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THE INFLUENCE OF STAGE OF HOST DEVELOPMENT ON VESICULAR-ARBUSCULAR MYCORRHIZAE AND ENDOGONACEOUS SPORES IN FIELD-GROWN VEGETABLE CROPS
II. WINTER-GROWN CROPS

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Abstract

The pattern of mycorrhizal infection of roots of the fully grown vegetable crops studied showed three phases, a lag phase, a phase of rapid development and a constant phase. Twenty eight days after sowing or transplanting 12-21% of the root length was infected, but the proportion then increased progressively to a more or less constant value which ranged between 58 to 90% depending upon the host species. With carrot (*Daucus carota* L.), coriander (*Coriandrum* sativum L.), onion (*Allium cepa* L.) and fenugreek (*Trigonella foenumgraecum* L.). No constant phase was observed within the sampling period. No VA mycorrhizae were found in cauliflower (*Brassicaot racea* L. var. *botrytis* L.), radishal (*Raphanus sativus* L.), turnip (*Brassica napobrassica* Mill), Spinach beet (Beta vulgaris L. spp *maritima*) and beet (*Beta vulgaris L.*). During the first 4-8 weeks of crop growth, the Endogonaceous spore population of the soil decreased, but then increased to final harvest time.

Introduction

Vesicular-arbuscular (VA) mycorrhizae are formed by a wide range of crop plants in associa ion with fungi belonging to the Endogonaceae (Daft & El-Gheahmi, 1975; Khan, 1972, 1975; Hayman, 1975; Strsemska, 1975) Various factors affecting spore populations of Endogonaceae & development of VA mycorrhizae have been studied by many workers (Furlan & Fortin, 1973; Hayman, 1970, 1974; Kruckelman, 1975; Saif, Sheikh & Khan, 1975; Sheikh, Saif & Khan, 1975, Sutton, 1973), but there had been few attempts to relate these to growth and differentiation in the host under field conditions.

In the present study the relationship between spores of Endogonaceae and mycorrhizal development with host development was examined in a wide range of field-grown winter vegetable crop plants. The results about field-grown summer vegetable crops have already been reported (Saif, 1977).

Materials and Methods

The crop plants used for these studies were grown in field plots at Agriculture Research Station, Murree Road, Rawalpindi, Sohan Village and Islamabad University Nursery in 1974-1975. Carrot (*Daucus carota* L.), garlic (*Allium sativum L.*) turnip (*Brassica napobrassica* Mill) and radish (*Raphanus sativus* L.) were grown at Agriculture Research Station in plots of 3.5 m² with 4 replicate plots in each case. Fenugreek (*Trigonella foenum-graecum* L.) and spinach beet (*Beta vulgaris* L. spp. *maritima*) were grown at Sinala Slaughter House in plots of 3.5 m2 with 4 replicate plots each

whereas coriander (*Coriandrum sativum* L.), beet (*Beta vulgaris* L.) and cauliflower (*Brassica oleraceae* L. var. *botrytis* L.) were grown at Sohan Village with plot diamensions of 4-20 & 6-25 m replicated 2-4 times. Onion (*Allium cepa* L.) lettuce (*Lactuca sativa* L.) and pea (*Pisum sativum* L.) were grown at Islamabad University nursery with plot dimensions of 3-5.5 & 8.2–9.6 m replicated 3-4 times. Strips of fallow soil 0.5m in width separated the plots. All crops were direct seeded. Onion and cauliflower were grown in unsterile soil-sand-peat moss 3:1:1 mix in separate small field plots and then transplanted. Plants were spaced in rows according to recommendations for commercially grown crops. Weeds were periodically eradicated by hand.

All the vegetable crops were harvested at 5-6 intervals during the growth period. Plants were harvested by digging 3 plants from inner rows in each of the replicate plots. Each plant or plant cluster harvested included most of the roots and surrounding soil to a depth of 15-20 cm. Shoot weights of individual plants were obtained after 24h at 80°C (Sutton, 1973) Roots from sampled plants were bulked, gently washed under slow tap water and were cut into segments 1 cm in length and fixed in formalin-acetic-alcohol (FAA).

