated as percent of the total buried seeds. The remaining seeds were submerged in conc. sulfuric acid solution for 20 min to test their viability.

2. 5 *Depth of seedling emergence*

To see the depth of emergence of dodder seedlings 50 acid-pretreated-seeds were put 1, 2, 3, 4, 5, 6, 7 and 8 cm in depth in four replicates in wooden flats. Soils were sandy and clay soil. Emergence of dodder was recorded during the period from mid-June to the third week of July when there was no emergence. The wooden flats were kept outdoors and water was supplied when necessary.

2. 6 *Effect of burning on dormancy and viability of dodder seeds*

The effect of burning on the germination could not be conducted easily in the field, because experimental condition hardly adequately controlled. Fire temperatures, however, were measured at two sites of the general coastal meadow community, Koshimizu Gensei-Kaen, when buring (Noyaki) was practiced for the purpose of recovery of *Rosa rugosa* by Koshimizu Town office in the spring of 1986.

2. 6. 1 *Measurements of temperatures in field conditions*

Temperatures during and after burning at Koshimizu Gensei-Kaen was measured by using a computerized temperature recording system called Data Collecter (AM 7001, Kairyogata, Anritsukeiki CO. Ltd.) which can measure up to 1200°C. Sensors connected to this instrument were placed at the following depths: 0.0, 1.0, and 2.0 cm. Moreover, two sensors were placed at 5 and 15 cm above the soil surface. Then the instrument was adjusted to record the temperatures at 10 seconds interval.

2. 6. 2 *Burning in experimental conditions*

The effect of burning on the germination and on the viability was studied in the laboratory. In two kinds of experiments temperatures at different soil depths were measured during buring and their effects on buried seed germination and viability were examined. For this purpose wooden flats were used. The size of the wooden flats was $120 \times 30 \times 10$ cm, the flats were divided into small compartments of which size was $30 \times 30 \times 10$ cm. Each of them was assigned the following depths: 0.0, 0.5, 1.0, and 1.5 cm. The wooden flats were then filled with soil. The sensors were fixed at each depth and connected to the computerized temperature recording system. Temperatures at 5 and 15 cm above the soil surface were also measured at the same time. The flats were then covered with a layer of dry grass, which was ignited and burned.

In the first experiment 150 non-treated seeds were put at each depth and recovered for germination tests which were replicated three times.

In the second experiment 150 seeds from the non-treated and 150 seeds from acid-pretreated seeds were put at each soil depth in the wooden flats. In order to facilitate recovery, the seeds first were placed in 9 cm petri dishes filled with soil which was meshed to remove the gravels. The seeds were put into the wooden flats as follows :

Petri-dishes were marked by a line at 0.5 and 1.0 cm in depth. Then, soil was put to the marker and the seeds placed into the different depths of soil.

After burning, the seeds were washed through a series of meshes to remove soil particles and then subjected to germination and viability tests. These were replicated three times.

2. 6. 3 *Heat-pretreatment by exposure to contorolled oven temperature*

This experiment was conduced to determine the buring effect on the germination when fire passed on the ground which contain dodder seeds at different depths. The effect of heat-pretreatment on germination of a two-year-old dodder seeds was studied in the following : Seeds were washed in tap water to remove destroyed seeds ; dried ; and then divided into two groups. One of them was submerged in conc. sulfuric acid solution for 30 min to remove the hard seed coat. Seeds from the dormant (non-treated) and seeds from the acid-pretreated were incubated at 25°C in 4 replicates for four weeks to determine germinability. Then twenty five seeds taken from each group were placed in three discs with 9 cm filter paper moistened by 5 ml of water in petri-dishes. In the treatments replicated four times seeds were exposed to controlled oven in which temperatures were 40, 55, 70, and 85°C for 10, 20, 30, and 40 min, respectively. They were placed in an invubator at 25°C for four weeks. Non-germinated seeds were tested for viability by the mechanical scarification. The germination was recorded every two days and those which attained to 0.5 cm or more long were considered. Deformed and injured seedlings, unless recovered, were considered not to be viable. Some of seeds exposed to high temperatures were examined under the microscope for the observation of physical changes on the testa.

3. Results

3. 1 *Pretreatment of seeds and temperatures required for germination*

Table 1 shows the germination percentage of the non-treated, mechanically scarified and acid-pretreated seeds on the 15th day of incubation at three regimes of temperatures from 5, 15 and 30°C.

Table 1. Germination percentage of non-treated seeds and acid-pretreated seeds in response to temperature at-the 15th day after incubation*

Time of acid-pretreatment	Percent germination at temperatures of		
	5°C	15°C	30°C
Non treated	0.1	0.0	1.0
5 min	14.0	40.0	62.0
10 "	30.0	64.0	70.0
15 "	77.0	99.0	98.0
20 "	53.0	99.0	99.0
25 "	56.0	97.0	98.0
30 "	58.0	98.0	98.0

* The test was carried out by placing 25 seeds per petridish and replicated four times.

