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Population development of the symphylan, Scutigerella immaculata (Newport), was studied in laboratory cultures. Also symphylan injury to greenhouse plants was investigated. Plastic polyethylene containers partly filled with damp ground hemlock bark were used to culture symphylan in the laboratory. Fresh lettuce food was supplied to the cultures weekly.

Factors which appreciably affected symphylan population development included: moisture content of the bark medium, culture temperature, kind of food supplied and population density. Results indicated that the optimum conditions for symphylan culture population development were: moisture--30%, temperature--24°C, fresh lettuce for food and adequate space in the culture container.

Greenhouse experiments included the demonstration of plant

injury by symphylans and the population levels involved. Plant injury was determined by the dry weight of the above ground portion of the bean or corn plant. Planting delays of one to two weeks after symphylan infestation did not seem to influence the degree of injury. When bean or corn plants were given a good start before symphylan introduction little or no injury resulted. Conditions for the development of symphylan cultures for use in laboratory and greenhouse experiments were defined by the conclusions of this study.

Population Development of *Scutigerella immaculata* (Newport), in Laboratory and Greenhouse Cultures

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INTRODUCTION

An investigation of the population development of the garden symphylan *Scutigerella immaculata* was initiated to set forth conditions under which populations and their effect on plants could be examined more thoroughly in laboratory and greenhouse situations.

Injury to plants by the garden symphylan is brought about by feeding on root hairs or the new root shoot as it leaves the germinating seed. This feeding results in stunting the plant or killing it. Symphylans also will feed on plant leaves or ripened berries lying on the soil surface. Secondary invasion by fungi and bacteria may then destroy the plant tissue. Although chemical control has kept this pest in check in some situations, much work remains to be done towards this end. Effort has gone into the study of this soil arthropod and its control over a period of many years with varying degrees of success.

Although the type species for *Scutigerella immaculata* was described by Newport in 1845, the first indication of economic importance in California asparagus fields is noted by Woodworth (cited in Michelbacher, 1938, p. 105). The first reported occurrences in Oregon were reported by Lovett (cited in Michelbacher, 1938, p. 63)

on many kinds of sprouting seeds, especially peas and beans. Injury by this organism in the Eastern United States is confined more to greenhouse crops and on the Pacific Coast the garden symphylan is primarily injurious to outdoor crops including vegetables, ornamentals, field crops, and small fruits. The geographical distribution of the garden symphylan is almost cosmopolitan. It has been recorded in at least 30 states and in the District of Columbia.

Because of the erratic and unpredictable nature of natural soil populations a need for the study of laboratory and greenhouse cultures is imperative.

This work deals with culture techniques and with factors affecting population development in laboratory and greenhouse cultures. The subjects of plant damage in relation to numbers of symphylans and plant resistance to symphylan attack have been dealt with.

It is hoped that the suggestions made from the results of this investigation will aid future workers by allowing them to culture and maintain populations of symphylans with which to experiment.

LITERATURE REVIEW

Various references relating to symphylan population development and related environmental conditions have been mentioned in earlier papers.

F. H. Wymore (1924) noted factors affecting symphylan distribution. He stated that "the chief factors determining their distribution seem to be an atmosphere of great humidity, a moderate temperature, comparative darkness, and an undisturbed at least uncultivated soil." Wymore (1924) also noted that the vertical migration of natural soil populations was primarily dependent on the soil moisture content, and that symphylans seem to thrive on an abundance of succulent plant food but they can exist very well for several months where no succulent material is present. This fact was dealt with in greater detail in a later paper by Shanks (1966).

It was noted by Filinger (1928) that symphylans do not seem to be able to make their own burrows through the soil. Instead they have to depend upon other means for passage ways such as earthworm burrows, natural soil cracks and crevices or cavities left by decaying roots.

F. H. Wymore (1931) again mentioned that symphylans prefer a cool moist earth environment and he also made reference to a simple population holding method by stating that symphylans

remained alive for several months in a can of earth at room temperature. G. A. Filinger (1931) made reference to temperature affecting the vertical distribution of symphylans. He noted that the optimum temperature was 65°F and when the soil temperature reached 70°-75°F at the surface the symphylans would follow their runways down to the subsoil at a depth of two to three feet.

Included in the 1931 paper by Filinger was the first description of a technique for culturing the symphylan under laboratory conditions. Filinger's description of this technique is as follows.

> The rearing of garden symphylans was done in stender dishes into which was poured a muck plate, made by mixing 10 parts of plaster of paris and 3 parts of finely ground muck. This muck plate was kept moist and the symphylans lived on the surface. Lettuce leaves were laid on the surface to afford food and hiding places. Where large dishes were used and large portions of leaves introduced, it was necessary to make grooves in the muck plate to give the symphylans a chance to escape from the weight of the leaf and from being caught and smothered when the leaf decomposed and lay flat on the surface of the plate.

Michelbacher (1938) modified Filinger's original muck plate by adding one part animal charcoal to darken the mixture thus making the symphylans and molted skins easier to see. The muck plates mentioned by Filinger and Michelbacher were used for observing symphylans under varying conditions. It was Michelbacher (1938) who originally devised a mass culture technique for symphylans. These cultures consisted of silty clay subsoil placed in battery jars

and covered with a pane of glass. A lettuce leaf was placed over the surface and replenished as needed. The transfer of symphylans was accomplished by lifting out the lettuce leaf and jarring it over a glass container to free the symphylans from the leaf. The symphylans were then manipulated with a camel's hair brush.

Michelbacher (1938) investigated symphylan temperature tolerances in greater detail than did Filinger. Michelbacher found that molting frequency was influenced by temperature and was most frequent at 28°C. He also noted that 37°C was lethal to symphylans, but they could survive 2°C for long periods. Michelbacher also demonstrated that symphylans which were cold-conditioned at $4\frac{1}{2}$°C may withstand 0°C for months. He reported the optimum temperature range for symphylan survival to be between 12°C and 20°C.

A later paper by Michelbacher (1949) related several observations relevant to the culturing of symphylans in the laboratory and greenhouse. He noted as did Filinger earlier, that symphylans apparently were unable to construct their own runways in the soil. Soil texture and structure were therefore an important influence on symphylan distribution and abundance in specific areas. Michelbacher (1949) found, as did Wymore (1924), that symphylans prefer succulent vegetation for food. They do, however, feed on lower plant life and soil microflora (Michelbacher, 1949) which would probably account for their living in the absence of apparent food.

