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Interaction of Meloidogyne hapla and Rhizoctonia solani in alfalfa

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INTERACTION OF MELOIDOGYNE HAPLA
AND RHIZOCTONIA SOLANI IN ALFALFA

by

William Arden Irvine

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INTRODUCTION

The root-knot nematodes, *Meloidogyne* spp., have as hosts over 1865 species of plants, both monocotyledonous and dicotyledonous. One of the hosts for *Meloidogyne hapla* Chitwood 1949, is alfalfa which may be severely damaged by this nematode. Approximately 2.2 million acres of land are planted to alfalfa for hay in Iowa and some of this acreage is known to be infested with *M. hapla*. Since alfalfa is a perennial used for hay and pasture for at least two years, it is possible that large infestations of a root-knot nematode could cause significant yield reductions of this crop. Specific loss figures for Iowa are lacking but this nematode is considered to be a problem to alfalfa elsewhere.

*Rhizoctonia solani* Kühn is an ubiquitous soil fungus and causes injury to a large number of plants under various conditions and in many different types of soil. The apparently universal presence of this facultative parasite in the soil, makes it a potentially damaging fungus. Some strains of *R. solani* are weak parasites but epidemics may result where a favorable combination of factors exist such as the presence of a virulent strain of the fungus, a susceptible variety of plant and optimum conditions of temperature and moisture for infection and disease development.

There are apparently two species of *Rhizoctonia* in this country able to attack alfalfa. One is *R. solani* which is widely distributed and causes mainly damping-off of seedlings. The other is *R. coccocum* DC ex Fr with a more limited distribution and it attacks, as a rule, only mature plants in the field (Peltier 1916).

Although *R. solani* has a wide host range and occurs in every state,
its economic importance in many cases is not well defined. Alfalfa stands infested with this fungus are often short lived. It is possible, however, that death of young alfalfa plants is not due entirely to \textit{R. solani}. The fungus is responsible for the death of many small and large roots and thus contributes to factors causing plant mortality (Smith 1943). Other factors, such as plant parasitic nematodes, may also be contributing to the death of the host. Therefore, the relative amount of resultant damage in an area should await a more thorough knowledge of the entire disease complex.

The primary purposes of this investigation were: 1) The study of the effect of \textit{Meloidogyne hapla} and \textit{Rhizoctonia solani} alone and in combination, on alfalfa yields as influenced by temperature; and 2) the investigation of the pathological anatomy of alfalfa infected with \textit{M. hapla} and \textit{R. solani}, alone and in combination under variable and constant temperatures.
LITERATURE REVIEW

General Life History of the Root-Knot Nematode

Based on the work of Christie (1946), Godfrey and Oliveira (1932) and other workers, the general life history of the root-knot nematodes has been fairly well investigated. The taxonomy was clarified by Chitwood (1949) when he erected several species in place of the generally accepted single species Heterodera marioni (Cornu, 1879) Goodey, 1932 and reinstated Meloidogyne as a valid genus. This work has been accepted by nearly all nematologists and it has helped to clear up some of the puzzling biological problems existing before 1949. Prior to this date, life history studies were no doubt made on several species of root-knot nematodes but were all attributed to one. Thus, parasite as well as host and physical environment differences may account for some of the discrepancies as reported by various authors. As more studies are made of known species of root-knot nematodes on given hosts, some of these discrepancies may be more clearly resolved.

Penetration

One of the accepted points of invasion by the second stage juvenile is at the root tip (Byars, 1914; Godfrey and Oliveira, 1932; Christie, 1936; Tarjan, 1952; Krusberg and Nelsen, 1958; Schilke and Crittenden, 1959; and Loewenberg et al., 1960). Linford (1939), Tarjan (1952), and Schilke and Crittenden (1959), however, reported that penetration could also occur in the region of cell elongation in snapdragon and soybean. Krusberg and Nelsen (1958) found that invasion of sweet potatoes by M. incognita acrita Chitwood, 1949 can also occur through lateral root wounds.
and at mechanical breaks in the epidermis.

Once invasion has occurred, migration to the feeding site occurs intercellularly (Christie, 1936) or both intracellularly and intercellularly (Krusberg and Nelsen, 1958). They found that the juveniles came to rest primarily in the plerome, in the region of cell elongation, in the cambial zone, or in the parenchyma, depending on whether the infection court was young root tips, lateral root wounds or mechanical breaks in the epidermis, respectively. The most generally accepted final position of rest and feeding is in the plerome or stelar initials of the root (Christie, 1936; Linford, 1937; Davis and Jenkins, 1960). The position of orientation may be entirely within the plerome parallel to the longitudinal axis of the root (Christie, 1936) or the anterior end may lie between the cells of the plerome with the posterior end of the body extending into the periblism and later tissue arising from these initials (Christie, 1936; Godfrey and Oliveira, 1932).

Host Reaction to Penetration

Various host reactions are produced as the result of penetration of the root-knot nematode into plant roots. One of the most obvious ultimate reactions, macroscopically, is the production of galls. Galls caused by different species of *Meloidogyne* are not all of the same size. It is well known that galls produced by *M. hapla* are smaller than those produced by *M. incognita incognita* (Kofoid and White, 1919) Chitwood, 1949 or *M. incognita acrita* on a given host. Gall formation is caused by the hypertrophy of cortical cells and cortical and stelar proliferation (Christie, 1936; Davis and Jenkins, 1960) on tomato and gardenia. The production of
numerous lateral roots from the galled areas is a common symptom resulting from infection by the root-knot nematodes (Christie, 1936; Schilke and Crittenden, 1959; Davis and Jenkins, 1960). Reynolds and O'Eannon (1960) showed this to be true in alfalfa. This production of lateral roots may depend upon the species of *Meloidogyne* used. Schuster and Sullivan (1960) found that *M. hapla* induced root hair formation on galls in agar tests and the proliferation of lateral roots in soil tests. *Meloidogyne incognita* on the other hand did not produce this effect. Loewenberg et al. (1960) observed that gall formation began before the nematodes penetrated the root and a few instances were observed where gall formation occurred without the nematode entering at all. Death of the root apical meristem due to penetration by large numbers of nematodes is often produced (Christie, 1936 and Schilke and Crittenden, 1959).

Microscopically one of the most constant and outstanding features of the galls is the presence of giant cells, although they were often overlooked in the older literature. Davis (1959) and Davis and Jenkins (1960) found from two to five and from ten to 20 giant cells around a single female. The young giant cells were vacuolate and contained 30 to 40 nuclei. In the older giant cells, there were fewer nuclei and they appeared to be coalesced. The stimulus which ultimately results in the formation of giant cells becomes operative before the cells of the perisome have proceeded far in their normal differentiation. In tomato, giant cells are usually initiated in the vascular cylinder and frequently are derived from cells that normally would have taken part in the formation of vessels (Christie, 1936). Davis (1959) found that giant cells in the differentiated tissue were formed from a group of vascular parenchyma cells which
were first enclosed by a thick cell wall.

Occasionally, giant cells are formed in the cortex but there is usually no tendency for them to grow inward and push aside the vascular elements as is the case with some of the cyst nematodes (Christie, 1936).

There seems to be general agreement as to the form and structure of the giant cells. They usually begin by coalescences of several members of a row of undifferentiated cells in the central cylinder with the ultimate dissolution of the separating cross walls (Christie, 1936). The cytoplasm is dense and deeply staining, the cell walls are thick and the nuclei become enlarged (Schilke and Crittenden, 1959). The nuclei increase in number by simultaneous mitosis throughout a single giant cell (Bird, 1961). The nuclei are large and irregular in shape and contain large nucleoli (Bird, 1961). According to Christie (1936) the galls and cell division are believed to be caused by a secretion from the anterior end of the root-knot nematode.

Factors Affecting Root-Knot Nematode Disease Development

Temperature and moisture appear to be important with regard to root-knot nematode development. Bloom and Couch (1959) and Couch and Bloom (1960) concluded that eggs of 

\( \textit{Hapla} \)

did hatch equally well at soil moistures of field capacity and permanent wilting percentage. They suggested that the hatched nematodes were unable to migrate at the higher moisture. Godfrey (1928) and Kincaid (1948) are of the opinion that soil moisture plays only a small part in the root-knot development as long as the water content of the soil was favorable to the growth of the host. The former found that there was little difference in root-knot development between 49
per cent and 80 per cent moisture holding capacity and that considerable
galling also occurred outside of this range.

Kincaid (1946) reported that the optimum temperatures for root-knot
development was between 20°C and 30°C with most rapid development occurring
at 27°C. Root penetration occurred between 12°C and 35°C. Godfrey (1926)
reported that infections were very rare at 10°C to 12°C. Tyler (1933)
found that the minimum time required for the life cycle of *H. marioni* in
tomato was 25 days at 27°C and increased to 87 days at 16.5°C. Tyler
(1933) also found that root penetration by juveniles occurred at tempera­
tures as low as 12°C and that no eggs were laid below 14.5°C. Thomason
and Lear (1961) found that populations of *M. hapla* differed significantly
from those of *M. javanica javanica* (Treub, 1885) Chitwood, 1949 and *M.
incognita acrita* in their ability to produce egg masses at high soil tem­
peratures. The maximum temperature at which egg masses were produced in
*M. hapla* was 32.6°C. Daulton and Musbaum (1961) found that *M. hapla* eggs
can survive longer than those of *M. javanica* in soil at -2°C. Conversely
they found the eggs of *M. hapla* were less tolerant to high temperatures
than those of *M. javanica*. Bird (1959) suggested that the higher tempera­
tures might affect the ability of the second stage larvae to penetrate
rather than influence growth rates once it is in the host.

