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Studies on the effect of heavy oil spilled from the wrecked Russian tanker "Nakhodka" on the growth of plants

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ABSTRACT

The effects of the heavy oil spilled from "Nakhodka" on germination and shooting of plants were investigated using the seed of morning glory and Japanese radish, the roots of dandelion, and the branches of camellia. The results showed that the volatile matter from the heavy oil had inhibitory effect on the early germination stage of morning glory seeds; the heavy oil mixed with soil inhibited the germination of Japanese radish, the elongation of leaves germinated from dandelion roots, and the root formation from camellia branches. The inhibitory effect could be explained by two mechanisms: the volatile matter from heavy oil inhibits the growth of plant, and the contamination of the heavy oil changed physical property of soil, and so the plant growth is inhibited.

INTRODUCTION

On January 2, 1997, a Russian tanker "Nakhodka" had an accident at the Sea of Japan. Then, massive amounts of crude oil were spilled from the "Nakhodka", and the contaminations spread as far as coastal region of Japan. The accident raised keen realization of the need for new studies on the effect of heavy oil on plants, because the spilled heavy oil did serious damage to plants growing on the coastal region of Ishikawa Prefecture in Japan.

Two mechanisms of inhibition in plant growth have been considered: (1) the volatile matter from heavy oil inhibits the growth of plant, and (2) the contamination of the heavy oil changed physical property of soil, and so the plant growth is inhibited. However, there are few studies clarifying the effect of heavy oil on the growth of plants. Thus, it is necessary to study the effect of heavy oil on the growth of plants, and such study will help to understand how heavy oil does damage to plants. Dandelion and camellia are distributed widely in Japan including at seacoast. Morning glory and Japanese radish are commonly used as laboratory plants. Hence, the present study aimed to clarify the effects of the heavy oil spilled from "Nakhodka" on the germination of morning glory and Japanese radish seed, on the growth of the roots of dandelion and of the branches of camellia.

1. Effect of volatile matter from heavy oil on germination of seeds of morning glory (*Ipomoea nil* (L.) Roth)

Materials and Methods

In autumn 1996, forty seeds were collected from morning glory growing in Kanazawa, Japan.

To study the effect of the volatile matter from heavy oil, thirty seeds were put in the airtight container filled with the volatile matter from C-heavy oil. The heavy oil was that spilled from a Russian tanker "Nakhodka". Ten seeds were exposed to the volatile matter for 3, 10 and 14 days, respectively. Another ten seeds were not exposed to the volatile matter, and they were used as the control experiment. The seeds were sown in seedbeds containing sand, and were grown in a growth chamber with 25。C day/night temperature for 25 days. To keep the suitable humidity of the germination, they were watered occasionally and the pots were covered with plastic wrap.

To analyze the effect of volatile matter on the germination, we defined the seeds with embryo appearing outside of the seeds as "germinated seeds", and number of germinated seeds was counted on 10th and 25th days after sowing.

Results and Discussion

On the 10 day after the start of experiment, germinated seeds were observed in all four experiments (Table 1-1). Germinated seeds occurred most frequent in control experiment (90%), and the frequency in other three experiments was fairly low (20-30%). Thus, ratio of germinated seeds was quite different between control and heavy oil experiments. On the 25 day from the start of experiment, in contrast, no difference was found among control and heavy oil experiments (Table 1-1). These results may indicate that the volatile matter from the heavy oil has inhibitory effect on the early germination of morning glory seeds.