Michelbacher (1949) also pointed out that moisture is one of the most important environmental factors affecting symphylans because these soil organisms have relatively soft bodies and are unable to withstand desiccation. Symphylans therefore do best in moist soils where humidity is close to 100%. Michelbacher (1949) also noticed that Scutigerella immaculata has feeding and non-feeding phases which are associated with the molt. Waterhouse (1963) also observed these feeding and non-feeding periods. Michelbacher found that the non-feeding phase occurs during the critical premolting and molting period. Symphylans thus consume most of their food prior to the premolting period.

More recent work would include that presented by M. G. Savos (1958) at Oregon State College. Savos used two pound coffee tins for stock symphylans cultures during the course of his investigations. The symphylans plus the soil in which they were collected were placed into the cans. Water was added as needed and the culture were supplied with lettuce leaves for food. Savos also used muck plates similar to those used by Michelbacher but modified them by the incorporation of sand. Two ounce salve tins were used for culture containers. Edwards (personal communication) maintained stock cultures in small wide mouth jars with screw caps. The medium consisted of fine soil particles and plaster of paris. A glass tube was embedded into the medium through which water was added to keep the surface

moist. Waterhouse (1963) used quart jars and large cans filled with earth for storage cultures. Fresh lettuce leaves were supplied for food. He also used muck plates similar to Michelbacher's (1938) for laboratory rearing cultures. Shanks (1966) reared symphylans in wide-mouth quart glass jars. One inch of "pea" gravel was placed in the bottom. Silt loam was used to fill the jar and a glass tube was inserted down to the gravel. Water was added through the glass tube to moisten the medium.

A very thorough and complete study of the ecology of the symphyla was made by C. A. Edwards and published in three parts. Part I (1958) dealt with soil characteristics and sampling techniques. Part II (1959) dealt with the seasonal soil migrations including vertical distribution and the attraction of symphylans to growing plants. Part III (1961) dealt with the factors controlling soil distributions and was most relevant to the work presented in this paper. Included in Part III were studies involving moisture and temperature influences on symphylan soil populations. Edwards (1961) found that symphylans apparently have a positive reaction to high soil moisture content in both a horizontal and vertical direction. He also demonstrated that symphylans prefer a temperature range of 15°-21°C. It was pointed out that temperature and moisture factors are intimately linked in influencing symphylan distribution in the soil. It is difficult to assess the relative importance of each factor but it

seems likely that temperature plays a major part in determining the vertical distribution of symphylan populations. Studies involving relative humidity (RH) and symphylan mortality by Waterhouse (1963) revealed that above 88% RH there was a marked increase in longevity. Symphylans in cultures held below 88% RH migrated to the bottom of the culture medium and did not feed. A RH value below 98% was unsuitable for culturing symphylans. Temperature studies by Waterhouse (1963) indicate that an optimum temperature range lies between 55° and 70°F on the basis of symphylan activity and mortality.

Factors affecting symphylan reproduction were investigated by Shanks (1966). He found that symphylans reproduced only in cultures which had fresh vegetable material for food. Shanks found also that non-living organic matter or microorganisms do not directly influence the buildup of large symphylan infestations by serving as food. Further results from Shanks (1966) indicate that light is not detrimental to symphylan cultures. Shanks also showed that reproduction apparently slows as competition increases in the confines of the culture container.

Scutigerella immaculata is susceptible to attack by nematodes as reported by K. G. Swenson (1966). It was demonstrated that the DD-136 nematode could invade and kill active symphylans.

Another natural enemy of the symphylan, the entomogenous

Phycomycete, Entomophthora coronata (Costantin), was reported on by Stimmann (1968). This fungus will parasitize symphylans and can retain its pathogenic viability for at least 30 days (Stimmann, 1968) on a non-nutrive substrate. These natural enemies of the symphylan could be very detrimental to laboratory and greenhouse cultures.

MATERIALS AND METHODS

Materials

Maintenance of Stock Cultures

Stock cultures of Scutigerella immaculata were started in 1966 from field collections made in the Corvallis area. The cultures were maintained in plastic buckets and were provided weekly with fresh lettuce.

Three different sizes of plastic containers with moisture-tight covers were used. The largest container measured $10\frac{1}{2} \times 7\frac{1}{2} \times 2\frac{1}{2}$ inches. The dimensions of the smallest container (one pint) were $4 \times 4 \times 3$ inches. A container of intermediate size measuring $8 \times 5\ 3/4 \times 3$ inches was used only for an experiment comparing the suitability of roots of different plants as food. The pint containers were utilized for an experiment on the effects of crowding. The large containers were used for the remainder of the culture investigations.

Ground hemlock bark was used as the culture medium for both the stock cultures and the experimental containers. The ground hemlock bark used for the culture medium was obtained from local sources and thoroughly air-dried before being weighed and used in the culture containers.

Four temperature control cabinets[1] provided the necessary controlled temperature conditions for the laboratory culture experiments.

Greenhouse Experiments

Plastic pots, $5\frac{1}{2}$ inches in diameter, were used for the greenhouse experiments. Precautions had to be taken to prevent the escape of symphylans from infested pots. The plastic pots lent themselves quite easily for this modification. A fine, 50 mesh, brass screen patch was heated with a soldering iron and pressed into the plastic to cover the drain holes in the bottom of the pot. As a further precaution a coating of a sticky substance, Deadline,[2] was applied to the top rim of the pot, although no symphylans were observed caught in this fashion.

Methods

Experimental Procedure

The ground hemlock bark medium, which was used throughout the laboratory cultures, was prepared in the polyethylene containers

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[1] Lab-Line Instruments, Inc., Melrose Park, Ill.

[2] Chevron Chemical Co., Ortho Div., San Francisco, Calif.

as prescribed by the particular experiment. Previous results (Swenson, Unpublished) showed a significant difference in symphylan populations between the moisture levels of 40% and 50%. On this basis, all of the laboratory experiments except the moisture percentage trial were set up and maintained at a 40% moisture level by weight. The symphylans were counted and placed into the trays from the stock cultures. A small spatula and hand counter were used to transfer and count the symphylans respectively.

A test was set up to determine the efficiency of this transfer method. Five large culture trays were each infested with 100 symphylans. The difference between the initial population and the number of symphylans present after two weeks averaged over 24 per container. Therefore it was expected that approximately a 25% mortality would be suffered from this transfer method. All of the experiments were designed on the randomized block concept. The treatments in any particular experiment were dealt with in a random order.