**Effect of *M. hapla* on Yield of Alfalfa**

Chapman (1959, 1960, 1963) found that 3000 and 1000 nematodes of *M.
hapla* per half gallon of soil reduced the growth of alfalfa 58 and 42 per
cent in 175 and in 381 days, respectively. Using 1000 juveniles in 500 gm.
of soil and comparing cut and uncut treatments, he found that the dif-
ferences in top weights between treated and control plants were significant in both cut and uncut tests. In this latter test he found no corresponding effect of the nematodes on the weight of the roots.

Factors Affecting *Rhizoctonia solani*

Peltier (1916) found that the greatest pathogenicity by *Rhizoctonia* sp. causing stem rot of *Dianthus caryophyllus* was above 88°F and at soil moistures unsuitable for best development of the plant. Blair (1943) found that *R. solani* grew fastest at 30 per cent saturation which was the lowest soil moisture content tested. Papavizas and Davey (1961) observed the saprophytic activity of *Rhizoctonia* sp. to be significantly higher when the soil moisture was maintained at 60 to 90 per cent. Sanford (1938a) found that between 16°C and 23°C, *R. solani* appeared equally virulent at soil moistures between 19 and 40 per cent of the moisture holding capacity.

Benedict (1954) found that periods of high temperatures and dry weather appeared to be conducive to the destructiveness caused by pathogenic strains of *R. solani*. Smith (1946) found most rapid growth of *R. solani* on potato dextrose agar occurred at 25°C and 30°C with no growth at 5°C, 10°C and 40°C during the three day period. Cankers did not develop on alfalfa at soil temperatures of 15°C and 40°C but an average of 29.4 cankers developed on each tap root at 30°C. Kernkamp (1949) found damping-off of alfalfa cuttings caused by *R. solani* to be especially severe at temperatures of 75°F to 85°F but less severe at temperatures below 60°F to 65°F. Cuttings failed to root at 80°F to 85°F. Smith (1943) found that *R. solani* was very pathogenic on alfalfa at temperatures between 25°C and 30°C but very little at 16°C to 18°C. Montsith and Dahl (1928), Newton
(1931), and Wellman (1932) all agree that development of *R. solani* is favored by temperatures between 23°C to 30°C with the optimum being 24°C to 27°C and with the most common temperature for best growth being 25°C. Gratz (1925), on the other hand, reported no infection on potato stem by *R. solani* between 22°C and 25°C. Richards (1923) found greatest destruction of the growing tips of potato to occur between 12°C to 18°C and that above 18°C this type of injury became less and disappeared entirely at approximately 21°C.

Storey (1941) found the existence of biological races, some isolates having a wide host range while others were very specific. Smith (1945) found that isolates of *R. solani* from alfalfa caused numerous root lesions after reinoculation into alfalfa. Isolates from other plant species failed to produce lesions on alfalfa.

**Nematode-Fungus Interactions**

Although "complexes" exist between other species of nematodes and fungi the literature reported here will be confined to root-knot fungus complexes, especially those involving *Meloidogyne hapla*.

Atkinson (1892) was the first to report an interaction existing between fungi and nematodes. He observed that infection by a root-knot nematode species increased the incidence and severity of *Fusarium* wilt of cotton in the field.

Jenkins and Coursen (1957) found that when *Fusarium oxysporum f. lycopersici* and *Meloidogyne hapla* were present together, 100 per cent wilt occurred in wilt susceptible Rutgers and 60 per cent in wilt resistant Chesapeake tomato, as compared with 60 per cent and zero per cent, respec-
tively, with the fungus alone. McGuire et al. (1958) showed that, in Buffalo alfalfa, the combination of *M. hapla* and *F. oxysporum f. vasinfectum* gave a significantly higher percentage of wilt and many more dead plants than that caused by the fungus alone or by the combination of the fungus and any of the other four species of *Meloidogyne* used. However, three of those other four species of *Meloidogyne* used in combination with the fungus gave significantly more wilting than when the fungus was present alone.

Wyllie and Taylor (1960) found that soybean plants growing in soil infested with both *M. hapla* and *Phytophthora sojae* Drechs. were more severely affected than in treatments inoculated with the fungus alone. They considered, however, that *M. hapla* was only contributory to the effect of the fungus and that the fungus caused most of the damage.

Taylor and Wyllie (1959) studied the effect of *M. javanica* and *M. hapla* alone and in combination with *R. solani* on pre-emergence damping-off of Chippewa soybeans in tests conducted at 75°F. Three weeks after planting the average emergence for each treatment expressed as a percentage of the checks was: *M. javanica* alone, 98 per cent; *M. hapla* alone, 83 per cent; *R. solani* alone, 50 per cent; *M. javanica* plus *R. solani*, 17 per cent; *M. hapla* plus *R. solani*, two per cent. Reynolds and Hanson (1957) found that the presence of the cotton root-knot nematode *Meloidogyne incognita* acrita increased the incidence of post-emergence damping-off of cotton caused by *R. solani*. They further showed that in fumigated plots where nematodes were controlled the loss in stand due to *R. solani* was less than in unfumigated plots. Brodie and Cooper (1964) showed that the presence of *Meloidogyne* spp. in soil in which cotton seedlings were planted prolonged the susceptibility of these seedlings to postemergence damping-off, caused by *R. solani*,
for a longer period of time than seedlings grown in nematode free soil.

Powell and Nusbaum (1960), in a histological study, found that the black shank fungus *Phytophthora parasitica var. nicotianae* (Breda de Haan) Tucker in tobacco apparently had an affinity for the hypertrophied and hyperplastic areas of the root-knot galled tissue. The mycelium was found to be more abundant in the galled than in the non-galled areas. To the writers knowledge a histological study involving the combination of *R. solani* and *M. hapla* has not been done for alfalfa.
MATERIALS AND METHODS

Source of Inoculum

*Meloidogyne hapla* was originally obtained from dandelion roots in an alfalfa field. The stock populations were increased from an initial source of 25 hand picked juveniles, rinsed in sterile water, and maintained in 12 inch pots in the greenhouse on tomato and subsequently Ranger alfalfa for several months prior to use. New populations were started from mass transfers of old ones. The method of extraction was adapted from Godfrey (1931). Essentially, when inoculum was desired, alfalfa plants were carefully removed from the soil, the roots were washed thoroughly, and lateral roots containing galls were placed on raised wire screens in petri plates. Distilled water was added to just bathe but not submerge the roots. The juveniles which emerged were poured off daily into a flask and fresh water was then added to the plates. If the nematodes were not used immediately they were stored in an Erlenmeyer flask containing continuously aerated tap water. The storage period varied from two to four days.

*Rhizoctonia solani* was obtained from stock culture A-22 which had been isolated from alfalfa roots.

Testing Varieties of Alfalfa for *M. hapla* Resistance

The following eight varieties of alfalfa were tested for resistance to *Meloidogyne hapla*: Alfa, Buffalo, Cody, DuPuits, Lahontan, Narragansett, Ranger, and Vernal. Eight inch pots were filled to within two inches of the top with a 1:1 sand-soil mixture which had been steam sterilized for five hours at 15 pounds pressure. About 250 cc of nematode infested soil from the stock cultures was spread evenly over the soil surface. This
was covered with a layer of the 1:1 sand-soil mixture.

Thirty-five seeds were planted in each of four pots for each variety tested. The plants in each pot were thinned to 20 one to one-and-a-half-weeks after emergence. Root-knot indexes were taken after eight weeks.

Effect of Temperature on Rhizoctonia solani

The same isolate of Rhizoctonia solani was used throughout the study. The effect of temperature on the growth of this fungus was studied in petri plates using incubators set at the same temperatures as used in the constant temperature tanks in some of the experiments in the greenhouse, i.e. 15°, 20°, 25° and 30°C. A two per cent water agar was used as the growth medium. Water agar was used because it would be comparable to the R. solani medium used in later soil infestation experiments.

A 0.2 cm piece of inoculum was transferred from a stock culture tube to the center of a petri plate containing the water agar. Measurements were made at 12 hour intervals, at right angles across the growth pattern, until the fungus reached the edge of the petri plates. The same line of measurement was taken each time. The experiment was repeated twice with four and three replications being used, respectively, for each temperature.

Yield Data Experiments

Two separate experiments were conducted to study the effect of Meloidogyne hapla and Rhizoctonia solani alone and in combination on alfalfa. In one experiment, carried out on the greenhouse bench, six inch clay pots were filled with steam sterilized potting soil. The treatments were as follows: 1) M. hapla alone; 2) R. solani alone; 3) a combination of M. hapla and R. solani; and 4) the check which contained neither
M. hapla nor R. solani. Five replications were used, for each treatment. The total number of nematodes were obtained by a standard counting method and dilutions were made to obtain 700 M. hapla juveniles for each appropriate pot. The nematodes were added to the pots in five cc of tap water. Soil treatments receiving R. solani were infested with one-half-petri plate of the fungus homogenized with 50 cc of water in a Waring Blender. Five cc of the nematode wash water was added to the check and Rhizoctonia treatments after a thorough examination and settling to ensure that no nematodes were still present. One-half petri plate of two per cent water agar, homogenized in 50 cc of tap water was added to each of the pots containing the checks and Meloidogyne alone.

The M. hapla and R. solani inoculum was placed in the center of the appropriate pot in a hole 2.5 cm in diameter and five cm deep. Pipettes were used to place the inoculum at the bottom of the hole and care was taken not to splash any of the inoculum on the sides of the hole. The holes were then filled with sterile soil.

Ranger alfalfa seeds were surface sterilized in a 27 : 1 dilution of Zephiran Chloride and rinsed in two changes of sterile distilled water. Ten seeds were then placed seven mm below the soil surface in each pot. The infestation of the soil with the fungus and nematodes and the planting of the seeds was all done on the same day. Two weeks after seeding, the soil was infested with a suspension of Rhizobium bacteria and the plants thinned to five per pot. Emergence counts were made during the two weeks period. The plants were first cut at two months after seeding and at monthly intervals thereafter for a total of eight months. Cuttings were made at five cm above the soil level. Oven dry weights were taken after
the cuttings had been dried at 60°C for 48 hours.

