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THE EFFECTS OF THE CONCENTRATION OF  
POWDERED OATS IN THE MEDIUM  
ON THE GROWTH OF SYMBIOTIC  
SEEDLINGS OF *SPIRANTHES SINENSIS* AMES  
AND *LIPARIS NERVOSA* LINDL.

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Introduction

In a previous paper (6), the authors reported that the germination and seedling growth of *Spiranthes sinensis* Ames were remarkably enhanced in symbiotic culture with a compatible *Rhizoctonia* isolate. During the experiments, we noticed that the growth of seedlings could be retarded at the early stages of development when the population density was high. Thus the effect of population density on the growth of seedlings of *Spiranthes sinensis* and *Liparis nervosa* Lindl. was investigated using an oat medium prepared with a decoction of 25 g/l oat grains. The results, which will be reported elsewhere in details, showed that the effect of population densities on the growth of seedlings was remarkable and the growth was almost proportional to the quantity of oat medium per individual seedling, and that the point where the maximum seedling growth was found was at a very low population density: it was 60 ml/seedling of medium for *S. sinensis* and 30 ml/seedling for *L. nervosa*, respectively.

Since these results suggested that the growth of seedling depended on the nutrient quantity per individual plant, it was considered necessary to investigate further the effect of nutrient concentration in medium on the growth of seedlings. The present paper describes the results of experiments in which the effect of the concentration of powdered oat grains added to the medium instead of a decoction of oat grains on the growth of symbiotic seedlings of *S. sinensis* and *L. nervosa* was investigated.

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Propagation of orchids by symbiotic culture. IV.

An outline of this paper was read at the annual meeting of the Japanese Society for Horticultural Science, Autumn 1987.

### Materials and Methods

The seeds of *S. sinensis* and *L. nervosa* dry stored at 4°C were used. Surface-sterilized seeds were sown on a slope of 30 ml of oat medium in a 30 mm × 150 mm test tube. The oat medium was prepared by a decoction of 25 g/l whole oat grains boiled for 1 hour and filtrated and solidified with 1% agar. One week after seeding a small fungal inoculum was placed onto the upper end of slope. Unless otherwise specified, binucleate *Rhizoctonia* AG-C Iso. No. 706 for *S. sinensis* and *Rhizoctonia repens* Iso. No. 624 for *L. nervosa* were used as the inocula, which were previously confirmed to be most effective. Three weeks after the inoculation of *S. sinensis* or 5 weeks after inoculation of *L. nervosa*, the just sprouting protocorms chosen in such a way as to be equal in size were transplanted onto 30 ml media to be examined in 100 ml Erlenmeyer flasks. All the cultures were kept at about 25°C in a 16-hour light and 8-hour dark regime. The growth of seedlings and dry weight of fungus per flask were measured 10 weeks after inoculation for *S. sinensis* and 16 weeks after inoculation for *L. nervosa*.

**Experiment 1.** The above mentioned oat medium, the oat medium of double strength prepared in the same manner and a powdered oat medium prepared by boiling 25 g of ball-milled fine powder of oat grains in 1 litre of distilled water for 30 minutes were compared. Three symbiotic protocorms of *S. sinensis* were transplanted into a flask. The germination percentages were obtained by counting germinated seeds and the total number of seeds directly sown on the slope of media to be examined in test tubes 7 weeks after inoculation.

**Experiment 2.** Three symbiotic protocorms of *S. sinensis* or *L. nervosa* per flask were transplanted onto the media containing 5, 10, 20, 30, 40 and 50 g/l of powdered oat, respectively.

**Experiment 3.** Varying numbers of symbiotic protocorms of *S. sinensis* were transplanted onto the media of 3 different concentrations of powdered oats of 5, 10 and 20 g/l to obtain quantities of oats per seedling 15, 30 and 150 mg; and in another series, of 10, 20 and 30 g/l of oats to obtain quantities of oat per seedling 50, 100, 200 and 300 mg, respectively.

**Experiment 4.** Three symbiotic protocorms of *S. sinensis* grown by inoculating with Iso. No. 706 or No. 624 were transplanted onto media containing 5, 10, 20 and 30 g/l powdered oats, respectively.

### Results

**Experiment 1.** The result of the comparison between oat media and

TABLE 1. Effect of culture media on the symbiotic germination and growth of *Spiranthes sinensis* 10 weeks after inoculation with *Rhizoctonia*

Medium		Germination percentage (%)	Proto-corm diameter (mm)	Leaf length (mm)	Root length (mm)	Fresh weight of seedling (mg)	Dry weight of mycelia (mg/flask)
Oat medium	25 g/l	60.3 <sup>b</sup>	2.78 <sup>cd</sup>	12.7 <sup>bc</sup>	9.3 <sup>a</sup>	47.3 <sup>c</sup>	17.8 <sup>c</sup>
Oat medium	50 g/l	62.4 <sup>b</sup>	3.58 <sup>b</sup>	17.0 <sup>b</sup>	10.1 <sup>a</sup>	78.4 <sup>b</sup>	25.9 <sup>bc</sup>
Powdered oat medium	25 g/l	72.0 <sup>a</sup>	5.73 <sup>a</sup>	26.8 <sup>a</sup>	9.5 <sup>a</sup>	235.1 <sup>a</sup>	75.2 <sup>a</sup>

Mean separation within columns by Duncan's multiple range test at 5% level.