Adult symphylans were used in all of the laboratory culture experiments and greenhouse investigations except one experiment in which young immature symphylans were an experimental variable. A symphylan is sexually mature in the sixth instar but continues to molt throughout its life without appreciably increasing in size (Waterhouse, 1963). It is difficult to distinguish between instars

without careful examination with a binoucular scope.

Fresh food was supplied to each container weekly. Every two weeks the containers were weighed to curb a possible weight gain due to moisture adsorption by the culture medium. If a weight gain was noted, dry paper towels were placed on the surface of the medium to absorb moisture. The towels were renewed weekly as needed. Most of the laboratory experiments were carried for six months which allowed from two to three symphylan generations to develop.

Assessment of Plant Injury

Accurate evaluation of symphylan injured greenhouse plants required a standardized procedure. The dry weights from the above ground portion of the plants were used for this evaluation. The values obtained from weighing the above ground portion of the plant will hereafter be referred to as the top dry weight. The plant root system was not included in the weight analysis because the incorporation of the sphagnum moss with the greenhouse soil made complete recovery of the roots very difficult.

At the termination of a greenhouse experiment the plant stems were cut at the soil level. The plant material was then tagged for identification and thoroughly dryed at 50° C for four days. Dry weight values were then recorded from a Mettler electric balance. Symphylans recovered from the pots were also counted and recorded.

DESCRIPTION OF EXPERIMENTS AND RESULTS

Population Development in Laboratory Cultures

Effect of Various Treatments of the Culture Medium

Earlier experimental results (Swenson, Unpublished) showed a lower symphylan population when boiled bark was used for the culture medium than when unboiled bark was used. This finding suggested that the adverse effects of boiling the bark might be attributed to elimination of microorganisms beneficial to a symphylan population, or to the release of toxic chemicals from the bark. An experiment was designed to investigate these possibilities.

Three culture medium treatments with five replications each were arranged as follows: 1) untreated bark, 2) boiled bark, and 3) autoclaved bark. The boiled bark treatment was boiled for 15 minutes then strained and spread over the surface of a bench to dry. The autoclaved bark was treated for two hours at 110°C. The latter two treatments were designed to separate the effects of an initial sterile medium with released bark compounds, including tannins, and an initial sterile medium without the released bark compounds represented by the boiled bark treatment and the autoclaved bark treatment respectively. Each of the 15 containers in this experiment were infested with 100 symphylans. These cultures were kept at

15° C for 26 weeks.

The pH value of the bark medium in each treatment was recorded after the experiment was in progress for 12 weeks. The average of three pH readings all taken within 24 hours, follows; untreated bark 4.55, boiled bark 4.34, and autoclaved bark 4.29. The differences among the substrate pH values were negligible.

At the termination of the experiment an analysis of variance was made of the number of symphylans. The results as indicated in Table 1, showed no significant differences among the treatments.

Table 1. Development of symphylan populations in untreated, boiled and autoclaved bark culture medium.

Replicates	Number of symphylans per medium treatment		
	Untreated	Boiled	Autoclaved
1	839	181	104
2	440	276	145
3	192	338	98
4	202	119	107
5	99	256	153
Total	1772	1170	607

Summary of Main Factor

Source of variation	Degrees of freedom	F	Significance
Replicates	4	.76	NS
Culture medium treatments	2	1.93	NS
Error	8		

Effect of Moisture Content of the Culture Medium

This experiment was designed to determine the effect of varying amounts of substrate moisture on the development of symphylan populations. Three moisture levels 30, 35 and 40% with five replications each were included in the experiment. The moisture variations were calculated on a weight basis, thus a 30% moisture level would represent a culture medium which would consist of 30% water and 70% bark by weight. The correct moisture level was maintained by weighing the culture trays every two weeks. An initial 50 symphylans were introduced into each tray. The experiment was held at 15° C for 26 weeks before an evaluation was made.

The number of symphylans noted at the termination of the experiment showed an inverse correlation to the percent moisture of the bark culture medium. The results of the analysis of variance showed a highly significant treatment difference as shown in Table 2. The recovery data verified this by showing progressively greater totals at lower moisture percents.

Table 2. Effect of moisture content of the bark medium on symphylan population development.

Replicates	Number of symphylans per moisture treatment		
	30%	35%	40%
1	376	355	279
2	303	210	288
3	291	137	192
4	468	259	146
5	476	276	219
Total	1914	1237	1124

Summary of Main Factor

Source of Variation	Degree of freedom	F	Significance
Replicates	4	1.63	NS
Moisture treatments	2	7.46	S-.05
Error	8		

Effect of Temperature on Cultures

The purpose of this experiment was to determine the effects of temperature on the population development of laboratory cultures.

Large polyethylene containers were used and the culture medium was maintained at a 40% moisture level. Four treatments with five replications each, were set up. An initial 50 symphylans were introduced onto the culture medium of each of 20 trays. Four constant temperature cabinets were utilized to retain the four treatments. The cabinets were set at 15°C, 18°C, 21°C and 24°C.

The experiment was terminated after 24 weeks. The number of symphylans indicated a direct correlation between temperature

and population development. An analysis of variance showed a highly significant treatment difference. The results are presented in Table 3.

Table 3. Effect of culture temperatures on symphylan population development.

Replicates	Number of symphylans per treatment			
	15°C	18°C	21°C	24°C
1	678	532	760	1004
2	298	552	927	1220
3	598	788	1015	800
4	274	591	543	1128
5	191	203	613	812
Total	2039	2666	3858	4964

Summary of Main Factor

Source of variation	Degree of freedom	F	Significance
Replicates	4	2.53	NS
Temperature treatments	3	11.20	S-.001
Error	12		

Effect of Different Kinds of Food and of Symphylan Age on Cultures

This experiment was designed to determine the effects of food type and initial symphylan size on the development of symphylan populations. Symphylan size was distinguished by the length of the organism. Those termed immature were approximately one-half the length of the adults. The four treatments were made up as follows: 1) immature symphylans with carrot for food, 2) immature symphylans with lettuce for food, 3) mature symphylans with carrot for food

and 4) mature symphylans with lettuce for food. Each of these four treatments were replicated five times. The initial number of symphylans introduced per container was 50. This experiment was maintained at 18°C for 26 weeks. Large trays with a 40% moisture culture medium were used. Food was replenished weekly and culture weights checked every two weeks.