After eight months, the plants were soaked gently out of the pots. After cutting off the five cm stubble at the root crown, the roots were washed carefully and thoroughly, blotted and fresh weights taken. One-half gm of roots from each treatment was taken, surface sterilized in ten per cent chlorox for one minute and rinsed in two changes of sterile tap water. These roots were plated on two per cent water agar for recovery of fungi. Root-knot indexes were made on the M. hapla and the M. hapla -- R. solani treatments. The remainder of the roots from each treatment were dried in the oven at 60°C for 48 hours and dry weights were taken.

The second set of two tests was similar to that described except that constant temperature tanks were employed. The temperatures used were: 15°C, 20°C, 25°C, and 30°C with a variation of ± 1°C. The four inoculum treatments as previously described for the bench experiment were used. There were six replications in the first test set and five in the second.

One quart plastic containers, hereafter referred to as containers, were painted on the outside with a semi-gloss black enamel. These containers were nearly filled with a steam sterilized 3:1:1 peat:loam:sand mixture. The nematode inoculum in the first temperature tank test was obtained as previously described except that 1200--1300 nematodes were used to infest the soil where required. Five cc of the wash water was added to the non-nematode treatments. The soil in the containers was infested with three one cm plugs of the fungus which was grown on two per cent water agar in petri plates. All of the inoculum was placed in the bottom of a hole 6.5 to 7.5 cm deep and 2.5 cm in diameter. The first cuttings were taken eight weeks after seeding and then at four week intervals thereafter.
for a period of 20 weeks. Dry weights were taken as previously described. At the conclusion of the experiment roots from two of the six replications were used for sectioning and reisolation of fungi. The roots from the other four replications were used for dry weights. Both tap and lateral roots were used for reisolation on two per cent water agar.

The experiment in the second set of temperature tanks was similar to that of the first with the following exceptions: 1) 800 nematodes were used to infest the soil where applicable; 2) the hole in the soil was only five cm deep; 3) the duration of the experiment was 16 weeks; and 4) one root from each container was used for sectioning and reisolation of fungi at the conclusion of the experiment.

At the conclusion of all experiments counts were made on the number of plants still alive and visual root-knot indexes were made in each treatment.

Histological Studies

At the same time as the experiments for the yield data were started, parallel experiments were begun on the greenhouse bench and in the constant temperature tanks from which the plant roots were used for sectioning. Four treatments, as described previously, were used with five plants per pot. The infestation of the soil with _M. hapla_ and _R. solani_, and inoculation with the _Rhizobium_ bacterial suspensions was the same as previously described.

In the bench experiment, roots were gently removed and examined for galls at daily intervals, from the third to the twelfth day after seeding. Thereafter sampling was extended to four day intervals up to and including
28 days after seeding. One sample for sectioning was taken at two months.

In the temperature tanks, roots for sectioning from each treatment were soaked gently out of the containers at six day intervals for a period of 30 days after seeding. At the end of the 20 and 16 week periods of growth, roots were taken from two of the six replications in the first set of temperature tanks and one root from each container in the second set of temperature tanks and prepared for sectioning.

Root measurements of the tap roots were made up to 20 days after seeding. Further measurements were not made after this period since the roots became too branched in the galled areas to tell which was the tap root or which were lateral roots.

Root pieces for sectioning were killed and fixed in Craf III, dehydrated, and prepared for embedding in wax by the ethyl alcohol-xylene method of Sass (1951). The pieces of tap root used for sectioning were taken at measured intervals along the root; measurements being made from the base of the cotyledons. Lateral roots below the five cm depth in the soil were also sectioned. Only root tips were sectioned from the three, four, and five day material in the bench experiment. Depending on the age and woodiness of the material, sections were cut at a thickness of ten or 12 microns on the rotary microtome. The sections were affixed to microscope slides with gelatin and stained with safranin and fast green.
RESULTS

Alfalfa Variety Tests

All eight varieties of alfalfa tested were found to be susceptible to *Meloidogyne hapla* (Table 1). Ranger alfalfa had the most while Vernal and DuPuits had the fewest knotted plants. The visual knotting index was low, ranging from one to two in all varieties. This is attributed to the infestation method employed. All knots were small and many lateral roots emerged from the galled areas. The root systems were not appreciably stunted due to the low root-knot infestation.

Table 1. Percentage of plants galled and the root-knot index of roots infected with *Meloidogyne hapla* after two months

<table>
<thead>
<tr>
<th>Variety</th>
<th>Number of plants galled out of eighty</th>
<th>Per cent of plants galled</th>
<th>Average root-knot index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranger</td>
<td>80</td>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>Alpha</td>
<td>77</td>
<td>96</td>
<td>1.5</td>
</tr>
<tr>
<td>Buffalo</td>
<td>76</td>
<td>95</td>
<td>1.5</td>
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<tr>
<td>Cody</td>
<td>74</td>
<td>92</td>
<td>1.5</td>
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<tr>
<td>Lahontan</td>
<td>72</td>
<td>90</td>
<td>1.5</td>
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<tr>
<td>Narragansett</td>
<td>70</td>
<td>87</td>
<td>1.5</td>
</tr>
<tr>
<td>DuPuits</td>
<td>68</td>
<td>85</td>
<td>1.5</td>
</tr>
<tr>
<td>Vernal</td>
<td>59</td>
<td>73</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>0 = no galls
1 = 1-25 per cent galled
2 = 26-50 per cent galled
3 = 51-75 per cent galled
4 = 76-100 per cent galled
Effect of Temperature on *Rhizoctonia solani*

The most rapid growth of *Rhizoctonia solani* on two per cent water agar occurred at 30° and 25°C. At both temperatures the mycelium had reached the edge of the petri plates within 48 hours after the transfer was made. It took more than 60 hours at 20°C and approximately 96 hours at 15°C for the mycelial growth to reach the edge of the petri plates (Figure 1). The most luxuriant and thickest growth of the mycelial mats occurred at 30° and 25°C with the least at 15°C.

**Macroscopic Examination of the Roots**

*Bench experiment*

Obvious swelling of the *M. hapla* infected root tips did not occur until the sixth day after seeding. Although, as will be shown later, penetration had occurred by the fourth day. The majority of the roots with the swollen tips usually occurred at approximately five cm in length which was the depth at which the nematodes were placed in the soil. Since a swelling occurred in the majority of cases at this depth, these knots along with those on lateral roots were used for histological studies. Other root pieces at measured intervals along the root were also used for sectioning.

In the early stages of infection by *M. hapla*, the root tips usually curved within five to ten days after seeding (Figure 2). In some cases only a slight curvature was observed while in others the root made a complete circle. On the inside of the curve and extending proximally one to two centimeters was a line which appeared as a soft looking water soaked area or as a brownish line, the latter being reminiscent of a suture line.
Figure 1. Averages of mycelium growth of *R. solani* on two percent water agar at four temperatures.
Figure 2. Alfalfa roots eight days after seeding. Note the swelling and curvature at the tip of the tap root of Meloidogyne treatments.

Top left — Meloidogyne hapla
Top right — Rhizoctonia solani
Bottom left — Check
Bottom right — M. hapla — Rhizoctonia solani
This line also appeared on the non-curved roots of the plants of the *M. hapla* and the *M. hapla -- R. solani* treatments.

By eight days after seeding the lateral roots had emerged in fairly large numbers from the tap root and these laterals showed swelling of the root tips indicating nematode penetration. Also in the nematode treatments lateral roots were observed to arise extremely close to the tap root tip. By approximately ten days, the lateral roots were severely galled. This swelling of the lateral root tips was particularly evident at approximately the five cm depth in the soil. As may be noticed in Figure 2 some of the root tips of the check plants were slightly swollen. This was due to the fact that the roots had reached the bottom of the pots by eight days after seeding and swelled slightly, a common phenomenon with some plants grown in pots.

Cessation of root growth in the *M. hapla* and the *M. hapla -- R. solani* treatments was evident very early as shown in Table 2. This was also evident later when the roots were soaked out of the pots and appeared only as short stubs of approximately five to six cm long. In other cases there would appear a larger knot at approximately five to seven and one-half cm in the soil but then the tap root appeared to have kept growing. This, however, may have been a lateral root put out from the gall and not the tap root at all.

The root lengths in the nematode treatments changed little between three and 11 days of growth as compared with the non-nematode treatments (Table 2). Lateral root development was much greater in the *M. hapla* than in the non-*M. hapla* treated plants, giving the root system of the former treatments a bunchy or witches broom appearance. The number of lateral
Table 2. Alfalfa root lengths in centimeters after 20 days treatment. Average of five plants

<table>
<thead>
<tr>
<th>Days</th>
<th>M. hapla — R. solani alone</th>
<th>R. solani alone</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.2</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>5.6</td>
<td>6.4</td>
</tr>
<tr>
<td>5</td>
<td>6.1</td>
<td>5.0</td>
<td>8.5</td>
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<tr>
<td>6</td>
<td>5.0</td>
<td>5.0</td>
<td>8.5</td>
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<tr>
<td>11</td>
<td>6.1</td>
<td>4.8</td>
<td>11.0</td>
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<tr>
<td>12</td>
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<td>5.7</td>
<td>20.1</td>
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<tr>
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<td>9.5</td>
<td>5.1</td>
<td>15.4</td>
</tr>
<tr>
<td>30</td>
<td>11.2</td>
<td>12.5</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Roots arising from a galled area on the tap root varied from one to 12 except in one instance where approximately 20 were observed. The lateral roots on the check plants were fibrous whereas those from the root-knot treated plants were much thicker. The knots produced on the tap roots were usually larger than those produced on the lateral roots. The bunchy appearance of the nematode infected roots at 20 days made it difficult to measure root lengths due to the difficulty in distinguishing the lateral from the tap root (Figure 3). Little difference in the top growth was observed between the four treatments at 20 days.