Table 1-1 Ratio of germinated seeds of morning glory on 10th and 25th day after sowing

Day of exposure to the volatile matter	Ratio of germinated seed % *n* =10	
	the 10th day	the 25th day
0	90	100
3	20	30
10	30	100
14	30	60

2. Effect of heavy oil on germination of seeds of Japanese radish (*Raphanus sativus* L. var. *hortensis* Backer)

Materials and Methods

To study the difference in germination depending on the concentrations of the heavy oil, four groups that consist of 100 seeds were sown in four seedbeds filled with four different media. The media were mixtures of sand and C-heavy oil spilled from a Russian tanker “Nakhodka” at difference concentrations (Table 2-1: seedbeds A, B, C and D). The C-heavy oil contained 47-60% seawater (Matsumoto *et al.*, 1997). Another group of 100 seeds was sown in a seedbed (seed bed X) containing only sand, and it was used as the control experiment. Moreover, to study the difference in germination depending on the type of admixture, the blend oil was used as admixture instead of C-heavy oil (Table 2-1: seedbeds A', B', C' and D'). The blend oil was the mixture of linseed oil and tung oil (3:2) and was oxidized by heating, in order to equalize the viscosity to that of C-heavy oil. The seedbeds were put in a growth chamber with 25。C day/night temperature for 10 days. To keep the suitable humidity of the germination, they were watered occasionally and covered with plastic wrap.

We defined the seeds with the embryo appearing outside of the seeds, as 'germinated seeds', and the number of germinated seeds was counted every day. In non-germinated seeds, two types were distinguished by appearances on the basis of the observation: one is rotten and with tearing seed coat (Type a), and another remains without changes (Type b). Number of the Type a and Type b were measured every day.

Results and Discussion

Germinated seeds were observed in all nine seedbeds (Table 2-1 and Fig. 2-1). The germination pattern was similar between heavy oil seedbeds, seedbed A, B and C, and blend oil seedbeds, seedbed A', B' and C'. In contrast to these seedbeds, the germination was quite different between the seeds of seedbed D and D' to the other seedbeds (Fig. 2-1). The ratio of germinated seeds in seedbed D and D' was fairly lower than those of the other seedbeds including control experiment (Fig. 2-1). Furthermore,

Table 2-1 Ratio of germinated seeds and non-germinated seeds of Japanese radish on the 10th day after the start of experiment

Symbol of seedbed*	Concentration of oil w/v %	Ratio of germinated seeds % n =100	Ratio of non-germinated seeds % n =100	
			Type α **	Type β **
X	0	92	8	0
A	0.4	99	1	0
B	1.3	100	0	0
C	4.0	96	1	3
D	13	77	4	19
A'	0.4	100	0	0
B'	1.3	100	0	0
C'	4.0	96	4	0
D'	13	76	14	10

* Seedbed X contains only sand. Seedbeds A, B, C and D are consisted of the mixture of sand and C-heavy oil. Seedbeds A', B', C' and D' are consisted of the mixture of sand and blend oil.