3. 1. 1 *Effect of mechanical scarification and acid pretreatment*

Non-treated seeds germinated poorly at higher temperatures from 20 to 30°C and gave only from 1 to 3% in germination within 15 days. When the incubation at 25°C continued for 16 weeks no seeds germinated (Zaroug and Ito 1987). The mechanical scarification and the acid pretreatment broke the dormancy and increased the germination significantly. In the former pretreatment at least 70% of the seeds imbibed water and radicle emerged at low temperatures of 5°C within 15 days in the incubator.

Acid pretreatment, in which seeds were submerged in conc. sulfuric acid for 15 min was adequate in the break of dormancy and resulted in high percentage of germination up to 98%. Figure 2 shows that an increase of the time of acid pretreatment improved the percentage of germination, and that even at relatively low temperatures it was effective in the increase of germination. Seeds submerged in the acid solution for 5, 10, and 15 min and incubated at 5°C germinated by 14%, 30%, and 77%, respectively. The increase of the time of the acid pretreatment for more than 15 min did not increase the germination.

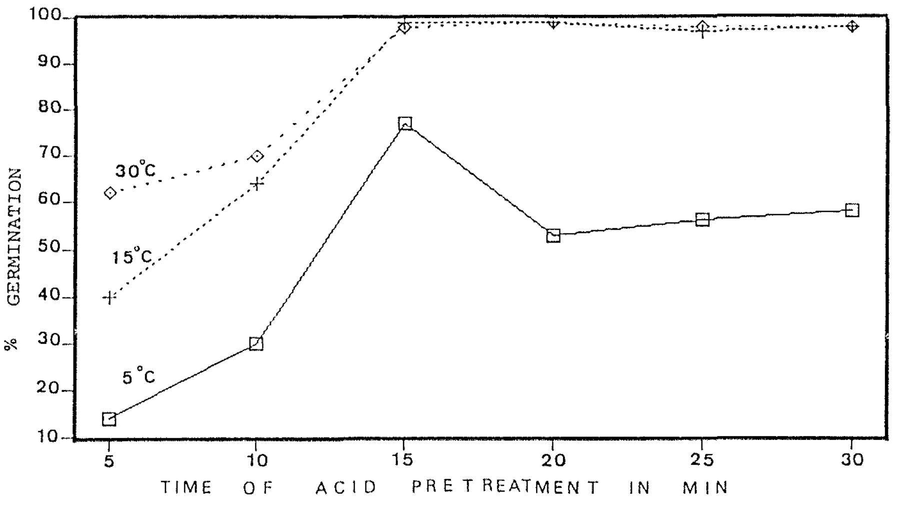

Figure 2. Effect of acid pretreatment and temperature on germination of dodder (*C. japonica* choisy).

3. 1. 2 *Temperatures required for germination of pretreated seeds*

As alredy mentioned the mechanical scarification and the acid pretreatment had apparently removed some parts of the seed coat layer and accelerated germination. The results presented in Table 1 and Figure 2 show that most of the seeds which were subjected to these treatments germinated at temperatures from 5 to 30°C within 15 days. There were no apparent differences in percentage of germination obtained at the 15th day after incubation.

Figure 3 shows that a decrease of temperatures was related to an increase of days which required for germination in acid-pretreated seeds. In this case seeds

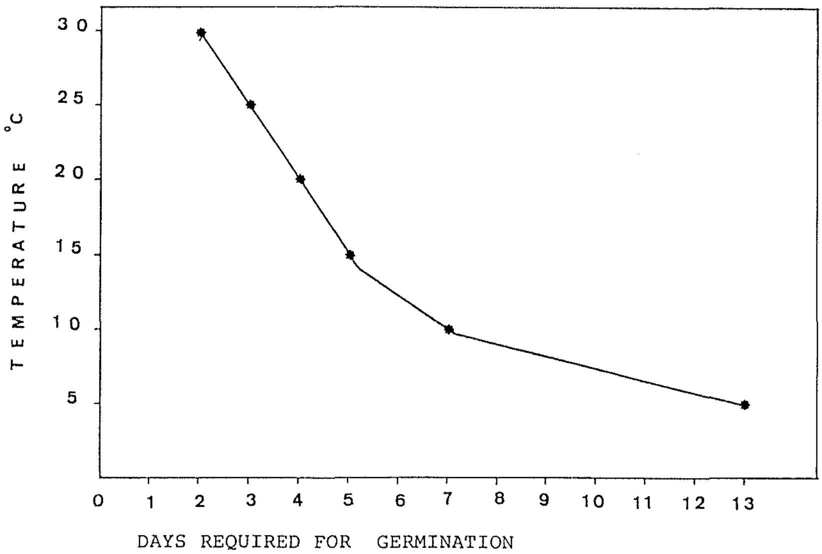

Figure 3. Relationship between temperatures and days required for germination.

submerged in conc. sulfuric acid for 30 min germinated by 58% in 13 days at 5°C, while the same seeds germinated by 98% in 48 hours when incubated at 30°C.

3. 2 *Depth of seedling emergence*

Table 2 shows the emergence pattern of *C. japonica* seedlings at various depths in clay and sandy soils. Seeds which placed at 1 to 8 cm in depth in mid-June started to emerge within 4 to 5 days from 1 to 2 cm depths. The emergence continued by the third week of July and it was dependent on the depth of placement. The delayed emergence of seedlings in the presence of favourable moisture and temperature conditions was apparently due to the different depths of placement. The drying of the upper soil layers during the period was partially responsible for the low percentage emergence which was prominent in sandy soil.