The tap root appeared to have ceased linear growth after infection by 16 to 20 days in some cases, while in others it continued. In the latter instances galls were some distance back from the root tip. It is possible, however, that what was assumed to be the tap root may actually have been a
Figure 3. Alfalfa roots 20 days after seeding showing lateral root development in nematode infected roots. Left to right: Meloidogyne hapla (2 plants); check (1 plant); Meloidogyne hapla -- Rhizoctonia solani (2 plants); Rhizoctonia solani (1 plant).
lateral root initiated by the nematode. Therefore, little emphasis should be placed on the measurements of the 16 and 20 day samples.

In the 24 day sample, galls were observed on the roots from 1.2 cm to 6.5 cm below the cotyledonary node. One knot, which was later shown to contain nematodes, occurred approximately at the root-stem transition zone. In this case either a mistake was made in infestation or splashing had occurred from one pot to another. Another possibility would be upward migration of the nematodes.

Bacterial nodules were not evident until approximately 28 days after seeding. Necrotic lesions were not observed in the *R. solani* treatments at 28 days or later, but there was a general browning of the roots.

At 59 days after seeding, in the *M. hapla* and *M. hapla - R. solani* treatments the tap root had ceased to grow at 3.5 to 5.5 cm below the soil in three of the five plants in each nematode treatment. All plants in the *R. solani* alone and check treatments had well developed tap root and fibrous root systems on all sampling dates.

*Rhizoctonia solani* was reisolated from the roots receiving this treatment in only two cases. These were at the 16 and 20 day samples. This low reisolation rate may have been due to the fact that only the tap root was used in the reisolation procedure.

**Temperature tank experiment**

Macroscopic examination of the roots from the temperature tanks revealed approximately the same results as those from the bench experiment especially with regard to 30°C, 25°C and 20°C treatments. Swollen and curved root tips occurred at six days after seeding at all temperatures.
except 15°C at which temperature plant growth was much slower (Table 3). Also, *R. solani* may have delayed germination as shown at six days in Table 3. All roots in the *M. hapla* and the *M. hapla -- R. solani* treatments exhibited swollen root tips at all four temperatures by 12 days. Curvature of the roots was noted as was the brownish seam previously described for the roots from the bench experiment.

By 12 and 18 days numerous lateral roots emerged from the galls in the 30°C, 25°C and 20°C tanks. Also at 18 days root growth of the check plants was greater at 20°C and 25°C than at 15°C and 30°C (Table 3). At 24 days, bacterial nodules were observed only on the check and *R. solani* alone treatments in the 15°C and 20°C tanks.

The severity of root-knotting and root-stunting was in the order of 30°C > 25°C > 20°C > 15°C at 24 and 30 days after seeding. There was less lateral root proliferation and fewer knots produced at 15°C. Root growth in the check treatments was greatest at 25°C and 20°C. The top growth was greater at 25°C and 20°C than at 30°C and 15°C at 24 days after seeding (Figures 4, 5, 6 and 7). The knots that occurred on the tap root by 30 days were usually larger than those which occurred on the lateral roots.

At the conclusion of the experiment, *R. solani* was recovered from the tap root only at 20°C from the *M. hapla -- R. solani* and *R. solani* alone treatments.
Table 3. Alfalfa root lengths in centimeters, average of five roots, after 18 days at four temperatures

<table>
<thead>
<tr>
<th>Days after seeding</th>
<th>15°C</th>
<th></th>
<th>20°C</th>
<th></th>
<th>25°C</th>
<th></th>
<th>30°C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MR</td>
<td>M</td>
<td>R</td>
<td>C</td>
<td>MR</td>
<td>M</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>---^</td>
<td>4.8</td>
<td>---^</td>
<td>3.7</td>
<td>3.6</td>
<td>3.5</td>
<td>7.0</td>
<td>8.7</td>
</tr>
<tr>
<td>12</td>
<td>9.5^</td>
<td>5.4</td>
<td>10.0</td>
<td>7.8</td>
<td>5.4</td>
<td>5.0</td>
<td>---^</td>
<td>---^</td>
</tr>
<tr>
<td>18</td>
<td>6.2</td>
<td>5.8</td>
<td>11.6</td>
<td>13.0</td>
<td>10.3</td>
<td>7.6</td>
<td>21.0</td>
<td>21.5</td>
</tr>
</tbody>
</table>

^MR = M. hapla --- R. solani

<table>
<thead>
<tr>
<th>Days after seeding</th>
<th>15°C</th>
<th></th>
<th>20°C</th>
<th></th>
<th>25°C</th>
<th></th>
<th>30°C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MR</td>
<td>M</td>
<td>R</td>
<td>C</td>
<td>MR</td>
<td>M</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>---^</td>
<td>4.8</td>
<td>---^</td>
<td>3.7</td>
<td>3.6</td>
<td>3.5</td>
<td>7.0</td>
<td>8.7</td>
</tr>
<tr>
<td>12</td>
<td>9.5^</td>
<td>5.4</td>
<td>10.0</td>
<td>7.8</td>
<td>5.4</td>
<td>5.0</td>
<td>---^</td>
<td>---^</td>
</tr>
<tr>
<td>18</td>
<td>6.2</td>
<td>5.8</td>
<td>11.6</td>
<td>13.0</td>
<td>10.3</td>
<td>7.6</td>
<td>21.0</td>
<td>21.5</td>
</tr>
</tbody>
</table>

^Just germinated.

fOnly one plant had emerged, the others had not germinated. This one had no swelling, not visibly infected.

^Root tips lost. Stub of root was at least 8 to 9 cm long.
Figure 4. Top left. Alfalfa plants 24 days after seeding at 15°C.

Figure 5. Top right. Alfalfa plants 24 days after seeding at 20°C.

Figure 6. Bottom left. Alfalfa plants 24 days after seeding at 25°C.

Figure 7. Bottom right. Alfalfa plants 24 days after seeding at 30°C.

Legend for Figures 4 through 7. Left to right: Check (1 plant); R. solani (1 plant); H. hapla (2 plants); H. hapla -- R. solani (2 plants).
Microscopic Examination of the Roots

Bench experiment

Sections made from roots killed and fixed three days after seeding were found to be free of nematodes. The roots of these three day old seedlings were approximately four cm long and, therefore, had not reached the depth at which the nematodes were placed in the soil. Root-knot nematodes were first observed, in both *Meloidogyne* treatments in the root tip sections four days after seeding.

Penetration by the second stage juveniles was observed to occur 1) directly through the root cap and tip, 2) behind the root tip but still in the region of the meristematic zone (Figure 8) or, 3) between the root cap and the dermatogen layer. In the latter instance, the nematodes were seen to penetrate at an angle then curve up towards the root histogens (Figure 9). When penetration occurred behind the root tip the anterior end faced towards the tip of the root. Under the conditions of these experiments no penetration was observed to occur at lateral root ruptures.

Nematodes were observed in or at the edge of the plerome within five to six days after seeding (Figures 10 and 11). At six days nematodes were observed not only in the first 0.5 cm piece of root which included the root tip but also in the 0.5 cm piece immediately behind the root tip. By seven days individual nematodes were observed in the central cylinder in the second cm from the tip and later than this occasionally in the third cm from the root tip. Since the plerome gives rise to the stele, the nematodes penetrated into this tissue at a very early date. Occasionally the posterior end of the nematode was seen to extend into the cortex and later
Figure 8. *Meloidogyne hapla* juvenile behind the root tip four days after seeding. Arrow indicates nematode (400X).

Figure 9. *Meloidogyne hapla* juvenile in the root cap five days after seeding. Nematode is curving up towards the root histogens. Arrow indicates nematode (400X).
Figure 10. Several juveniles in the plerome at six days after seeding (160X).

Figure 11. Crosssection of juveniles in the plerome at six days after seeding (400X).

A = nematodes

B = protophloem cell
they were also observed to be entirely within the cortex. In this latter case no obvious changes in cellular structure were observed.

Mass as well as individual penetration by the nematodes occurred. In the former case, an obvious path of entry was present, but in the latter case tunneling was not observed (Figure 12). Ultimately the nematodes came to rest and began feeding in the central cylinder.

The root curvature observed macroscopically resulted when one or several nematodes invaded the root tip. The curvature was apparently due to suppression of growth on the side of invasion (Figure 13). The degree of curvature was often severe as shown in Figure 14.

Another effect produced on the host by the nematodes was observed in the root cap. In general the plants grown in the soil infested with nematodes appeared to have less root cap than plants grown in non-nematode infested soil.

Macroscopically, the knotted areas of the roots showed great proliferation of lateral roots. Sections of these areas showed, in practically every case, a nematode and giant cells in the area of the origin of the lateral root (Figure 15). In roots six days or older, lateral root proliferation, with nematodes and giant cells at the base, frequently occurred close to the root tip as well as farther back. The presence of the nematode appeared to have stimulated lateral root initiation. This lateral root initiation can probably be caused by both males and females. As shown by later sections, however, females were predominant at the base of the laterals.

After penetration had occurred and feeding had begun, the ultimate effects of the presence of the nematodes on the cells was hypertrophy and
Figure 12. Mass penetration by the juvenile nematodes through the root tip. Note the space with nematodes (160X).

Figure 13. Longitudinal section showing curvature of the root at the tip six days after seeding. Arrow indicates nematodes (60X).
Figure 14. Longitudinal section showing the severity of curvature of the root at nine days after seeding. Arrows indicate nematodes in the space (60X).