An analysis of variance was applied to the numbers of symphylans observed at the end of 26 weeks. Treatment totals showed that the treatment containing mature symphylans with lettuce for food was greater than the other treatment totals. The results are presented in Table 4.

Table 4. Effect of carrot and lettuce food and symphylan maturity on culture population development

Replicates	Number of symphylans per treatment			
	Initial immature		Initial mature	
	Carrot	Lettuce	Carrot	Lettuce
1	42	94	143	339
2	62	209	76	329
3	42	0	38	45
4	113	54	103	73
5	53	66	103	69
Total	312	423	463	855

Summary of Main Factors

Main Factor	Number of symphylans	Significance
Symphylans		NS
Immature	735	
Mature	1318	
Food		NS
Carrot	775	
Lettuce	1287	

Effect of Different Kinds of Plant Roots

Two experiments were conducted to determine if symphylans would increase their numbers at a more rapid rate by feeding on one kind of root foot as compared to another population exposed to another kind of root food.

The first experiment was set up in large culture trays with the bark substrate at the standard 40% moisture level. Two treatments were involved and were replicated five times. In one treatment the roots of the Perfected Wales pea, Pisum sativum Linn. were used and in the other treatment the roots of the Ruby Dwarf Horticulture bean, Phaseolus vulgaris Linn. were used. The source plants for the roots were grown in the greenhouse. After five weeks the tops were discarded and the soil washed from the roots. The culture trays were replenished with this root material at weekly intervals. This experiment was evaluated after 25 weeks at 18°C.

The analysis of variance of the numbers of symphylans observed at the termination of the experiment showed a significant difference between the two treatments. The treatment in which pea roots were fed totaled nearly twice the number of symphylans than the treatment employing the bean roots. The results are presented in Table 5.

Initially 50 symphylans were introduced into each culture tray.

The lowest number recovered was 11 while the highest number recovered was only 39. No young, first, second, or third-instar stages were noticed in the bark medium. Also a culture from each treatment was lost during the course of the experiment. The cause was undetermined but it was evident that the conditions for a thriving culture were marginal. Therefore it may be suggested from these observations that the non-living root material was an inadequate food base for the support of a thriving culture.

Table 5. Comparison of pea and bean roots fed to symphylan cultures.

Replicates	Number of symphylans per food treatment	
	Pea root	Bean root
1	39	19
2	23	11
3	30	25
4	34	16
Total	126	71

Summary of Main Factor

Source of variation	Degrees of freedom	F	Significance
Replicates	3	2.51	NS
Food treatments	1	16.59	S-.05
Error	3		

The next experiment included three treatments replicated five times. Two closely related plant varieties were chosen to be compared with an unrelated plant. The two bean varieties included the Ruby Dwarf Horticulture bean, Phaseolus vulgaris Linn. and the Blue Lake Stringless bean, also, P. vulgaris Linn. Roots of the sweet corn plant, Zea mays v. rugosa, were used for the third treatment.

Intermediate sized containers were used, each receiving 50 symphylans on a 40% moisture bark medium. This experiment was held at 18°C and was carried 19 weeks.

The number of symphylans per tray observed at the completion of this experiment varied between a low count of 41 and a high count of 84. An analysis of variance indicated no significant difference among treatments. The results are presented in Table 6. The population counts per culture were somewhat higher than for the previous experiment even though the duration of this latter experiment was six weeks shorter. Generally, numbers of symphylans per culture, mentioned above, were very low when compared to cultures which had received fresh lettuce or carrot food.

Table 6. Comparison of two varieties of bean roots and a corn root fed to symphylan cultures.

Replicates	Number of symphylans per food treatment		
	Bush bean	Corn	Pole bean
1	80	61	49
2	41	44	54
3	62	69	51
4	55	84	57
5	59	56	56
Total	297	314	267

Summary of Main Factor

Source of variation	Degree of freedom	F	Significance
Replicates	4	1.29	NS
Food treatments	2	.86	NS
Error	8		

Effect of Culture Population Density on Rate of Population Increase

Previous experimental results (Swenson, Unpublished) showed that the culture container size influenced the rate of increase of the symphylan population. After a certain culture density, the rate of increase was inversely proportional to the number of symphylans per given volume. The following experiment was conducted to obtain supporting data for this effect.

Pint containers were used, each with 60 grams of bark and 40 ml of water. The weight was checked twice monthly and care was taken to maintain an ample supply of fresh lettuce in the containers.

Five treatments were set up, each replicated five times. The treatments consisted of the following number of symphylans per container: 5, 10, 25, 50 and 100. The experiment was duplicated and maintained at 15°C. The first experiment ran for 26 weeks and the second for 24 weeks. The results are presented in Table 7.

The number of symphylans observed at the termination of these experiments indicated that the rate of increase per initial symphylan increased through the fourth treatment. At this point 50 symphylans per container were initially introduced. The rate of increase decreased sharply in the fifth treatment representing an initial introduction of 100 symphylans.

Table 7. Effect of increasing population density on rate of population increase.

Treatment number	Initial symphylans per container	Rate of population increase per symphylan	
		Rep 1	Rep 2
1	5	1.8	1.8
2	10	4.1	5.2
3	25	5.3	4.0
4	50	7.0	6.7
5	100	4.4	5.0

Greenhouse Experiments on Symphylan Injury to Potted Plants

General Considerations

Many greenhouse trials were made in attempts to show

symphylan damage to plants under controlled conditions. Plastic pots were preferred to clay pots for two reasons. First, there was no moisture loss through the sides of the plastic pot; and second, the drain holes were easily screened to prevent symphylans from escaping. One preliminary trial involved comparing the plant growth rates in plastic pots using two different soil mixtures with the growth rate in clay pots filled with greenhouse soil. One of the two soil mixes used in the plastic pots consisted of one half sphagnum moss and one half greenhouse soil. The other soil mixture consisted of one half vermiculite and one half greenhouse soil. Both Perfected Wales peas and Dwarf Hortculture beans were planted in this trial. Height measurements of the plants were taken after a growth period of 20 days. The differences in these values were slight and not significant. It was concluded that the type of pot and soil mixture would not affect results.