Table 2. Emergence of dodder seedlings at various depths in clay and sandy soil

Depth of placing seeds	Percentage of emergence from		Depth of placing seeds	Percentage of emergence from	
	clay soil	sandy soil		clay soil	sandy soil
1 (cm)	56.0±4 *	0.0	5 (cm)	56.5±7.7	20.0±4.3
2	53.0±5.9	30.0±8.6	6	50.0±5.9	20.0±6.2
3	58.5±7.7	34.0±5.9	7	43.5±5.9	14.0±3.7
4	59.0±8.3	34.0±7.1	8	34.5±6.6	10.0±3.7

* The mean±SD

3. 3 *Effect of burial depth on dormancy and viability of seeds*

Table 3 shows the monthly average air and soil temperatures at 1 and 5 cm depths during the time of burial near the experimental plots.

Table 3. Monthly average of air and soil temperature at 1 and 5 cm depth during the period from June 1985 to June 1986 near the burial experiment site, Forest Nursery, Faculty of Agriculure, Hokkaido University

Month	Air Temperatures Maximum (°C)	Air Temperatures Minimum (°C)	Soil temperatures at 1.0 cm (°C)	Soil temperatures at 5.0 cm depth (°C)
June 1985	26.6	10.9	14.4	13.6
July 〃	24.5	16.5	18.9	18.5
August 〃	30.8	20.8	22.5	21.9
September 〃	22.2	12.8	16.3	16.7
October 〃	16.9	6.5	10.6	10.8
November 〃	8.8	0.5	4.6	5.0
December 〃	2.0	−8.2	−0.7	0.1
January 1986	−0.1	−10.5	−0.7	−0.1
February 〃	2.3	−11.8	−1.0	−0.6
March 〃	5.5	−3.9	−0.1	−0.1
April 〃	13.2	1.7	5.6	5.1
May 〃	17.4	5.0	11.2	10.3
June 〃	21.8	11.3	14.9	14.2

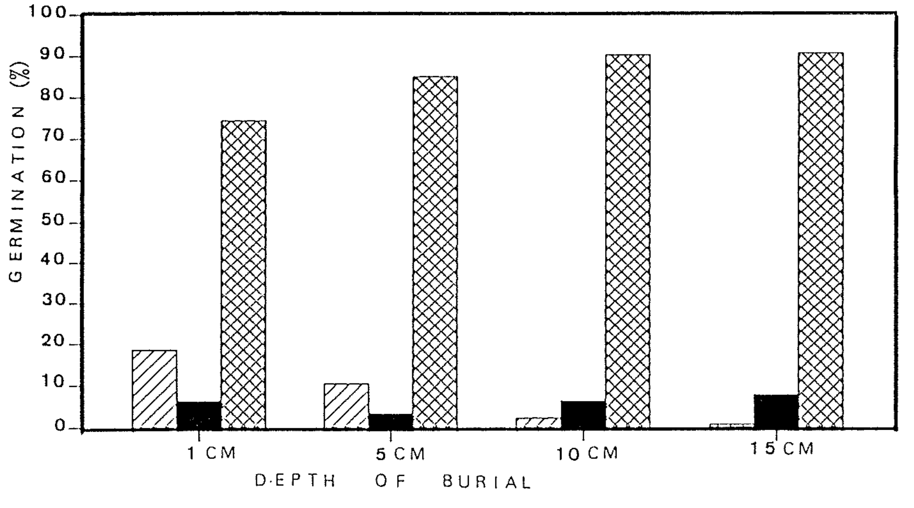

Figure 4. Effect of burial depth on dormancy and viability of dodder seeds recovered after 10 months of burial at different soil depths (recovered April 1986).

hatched: seeds imbibed water; solid: seeds enforced dormancy
netted: dormant seeds.

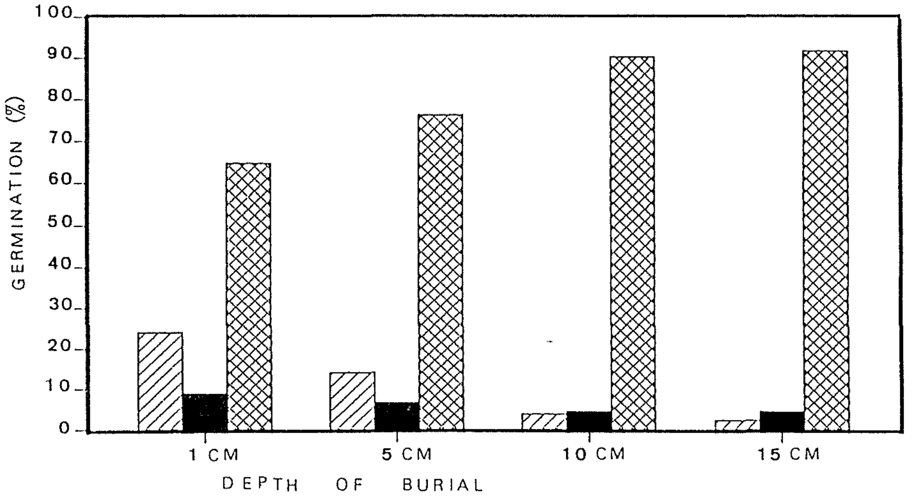

Figure 5. Effect of burial depth on dodder seed dormancy and viability after one year of burial at different depths (recovered June 1986).
hatched: seeds *in situ*; solid: seeds enforced dormancy; netted: dormant seeds.