Figure 15. Lateral root proliferation with nematodes and giant cells at the base. Arrows indicate the nematodes (60X).
the eventual production of giant cells. The giant cell development ap­
peared to correspond with the development of the nematode in most cases. 
The giant cells began to form early in the central cylinder by a coalescing 
and dissolution of the cross walls of a number of cells to produce a multi­
nucleate condition (Figure 16). The nematode present here is probably a 
second stage juvenile. In the early stages of development the protoplasm 
was lightly stained, but at approximately 10 to 11 days after seeding it 
appeared more darkly stained and granular. Also by 10 to 11 days after 
infestation of the soil, the nematodes in the root sections appeared larger 
than at the beginning of the experiments and, therefore, may have been ap­
proaching the third stage juvenile (Figure 17). By 16 days after seeding 
the nematodes were large and were third or fourth stage juveniles. Corre­
spondingly the giant cells were larger, the protoplasm still deeply stained 
but at this stage was becoming very vacuolate. Also present in the cells 
was a large deep blue stained area which was probably a number of coalesced 
nuclei (Figure 18). At 20 days, the nematodes were pear shaped and the 
vulva was evident (Figure 19). This may be an adult or a fourth stage 
juvenile. A molted cuticle was not evident until 24 to 28 days after seed­
ing at which time the nematodes were definitely adults, (Figure 20). Be­
tween 20 and 28 days after seeding the giant cells gradually became more 
vacuolate. However, not all giant cells in a section were in the same 
stage of development. This was also true of the nematode development, 
particularly where mass penetration had occurred. This was probably due 
to a food factor.

At 24 days after seeding, the females in the root sections were large, 
pear shaped and a molted cuticle was evident (Figure 21). Due to the pres-
Figure 16. Beginning of giant cell production in the vascular tissue, six days after seeding (400X).

A = nematode
B = giant cell
C = lateral root

Figure 17. Multi-nucleate giant cells with deep staining, granular protoplasm at the base of a lateral root 11 days after seeding (160X).

A = nematode
B = multi-nucleate giant cell
C = lateral root
Figure 18. Juvenile at 16 days after seeding. The giant cells are becoming vacuolate and there are large blue staining areas which are probably coalesced nuclei (160X).

A = nematode
B = giant cell

Figure 19. Obese female showing vulva and nearly empty giant cells at 20 days after seeding. Note that the xylem ends at the giant cells (160X).

A = nematode
B = giant cell
C = coalesced nuclei in the giant cell
Figure 20. Nematode with molted cuticle 28 days after seeding, (400X).

A = cuticle

B = egg
Figure 21. Large female nematode with large giant cells devoid of protoplasm at 24 days after seeding (160X).

A = nematode

B = giant cell
ence of this cuticle and vulva the nematodes were probably adults. At this stage of development the egg sac was evident as a lightly staining area at the posterior end of the female. The giant cells were devoid of protoplasm. The egg sac contained a few undifferentiated eggs at 28 days (Figure 22) and at 59 days numerous eggs at various stages of development were present in the egg sacs which were oriented toward the outside of the root. At 28 days the giant cells were almost completely devoid of protoplasm. The number of giant cells was usually three to five around the anterior end of the female.

The effect of the giant cells on the vascular tissue was one of interruption and displacement of the vascular elements. Also evident were short pieces of xylem tissue which were irregularly arranged with no definite orientation. These could possibly be new xylem vessels formed from parenchyma cells.

The development of the nematodes and giant cells in the M. hapla --- R. solani treatments showed a similar developmental pattern to those in the M. hapla alone treatments described above. R. solani mycelium was observed in only one slide of the M. hapla --- R. solani treatments. In this case the mycelium was observed congregated in a single area around some giant cells (Figure 23).

In the sections of the R. solani alone treatments, the mycelium was observed in the nine through 24 day material. The fungus was both inter- and intracellular, completely encircling the root at the point of penetration. The tissue in the area of penetration was very necrotic.
Figure 22. Adult female with a few eggs in the egg sac at 26 days after seeding (160X).

A = nematode
B = cuticle
C = egg in egg sac
Figure 23. Mycelium of *R. solani* present in the vicinity of the giant cells at 24 days after seeding (400X).

A = hyphae

B = giant cell
Temperature tank experiment

The roots grown at all temperatures had been penetrated by nematodes at six days after seeding. The rapidity of development of the nematodes to the egg-producing stage was in the following descending order: 30°C, 25°C, 20°C, 15°C (Figures 24, 25, 26, and 27). Eggs first appeared in the roots at 30 days in the 30°C tank. The nematodes in the root sections from the 15°C tank showed very little difference in development between six and 30 days. The life cycle of *M. hapla* progressed much more rapidly at 30°C and 25°C than at 20°C and 15°C.

The *M. hapla* -- *R. solani* treatment followed the same developmental pattern as described above for *M. hapla* alone at all temperatures. No *R. solani* mycelium was found in the sections from these treatments.

Yield Data

Bench experiment

The only significant differences in yield at three months were between the *M. hapla* and the check treatments (Table 4). At the third cutting, four months after seeding, the difference between the nematode and check treatments was highly significant. This difference remained until the conclusion of the experiment with two exceptions in the *M. hapla* treatment. One was at six months at which time the difference was significant at the five per cent level. The other exception was at eight months where no significant difference was observed.

The differences between the top weights of the *R. solani* alone and check treatments were not significant until the seventh month. The roots of the *R. solani* alone and check treatments completely filled the pots
Figure 24. Eggs present in egg sacs 30 days after seeding at 30°C. Eggs to outside of root (160X).

A = nematode

B = eggs in egg sac

Figure 25. Large adult female but no eggs 30 days after seeding at 25°C (60X).

A = nematodes

B = giant cell
Figure 26. Nematodes 30 days after seeding at 20°C. Arrows indicate nematodes (60X).

Figure 27. Nematode has not progressed far in development 30 days after seeding at 15°C. Arrow indicates nematode (60X).
Table 4. Dry weights in gms of the top growth of alfalfa. Average of five replications for a period of eight months

<table>
<thead>
<tr>
<th>Time in months</th>
<th>M. hapla — R. solani</th>
<th>M. hapla</th>
<th>R. solani</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.54</td>
<td>4.33</td>
<td>4.83</td>
<td>4.73</td>
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<td>4</td>
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</tr>
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<td>3.96</td>
</tr>
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<td>3.76</td>
</tr>
<tr>
<td>8</td>
<td>1.03</td>
<td>1.65</td>
<td>1.96</td>
<td>2.35</td>
</tr>
</tbody>
</table>

LSD at .05 = 1.981

LSD at .01 = 2.621

and the decline in top weights of these treatments after four months was believed due, at least in part, to pot binding. No necrotic lesions were observed on the R. solani treatments, however, a general browning was observed.

The M. hapla — R. solani and the M. hapla treatments were severely stunted after five months (Figure 28). The foliage was chlorotic, the stands thin and the stems smaller in diameter and were weaker than the check or R. solani alone treatments.

Flowering did not follow a set pattern. When flowering did occur, however, the check plants usually flowered first and produced the most abundant blooms. The flower heads produced on the check and R. solani alone treatments were usually larger in size than the treatments containing M. hapla.
Figure 28. Alfalfa plants from the bench experiment five months after seeding. Left to right: R. solani alone; R. solani — M. hapla; Check; M. hapla alone.
At the conclusion of the experiment, the dry weights of the roots exhibited the same pattern as shown by those of the tops with the checks being the heaviest and the nematode treatments being the lightest (Table 5).

In all treatments exposed to the root-knot nematode, the root system, after eight months was severely stunted and exhibited a witches broom effect. Numerous small knots were observed on the lateral roots with larger ones on the tap root. Many lateral roots were observed to emerge from the galls and galled areas. The root system had grown very little in length and in some instances the tap root was almost non-existent as compared to that of the check and *R. solani* alone treated plants.

Rhizoctonia was reisolated from each treatment that had originally been inoculated with this fungus. The number of plants alive and the root-knot index after eight months is given in Table 5.

**Temperature tank experiment**

At six weeks after seeding the growth of the alfalfa plants in the 20° and 15°C containers appeared to be uniform in all treatments (Figures 29 and 30). Differences between treatments, at this time, were most evident at 30°C (Figure 32). At this temperature the main difference occurred between the check plants and those containers infested with nematodes with little difference visible between the other treatments.

The first cutting of the tops was made at eight weeks after seeding. The dry weight data averages for each cutting are presented in Table 6. As can be seen from this table, at eight weeks the lowest weights occurred in the nematode treatments, with these differences being greatest at 30°C and 25°C. At 20 weeks in these latter temperatures the plants in the nema-
Table 5. Dry weights of the alfalfa roots in gms, root-knot disease index and plant survival after eight months

<table>
<thead>
<tr>
<th>Average root weights</th>
<th>Reisolation of R. solani</th>
<th>Disease index</th>
<th>No. of plants alive&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR&lt;sup&gt;b&lt;/sup&gt; M&lt;sup&gt;c&lt;/sup&gt; R&lt;sup&gt;d&lt;/sup&gt; C&lt;sup&gt;e&lt;/sup&gt;</td>
<td>MR M R C</td>
<td>MR M R C</td>
<td>MR M R C</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of plants alive over total plants.

<sup>b</sup>MR = M. hapla -- R. solani
<sup>c</sup>M = M. hapla
<sup>d</sup>R = R. solani
<sup>e</sup>C = check
Figure 29. Alfalfa six weeks after seeding at 15°C. Left to right: Check; *R. solani*; *M. hapla*; *M. hapla* — *R. solani*; *M. hapla*.

Figure 30. Alfalfa six weeks after seeding at 20°C. Left to right: Check; *R. solani*; *M. hapla* — *R. solani*; *M. hapla*. 
Figure 31. Alfalfa six weeks after seeding at 25°C. Left to right: Check; *R. solani*; *M. hapla* — *R. solani*; *M. hapla*.