Endogonaceous spores were recovered from the plot soils before and after planting at regular intervals. These spores were recovered from the sieved soil samples by taking five subsamples, weighing 50g from each soil sample. Each subsample was placed in 200 ml of water contained in 500 ml beaker, stirred well with magnetic stirrer for about 2-4 min and allowed to stand for about 3-5 min to permit large soil particles to settle at the bottom. The supernatant was then poured from the beaker which was simultaneously rotated on to a filter paper placed in the glass funnel. The extraction procedure was repeated again. The spores along with small amount of organic debris thus collected on the filter paper were counted on a stereoscopic microscope as described by Khan (1971).

For mycorrhizal estimations, the fixed root segments were cleared in KOH and stained with 0.05% trypan blue as described by Phillips & Hayman (1970). Fifty to eighty root segments randomly sampled from each plot were examined at 100 and 400 magnifications. Each 1 cm segment was examined in detail for presence, length and type of infection. The results are expressed as percentages of true roots that were mycorrhizal

Results

VA mycorrhizae developed in most of the vegetable crops used (Fig 1-2). After four weeks from sowing or transplanting, infection of the root system by mycorrhizal fungi was generally low and ranged from 12% in fenugreek (Fig. 2c), to 21% in pea (Fig. 1b). Mycorrhizal infection subsequently increased progressively to a maximum between 58% in fenugreek (Fig. 2c) to 88% in lettuce (Fig. 1a). In most cases 58-88% of the root system was infected until the flowering/fruit stage of the host. No VA mycorrhizae were found in cauliflower, radish, spinach beet and turnip.

Three distinct phases sequentially, a lag phase, a phase of extensive development and a constancy phase of mycorrhizal development were observed in garlic, lettuce and pea whereas in onion, coriander, carrot and fenugreek a constancy phase was not observed within the sampling time (Fig. 1-2). Few roots were mycorrhizal during the initial lag phase lasting 30-50 days. The proportion of mycorrhizal root increased steadily during a subsequent phase of extensive mycorrhizal development of 45-60 days duration but remained more or less constant thereafter (Fig. 1-2).