The texture of soil used in experiments with symphylans is very important. The soil in the pots must be loosely packed leaving many natural cracks and crevices to allow free symphylan movement and access to the plant roots. The relative humidity of the soil environment is also critical to symphylan survival (Edwards, 1961). The moisture holding capacity of the soil must be such that a 100% RH is maintained. These conditions were met by mixing sphagnum moss with the greenhouse soil in a cement mixer on an equal volume

basis. A supply of this soil mixture was prepared at one time and used as needed for the remainder of the greenhouse experiments.

A trial plant-damage experiment was made in February 1968. The 15 pots in the experiment were divided into three replications of five pots each. Each replication consisted of three treatments and two controls. The pots of the first treatment contained 50 symphylans each, the second treatment 100 each, and the third 200 each. Four days later Dwarf Horticulture beans were planted.

The results showed a difference between the emergence dates of the control plants as compared to the pots infested with symphylans. The plants of the control group emerged from three to eight days before the treated group. Differences in emergence dates among the infested plants were not significant. Although the top dry weights of the plants were not recorded this trial demonstrated that the presence of symphylans in potted greenhouse plants was manifest by their effects on emergence of the plants.

Greenhouse temperatures are at times subject to wide variations depending on the outside weather conditions and mechanical functioning of the heating and cooling equipment. Although it has been shown that symphylans can survive a wide range of natural soil temperatures (Edwards, 1961), the temperature in greenhouse rooms can rise above the upper limit for survival. Consequently thermocouple temperature readings were recorded from the potted

soil of the greenhouse experiments. Although the desired room temperature was 70° F, a variation of plus or minus five degrees or more was normal and not detrimental to the symphylan experiments. All greenhouse plants were subject to a 16 hour photoperiod.

The following two experiments were designed to improve the reliability of greenhouse experimentation to determine plant injury by symphylans.

Effect of Delayed Planting Following Infestation on Symphylan Injury to Plants

This experiment was designed to determine the effect of delayed planting following symphylan infestation. Two factors involved were the effect of symphylan mortality after transfer and the effect of the establishment of runways by the symphylans in the new environment. Dry weights of the plant tops, cut at ground level, were used to determine the extent of damage to bean and corn plants.

The treatments for both experiments (delayed planting effects and delayed infestation effects) numbered 18, each replicated five times. In each replication one half of the 18 pots were planted with Golden Bantam corn, Zea mays v. rugosa, and the other half with Dwarf Horticulture bean, Phaseolus vulgaris Linn. Within each replication, in both this and the following experiment, each treatment was processed in random order, and also placed on the

greenhouse bench in random order.

Summarized in Table 8 below and in reference to the delayed planting experiment are: the treatment number, the kinds of plants, the numbers of symphylans introduced and the length of time following the infestation of the pots till the germinated seed was planted.

Table 8. Arrangement of the delayed planting experiment.

Treatment number	Plant	Number of symphylans	Delay in planting (weeks)
1	Bean	0	0
2	Bean	0	1
3	Bean	0	2
4	Bean	25	0
5	Bean	25	1
6	Bean	25	2
7	Bean	50	0
8	Bean	50	1
9	Bean	50	2
10	Corn	0	0
11	Corn	0	1
12	Corn	0	2
13	Corn	25	0
14	Corn	25	1
15	Corn	25	2
16	Corn	50	0
17	Corn	50	1
18	Corn	50	2

Thus, all of the 60 pots were infested at the same time, excluding the controls. One third of the planting was delayed two weeks, one third one week and one third or 30 of the seeds were planted at the time of infestation. Before the bean and corn seeds were planted they were germinated on wet paper towels then selected for planting on the basis of uniform development.

The evaluation of each of the three delayed planting sections of the experiment was made 17 days after the respective planting of each section. The data which consisted of the dry weights of the plants and the numbers of recovered symphylans were subjected to a factorial analysis of variance.

Two of the main factors from the analysis of the dry plant material weights proved significant at the 0.1% level. These were plants and symphylans. The only nonsignificant main factor was the planting delay. Plant and symphylan interaction was significant at the 5% level. All other interactions were not significant. The plant main factor in the dry weight analysis, was significant, probably because of the greater size of the bean plant compared to the corn plant at this stage in growth. The main factor of introduced symphylans was highly significant which indicated that the greater the symphylan population per pot the more extensively the plant was injured. Results from the factoral analysis of the planting delay experiment revealed significant differences, many of which were

not unexpected. The results are presented in Table 9.

The analysis of variance calculations for the number of symphylans recovered at the termination of the experiment showed the significant main factors to be symphylans and planting delay. No other main factors or first order interactions proved to be significant. Regarding the time delay factor, examination of the data reveals that most symphylans were recovered from the pots which were planted immediately after they were infested. The next highest numbers were recovered from pots which were planted one week after infestation, and the least numbers were recovered from the pots planted two weeks after infestations. The average percent recovery from the population level of 25 was 67% and from the 50 level, 63%. The results are presented in Table 10.

Table 9. Plant dry weight analysis from delayed planting in Symphylan infested soil.

	No Delay			1 Week Delay			2 Week Delay		
Replicates	0	25	50	0	25	50	0	25	50
Beans									
1	.519	.492	.220	.410	.330	.287	.316	.402	.091
2	.399	.310	.414	.528	.161	.146	.312	.110	.040
3	.361	.260	.375	.149	.400	.192	.330	.356	.039
4	.071	.367	.000	.949	.149	.000	.584	.200	.227
5	.502	.554	.184	.718	.184	.446	.558	.000	.350
Total	1.852	1.983	1.193	2.754	1.224	1.071	2.100	1.068	0.747
Corn									
1	.056	.054	.047	.163	.031	.056	.162	.031	.018
2	.080	.076	.145	.193	.110	.034	.105	.051	.022
3	.177	.070	.128	.191	.055	.060	.146	.072	.023
4	.089	.000	.071	.218	.023	.056	.161	.023	.040
5	.090	.167	.046	.112	.039	.059	.112	.059	.074
Total	0.492	0.367	0.437	0.877	0.258	0.265	0.686	0.236	0.177

Summary of Main Factors

Main Factor	Dry Weight (Grams)	Significance
Plant		S-.001
Bean	13.992	
Corn	3.795	
Planting Delay		NS
No delay	6.324	
1 week delay	6.449	
2 week delay	5.014	
Symphylans		S-.001
0	8.761	
25	5.136	
50	3.890	

Summary of Interactions

Plants x Planting delay		NS
Bean x No delay	5.028	
Bean x 1 week delay	5.049	
Bean x 2 week delay	3.915	
Corn x No delay	1.296	
Corn x 1 week delay	1.400	
Corn x 2 week delay	1.099	

(continued)

Table 9. Continued.