Seeds recovered at different times in various burial depths were analyzed as follows: (1) Seeds germinated *in situ*, (2) Seeds germinated in four weeks of incubation at 25°C after recovery in dark condition during 4 weeks; and (3) Seeds germinated only after acid pretreatment condition for 20 min. Figures 4 and 5 shows the effect of burial depth on the dormancy and viability of seeds after 10 and 12 months of burial at the different soil depths. These show that viability of dodder seeds was not affected by the burial after 10 and 12 months at all depths investigated. Seeds imbibed water, that is, seeds which were broken dormancy were highest in the 1 cm depth in percentages of germination *in situ* and reduced gradually to deeper parts of soil.

Table 4 shows percentage of germination of seeds *in situ* which recovered three times in one-month intervals after being buried in the soil at 1, 5, 10 and 15 cm depth for 10, 11, and 12 months. The first recovery was carried out on 17 April 1986 when snow melted and soil temperature was about 3.3°C (the average of the first half of April) at one and 5 cm depth. During that time 18% of the seeds (Table 4) imbibed water (initiation of germination) and they germinated at 25°C in the incubator after two days. In seeds recovered after one month, the germination *in situ* increased slightly by 2% at 1 cm depth. Seeds recovered in June after one year germinated by 23.9% *in situ*, and produced longer seedlings and increased in size significantly more than those recovered earlier. In the laboratory, however, it has been consistently observed that dodder seeds germinated at 5°C remained unchanged in size for the first four to five weeks after the radicle emergence, and that resumed normal growth when in higher temperatures. Generally, percentage of germination *in situ* decreased significantly by an increase of depth of burial in the soil from 1 to 10 cm. At the 10 and 15 cm depth germina-

Table 4. Percentage of germination *in situ* of seeds buried at different soil depths and recovered after 10, 11, and 12 months, in April, May and June

Soil depth (cm)	Percentage of germination *in situ* after			
	10 (April)	11 (May)	12 (June)	Months
1	18.3*	20.2	23.9	(4.09)**
5	10.4	13.3	14.1	(2.11)
10	2.3	3.1	3.6	
15	0.7	2.2	2.1	
	(3.98)	(6.62)	(4.73)	Lsd.

* These seeds imbibed water at time of recovery and germinated at 25°C in the laboratory within two days.
** Figures in parenthesis indicate Lsd. (least significant difference at 5% level of probability).

Table 5. Percentage of enforced dormant seeds which buried and recovered after 10, 11, and 12 months at different soil depths

Soil depth (cm)	Percentage of enforced dormant seeds after		
	10	11	12 Months
1	6.2	6.4	8.7
5	3.4	4.9	6.6
10	6.4	3.1	4.5
15	7.6	2.5	4.4
	N. S	N. S	(2.66) Lsd. (5%)

tion *in situ* remained unchanged during the three times of recovery. The germination of seeds *in situ* recovered in June 1986 was significantly larger than that in April 1986 at 5% level of probability. These facts suggest that the dormancy was broken in response to the sudden changes of environmental factor near the soil surface, and that the germination increased gradually in accordance with the increase of soil temperature during the season.

Table 5 shows the precentage of seeds in enforced dormancy, which were buried and recovered after 10, 11, and 12 months at different depths. As seen in Table 5 there were no significant changes at different soil depths and also at the first and second recovery. In seeds recovered in June after one year, seeds under enforced dormancy at 1 cm depth was significantly larger than those at 10 and 15 cm soil depths at 5% level of probability. It suggests that the germination and consequently the emergence of seedlings in *C. Japoncia* differs from other species which are known for their continuous germination and prolonged emergence (Dawson 1964).

3.4 *Effect of burning on dormancy and viability of seeds*

3.4.1 *Burning temperatures*

Table 6 shows soil temperatures at the soil surface and 1 cm depth ; and air

Table 6. Temperatures during burning in field conditions at Koshimizu Gensei Kaen in the spring of 1987

Position of sensors	Maximum temp. (°C)	Range of temp. (°C)	Duration	
			(min.)	(sec.)
5 cm above SS*	430	51–430	8	40
on the SS	256	40–256	4	40
on the SS	184	40–184	8	30
1 cm depth	39	25–39	5	30

* SS means the soil surface.

Table 7. Temperatures rise during burning in experimental conditions

Position of sensors	Maximum temp. (°C)	Range of temp. (°C)	Duration	
			(min.)	(sec.)
5 cm above SS*	451	45–451	6	40
on the SS	187	45–187	8	50
0.5 cm depth	77	45–77	11	
1.0 cm depth	45	40–45	9	

* SS means the soil surface.

temperature at 5 cm above the soil surface at Koshimizu Kaen. The highest temperature was 256°C at the soil surface and temperatures continued from 40 to 256°C for 4 min 40 seconds. But the temperatures above 100°C continued for only 2 min. Temperatures at 1 cm depth rose slightly from 15°C before burning to 39°C during burning, and the temperatures continued from 25 to 39°C for 5 min 30 seconds after burning.