** Type α is the rotten seed and the seed with tearing seed coat, and Type β is the seed remains without change.

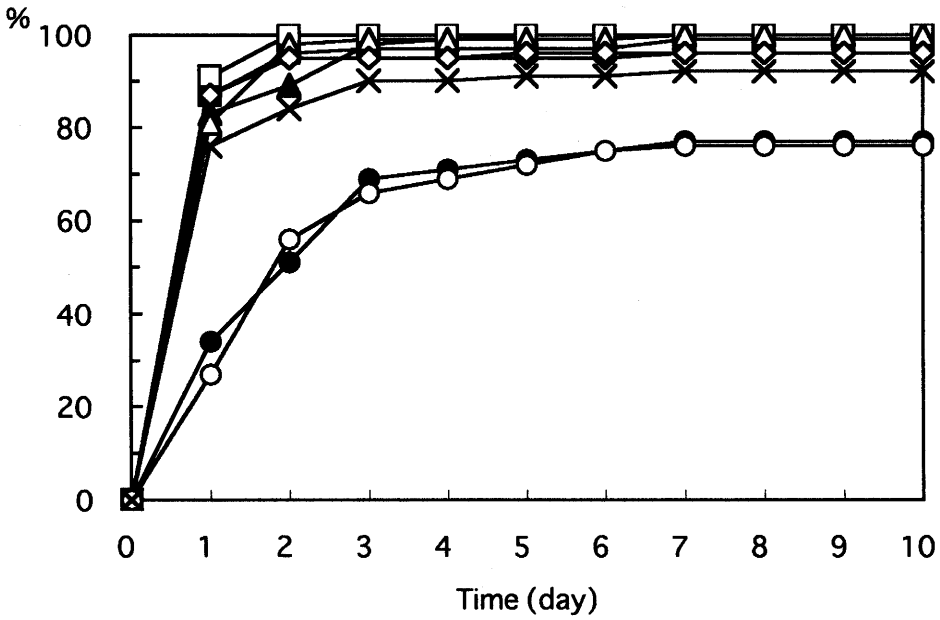

Figure 2-1 Time course of germination of nine different experiments in the initial 10 days after sowing; Seedbed X (crisscross) contains only sand, control experiments. Seedbeds A (closed square), B (closed triangle), C (closed rhombus) and D (closed circle) are consisted of the mixture of sand and C-heavy oil. Seedbeds A' (open square), B' (open triangle), C' (open rhombus) and D' (open circle) are consisted of the mixture of sand and blend oil.

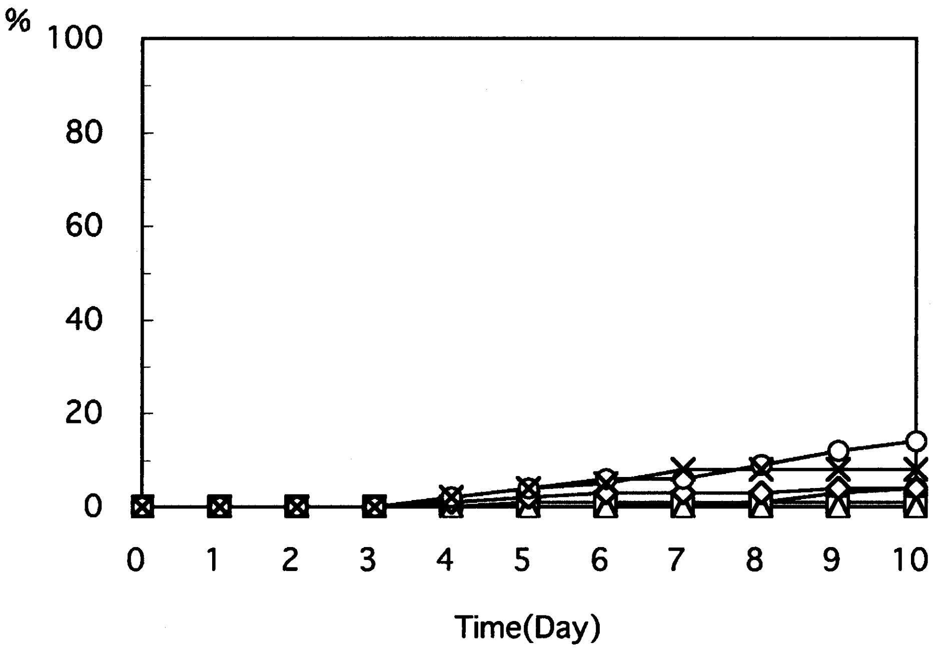

Figure 2-2 Time course of rotten seed appearance of nine different experiments in the initial 10 days after sowing; Seedbed X (crisscross) contains only sand, control experiments. Seedbeds A (closed square), B (closed triangle), C (closed rhombus) and D (closed circle) are consisted of the mixture of sand and C-heavy oil. Seedbeds A' (open square), B' (open triangle), C' (open rhombus) and D' (open circle) are consisted of the mixture of sand and blend oil.

in seedbed D and D', the seeds germinated four days later than the seeds of the other seedbeds (Fig. 2-1). These results suggest that the oil at the highest concentration (13 v/w %) inhibits the germination of Japanese radish.

In non-germinated seeds, the seed started rotting on the 4th day after sowing (Fig. 2-2). Comparing seeds in seedbed D and D', the ratio of rotten seeds was fairly lower in seedbed D than seedbed D' (Fig. 2-2). These results may indicate that the inhibition of seed germination is different in heavy oil from that in blend oil.

3. Effect of heavy oil on germination of roots of dandelion (*Taraxacum officinale* Weber)

Materials and Methods

In May 1997, ten roots of dandelion growing in the Takaramachi campus of Kanazawa University, Kanazawa, Japan were collected.