Figure 32. Alfalfa six weeks after seeding at 30°C. Left to right: Check; *R. solani*; *M. hapla* — *R. solani*; *M. hapla*. 
Table 6. Dry weights of the tops at four cuttings and four temperatures for a period of 20 weeks. Average of six replications

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (wks.)</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
</tr>
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<tbody>
<tr>
<td><em>M. hapla</em></td>
<td>8</td>
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<td>1.55</td>
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<td>1.81</td>
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<td></td>
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<td>1.49</td>
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<td><em>R. solani</em></td>
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<td>8</td>
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<td>2.44</td>
<td>2.69</td>
<td>3.01</td>
<td>2.17</td>
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</tbody>
</table>

tode treatments were only approximately one-half those of the check plants.

From Figure 33, the temperature for optimum growth of the alfalfa was 20°C, however, growth at 15°C and 25°C was not significantly different from the plants grown at 20°C. Growth at 30°C was reduced as compared to the growth at the other temperatures.

Using the Duncan Multiple Range Test calculated on treatment means at each temperature, differences in dry weights between treatments were not significant at 15°C (Table 7 and Figure 33). At 20°C significant differences at the 5 per cent level were obtained between the check and root-
Figure 33. Graph of the combined means of yields at four temperatures and four treatments.
Table 7. Comparison of overall treatment means at each temperature.
Any two means not underscored by the same line are significantly different. For significance levels refer to Table 2 of the Appendix

<table>
<thead>
<tr>
<th>Temperature</th>
<th>M&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MR&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C</td>
<td>1.80</td>
<td>1.90</td>
<td>1.92</td>
<td>1.92</td>
</tr>
<tr>
<td>20°C</td>
<td>1.67</td>
<td>1.75</td>
<td>1.94</td>
<td>2.11</td>
</tr>
<tr>
<td>25°C</td>
<td>1.29</td>
<td>1.39</td>
<td>1.62</td>
<td>1.91</td>
</tr>
<tr>
<td>30°C</td>
<td>0.70</td>
<td>0.81</td>
<td>1.22</td>
<td>1.32</td>
</tr>
</tbody>
</table>

<sup>a</sup>M = *Meloidogyne* hapla.
<sup>b</sup>R = *Rhizoctonia* solani.
<sup>c</sup>MR = *Meloidogyne* hapla — *Rhizoctonia* solani.
<sup>d</sup>C = Check.

I also treatments. At 25°C the difference in dry weights between the check and root-knot treatments were significant at the 1 per cent level. Also at this temperature the differences between the check and *R. solani* treatments were significant at the 5 per cent level, however, this was not true in the 30°C tank. In the 30°C tank the dry weight differences between the check and nematode treatments were significant at the 1 per cent level. Also at this temperature the differences between the *R. solani* and
and *M. hapla* — *R. solani* and the *M. hapla* treatments were significant at the 1 per cent and 5 per cent levels, respectively. The differences between *M. hapla* — *R. solani* and *M. hapla* alone treatments were not significant at any of the temperatures (Table 7 and Figure 33).

An analysis of variance calculated for each of the four cuttings, showed no interaction between temperature and treatment for the first three cuttings (Tables 4, 5, and 6 in Appendix), but a significant interaction between temperature and treatment occurred at the fourth cutting (Table 7 in Appendix). In the combined means, significant interactions were obtained between cuttings by temperature by treatment; cuttings by treatment; and cuttings by temperature where cuttings is the time factor. Also in the combined means the interaction between treatment and temperature was not significant (Table 8 in Appendix).

At 20 weeks the *M. hapla* and the *M. hapla* — *R. solani* treatments exhibited stunting of the plants as well as marked chlorosis of the foliage. This was especially true at 30°C and to a lesser extent at 25°C (Figures 36 and 37). These symptoms were not evident at either 20°C or 15°C (Figures 34 and 35).

The time of flowering between treatments was variable. However, the plants in the 30°C and 25°C tanks formed flowers first followed by the plants in the 20°C and 15°C tanks.

At the conclusion of the experiments, the dry weights of the roots exhibited a similar pattern to the dry weights of the tops in that the root-knot infected roots weighed less than those of the check plants (Table 8 and Figures 38, 39, 40, and 41). As shown by the root-knot indexes most galling occurred at 30°C and 25°C. The greatest number of plants killed were at
Figure 34. Alfalfa 20 weeks after seeding at 15°C. Left to right: Check; R. solani; M. hapla; M. hapla — R. solani.

Figure 35. Alfalfa 20 weeks after seeding at 20°C. Left to right: Check; R. solani; M. hapla; M. hapla — R. solani.
Figure 36. Alfalfa 20 weeks after seeding at 25°C. Left to right: Check; R. solani; M. hapla; M. hapla -- R. solani.

Figure 37. Alfalfa 20 weeks after seeding at 30°C. Left to right: Check; R. solani; M. hapla; M. hapla -- R. solani.
Table 8. Dry weights of the roots, root-knot index and the number of plants alive after 20 weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry wgt. roots (gm)</th>
<th>Root-knot index&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plants alive&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. hapla ---</td>
<td>3.66</td>
<td>2.47</td>
<td>1.61</td>
</tr>
<tr>
<td>R. solani</td>
<td>2.72</td>
<td>2.80</td>
<td>2.17</td>
</tr>
<tr>
<td>M. hapla</td>
<td>2.89</td>
<td>4.13</td>
<td>3.95</td>
</tr>
<tr>
<td>R. solani</td>
<td>3.05</td>
<td>5.12</td>
<td>4.78</td>
</tr>
</tbody>
</table>

<sup>a</sup>Root-knot index based on: 1 = 1-26%: 2 = 26-51%; 3 = 51-76%; 4 = 76-100% of the roots have galls.

<sup>b</sup>Number of plants alive from the original 30 in each treatment.
Figure 38. Alfalfa roots 20 weeks after seeding at 15°C.

A = Check
B = R. solani
C = M. hapla
D = M. hapla — R. solani

Figure 39. Alfalfa roots 20 weeks after seeding at 20°C.

A = Check
B = R. solani
C = M. hapla
D = M. hapla — R. solani
Figure 40. Alfalfa roots 20 weeks after seeding at 25°C.

A = Check
B = R. solani
C = M. hapla
D = M. hapla -- R. solani

Figure 41. Alfalfa roots 20 weeks after seeding at 30°C.

A = Check
B = R. solani
C = M. hapla
D = M. hapla -- R. solani
30°C in the combination of *M. hapla* -- *R. solani* treatments (Table 9).

The reisolation of *R. solani* was low in comparison to the number of root pieces transferred onto two per cent water agar. *Fusarium* sp. bacteria and occasionally *Trichoderma* spp. were also reisolated from all treatments.

In the second set of temperature tanks the dry weight trends appeared to be similar to those shown in the first set in that the greatest reduction in yield occurred in the nematode treated plants at 30°C and 25°C (Table 10 in Appendix). One difference, however, was obvious in the check plants. In this set of tanks the dry weights of the check plants at 30°C were as high as those at the other three temperatures. At 12 and 16 weeks at 30°C and at 16 weeks in the 25°C tank the *M. hapla* -- *R. solani* dry weights were greater than the *M. hapla* treatment. The same effect was true at 30°C and 25°C with the dry weights of the roots (Table 11 in Appendix).

In general the plants in the second set of tanks exhibited taller growth at 20°C and 15°C, but, on the other hand, the plants grown at 30°C and 25°C were shorter but more bushy.
DISCUSSION

The low root-knot index obtained in the variety tests was believed to be due, in part, to the method of infesting the soil. Had the soil been infested throughout, more galling might have resulted.

The growth rate studies of Rhizoctonia solani were found to be in close agreement with those of Monteith and Dahl (1928), Newton (1931), Wellman (1932), Le Clerg (1938) and Smith (1946) who found the optimum temperatures for growth of R. solani on nutrient agar varied between 23°C to 30°C. These higher temperatures were in agreement with the work done on the greenhouse bench in which reduction in yield was evident by seven months. Periodic checks of the soil in the pots showed the temperature to range between 27°C to 29°C. In the temperature tanks any reduction in yield which occurred due to the presence of Rhizoctonia was generally at 25°C.

The method used for infesting the soil with nematodes was very effective. The galls developed early after seeding and, as observed later, numerous lateral roots were seen to emerge from the galled areas of the Meloidogyne hapla infected roots. The nematode and fungus inoculum was placed at the specified depths in the soil in order to give both pathogens an equal opportunity for penetration at the same time. The R. solani was grown on two per cent water agar because it was not known what effect the high sugar content of potato dextrose agar might have on the nematodes.

Microscopically, penetration of the roots by the nematodes occurred within four days after seeding and infestation of the soil. If the nematodes had been placed closer to the soil surface, penetration probably would have occurred sooner as shown by Christie (1936). He obtained pene-
tration within 24 hours by placing the nematodes directly in contact with the tomato root tips and then planting the tomato seedlings in the soil.

Since nematodes were found in the roots four days after seeding, penetration of the roots by the nematodes must have been rapid. Allowing two to three days for seed germination and radicle growth to a five cm depth would mean that penetration of the roots occurred within one to two days. Probably less time than this was required for penetration since in this one to two days root contact by the nematode had to be made.

In Iowa, alfalfa is planted about the middle of April. At this time the soil temperature at about the five cm depth is approximately 10°C to 15°C. As shown by the temperature tank experiments, penetration by the nematodes will occur at 15°C but development or at least a more rapid development and completion of the life cycle occurs at the higher temperatures of 20°C, 25°C and 30°C. These latter temperatures occur about the last week in May to the middle of June. Therefore, a more rapid penetration of new roots, completion of the life cycle and root destruction will probably occur at these latter dates. This is also true of *R. solani* in that the higher temperatures would favour penetration and reduction in yield due to this fungus.

At six days nematodes were found in the root tip and in the half cm piece immediately behind the root tip. At seven days nematodes were seen in the second cm from the root tip. The position of these nematodes is believed not to be entirely attributable to migration or penetration further up the root from the tip. Migration may have played a part where mass penetration was concerned so that by sheer numbers the nematodes destroyed tissue and pushed cells apart in front of them. This was evident by the
tunneling effect. Also mass penetration seemed to cause the root tip to cease growing. Individual nematodes, on the other hand, may have migrated as far as the plerome and become sedentary. Meanwhile the cells in the immediate vicinity kept dividing and the root grew around the nematode while it fed on a few cells. If migration does occur it is only for very short distances, and tunnels were not evident with individual nematodes.