Table 7 shows temperatures measured in the experimental conditions at the surface, 0.5 cm and at 1.0 cm depths. The temperatures measured in the experiment were nearly similar to those measured in the field conditions. In the experimental condition the temperature at 0.5 cm depth was 77°C and temperatures from 45 to 77°C continued for 11 min.

3. 4. 2 *Effect of burning on dormancy and viability of seeds*

Table 8 shows the effect of burning on dormancy and viability of seeds put

Table 8. Effect of burning on dormancy and viability of dodder seeds buried at different soil depths in experiment 1*

Soil depth (cm)	Germination (%)	Viability (%)
0.0	0.0	42.6
0.5	5.7	87.3
1.0	3.3	94.0
1.5	0.0	95.0

* 50 seeds in three replicates at each soil depth.

Table 9. Effect of burning on dormancy and viability of dormant and non-dormant seeds buried at different soil depths in experiment 2*

Soil depth	Dormant seeds		Non-dormant seeds
	germination	viability	germination**
(cm)	(%)	(%)	(%)
0.0	0.6	74.0	13.3
0.5	2.6	94.6	76.0
1.0	1.3	95.3	81.3

* 50 seeds in three replicates at each depth.
** Acid-pretreated seeds.

at different soil depths. Seeds of which dormancy was broken were by 5.7% at 0.5 cm depth where soil temperature rose to 77°C during burning. As seen in Table 8, 57.4% of the seeds lost viability at the soil surface. Table 9 shows effect of burning on both dormant and non-dormant seeds. In dormant seeds max. percentage of germination was by 2.6% at 0.5 cm depth and 26% of seeds lost viability at the surface. In non-dormant seeds 86.7% of seeds lost viability at the surface, but those buried at 0.5 and 1.0 cm depths were safe from high temperature caused by burning.

3. 4. 3 *Effect of heat-pertreatment*

Figure 6 shows effect of heat pretreatment on the dormancy and viability. Germination percentages of dormant seeds which heat- pertreated at temperatures of 70°C for 10-40 min was 7-17%. Similar germination percentages were obtained in seeds which heat- pretreated at temperatures of 85°C for 10 and 20 min. As seen in Figure 6 the heat pretreatment at temperatures of 85°C for 30-40 min destroyed considerably the viability of seeds.

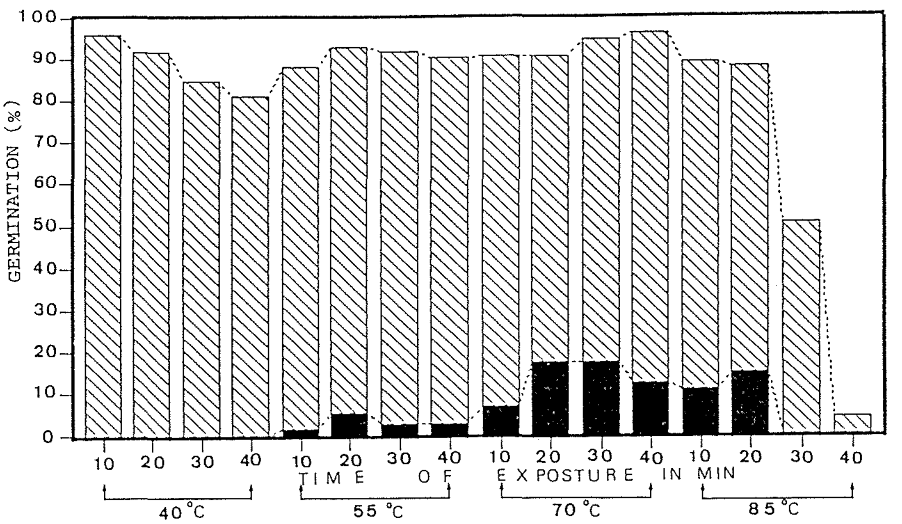

Figure 6. Effect of heat-pretreatment on dormancy and viability of seeds. hatched: dormant and viable seeds; solid: seeds germinated by heat-pretreatment.

Table 10. Effectof heat pretreatment on viability of acid-pretreated seeds*

Oven temp. (°C)	Duration (min.)	Germination (%)	Oven temp. (°C)	Duration (min.)	Germination (%)
40	10	100	70	10	100
40	20	98.7±2.3	70	20	93.3± 8.3
40	30	97.3±2.3	70	30	64.0±10.6
40	40	98.7±2.3	70	40	21.3±12.2
55	10	100	85	10	93.3± 6.1
55	20	100	85	20	52.0± 3.6
55	30	98.7±2.3	85	30	0.0
55	40	94.7±2.3	85	40	0.0

* 50 seeds replicated three times in each treatment.

Table 10 shows the effect of heat-pretreatment on viability of acid-pretreated seeds. The results show that heat-pretreatment at 40–55°C for 10–40 min and at 70–85°C for 10–20 min did not destroy viability of acid-pretreated seeds. However, the heat-pretreatment at 85°C for 30–40 min destroyed completely viability.