To study the difference in growth of root depending on the concentrations of the heavy oil, five pieces were dissected from each root in equal quantity, and were grown into five plastic pots (6L) in five different mixtures of sand and C-heavy oil spilled from a "Nakhodka". The C-heavy oil contained 47-60% seawater (Matsumoto *et al.*, 1997). The mixture contained at different concentration of heavy oil: 0, 0.4, 1.3, 4.0 and 13 w/v %. Five experiments were set on the basis of the different concentration of the heavy oil: control experiment, heavy oil I experiment, heavy oil II experiment, heavy oil III experiment and heavy oil IV experiment. These roots were grown in a greenhouse for 88 days.

To analyze the effect of heavy oil on the growth of roots, number of germinated roots, number and the length of leaves germinated from each root were measured at intervals of about seven days.

Results and Discussion

Figure 3-1 shows that ratio of germinated roots in five different experiments. Germinated roots occurred most frequent in control and heavy oil II experiment (60%), and the germination was not observed entirely in roots of heavy oil I experiment (Fig. 3-1). The frequency in other experiments was fairly low (20-40%).

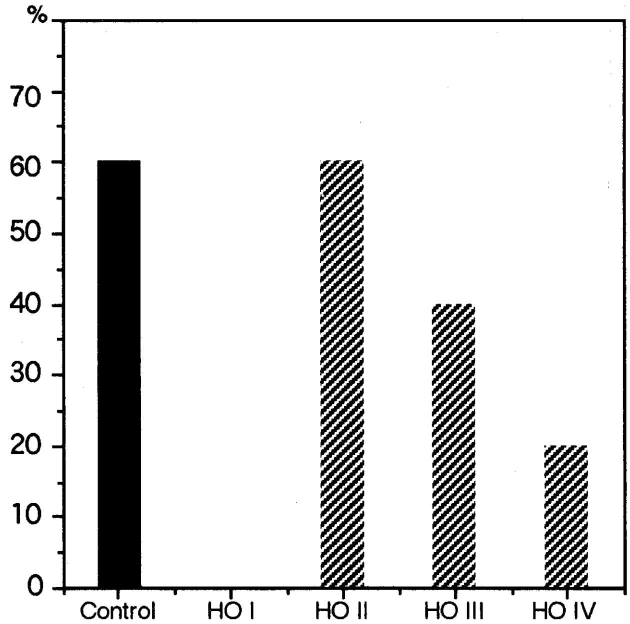

Figure 3-1 Ratio of germinated roots of five different experiments; Control: control experiment; HO I, heavy oil I experiment; HO II, heavy oil II experiment; HOIII, heavy oil III experiment; HO IV, heavy oil IV experiment.

The leaves were observed in roots of control and other three experiments on the 24th day after start of experiment (Fig. 3-2). In heavy oil II experiment, the maximal length of leaves increased from the third through the sixth week, attained 21 mm on the 45th day, and then stabilized from the sixth through the twelfth week with a little variation (16-23 mm). In heavy oil III experiment, the maximal length of leaves increased from the third to the sixth week, attained 12 mm on the 45th day, and then it remained constant from the sixth to the ninth week (Fig. 3-2). Afterwards the length increased again in the tenth week and then it remained constant during eleventh to the twelfth week (Fig. 3-2). In heavy oil IV experiment, the maximal length of leaves gradually increased from the third through the seventh week, attained 4 mm on the 53th day, and then it remained constant from the eighth through the tenth week (Fig. 3-2). Afterwards the length decreased from the eleventh through the twelfth week and leaves were not observed entirely on the 88th day (Fig. 3-2). In control experiment, the maximal length of leaves increased from the third to the sixth week, attained 30 mm on the 45th day, and then it remained constant from the sixth through the ninth week (Fig. 3-2). Afterwards the length increased again in the tenth week, attained 42 mm on the 72th day, and then decreased in the twelfth week (Fig. 3-2). The leaves appear to have more length in control experiment than those in heavy oil experiment (Fig. 3-2). In contrast to the leaf length, the number of leaf varied widely throughout the experiment (Fig. 3-3), and no difference was found among control and heavy oil experiments. These results suggest that the heavy oil inhibits the elongation of leaves germinated from dandelion roots.