Summary of Interactions

Main Factor	Dry Weight (Grams)	Significance
Plants x Symphylans		S-.05
Bean x 0	6.706	
Bean x 25	4.275	
Bean x 50	3.011	
Corn x 0	2.055	
Corn x 25	0.861	
Corn x 50	0.879	
Planting Delay x Symphylans		NS
No delay x 0	2.344	
No delay x 25	2.350	
No delay x 50	1.630	
1 week delay x 0	3.631	
1 week delay x 25	1.482	
1 week delay x 50	1.336	
2 week delay x 0	2.786	
2 week delay x 25	1.304	
2 week delay x 50	0.924	

Table 10. Analysis of the number of symphylans recovered from the delayed planting experiment.

Replicates	Beans								
	No delay			1 Week delay			2 Week delay		
	0	25	50	0	25	50	0	25	50
1		20	41		18	34		14	33
2		11	21		13	33		22	34
3		23	38		17	42		12	26
4		16	37		24	34		15	24
5		13	29		12	32		11	17
Total		83	166		84	175		74	134
				Corn					
1		19	39		19	31		21	38
2		16	34		17	33		9	30
3		15	25		21	23		12	31
4		21	43		19	24		20	23
5		22	36		14	33		19	24
Total		93	177		90	144		81	146

Summary of Main Factors

Main factor	Number of symphylans	Significance
Plant		NS
Bean	716	
Corn	731	
Planting delay		S-.05
No delay	519	
1 Week delay	493	
2 Week delay	435	
Symphylans		S-.001
0		
25	505	
50	942	

(Continued)

Table 10. Continued

Summary of Interactions

Main factors	Number of symphylans	Significance
Plants × planting delay		NS
Bean × no delay	249	
Bean × 1 week delay	259	
Bean × 2 week delay	208	
Corn × 1 week delay	270	
Corn × 1 week delay	234	
Corn × 2 week delay	227	
Plants × symphylans		NS
Bean × 0		
Bean × 25	241	
Bean × 50	475	
Corn × 0		
Corn × 25	264	
Corn × 50	467	
Planting delay × symphylans		NS
No delay × 0		
No delay × 25	176	
No delay × 50	343	
1 week delay × 0		
1 week delay × 25	174	
1 week delay × 50	319	
2 week delay × 0		
2 week delay × 25	155	
2 week delay × 50	280	

Effect of Delayed Infestation Following Planting on Symphylan Injury to Plants

This experiment was similar to the preceding one except that the symphylan infestation of the pots was delayed instead of the planting. The same number of treatments were involved. These can be reviewed in Table 8. All 90 pots were planted with pregerminated bean and corn seed on the same date. At this time 20 pots were infested. The following week another 20 were infested and the remaining 20 were infested at the end of the second week. Evaluations of this experiment were made 17 days after each infestation which represented plant growth periods of 17, 24 and 31 days for the three groups.

The recorded top dry weights and symphylan recovery values were subjected to an analysis of variance. The dry weight analysis of variance showed a very high significant variation due to infestation delay, plant, and replication as indicated in Table 11. The source of variation in regards to the infestation delay can mainly be attributed to the length of growth period. Both bean and corn plants were divided into three growth periods 17, 24 and 31 days. These periods allowed wide variation in the dry weights among the three groups. The significant variation due to the plant factor could be attributed to the growth habit of the bean and corn plants involved especially in conjunction with the longer growth periods. The variation among

replications was probably due to the positioning of the replicates on the greenhouse bench.

Variation due to the introduction levels of symphylans was not significant and probably attributed to the delayed infestations which allowed the plants to get a good start and out-grow any damage sustained by the symphylans. The analysis of variance of the symphylan recover data show the number of introduced symphylans to be significant at the 0.1% level. All other main factors and interaction were not significant. The results are presented in Table 12.

Table 11. Plant dry weight analysis from delayed infestation of bean and corn plants.

	Beans								
	No Delay			1 Week Delay			2 Week Delay		
Replicates	0	25	50	0	25	50	0	25	50
1	.275	.420	.436	.790	.827	.798	1.166	1.022	1.035
2	.365	.360	.305	.528	.740	.372	.700	1.099	1.254
3	.414	.412	.322	.643	.490	.913	1.590	1.150	1.177
4	.221	.267	.191	.426	.423	.425	.958	.981	.861
5	.132	.320	.260	.575	.562	.661	1.135	.963	.904
Total	1.407	1.779	1.514	2.962	3.042	3.169	5.549	5.215	5.231
	Corn								
1	.150	.135	.165	.340	.413	.460	.755	1.042	.988
2	.161	.112	.170	.325	.380	.270	1.291	1.080	.420
3	.108	.087	.082	.452	.345	.271	.737	.893	.639
4	.070	.026	.047	.260	.122	.135	.492	.000	.406
5	.077	.084	.060	.249	.252	.213	.537	.194	.432
Total	.566	.444	.524	1.626	1.512	1.349	3.812	3.209	2.885

Summary of Main Factors

Main Factors	Dry Weight (Grams)	Significance
Plant		S-.001
Bean	29.868	
Corn	15.927	
Infestation Delay		S-.001
No delay	6.234	
1 week delay	13.660	
2 week delay	25.901	
Symphylans		NS
0	15.922	
25	15.201	
50	14.672	

Summary of Interactions

Plants x Infestation Delay		NS
Bean x No delay	4.700	
Bean x 1 week delay	9.173	
Bean x 2 week delay	15.995	
Corn x No delay	1.534	
Corn x 1 week delay	4.487	
Corn x 2 week delay	9.906	

(continued)

Table 11. Continued.

Summary of Interactions

Main Factor	Dry Weight (Grams)	Significance
Plants x Symphylans		NS
Bean x 0	9.918	
Bean x 25	10.036	
Bean x 50	9.914	
Corn x 0	6.004	
Corn x 25	5.165	
Corn x 50	4.758	
Infestation Delay x Symphylans		NS
No delay x 0	1.973	
No delay x 25	2.223	
No delay x 50	2.038	
1 week delay x 0	4.588	
1 week delay x 25	4.554	
1 week delay x 50	4.518	
2 week delay x 0	9.361	
2 week delay x 25	8.424	
2 week delay x 50	8.116	

Table 12. Analysis of the number of symphylans recovered from the delayed infestation experiment.