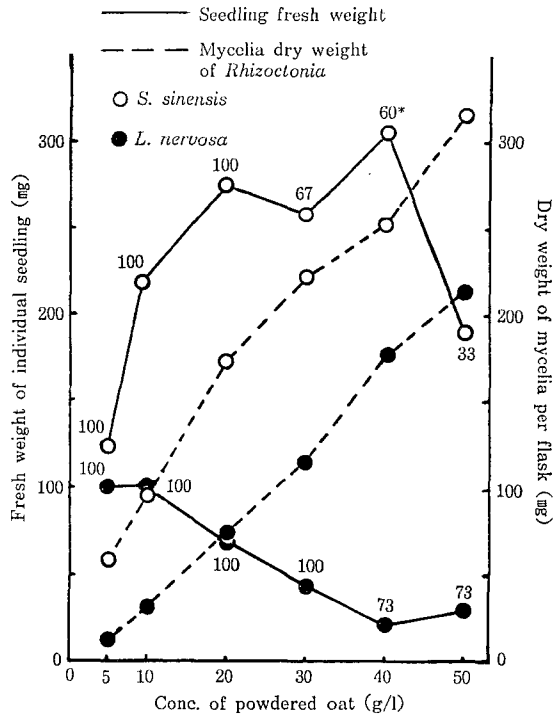


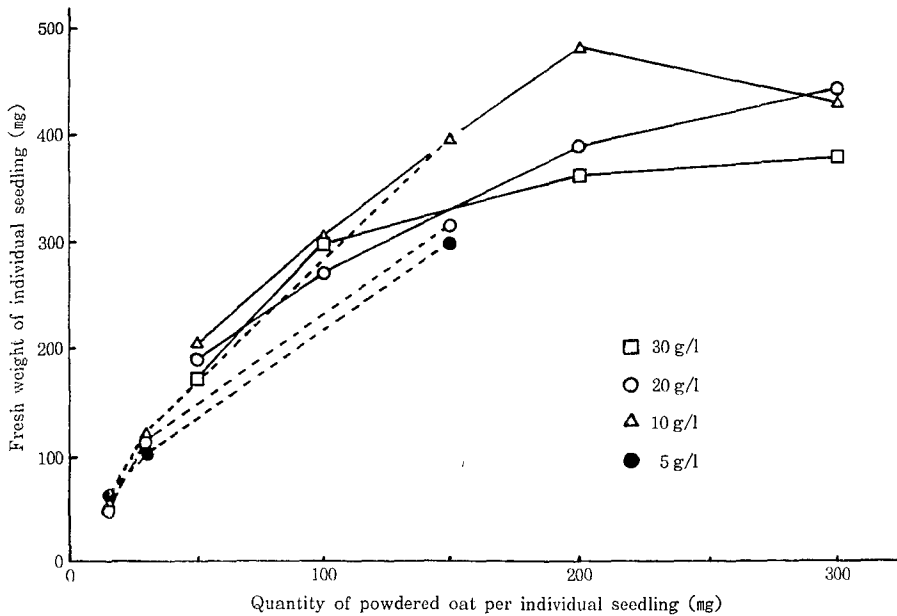
Fig. 1. Effect of the concentration of powdered oat in medium on the growth of symbiotic seedlings of *Spiranthes sinensis* and *Liparis nervosa*. (Three seedlings were planted on 30 ml medium; *S. sinensis*, 10 weeks after inoculation; *L. nervosa*, 16 weeks after inoculation; )

\* The figures on the lines indicate survival rate of each treatment.

powdered oat medium is shown in Table 1. Although the germination percentage was highest by powdered oat medium, the difference among these 3 treatments was not so remarkable. But the growth of seedlings, especially the fresh weight of seedlings differed markedly among the 3 treatments: the growth of seedlings was additionally stimulated by increasing the concentration of oat decoction and it was greatly enhanced with powdered oat medium than oat decoction media. The dry weight of fungus showed a similar tendency.

**Experiment 2.** The result of experiment in which the effect of concentrations of powdered oat was examined by transplanting 3 protocorms of *S. sinensis* or *L. nervosa* into a flask is summarized in Fig. 1.