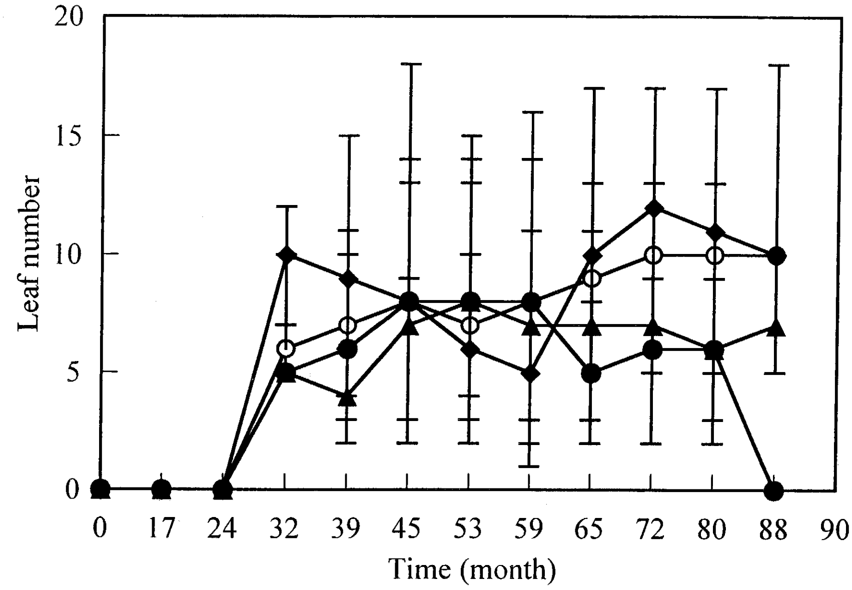

Figure 3-2 Time-dependent changes in maximal length of leaves germinated from roots; control experiment (open square), heavy oil II experiment (closed triangle), heavy oil III experiment (closed rhombus), heavy oil IV experiment (closed circle). Bars indicate deviations from minimum to maximum.

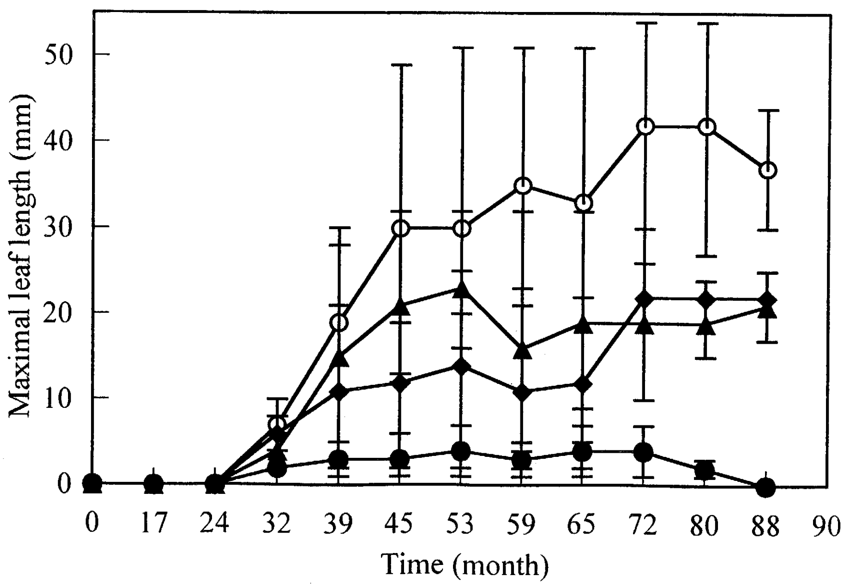

Figure 3-3 Time-dependent changes in the number of leaves germinated from roots; control experiment (open square), heavy oil II experiment (closed triangle), heavy oil III experiment (closed rhombus), heavy oil IV experiment (closed circle). Bars indicate deviations from minimum to maximum.