	Bean								
	No delay			1 Week delay			2 Week delay		
	0	25	50	0	25	50	0	25	50
1		7	13		11	14		7	23
2		6	12		12	17		0	10
3		11	19		7	19		5	16
4		14	19		3	8		4	23
5		5	14		10	14		7	5
Total		43	77		42	72		23	77
	Corn								
1		9	15		9	9		8	26
2		7	30		5	17		5	13
3		4	12		5	7		11	11
4		9	12		6	14		6	10
5		7	10		8	14		6	25
Total		36	79		33	61		36	85

Summary of Main Factors

Main factor	Number of symphylans	Significance
Plant		NS
Bean	334	
Corn	330	
Infestation delay		NS
No delay	235	
1 week delay	208	
2 week delay	221	
Symphylans		S-.001
0		
25	213	
50	451	

(Continued)

Table 12. Continued

Summary of Interactions

Main factors	Number of symphylans	Significance
Plants × infestation delay		NS
Bean × no delay	120	
Bean × 1 week delay	114	
Bean × 2 week delay	100	
Corn × no delay	115	
Corn × 1 week delay	94	
Corn × 2 week delay	121	
Plants × symphylans		NS
Bean × 0		
Bean × 25	108	
Bean × 50	226	
Corn × 0		
Corn × 25	105	
Corn × 50	225	
Infestation delay × symphylans		NS
No delay × 0		
No delay × 25	79	
No delay × 50	156	
1 week delay × 0		
1 week delay × 25	75	
1 week delay × 50	133	
2 week delay × 0		
2 week delay × 25	59	
2 week delay × 50	162	

Intraspecific Differences Among Plant Varieties to Injury by Symphylans

Methods derived from the two previous experiments were utilized here to determine if evidence for plant resistance to symphylan attack could be demonstrated. The five bean varieties chosen for this experiment were: Phaseolus vulgaris Linn. v. Ruby Dwarf Horticulture, P. vulgaris Linn. v. Pencil Pod wax, P. limensis Macfadyen the lima bean, and P. multiflourus Willd. the ornamental Scarlet Runner bean. Five plants of each variety were infested immediately after planting with 50 symphylans in each pot. All seeds had been presoaked and germinated to assure germination and uniform stand. A control plant was set up for each infested plant. In this manner the resistance value, expressed in percentage, for each variety could be judged by comparing the dry weight of the five infested plants against the dry weight of the five noninfested plants.

The plants were allowed to grow for 16 days. Data consisted of the top dry weights and the symphylan recovery numbers. A comparison among Dwarf Horticulture, Kentucky Wonder and Scarlet Runner bean plants showed that the dry weights of the noninfested plants were greater than the dry weights of the infested plants. The amount of injury for the three varieties as determined by comparing dry weights of infested with noninfested plants was: 89% for Kentucky Wonder, 85% for Scarlet Runner and 62% for Dwarf Horticulture.

The remaining three varieties showed greater dry weight totals from the infested plants than from noninfested plants. Four of the five Lima bean control seeds and one seed from an infested pot failed to emerge and rotted in the soil. This helps to explain the irregular weight values from this variety. The unusual results for the remaining two varieties is unknown. The amount of injury for the three varieties as determined by comparing dry weights of infested with noninfested plants was: 501% for Lima, 153% for Wax and 103% for Blue Lake. These weight difference figures reveal a wide variation among the positive as well as negative results.

A statistical test for paired differences was applied to the evaluation data from this experiment. The results showed no significant difference at the 5% level among the dry weight totals of the six varieties.

DISCUSSION AND CONCLUSIONS

Laboratory Culture Experiments

The results of the bark culture treatments, although statistically not significant, are meaningful with respect to culturing symphylans in the laboratory. That is, apparently no advantage would be gained by sterilizing the bark culture medium. Also, some of the low numbers of symphylans recovered indicated that certain culture populations were severely suppressed. This effect may possibly be caused by toxic gases produced by the microflora in the substrate. I suggest that in order to avoid this dilemma an increase in the airspace in the culture tray might be tried. This would necessitate the use of deeper trays or less bark.

It was shown that the moisture content of the bark substrate clearly affected the culture populations. The treatment with the low moisture content of 30% resulted in the largest numbers of symphylans recovered. Apparently the relative humidity of the culture medium was favorable to symphylan population development. The reduced amount of water added to these cultures was thoroughly absorbed by the ground bark so no free water was present to restrict movement of the symphylans or cause drowning. It has been reported (Savos, 1958) that liquid water is definitely repellent to symphylans. In addition, a lower moisture content would

tend to retard microflora and mold growth thus diminish the accompanying detrimental effects to the culture.

The symphylan population increase seemed to correlate directly to the temperature at which the symphylan cultures were held. That is, a gradual increase in the population counts parallel the increase in temperature. The higher temperature seemed to increase the growth rate and shorten the development time, so more generations cycled during the duration of the experiment as compared to cultures held at lower temperatures. The fecundity may also have been enhanced in the higher temperature treatments resulting in a greater population. A combination of 24°C temperature and 30% moisture appears to be ideal for laboratory symphylan cultures.

The experiment involving the comparison of carrot and lettuce food with immature and adult symphylans was plagued with difficulties not unlike the bark treatment experiment. The fact that one culture was lost due to unknown causes and that the population counts in other cultures were low suggest again that possible toxic products from the microflora influenced the outcome. Aside from the effects of these unknown factors the number of recovered symphylans indicate that initial infestation of cultures with mature symphylans resulted in a greater population increase. The studies of effects of different kinds of plant roots provided as food point out the fact that Shanks (1966) demonstrated. That is, in order for a symphylan

population to thrive and reproduce fresh succulent food is needed. The culture populations which received the root food seemed to subsist but not reproduce to any significant extent. In many cases the numbers dwindled from an initial 50 symphylans down to a low of 11, while in other cases an increase to a high of 84 was noted. Further evidence of the stress the populations were under is verified by the loss of two cultures, one from each treatment in the pea and bean root feeding experiment. If the cultures were carried much longer than the 25 weeks, other cultures may have been lost.