The results indicated that dormant seeds could germinate after heat-pretreatment at temperatures up to 70–85°C for 10–20 min, and that 96% of the seeds lost their viability after heat-pretreatment at 85°C for 30–40 min (Figure 6). On the other hand, the acid-pretreated seeds were safe from heat pretreatment at 70–85°C for short time of 10 min.

4. Discussion and conclusion

4. 1 *Pretreatment of seeds and temperatures required for germination*

4. 1. 1 *Pretretment of seeds*

Dormancy of seeds of *C. japonica* was broken by mechanical scarification and by submerging the seeds in conc. sulfuric acid for 5 to 30 min. Mechanical scarification of the seed coat increased germination of dormant seeds of dodder to 70% at 5°C and to 94–99% at 10 to 30°C (Zaroug and Ito 1987). Barton (1965) reported that mechanical scarification promoted the germination of dormant seeds of the Leguninosae. He stated that the reason for the effects of mechanical scarification on the promotion of germination was ; the opening allows the movement of water through normally impermeable hard seed coats ; it might also make seed coats permeable to gas diffusion and it lessened mechanical restriction of radicle protrusion. Hutchison and Ashton (1979) found that mechanical scarification of seeds of *C. campestris* by sand paper increased germination to 98%. They demonstrated that the removal of the epidermal layer and portion of the hypodermal layer increased germination from 1 to 48% and that the removal of both layers increased germination up to 92%.

In a case of *C. japonica* the removal of the epidermal layer seemed not to promote the germination of dormant seeds. In seeds which were buried for 3 months and recovered, the germination was not improved despite the removal of the epidermal layer. It indicates that the removal of the epidermis, hypodermis and parts of the macrosclerieds allows seeds to enter water and initiate germination.

By the submersion of the seeds in conc. sulfuric acid solution for 5 min germination increased to 14% at 5°C, 40% at 10°C, and 62% at 30°C. By elongation of the time of acid submersion from 5 to 15 min germination increased from 14 to 77% at 5°C, 40 to 99% at 15°C, and 62 to 98% at 30°C (Table 1, and Figure 2). It suggests that the epidermal layer and part of the macrosclerieds layers were dissolved by the acid in the acid-pretreatment for 5 min. Elongation of the time of acid-pretreatment up to 15 min removed completely the impermeable layers and impreoved the absorption capacity of the seeds. As a result the germination improved and reached 98%, most of the seeds were able to germinate. Hutchison and Ashton (1979) found that acid-pretreatment broke the dormancy of seeds of *C. campestris* but it was not as effective as mechanical scarification by sand paper. Their results showed that the acid-pretreated seeds for 60 min germinated by 61%. Moreover, they demonstrated that by submerging the seeds in the acid for 5 min, the epidermis swollen and by extending the time of the pretreatment to 15 min, the epidermal and the exodermal layers were hydrolized. In the present study the acid-pretreatment for 5 min did not cause swelling of the epidermal layer but apparently caused the dissolution of it. Moreover, as a result of the extension of the acid-pretreatment, most of the layers were dissolved gradually and the germination rose (Figure 3). It sugge ts that the impermeable layer is not restricted to specific regions above the light line and at the junction of the hypodermis and the palisade layers as suggested by Hutchison and Ashton (1979). The acid-pretreatment was as effective as mechanical scarification in the break of formancy of seeds of *C. japonica*. The most effective was submersion of the seeds in conc. sulfuric acid for 15 min.

4. 1. 2 *Temperatures required for germination*

The mechanical scarification and the acid-pretreatment removed or dissolved parts of the impermeable layers of hard seed coat and consequently accelerated germination. Seeds submerged in the acid for 30 min, started to germinate at 5°C, giving 58% germination; by increasing temperature to 10–30°C, 83% to 98% of the pretreated seeds germinated within 15 days (Table 1). Days required for germination, decreased gradually according to an increase of temperatures from 5 to 30°C. The acid-pretreated seeds for 30 min began to germinate after 13 days in 5°C incubation, but after 48 hours in 30°C incubation (Figure 3). These facts showed that seeds of *C. japonica* required longer time at lower temperatures of 5°C in germination than at higher temperatures. The ability of seeds of *C. japonica* to germinate in a wide range of temperatures is characteristic of this species and services the chances of survival. Germination of acid-pretreated seeds of some other species behaved differently in response to temperatures. For example, Allred and Tingey (1964) found that germination in *C. approximata* Bab. was 25–30% at 1.5–

7.2°C, and maximum of 51% at 15.5°C; in *C. indecora* Choisy was 4% at 4.5–7.2°C, 75% at 10°C and maximum of 88% at 21.1°C; and in *C. campestris* Yunck. 7% at 10°C and maximum of 31% at 15.5–35°C. They demonstrated that *C. approximata* germinated rapidly at 1.4°C; *C. indecora* at 10°C; and *C. campestris* at 15.5°C. In Yugoslavia the seeds of *C. prodani* and *C. tinei* germinated best at 20°C, while seeds of *C. campestris*, *C. pentagona* Engel. and *C. monogyna* germinated at 30 to 33°C (Stojanoric and Mijatavic 1973). In Japan, Furuya *et al.* (1980) demonstrated that acid-pretreated seeds of *C. pentagona* germinated at both constant (25 to 30°C) and alternate (30 and 20°C) temperatures in light and dark conditions. They showed that seeds did not germinate at 10°C but did at 15°C.