4. Effect of heavy oil on shooting of branches of camellia (*Camellia japonica* L.)

Materials and Methods

In July 1997, 100 branches of about 10 cm long were collected from aerial parts of Camellia japonica growing at the seacoast of Kurosaki, Kaga, Japan.

To study the difference of growth depending on the concentrations of the heavy oil, 80 branches were grown into eight pots in four different mixtures of sand and C-heavy oil spilled from a Russian tanker "Nakhodka". The C-heavy oil contained 47-60% seawater (Matsumoto *et al.*, 1997). The mixture contained heavy oil at different concentrations: 0, 0.4, 1.3, 4.0 and 13 w/v %. Four experiments were set on the basis of the different concentration of the heavy oil: heavy oil I experiment, heavy oil II experiment, heavy oil III experiment and heavy oil IV experiment (Table 4-1). Another 20 branches were grown in two pots containing only sand, and they were used as control experiment. These branches were grown in the open air from July 1997 through April 1998.

To analyze the effect of heavy oil on the growth of branches, number of healthy branches was counted monthly, and number of branches with roots, diameter of callus, length and number of roots from each branch were measured at October 1997.

Results and Discussion

Figure 4-1 shows changes of ratio of healthy branches for nine months. In heavy oil I experiment, the ratio of healthy branches decreased during summer, to 20% in October 1997, and then it remained constant during winter (Fig. 4-1). Afterwards the ratio decreased again in March 1998 and to 10% in April 1998. In heavy oil II experiment, the ratio decreased during summer and autumn, to 40% in November 1997, and then it remained constant during winter (Fig. 4-1). Afterwards the ratio decreased again in spring 1998 and all branches died in April 1998. In heavy oil III experiment, the ratio of healthy branches decreased during summer and autumn, and to about 10% in November 1997 and then it remained constant during winter (Fig. 4-1). Afterwards the ratio decreased again during February 1998 and all branches died in March 1998. In heavy oil IV experiment, the ratio of healthy branches rapidly decreased during July

1997, and the branches did not survived in August 1997. In control experiment, the ratio of healthy branches gradually decreased during summer and autumn, to 40% in December 1997, and then it remained constant during winter (Fig. 4-1). Afterwards the ratio decreased again during March 1998 and to 30% in April 1998. The branches of heavy oil experiments have decreases during first three months (Fig. 4-1). It means that the heavy oil inhibits rooting of camellia branches.