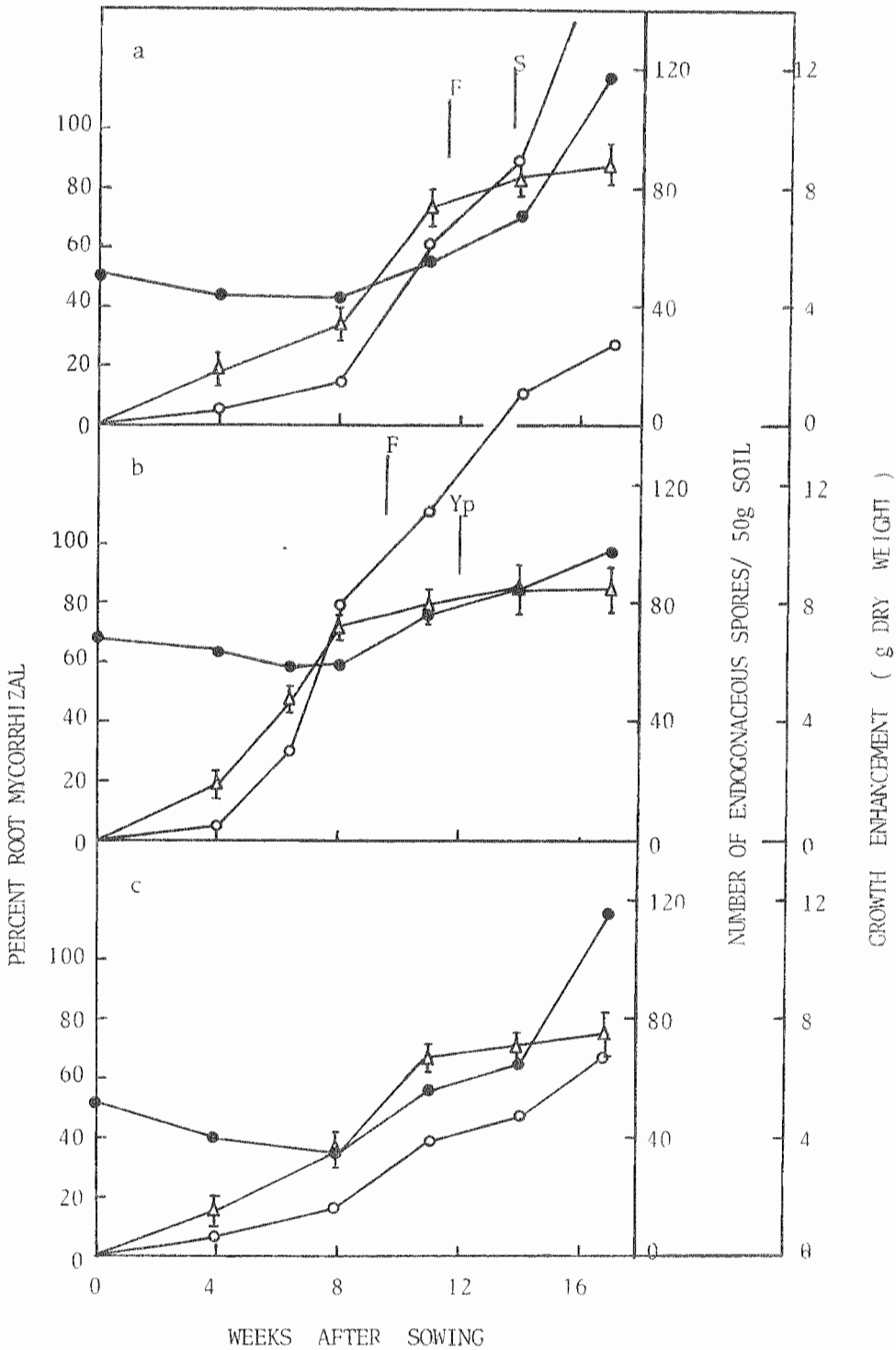


Fig. 1. Percentage roots mycorrhizal (—△—) number of Endogonaceous spores (—●—) and dry weights of shoot (—○—) at various times after sowing in a) lettuce b) pea c) garlic.