4.2 *Effect of burial depth on germination and viability of dodder seed*

The percentage of total viable seeds of *C. japonica* at various soil depths was not affected by one-year burial in the soil. Recovered seeds are divided into: (1) seeds germinated *in situ*; (2) seeds under enforced dormancy, which germinated after being incubated for four weeks; and (3) dormant seeds (hard coated seeds).

The seeds at shallower depths germinated *in situ* more than at deeper ones, when seeds were recovered three times in one month intervals from 17 April to 17 June. Seeds recovered on 17 April imbibed water at that time and could germinat easily within 48 hours at 25°C of incubation. The results showed that the seeds could begin to germinate at low temperature of 5°C. However, these seeds sometimes issued the radicle in a few mm during the period from 3 to 4 weeks and resumed normal growth when exposed to more favourable temperatures. It suggests that germination of non-dormant seeds begins early in the spring when soil temperature was lower about 5°C. When soil temperatures rose, the recovered seedlings showed pronounced emergence of radicle, but the germination *in situ* was not significantly different between the first and the second recovery. In the third recovery, germination *in situ* at 1 cm soil depth increased significantly than the first recovery. The results indicate that the dormancy in *C. japonica* is broken in response to soil-burial conditions. These changes show pronounced effect on the germination *in situ* at the upper 1 to 5 cm soil depth.

Barton (1965) suggested that dormancy of hard seeds can be borken by attack of soil microorganisms and by weathering. He also reported that in natural conditions or in dry storage for overwinter, 80 to 100% of the hard seeds of sweet clover was softened by the middle of the following April. He concluded that it is required to expose the seeds in fluctuation near 0°C for two or more months for effective softening of the seed coat. Hutchison and Ashton (1979) reported that cold storage at 4°C will break dormancy of *C. campestris*. They examined samples of seeds which showed 80% germination after storage under Stereoscan Electron Microscope but did not observe any morphological changes such as cracks or pores which might explain loss of impermeability. Hutchison and Ashton (1980) reported that the dormancy of seeds buried at various soil depths were nroken by overwintering; by 2–27% in new seeds and 18–42% in old seeds.

In this study seeds which were buried on 17 June were dormant when recovered three months later. Samples from these seeds examined under Scanning Electron Microscope showed morphological changes in seed coat, which shows partial removal of the epidermal layer. It indicates that soil conditions such as temperature, moisture and soil microorganisms help to remove the hard coat. However, the mechanism is not known in details.

The germination percentage of the seeds recovered after overwinter was 2-24% (Table 4). It suggests that removal of the hard seed coat was effective on softening of the seed coats. The changes were significant at 1 and 5 cm depths, and indicate that the fluctuation of temperatures during winter is effective on softening of the hard seed coats and breake of the dormancy.

Moreover, the rise of soil temperatures in summer season, provided that the experimental plots were kept bare throughout the period of burial, seemed to supply sufficient heat to the seeds buried near the soil surface. This in turn will provide the cumulative temperature required for the germination. Daeson (1965) showed that seeds of *C. acampestris* planted at various soil depths in bare plots germinated more than those in shaded plots, because the soil was warmer in the former than in the latter. He found that soil temperatures in bare plots rose twice as high as in shaded plots in summer. The facts mentioned above indicate that the heating of the soil during summer season can also facilitate the breake of the dormancy.

Some buried seeds were not dormant but did not germinate i. e. under enforced dormancy (Table 5). Carbon dioxide narcosis is seemed to be responsible for the enforced dormancy (Harper 1972).

4. 3 *Effect of burning and heat-pretreatment on dodder seeds*

Seeds of many plant species, if slightly covered by soil can survive a relatively intense fires (Weight and Baily 1982). These authors have also reported that the grassland fires would probably have only slight effect on the mortality of dormant seeds, even if they are lying on the soil surface. However, according to the present burning experiment, 26% to 57% of the dormant seeds which were placed artifically on the soil surface lost viability during the burning (Tables 8 and 9). Whereas the non-dormant acid-pretreated seeds lost viability by 87% (Table 9). Despite of such an unusal treatment for removing the seed coat, at least 13% of the non-dormant seeds were safe from high temperature of 187°C on the soil surface (Table 7). The seeds buried at 0.5 cm depth were not affected by high temperature in burning experiment (Table 9). Dormant seeds lost viability by 96% when exposed to heat-pretreatment at 85°C for more than 30 min. Non-dormant seeds lost viability 100% when exposed to at 70°C and 85°C for more than 30 min (Table 10). These facts suggest that the hard seed coat can to some extend protect the embryo from burning and high temperatures.