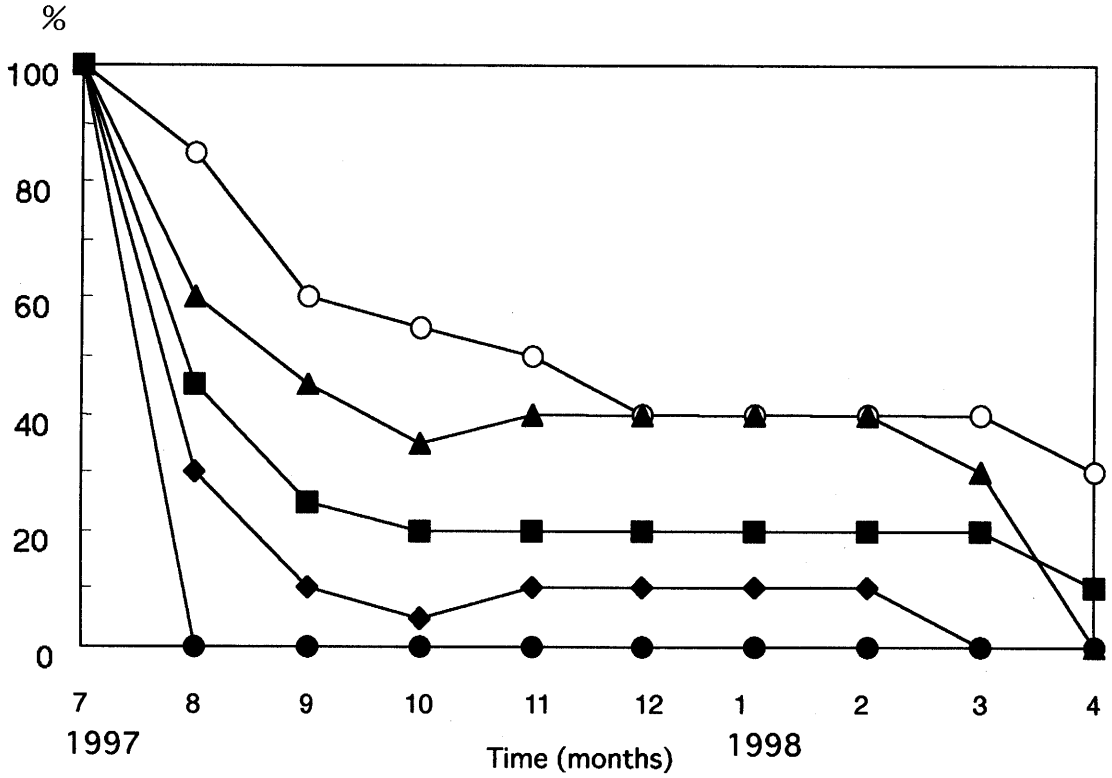

Figure 4-1 Time-dependent changes in ratio of healthy branches; control experiment (open circle), heavy oil I experiment (closed square), heavy oil II experiment (closed triangle), heavy oil III experiment (closed rhombus), and heavy oil IV experiment (closed circle).

The ratio of branch with roots varied from 50% (control experiment) to 0% (heavy oil IV) at three months after start of experiment, and it decreased depending on the heavy oil concentration (Table 4-1). These results suggest that the heavy oil inhibits the growth of roots.

In each branch with roots, diameter of callus ranged from 1 to 3 mm, and it was similar among the branches of four experiments (Table 4-1). However, the length of roots was quite different between control and other experiments (Table 4-1). In control experiment, the length of roots was 1.1 mm, and the length in other four experiments was fairly longer (4.0-6.0 mm) than that of control experiment. In contrast, the branches had more number of root in control experiment (3.0 roots) than those of other four experiments (1.0-1.5 roots). These results may indicate that the effect of the heavy oil is not for the inhibition of the root expansion but for the inhibition of root formation.

Table 4-1 Number of shoot with roots, diameter of callus, length and number of roots in October 1997.

Experiment	Concentration of heavy oil	Number of shoot with roots	Diameter of callus	Length of roots	Number of roots
				Average	Average
	w/v %	n =10	mm	mm	root(s)
Control	0	5	1-3	1.1	3.0
Heavy oil I	0.4	3	1-3	4.0	1.0
Heavy oil II	1.3	2	1-3	4.5	1.5
Heavy oil III	4.0	1	1-3	6.0	1.0
Heavy oil IV	13	0	1-3	-	-

As discussed above, the growth of branches differs significantly among the control experiment and heavy oil experiments. In particular, the difference in root formation from the branches is noteworthy. It has been considered that the contamination of the heavy oil changed physical property of soil, and, as a result, the plant growth is inhibited. In branches of camellia, we found that the formation of roots was inhibited by heavy oil. Therefore, the inhibition of the branch growth must be associated with the change of soil property. It is possible that the promotion of root expansion finding in this experiment is a result of adaptation of plant for water deficiency by the contamination of heavy oil.

CONCLUSION

This study confirmed that the volatile matter from the heavy oil had inhibitory effect on the early germination of morning glory seeds, and that the heavy oil inhibited the germination of Japanese radish, the elongation of leaves germinated from dandelion roots, and the root formation from camellia branches. There are two probable mechanisms for the inhibitory effect of heavy oil on growth of plants: (1) the volatile matter from heavy oil inhibits the growth of plant, and (2) the contamination of the heavy oil changed physical property of soil, and so the plant growth is inhibited. The inhibitory effect can be explained by both of two mechanisms.

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