The results of the experiment in which symphylans were fed on pea and bean roots showed that a greater population developed in the pea root cultures as compared to those in the bean root cultures. Since it is evident that the populations of both treatments were under stress and marginal nutritional conditions I would conclude that the pea roots were a more suitable food than were the bean roots under these conditions. Ideally, fresh root material could be supplied to a culture if the plants could be grown in the bark medium but the high acidic nature of the medium among other factors prevent this. Instead, fresh root material could be supplied at more frequent intervals.

The results of the crowding experiments conclusively demonstrated a decrease in reproduction rates when space is limited. The decrease in reproduction rate under crowded conditions may indicate

a mechanism for dispersal under natural conditions. The increase-per-symphylan values also indicate a correlation between greater initial populations and greater rates of increase. The following observation might possibly be explained by Michelbacher (1938) who suggested that the first and second instars demonstrated a more or less gregarious nature. Upon reaching the third instar stage the symphylans came out to feed and abandon their gregarious behavior.

Associated with the laboratory cultures of symphylans were large populations of collembola, also noted by Michelbacher (1938). They did not appear to interfere in any way with the symphylans.

Greenhouse Experiments

The greenhouse experiments demonstrated that plant injury can be duplicated under controlled conditions which is a basic step to further experiments with plant resistance or other greenhouse investigations.

The experimental methods used in the greenhouse dealt with four important factors: temperature, moisture, soil texture and symphylan infestation levels.

The temperature requirements are not narrowly limited for symphylan survival under greenhouse conditions. Temperature recordings should be maintained to prevent symphylan mortality due to high temperatures. The experiment room was set at 70° F, but

the actual soil temperature readings fluctuated as much as 15°F above and below the desired temperature. Since symphylans are relatively tolerant to wide temperature changes (Edwards, 1961) this range of greenhouse temperatures presented no difficulties. All greenhouse plants were set in saucers and watered from the bottom. A favorable moisture balance for plant growth and symphylan survival has to be maintained. Since the moisture is absorbed by the soil from the bottom, the soil surface may appear dry so care was taken not to over water.

The incorporation of sphagnum moss with the greenhouse soil on an equal volume basis provided a soil environment in which the symphylans were allowed free movement both horizontally and vertically. The spongy nature of the moss-soil mixture exposed the young tender rootlets to the symphylans and generally made the root tissue more vulnerable to the symphylans. The presence of the sphagnum moss also helped stabilize the moisture loss by its water holding properties and maintaining adequate relative humidity.

The total dry weight for both bean and corn control plants in the delayed planting experiment was 8.761 g. The total dry weight for the plants infested with 25 symphylans per pot was 5.136 g. The total dry weight for the plants infested with 50 symphylans per pot was 3.890 g. It was concluded that the greater the number of introduced symphylans the greater the plant damage as evidenced by the

dry weight. Also, 50 symphylans per plant would be the most suitable infestation level for plant resistance studies in this volume of soil.

Possibly, if smaller plants were used in similar experiments, 25 symphylans per pot would yield the same effects. Considering young bean and corn plants are robust with fast developing root systems, the transfer mortality factor and the premolt nonfeeding periods (Waterhouse, 1963), 50 symphylans may seem like a conservative amount with which to infest a pot to show injury.

The greenhouse experiment in which all of the pots were initially infested and the planting delayed indicated that there was no greater injury to the plants by symphylans when their primary food source was withheld them for several weeks than when the germinating seedling was available.

The succeeding experiment showed conclusively that when these plants were given a good start with an established root system the symphylans introduced did not cause any noticeable harm to the plant. Therefore plants are most vulnerable to symphylan attack when at a young and tender growth stage and before a root system becomes established. A successful method for symphylan control would have to effectively protect the plant root system during this vulnerable period.

The methods employed in the plant resistant experiment

obviously need refinement. No conclusions could be drawn from the results. A few suggestions and comments for future greenhouse experiments involving symphylans and potted plants are given below:

1. The pre-germinated seed should be planted in the potted soil prior to symphylan infestation. By this method the soil is disturbed before the symphylans are introduced thus reducing the chance for symphylan mortality due to mechanical injury.

2. When dealing with different varieties as well as different plants, many varied growth rates are encountered. Therefore it may be necessary to increase the number of introduced symphylans from 50 per pot which was adequate in these experiments to demonstrate plant injury. Also the volume of soil and pot size will be a factor in the number of symphylans introduced.

3. To establish differences among varieties of plant susceptibility an increased number of replications will be needed.

The significance of the greenhouse experiments is that favorable conditions were defined for evaluating symphylan damage to greenhouse grown plants under experimental conditions. It may seem absurd to go to great effort to demonstrate symphylan damage when commercial crop injury caused by symphylans is prevalent. However, this study of symphylan damage is of value in that: it reveals the number of symphylans necessary to injure a plant, also the most susceptible growth stage of plant growth was demonstrated.

Also, other experimental conditions were defined for use by future greenhouse investigators of symphylan damage and symphylan control.

SUMMARY

Factors affecting symphylan population development in laboratory and greenhouse cultures were studied. Symphylans were cultured in plastic polyethylene containers with moisture-tight lids in a ground hemlock bark medium. Fresh lettuce leaves were used as food. This culture method proved both simple and productive.

Treating the bark medium did not appreciably affect population development. The culture medium was tested at 30, 35 and 40% moisture by weight. The low 30% moisture content affected population increase the greatest. Temperature treatments included cultures at 15°C, 18°C, 21°C and 24°C. The high temperature value of 24°C affected population increase the greatest. Treatments with different kinds of food on population development revealed that cultures supplied with lettuce or carrots produced larger populations than did cultures supplied with plant root material. The rate of symphylan population increase declines as the population density increases.

Experiments conducted in the greenhouse demonstrated that a mixture of greenhouse soil and sphagnum moss allowed symphylans enough freedom of movement to damage potted plants. Further experiments with potted bean and corn plants indicated that symphylan population levels of 25 to 50 per pot were sufficient to damage young

plants. Also, no greater injury was sustained by potted plants when the initial symphylan infestation was followed by a one or two week delay in the planting dates. When the bean and corn plants were given a good start before the symphylans were introduced, little or no damage resulted. Although no conclusions were provided by the plant resistant experiment a few suggestions were made for future researchers. The conclusions defined conditions for the development of symphylan cultures for use in laboratory and greenhouse experiments.

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