Percentages of germination in seeds at 0.5 cm depth were 2.6-5.7% for dormant seeds in burning experiments, and 76% for non-dormant seeds (Tables 8 and 9). Percentages of germination of dormant seeds were 17 and 14% at 70°C for 20 min and 85°C for 10 min in heat-pretreatment, respectively. In samples from heat-pretreated seeds rupture of the seed coat was observed by naked eyes and examined under light microscope. Moreover, all the non-viable seeds which heated for more than 30 min at 85°C imbibed water. It indicates that heat-pretreatment ruptures the seed coat and improves permeability to water and lead to break the dormancy. But by extension of heat-pretreatment seeds lost viability.

The effect of heat-pretreatment on inducing germination of hard seeds has been reported for several species. For example, Mallik and Gimingham (1985) found that the heat-pretreatment of seeds of *Hypericum pulchrum* resulted in high germination at 100°C for 2 min. Takahashi and Kikuchi (1986) found that heat-pretreatment induced seed germination of *Rhus javanica*, *Purearia lobata* and *Lespedeza homoloba*. According to them these species were among the important species of the post-fire vegetation at Mankita Hill, Miyagi prefecture, Honshu. Examples about fire-induced germination of *Cuscuta* seeds are scarce. However, as already mentioned a fluch of *C. japonica* was observed after grassland fire. According to him, the phenomenon is very comparable to the prosperity of *Lespedeza bicolor* in eastern Hokkaido or *Epilobium angustifolium* after frequent fires in Alaska. Furthermore, *C. japonica* made an appearance again in 1987 following the burn in early spring at Koshimizu Gensei Kaen. Based on the results obtained in this investigation the flush of *C. japonica* after grassland fires could be attributed to the following:

(1) Germination of dodder seeds could be induced by fire or burning;

(2) Some non-dormant seeds buried in the soil escape high temperatures caused by firs. These can germinate successfully in the post-fire recovery period; and

(3) During the period of recovery after burning, a rise of soil temperatures near the soil surface may accelerated dodder seed germination.

The rise in soil temperatures during post-burning was reported by Philips (1919, 1930). He noted that large differences in soil temperatures were observed between burned and unburned grasslands during the period of recovery following the burn, and that the soil surface temperature on burned field might rise twice as high as on unburned one but that the temperatures might drop several degrees lower on burned one during cold nights than unburned one.

Temperatures required for the germination and effect of soil depths on the dormancy are serviceable to interpret the phenological pattern of *C. japonica* in Hokkaido. As seen in Figure 7 seeds on the soil surface or buried tend to lose the impermeability gradually in response to various environmental factors. Initiation of the germination begins in early spring. The seeds could remain in water absorption condition until soil temperatures rise to optimum levels for the germination. An increase of soil temperatures during late May and early June will accelerate the germination and seedlings emergence to above ground. The seedlings could survive for three weeks on ground, but they will die if they can not attach to a host. The seedlings which can attach to a host are tincted with red and increases in diameter. After vegetative growth flowering is from late July to September.

Finally, we can state the behaviour of dodder seeds dispersed from the parent. The most common course of dodder seed in soils is to germinate in soils in the

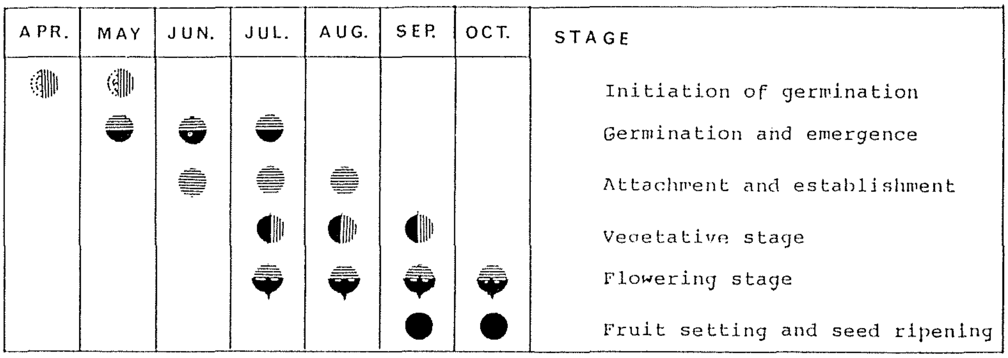

Figure 7. Phenology of *C. japonica* Choisy in Hokkaido.

next year and consective years ; and to emerge above the ground. After emergence, young seedling can grow and fruit when they are successful in contact with major hosts, but they can survive vegetatively when they attach to minor hosts. Among the seeds buried in the soil some are positioned in shallow depths, but others are deeper. Seeds at shallow depths and those at depths shallower than the maximum depth of the seedling emergence can emerge and look for a host. But deeper seeds can germinate but die owing to the exhausement of nutrients in seeds. Thus, there are 4 gates in order to complete dodder's life history as follows ; (1) the ability of germination in soils ; (2) the possibility of emergence above ground ; (3) the chance of attaching to major hosts ; and (4) the guarrantee of sound growth given by hosts.

Another course, although its occurence is small in chance. is the germination of seeds on the soil surface induced by burning. As a result. the emerging seedlings have an immediate chance to find a host and complete its life history. Generally speaking, the latter factor is more favourable to maintain the dodder population in an area than the former normal factors. This is one of the causes, which *Cuscuta* species are temporarily strongly harmful to crop plants in gardens and cultivated fields, but are not members of common harmful weeds in pest lists in agiculture.

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