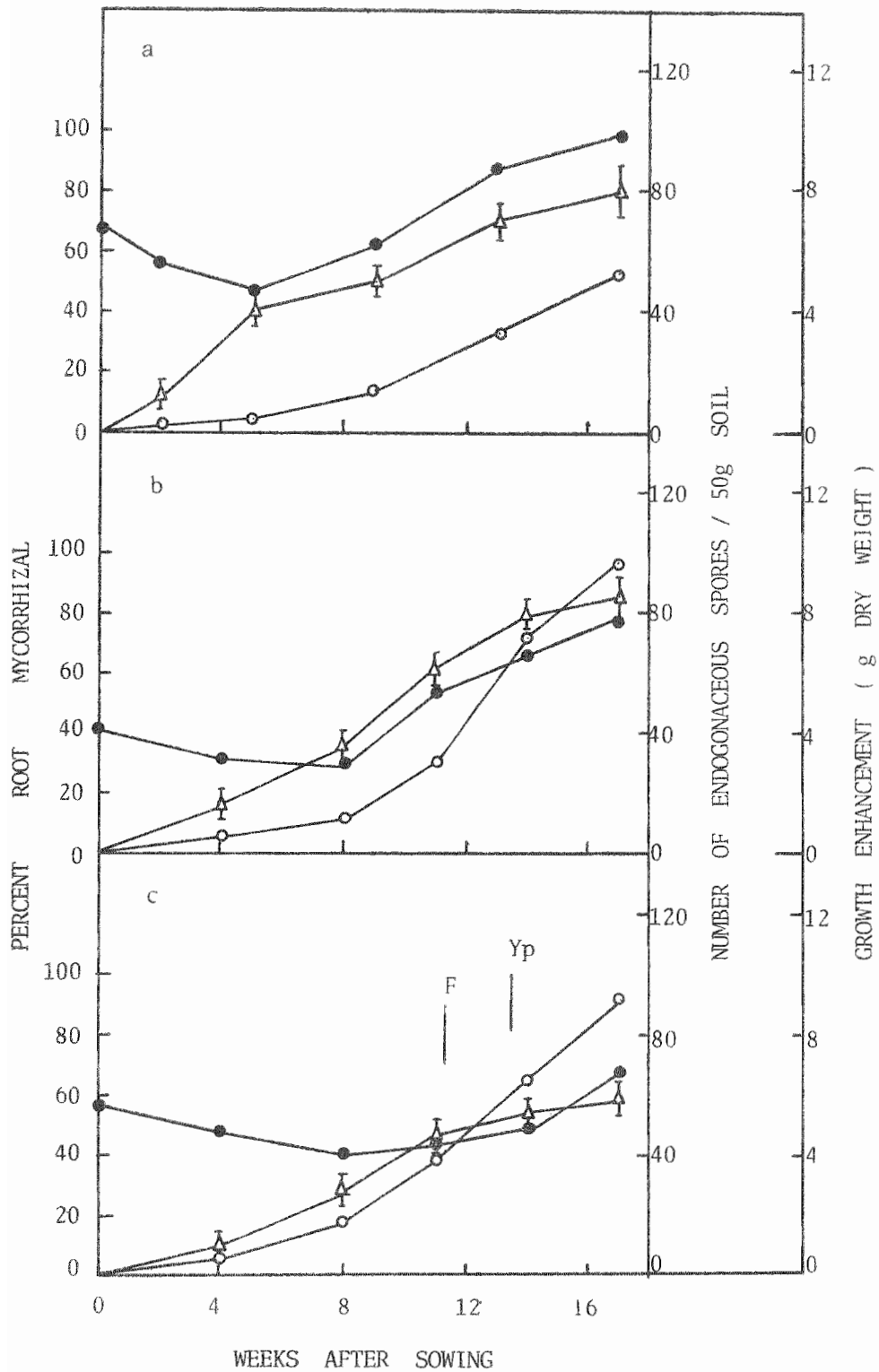


Fig 2. Percentage roots mycorrhizal (- △ -), number of Endogonaceous spores (—●—) and dry weights of shoots (—○—) at various times after sowing in a) coriander b) carrot c) fenugreek.

TABLE 1. The extent of VA mycorrhizal infection in *Lactuca sativa* L. at different stages of host development.

Sampling time, days from sowing	Stages of host development.	No. of root segments examined/ plot	Root segments infection %	Root length infected %	% infected root segments with no Ar	no V	few Ar	few V	many Ar	many V
28	L (4)	50	22±2*	19±2*	67	94	25	6	8	0
56	L (9)	50	43±3	35±2	39	67	27	27	34	6
76	L(15)+Fb	50	76±5	75±4	40	57	23	32	37	11
89	F	50	84±5	85±6	60	45	18	27	22	28
119	S	50	85±7	88±8	73	46	15	22	12	32

L. leaves; Fb, flower buds; F, flower; S, seeds; Ar, arbuscules; V, vesicles.
*Standard deviation.

The phase of extensive mycorrhizal development in lettuce, pea and coriander began after the completion of 5th, 7th and 6th leaf respectively until the appearence of flowers. In onion, carrot, garlic and fenugreek the phase of extensive mycorrhizal development began at 5th, 6th, 7th week and ended at 13th, 12th, 11th and 12th week respectively (Fig. 1-2).