The ultimate site of feeding after penetration was in the plerome with the resultant production of giant cells from the cells of this meristematic tissue. Occasionally nematodes were observed in the cortex, however, no cellular alteration was evident and it is doubtful that these nematodes would survive. The plerome would seem to be an ideal tissue in which to feed since the cells are young and succulent, actively dividing and will eventually give rise to the xylem and phloem.

The giant cell development advanced from a multi-nucleate condition with abundant protoplasm, when the juvenile was present, to one in which the cells were large and practically devoid of protoplasm as the nematode approached maturity and egg production. This may be a case of once the food supply begins to diminish then egg production begins. It should be pointed out, however, that various stages of giant cells and nematodes were present in the same sections. In cases where many nematodes were present slower development may have been due to crowding and food factors. A molted cuticle was not evident until 28 days after seeding at which time the nematodes were adults. Either the nematode does not slough off its cuticle at each juvenile stage or they were unable to do so due to their confinement in the root. Also, the shrinkage of the nematodes was probably due to the killing and fixing procedure.
Another factor evident in the galled areas was the abundant pro-
duction of lateral roots. In practically every case a nematode was found
at the base of the lateral root. Perhaps the same stimulus that caused the
production of the giant cells also caused the production of these lateral
roots. This phenomenon is somewhat difficult to explain since these same
nematodes often caused the cessation of growth of the tap root of alfalfa.
This latter case often occurred where large numbers of nematodes were in-
volved. Stimulation to produce lateral roots, on the other hand, usually
occurred where only one or two nematodes were involved. The root lengths
taken in the early stages of growth were further evidence of the fact that
this nematode can cause the cessation of growth of the tap root of alfalfa.

In the majority of the infections, the galls occurring on the tap root
were usually larger than those on the lateral roots. This could be due to
the fact that there is more storage tissue in the tap root and also the tap
root is older, or to an earlier more concentrated penetration of the tap
root. These larger galls on the tap root may also have been due to the
coalescing of a number of individual galls.

The completion of the life cycle is in agreement with those of Kincaid
(1946) who found most rapid development of root-knot to occur at 27°C with
penetration occurring between 12°C to 35°C. Tyler (1933), with Heterodera
marioni, also found most rapid development of the life cycle to occur at
27.0°C. Thomason and Lear (1961) found the maximum temperature at which
egg masses of M. hapla were produced was at 32.6°C. Since the common name
of M. hapla is the northern root-knot nematode one would expect its develop-
ment to be aided by cool temperatures, but this does not appear to be the
case. The common name may, however, have originated due to the fact that
this nematode can survive at relatively low temperatures.

The results obtained for _R. solani_ alone were not found to be significantly different from the check plants in any of the experiments, except at 25°C. The reason for this is unknown and only speculations can be made. Two factors which could have worked adversely on the fungus are temperature and moisture. Various soil moistures and temperatures favorable for the growth of _R. solani_ have been reported in the literature. Peltier (1916) reported 88°F and above with soil moistures unfavorable for best development of the plants as being favorable to _R. solani_. Blair (1943) reported moisture deficiency as favorable to the development of _R. solani_. The work of Peltier and Blair was confirmed by Benedict (1954). Sanford (1938a) found that between 16°C and 23°C, _Rhizoctonia_ appeared equally virulent at soil moistures between 19 and 40 per cent of the moisture holding capacity. Smith (1946) found that cankers did not develop on alfalfa at soil temperatures as low as 15°C, but an average of 29.4 cankers developed on each tap root at 30°C. Since in these experiments the writer used what appeared to be favorable soil temperatures then the soil moisture must have been one of the factors for poor development of _R. solani_. The soil may have been kept too wet. A different strain of the fungus may also have been used.

Other factors shown to be detrimental to _Rhizoctonia_ are carbon dioxide and certain fungi. Carbon dioxide was probably not too important in these experiments because the soil was kept loosened up and care was taken not to flood the soil. Papavizas and Davey (1962) found the saprophytic activity of _Rhizoctonia_ inhibited by 10 and 20 per cent with drastic inhibition at 30 per cent carbon dioxide. The pathogenic phase was even more sensitive to carbon dioxide than the active saprophytic stage. Durbin (1959) found
that clones of Rhizoctonia found in the soil were more tolerant to carbon dioxide than those found in aerial environments. Certain Fusarium and Trichoderma spp. of fungi have been found to be antagonistic to Rhizoctonia by Weindling (1934), Allen and Haenseler (1935) and Kommedahl and Young (1956).

Various isolates of R. solani apparently produce different effects. Wyllie (1962) found that isolates ran the gamut of complete necrosis of the root systems to almost no necrosis. This may account for the lack of necrotic areas with the strain of fungus used in these experiments. Stunting of the plants is not always produced by R. solani. This fact would account for the R. solani alone treatments being approximately the same height as the check plants. Wyllie (1962) further stated that a toxin produced by the fungus is translocated through the secondary roots to the primary root and that a "softening-up" process by the toxin is essential to the actual mycelial invasion. Deshpande (1961) found filtrates of R. solani to contain both polygalacturonase and dipolymerase. Bateman (1963) showed that the enzyme polygalacturonase was primarily responsible for the maceration of the tissue.

Reisolation of R. solani in the bench experiment was good. In the first set of temperature tanks reisolation was low and in the second set it was much better but not as good as in the bench experiment. The reason for this could be due to the length of time the experiments were run or since two different methods of infestation of the soil were used the fungus in the temperature tank experiments may not have had a large enough food base to get well established. In the first set of tanks tap roots were mainly used for the reisolation and this may account for the low recovery
From the results of the bench experiment there was a significant difference between the nematode and check treatments at three months. This difference was highly significant at four months. The difference between the fungus alone and check treatments was significant at seven months after seeding. The *M. hapla* -- *R. sclani* treatments were not significantly different from the *M. hapla* alone treatments. From this then it can be concluded that the fungus and nematode are acting independently with most of the effect being produced by the nematode.

Time proved to be an important factor. In the bench experiment by seven months the weights of the check plants had dropped significantly. This was probably due to pot binding and depletion of nutrients. This was probably not the case with the nematode treatments because the root system was greatly stunted and, therefore, there was plenty of room in the pots. The reduction of dry weights in this latter case was due to a large build up of nematodes and destruction of the roots. That the time factor was important also was shown by the analysis of variance of each cutting in the temperature tanks. Here a significant interaction between temperature and treatment did not occur until the fourth cutting. This was probably due to a gradual build up in the nematode populations with a gradual destruction of the root system until by 16 to 20 weeks these effects were becoming evident in the reduction of top growth.

The significant interaction shown by the analysis of variance for cuttings by temperature by treatment could be very well explained by the fact that vegetative regrowth would be slower in some cases than in others. The vegetative regrowth in plants infected with the nematodes would very likely
be slower than in the check plants due to the reduced root system. Also,
the number of axillary buds present would have an effect on the regrowth.

The effect of temperature was evident not only by the dry weights of
the tops but also with the sections made on the microtome. The life cycle
apparently proceeded much more rapidly at the higher temperatures. The
reduced growth at 30°C was not due to temperature but more probably due
to an illumination factor. The first set of temperature tanks were run
during the winter under artificial illumination. Since this tank was at
one end the bank of lights may not have been pulled far enough that way
and, therefore, the plants in this tank obtained less light. Further evi-
dence for this was shown in the second set of tanks which were run under
natural illumination in the spring. In this latter case there was very
little difference in the dry weights of the check plants at any of the
temperatures.

The effect of this nematode on a crop of alfalfa has not only the
immediate effect of a reduced yield and root system but also the crop is
predisposed to severe winter kill due to the latter condition.
The most rapid growth of *Rhizoctonia solani* on two per cent water agar was obtained at 30°C but good growth was also obtained at 24°C. The plates incubated at 20°C were intermediate in growth rate with the slowest rate occurring at 15°C.

The following alfalfa varieties were tested for resistance to *M. hapla*: Alfa, Buffalo, Codu, DuPuits, Lahontan, Narragansett, Ranger, Vernal. All varieties were susceptible. Ranger alfalfa had the most and Vernal had the fewest galled plants.

Macroscopic and microscopic observations were made periodically for 28 and 30 days from greenhouse and controlled temperature material, respectively. In these experiments, obvious swelling of the root tips occurred by the sixth day after seeding. Stained sections, however, showed that penetration by the nematodes had occurred by the fourth day. Penetration was observed to occur in the vicinity of the root tip in alfalfa.

Penetration and feeding by *M. hapla* in the root produced the following abnormal conditions: 1) Development of galls on the tap root and the lateral roots. The galls on the lateral roots were smaller than those on the tap root. 2) Curvature of the root tip occurred in the early stages of growth. This apparently was caused by the reduction of growth on the side of penetration and feeding. 3) Proliferation of lateral root occurred in the galled areas. At the base of every lateral in these galled areas, a nematode or giant cells, or both, were observed. The stimulation to produce these large number of laterals probably came from the feeding of the juveniles. 4) Cessation of linear root growth occurred in some cases.
This, in combination with the lateral root proliferation, gave the root systems a stunted, bushy appearance. 5) Giant cells were produced in the central cylinder. These cells from the cells of the central cylinder and are first obvious as a multi-nucleate cell with a lightly staining protoplasm and gradually enlarge until no protoplasm appears to be left in the cell. This appears to be correlated with the growth of the nematode in that when the nematode approaches the adult stage and egg production, the giant cells appear to be practically devoid of protoplasm.