In *S. sinensis* the dry weight of fungus increased continuously with the increase of oat concentration, but the fresh weight of seedling reached its maximum at a concentration of 20 g/l. At concentrations 30 g/l and over, some protocorms died at the early stage of growth resulting in a lowering of survival rate of seedlings; at a concentration of 50 g/l, even a decrease of fresh weight of seedling occurred. In *L. nervosa* the fresh weight of seedling reached its maximum at a concentration of 10 g/l. At concentrations

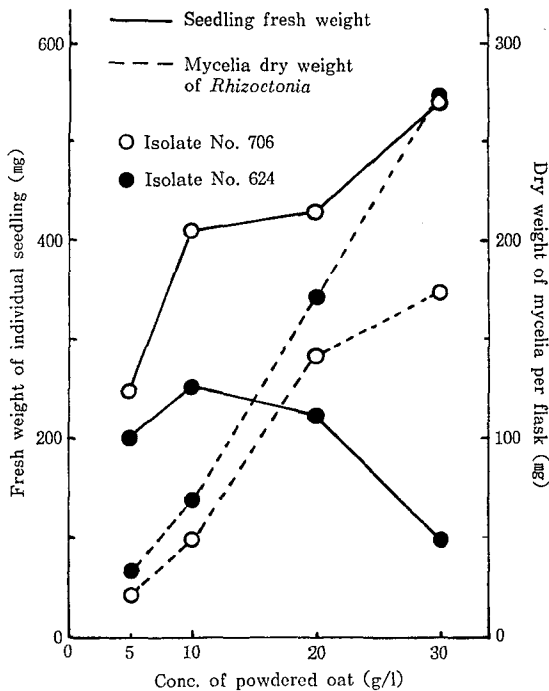


**Fig. 2.** Effect of the quantity of powdered oat per individual seedling on the growth of seedlings of *Spiranthes sinensis* under different concentrations of oat in the medium.

of 20 g/l and over, the fresh weight of seedling decreased with the increase of concentration although the dry weight of fungus increased continuously, and at a concentration of 40 g/l or over the survival rate of seedlings was lowered to about 70%.

**Experiment 3.** Since the response of seedlings to the concentration of powdered oats under a constant population density was clarified in the above experiment, the response of seedling growth to the simultaneous changes of both the concentration of powdered oats and population density was examined using the quantity of powdered oats per individual seedling as a parameter. the result is summarized in Fig. 2 regarding the fresh weight of seedlings.

In general, the fresh weight of seedling increased linearly with the increase of the quantity of powdered oats per individual seedling regardless of the concentration of oats in the medium and the growth reached its maximum at about 200 mg/seedling. Namely, the growth of seedling depended on the quantity of powdered oats per individual seedling rather than



**Fig. 3.** Differences in the effect of concentration of oat in medium on the growth of symbiotic seedlings of *Spiranthes sinensis* between 2 *Rhizoctonia* isolates 10 weeks after inoculation.

on the concentration of oats in the medium itself. However, there was a tendency that the fresh weight of seedling showed a slight decrease at the concentration of 20 g/l or higher in the range of quantity per individual seedling of 200 mg and more.

**Experiment 4.** In Experiment 2, a clear difference in the oat concentrations, at which the growth of seedlings reached their maximum and began to be retarded, was found between *S. sinensis* and *L. nervosa*. In order to decide whether this was due to the difference of orchids or that of the symbionts, the two fungal isolates the same as in Experiment 2, No. 706 and No. 624, were inoculated to *S. sinensis* seeds on which these 2 isolates were known to be effective.

As shown in Fig. 3, the fresh weight of seedlings inoculated with No. 706 increased with the increase of oat concentration up to 30 g/l in the same manner as in the other experiments described, while the fresh weight of those inoculated with No. 624 reached its maximum at 10 g/l and decreased rapidly thereafter. But the dry weight of fungus per flask increased throughout the experiment in both isolates. Thus the same *S. sinensis* responded differently to the oat concentrations according to the associated fungi and responded in the similar manner as *L. nervosa* when it was infected with identical fungus.

### Discussion

The fact that a better growth of seedlings was obtained in the powdered oat medium than in the oat decoction media suggests that the difference is due to the constituents of oat grains available for both fungus and orchids, which are not extracted by boiling water. This means that the symbiotic seedlings require both constituents of oat grains extractable and inextractable by hot water to obtain vigorous growth. HADLEY (3) reported that cellulose added to the medium was an excellent carbon source for the seedling growth of *Dactylorhiza purpurella* in symbiotic culture. It may be greatly possible that the principal ingredients of this effective insoluble substances are polysaccharides, which should be investigated further.