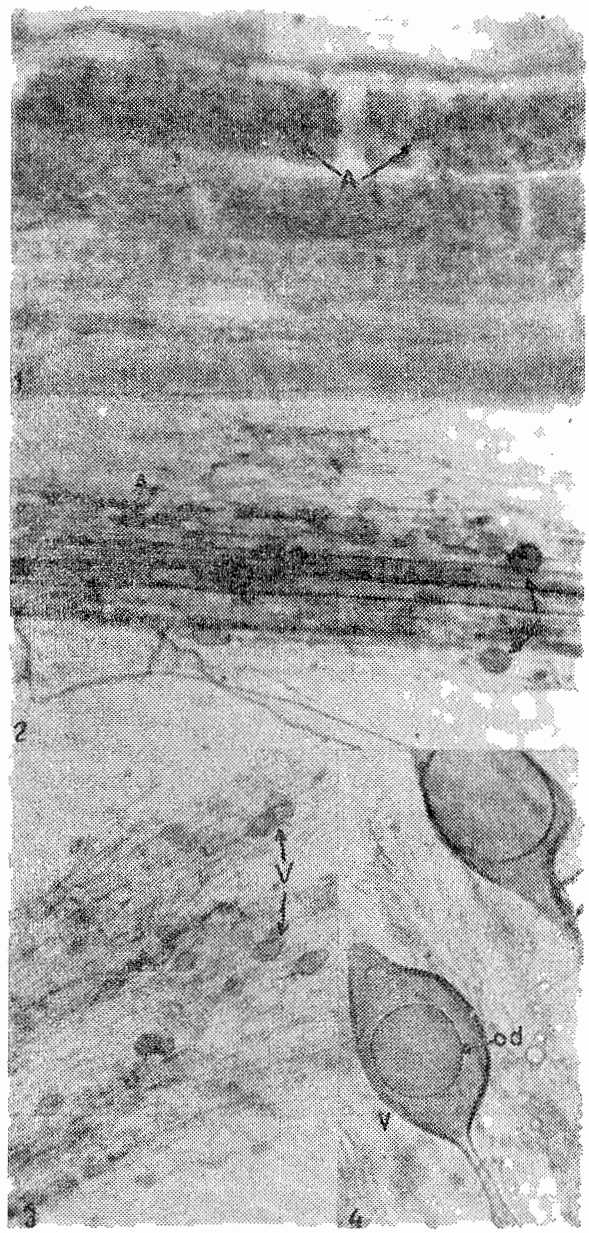
The phase of constancy in the proportion of mycorrhizal to non-mycorrhizal roots began after the period of extensive mycorrhizal development and was observed in lettuce, pea and garlic. This phase ontinued until host senescence (Fig. 1-2).

The number of indigenous Endogonaceous spores in the field plots decreased slightly upto 5-8 weeks of growth. In almost all the host plants the indigenous spore population did not decreaso much and even after the 8th week 80% of the initial spore population was recovered. In coriander 71% of the spores were recovered at 5th week growth stage. From the 8th week in most cases and 5th week in coriander spore numbers started to increase. With lettuce, pea and carrot this increase crossed the initial spore number in the 10th week, in onion and garlic in the 11th week and in corinader at the 12th week growth stage (Fig. 1-2).

TABLE 2. The extent of VA mycorrhizal infection in *Coriandrum sativum* L. at different stages of host development.

15	L(2)	50	18±2*	14±2*	67	82	28	18	5	0
33	L(6)	50	48±3	42±3	39	80	43	20	18	0
63	L(13)	50	73±2	52±3	34	52	40	33	26	5
93	F	50	81±6	73±4	61	54	27	35	12	11
119	S	50	79±5	79±7	83	48	15	39	2	13

Notations same as in Table 1



Nos. 1-4 Lettuce (*L. sativa*).

No. 1. Cortical cells after 8 weeks of field growth filled with large arbuscules (A). Hyphal branches from the main trunk hypha (h) entering the cortical cells are clearly visible X 450.

No. 2. Root after 14 weeks of field growth with few arbuscules (A) and vesicles (V) X 100.

No 3. Root after 17 week of growth with large number of vesicles (V) and very few arbuscules (A) X 100.

No 4. Portion of the No. 3 at greater magnification showing two vesicles (V) containing few small oil droplets a large central oil droplet (od) and also show open cytoplasmic connection with the parent hyphase X 450

Nos. 1-3. Coriander (*C. sativum*).

No 1 Portion of the root showing large number of infection hyphae on the surface X 100.

No. 2. Portion of No. 1 at higher magnification showing infection hyphae forming appressorium (ap) which sends branches towards different directions to colonize the host cells. Just below the appressorium host cells are filled with fungal coils (fc) X 400.

No. 3. Newly produced spore (s) lying near the root surface. Oil droplets (od) are also visible in the spore cytoplasm X 100.

Numbers of Endogonaceae spores increased with the increase in dry weight of host species. (Fig. 1-2). Large host species produced many spores whereas small plants produced fewer.

TABLE 3. The extent of VA mycorrhizal infection in *Trigonella foenum-graecum* L. at different stages of host development.