Egg production was observed to occur within 28 to 30 days after seeding. The life cycle of M. hapla from second stage juvenile to egg stage progressed most rapidly in the following descending order of temperatures: 30°C > 25°C > 20°C > 15°C. At 15°C the life cycle proceeded slowly with little apparent difference in development between six and 30 days.

The severity of root-knotting, root-stunting and lateral root proliferation was also in the order of 30°C > 25°C > 20°C > 15°C.

The greatest number of plants killed was in the M. hapla -- R. solani treatment. This was followed by M. hapla alone. No plants died in the R. solani alone and check treatments. The same situation was true in the temperature tanks, but the most plants killed were at 30°C while none were killed in any of the 15°C treatments.

In the M. hapla -- R. solani treatment, only sections on one slide were found which contained the fungus. On this slide, R. solani was found to be in close association with the giant cells.

In the bench experiment, significant differences in yield between nematode treated plants and check plants were observed three months after seeding. At four months these differences were highly significant. Sig-
significant differences between nematode treatments were not observed. The differences between *R. solani* alone and check treatments were found to be significant at seven months after seeding. In the temperature tanks, at the higher temperatures significant differences were observed between the nematode and check treatments, but not between the nematode treatments. The difference between *R. solani* alone and the check treatments was not significant at any of the temperatures, except at 25°C.
LITERATURE CITED


Thomason, Ivan J. and Bert Lear. 1961. Rate of reproduction of Meloidogyne spp. as influenced by soil temperature. Phytopathology 51: 520-524.


Weindling, R. 1934. Various fungi found to be parasitic on Rhizoctonia solani. Phytopathology 24: 1141.


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APPENDIX

Table 1. Duncan's multiple range test. Shortest significant ranges

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Table 2. Significance levels for each temperature using Duncan's multiple range test

15°C

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20°C

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<th>Significance</th>
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C - M     5%
C - MR 5%
C - R    N. S.
R - M    N. S.
R - MR   N. S.
MR - M   N. S.
Table 2 (Continued).

### 25°C

<table>
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<tr>
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<td>1.62</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>C - M</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - MR</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - R</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R - M</td>
<td>N. S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R - MR</td>
<td>N. S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR - M</td>
<td>N. S.</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### 30°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MR</th>
<th>M</th>
<th>R</th>
<th>C</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.70</td>
<td>0.81</td>
<td>1.22</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>C - M</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - MR</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - R</td>
<td>N. S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R - M</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R - MR</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR - M</td>
<td>N. S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Analysis of variance for the bench experiment

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>4</td>
<td>7.56</td>
<td>1.89</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>57.65</td>
<td>19.22</td>
</tr>
<tr>
<td>Cuttings</td>
<td>6</td>
<td>159.14</td>
<td>26.52</td>
</tr>
<tr>
<td>Treatments x cuttings</td>
<td>18</td>
<td>18.42</td>
<td>1.02</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>59.63</td>
<td>0.55</td>
</tr>
</tbody>
</table>
Table 4. Analysis of variance for the first cutting of the alfalfa in the temperature tanks

<table>
<thead>
<tr>
<th>Due to</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error A Reps. 5</td>
<td>20</td>
<td>0.473</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>Temp. x Reps. 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>3</td>
<td>18.791</td>
<td>6.264</td>
<td>1 %</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.324</td>
<td>0.275</td>
<td>1 %</td>
</tr>
<tr>
<td>Temperature x treatment</td>
<td>9</td>
<td>0.142</td>
<td>0.016</td>
<td>N. S.</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>2.766</td>
<td>0.046</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Analysis of variance for the second cutting of the alfalfa in the temperature tanks

<table>
<thead>
<tr>
<th>Due to</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error A Reps. 5</td>
<td>20</td>
<td>0.835</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>Temp. x Reps. 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>3</td>
<td>10.950</td>
<td>3.650</td>
<td>1 %</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.303</td>
<td>0.101</td>
<td>N. S.</td>
</tr>
<tr>
<td>Temperature x treatment</td>
<td>9</td>
<td>0.389</td>
<td>0.043</td>
<td>N. S.</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>4.308</td>
<td>0.072</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Analysis of variance for the third cutting of the alfalfa in the temperature tanks

<table>
<thead>
<tr>
<th>Due to</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error A Reps. 5 Temp. x Reps. 15</td>
<td>20</td>
<td>2.152</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>3</td>
<td>10.319</td>
<td>3.440</td>
<td>1%</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>2.658</td>
<td>0.888</td>
<td>1%</td>
</tr>
<tr>
<td>Temperature x treatment</td>
<td>9</td>
<td>1.259</td>
<td>0.140</td>
<td>N. S.</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>5.525</td>
<td>0.092</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Analysis of variance for the fourth cutting of alfalfa in the temperature tanks

<table>
<thead>
<tr>
<th>Due to</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error A Reps. 5 Temp. x Reps. 15</td>
<td>20</td>
<td>4.833</td>
<td>0.242</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>3</td>
<td>9.441</td>
<td>3.147</td>
<td>1%</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>15.466</td>
<td>5.162</td>
<td>1%</td>
</tr>
<tr>
<td>Temperature x treatment</td>
<td>9</td>
<td>6.149</td>
<td>0.633</td>
<td>1%</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>14.101</td>
<td>0.235</td>
<td></td>
</tr>
</tbody>
</table>

*aSignificant interaction,*
Table 8. Analysis of variance of the combined means for the temperature tanks

<table>
<thead>
<tr>
<th>Due to</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>3</td>
<td>47.60</td>
<td>15.87</td>
<td>1%</td>
</tr>
<tr>
<td>Error temperature</td>
<td>20</td>
<td>4.56</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>11.49</td>
<td>3.83</td>
<td>1%</td>
</tr>
<tr>
<td>Temperature by treatment</td>
<td>9</td>
<td>3.93</td>
<td>0.44</td>
<td>M.S.</td>
</tr>
<tr>
<td>Error replications</td>
<td>240</td>
<td>16.53</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Cuttings</td>
<td>3</td>
<td>38.49</td>
<td>12.83</td>
<td>1%</td>
</tr>
<tr>
<td>Temperature by cuttings</td>
<td>9</td>
<td>1.90</td>
<td>0.21</td>
<td>1%</td>
</tr>
<tr>
<td>Treatment by cuttings</td>
<td>9</td>
<td>7.78</td>
<td>0.86</td>
<td>1%</td>
</tr>
<tr>
<td>Temperature by treatment by cuttings</td>
<td>27</td>
<td>4.01</td>
<td>0.15</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table 9. Mean value of total plant dry weights of three treatments and the check at four temperatures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;R&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92</td>
<td>1.75</td>
<td>1.29</td>
<td>0.70</td>
</tr>
<tr>
<td>M&lt;sub&gt;B&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.80</td>
<td>1.67</td>
<td>1.39</td>
<td>0.31</td>
</tr>
<tr>
<td>M&lt;sub&gt;C&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.90</td>
<td>1.94</td>
<td>1.62</td>
<td>1.22</td>
</tr>
<tr>
<td>M&lt;sub&gt;D&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.92</td>
<td>2.11</td>
<td>1.91</td>
<td>1.32</td>
</tr>
</tbody>
</table>

<sup>a</sup><sub>M<sub>R</sub></sub> = Meloidogyne hapla – R. solani

<sup>b</sup><sub>M<sub>B</sub></sub> = Meloidogyne hapla

<sup>c</sup><sub>M<sub>C</sub></sub> = Rhizoctonia solani

<sup>d</sup><sub>M<sub>D</sub></sub> = Check
Table 10. Dry weights of the tops at four cuttings and four temperatures for a period of 16 weeks. Average of five replications

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (wks.)</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. hapla</td>
<td>3</td>
<td>3.21</td>
<td>3.74</td>
<td>1.82</td>
<td>1.86</td>
</tr>
<tr>
<td>R. solani</td>
<td>12</td>
<td>2.95</td>
<td>3.79</td>
<td>2.12</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>3.36</td>
<td>3.61</td>
<td>2.23</td>
<td>1.49</td>
</tr>
<tr>
<td>M. hapla</td>
<td>5</td>
<td>3.37</td>
<td>3.55</td>
<td>2.42</td>
<td>1.98</td>
</tr>
<tr>
<td>R. solani</td>
<td>12</td>
<td>3.28</td>
<td>3.59</td>
<td>3.10</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>3.36</td>
<td>3.59</td>
<td>2.03</td>
<td>1.36</td>
</tr>
<tr>
<td>R. solani</td>
<td>8</td>
<td>3.51</td>
<td>3.71</td>
<td>3.72</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.33</td>
<td>3.99</td>
<td>4.37</td>
<td>3.85</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>3.58</td>
<td>4.36</td>
<td>4.30</td>
<td>4.20</td>
</tr>
<tr>
<td>Check</td>
<td>3</td>
<td>3.58</td>
<td>3.87</td>
<td>3.63</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.32</td>
<td>4.04</td>
<td>4.28</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>3.72</td>
<td>4.41</td>
<td>4.46</td>
<td>4.85</td>
</tr>
</tbody>
</table>
Table 11. Dry weights of roots, root-knot index and the number of plants still alive after 16 weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry wt. roots (gm)</th>
<th>Root-knot index</th>
<th>Plants alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. solani</td>
<td>3.20</td>
<td>1.96</td>
<td>1.29</td>
</tr>
<tr>
<td>M. hapla</td>
<td>3.35</td>
<td>2.74</td>
<td>1.02</td>
</tr>
<tr>
<td>M. hapla--</td>
<td>2.03</td>
<td>4.50</td>
<td>3.82</td>
</tr>
<tr>
<td>Check</td>
<td>4.48</td>
<td>5.06</td>
<td>4.57</td>
</tr>
</tbody>
</table>

*R. solani* index based on: 1 = 1-26%; 2 = 26-51%; 3 = 51-76%; 4 = 76-100% of the roots have galls.

Number of plants alive of the original 25 in each treatment.