CLEMENTS *et al.* (2) also used a powdered oat medium for the symbiotic cultures of terrestrial orchids although a yeast extract was supplemented. However, no study on the quantity effect of nutrients in culturing media could be found. Thus the concentration effect of powdered oat medium was investigated in Experiment 2. The result demonstrated that the maximum growth of seedlings was obtained at the oat concentrations of 20 g/l for *S. sinensis* and 10 g/l for *L. nervosa*, and both growth and survival rate of

seedlings were lowered at concentrations higher than those. On the other hand, the dry weight of fungus per flask continued to increase. CLEMENTS and ELLYARD (1) pointed out that an exceptionally vigorous fungus in a high nutrient medium would overwhelm the orchid seed resulting in little or no germination. But no paper deals with this question quantitatively.

The present results may indicate that under a high nutrient condition fungus can exert a parasitic effect on seedlings. HARVAIS and HADLEY (5) pointed out that a depletion of nutrient in the medium destroyed the balance between orchid and fungus resulting in an increase of parasitism of the fungus. HADLEY (3) also demonstrated that supplementing the carbon source such as sugars or cellulose was effective in controlling the parasitism of fungus due to nutrient depletion. However, no evidence to support such a depletion effect could be found in the present investigation.

Since in the above mentioned experiment both the orchid species and fungal isolates were different, it was difficult to decide whether the difference in the concentrations of oats, at which the parasitism of fungus appeared, resulted from the difference of orchids or from that of fungi. Accordingly the difference in the response to the concentration of powdered oats in the medium between the seedlings of *S. sinensis* inoculated with Iso. No. 706 and that inoculated Iso. No. 624 was examined. The seedlings of *S. sinensis* inoculated with No. 624 responded to the oat concentrations in a similar manner to those of *L. nervosa*. This suggested that the difference in the effect of nutrient supply on the parasitism mainly depended on the characteristics of the fungus in question. It has been known that there is a wide range of variation from symbiosis to parasitism in the relation between and orchid species and various fungal isolates (4, 7). Even in an isolate of a fungal species or of a group of fungal isolates the parasitism on orchids is obviously affected by the nutrient condition for the fungus. In the screening practice for the symbiotic capacity of fungi, this must be taken into consideration.

As already described, in the oat decoction medium, the effect of population density on seedling growth was primarily dependent upon the quantity of medium or nutrient per individual plant. In order to investigate whether this relation is also true when both the concentrations of powdered oats in the medium and population density varied, it was examined at various combinations of concentrations and population densities. The result indicated that it can also be applied to the powdered oat medium and that the maximal seedling growth was obtained at 200 mg/seedling of oat regardless of the oat concentrations in the medium. This is equivalent to the population density



of 4.5 plants on 30 ml medium of 30 g/l of concentration or 1.5 plants on that of 10 g/l of concentration, respectively. In experiment 2, in which the effect of oat concentration was examined by planting 3 protocorms on 30 ml medium, the oat concentration of 20 g/l was equivalent to 200 mg per seedling. These results coincided with each other. Since in the case of oat decoction medium the maximum seedling growth was obtained at the low population density of 60 ml medium per seedling, the result means that the growing space may be utilized more efficiently by using the powdered oat medium.

However, the absolute fresh weight of seedlings was not completely independent from the concentration of powdered oat in the medium, but it showed a tendency that the fresh weight of seedlings decreased at higher concentration even when the quantities of oats per seedling were equal. This fact indicates that there is a limit in increasing the efficiency of propagation by increasing both the concentration of oats and population density. Thus it will be necessary to investigate the possibility of maintaining the seedling growth at higher densities by using liquid medium of lower oat concentration and by renewing it successively.

### Summary

The effect of concentration of powdered oats in the medium on the growth of seedlings was investigated using the protocorms raised by inoculating binucleate *Rhizoctonia* AG-C Iso. No. 706 on *Spiranthes sinensis* seeds and *Rhizoctonia repens* Iso. No. 624 on *Liparis nervosa* seeds. The powdered oat medium which was prepared by adding ball-milled fine powder of whole oat grains directly to the medium showed much higher stimulating effect on the seedling growth than the oat decoction medium. The comparison among various combinations of concentrations of powdered oats and population densities in *S. sinensis* showed that the growth of seedlings increased with the increase of the quantity of powdered oats in the medium per individual seedling regardless of the concentration of oats in the medium. It attained to the maximum growth at about 200 mg/seedling of oats. Thus the growth of seedlings was considered to be primarily dependent upon the quantity of powdered oat per individual seedling. Under the condition of higher oat concentration beyond this limit, the growth of seedling was retarded and the survival rate of seedlings was lowered with the increase of oat concentration in medium. This was considered to be due to the increase of parasitism of fungus. The threshold quantity of oat per individual seedling for *L. nervosa* was 100 mg which was much less than for *S. sinensis*. This difference in parasitism could be attributed to the difference

of the fungal isolates associated with the orchids.

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