28	L(3)	50	9±1*	12±1*	73	100	23	0	5	0
56	L(9)	80	23±1	29±2	58	97	21	3	21	0
76	F	80	48±2	45±2	43	68	18	26	39	6
98	F+YP	80	60±2	53±3	73	58	12	28	15	14
119	P	50	58±3	58±2	79	51	15	24	5	25

YP. young pod; P. pod; other notations same as in Table 1.

In the early stages of growth of all the hosts the roots showed progressive formation of arbuscules until the 11th week. After 4 weeks of growth percentage of root segments infected with no, few and many arbuscules was found with a range of 37-83, 15-28 and 2-8, respectively. Typical data for the extent, type and pattern of development of VA mycorrhizal infection in lettuce, coriander and fenugreek are given in Tabels 1, 2 and 3, respectively. Percentage of root segments with no and few arbuscules further decreased until 11th week as the root segments infected with many arbuscules continued to increase (Table 1-3). Root segments with few arbuscules after the 11th week remain almost same (Table 1-3).

After 4th week of field growth percentage of root segments infected by no, few and many vesicles was found in the range of 80-100, 6-20 and 0, respectively. Root segments with no vesicles after 4th week started to decrease with little fluctuations until the senescence of the host whereas the numbers of segments with many vesiclees continued to increase (Table 1-3). Root segments with many vesicles at the time of final harvest i.e. after 17 weeks of field growth ranged between 13 and 35%.

Roots of various hosts were infected intensively by mycorrhizal fungi (Plate 1-2). No mycorrhizal infection was observed in radish, turnip, beet, spinach beet and cauliflower. In lettuce after the 8th week the root cortical cells were filled with large and distinct arbuscules (Plate 1, No. 1). Hyphae running intercellulary produced branches which entered th cells and formed arbuscules (Plate 1, No. 1). After the 14th week arbuscular infection decreased and the existing arbuscules started to disintergrate and vesicles appeared in greater numbers (Plate 1, No. 2). After 17 weeks of growth, the root cortex contained many vesicles and very few arbuscules (Plate 1, No. 3). At this stage vesicles contained a large central oil droplet and had open cytpolasmic connection with the parent hypha (Plate 1, No. 4). In coriander large infection hyphae were observed on the root surface (Plate 2, No. 1) where they formed appressorial structures from which hyphae entered the cortical cells. These hyphae, after entering the cortical cells, formed coils and also spread from one cell to the other (Plate 2, No. 2). Ocasionally young spores were found on the surface of the root (Plate 2. No. 3).

Discussion

The lag phase in winter-grown vegetable crops lasted from 30 to 50 days whereas in summer-gown crops it ranges between 20-30 days duration (Saif, 1975). This may be interpreted as the result of low temperature in winter season. Fulrlan & Fortin (1973) showed in their pot experiments with onion that the lag phase at a low temperature regime (11/16°C) was longer than at a high one.

The phase of extensive mycorrhizal development lasted 45-60 days in winter-grown crops as compared to 30-45 days in summer-grown crops (Saif, 1975). In lettuce and pea, the phase of extensive development terminated with flowering. The longer period of extensive mycorrhizal development may be due to the slow formation of external mycelium by the mycorrhizal fungus in soil from which penetration structures are produced and initiate new infection.

During the initial stages of host there was a small decrease in the indigenous population of Endogonaceous spores. This decrease was about 20% as compared to 50% in summer-grown vegetable crops (Saif, 1975). The small decrease may be interpreted as the result of slow germination of spores due to low temperature in winter. Due to the slow germination of spores, process of infection was also much slower in the winter-grown crops as compared to summer-grown crops. However, spore numbers started to increase after 8 weeks of growth and crossed the intial number present in the field soil.

Host species with large shoot dry weights produced many spores of mycorrhizal endophytes (Fig. 1-2). This may be perhaps due to the large amount of root material produced by such species, which inturn provide greater surface for endophytic mycelia to produce spores. Similar results have also been reported by Daft & Nicolson (1972) who found a significant rank correlation between the numbers of spores and shoot dry weights for each plant. They also reported that large tomato plants produced more spores than small plants.

The results clearly show that the stage of host development influence the development of VA mycorrhiza and also affects the population of Endogonaceous spores in the soil around their roots. This also provide information about the period of extensive mycorrhizal development in the life cycle of host plants.

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