BIONOMICS OF THE ALFALFA STEM NEMATODE, *DITYLENCHUS DIPSACI*, IN ARIZONA

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ABSTRACT

The bionomics of the alfalfa stem nematode, Ditylenchus dipsaci (Kuhn 1857) Filipjev 1926, attacking alfalfa in Arizona was studied for a period of 11 months. During this time it was found that when the nematodes were inactive, as expressed by lack of symptoms in the host, they were concentrated in the crowns of the alfalfa plants and were almost exclusively pre-adult forms. Observations on nematode activity in relation to temperature and stage of development during this time indicate that the temperature range between 76 F and 38 F was the most favorable for nematode activity, reproduction, and development under the conditions of this study.

Time vs. temperature studies of dessicated pre-adults show that for any interval of time survival was greater at 4 C than at 25 C or 45 C. Survival at 45 C was very low at any time interval, never being higher than .2%.

Time vs. temperature studies of D. dipsaci in distilled water showed that after 2 days at 35 C or 45 C all the nematodes were killed. The highest percent of survival was again at 4 C. There is evidence that dessicated D. dipsaci can withstand higher temperatures than D. dipsaci in water.

Fresh fecal samples collected from sheep pastured on an alfalfa field heavily infested with stem nematodes were checked for the presence of *D. dipsaci*. No eggs, larvae, or adults were found and it was concluded that *D. dipsaci* could not survive digestive action.

INTRODUCTION

The alfalfa race of the stem and bulb nematode, *Ditylenchus dipsaci* (Kuhn 1857) Filipjev 1926, has long been recognized as an important pest of alfalfa especially in areas of high rainfall and in areas where irrigation is practiced (4).

Stem nematode attack in alfalfa is usually apparent during the spring of the first year of the crop and is characterized by small circular patches of plants which are stunted. On closer observation these plants usually have swollen bases and some distortion of the lower leaves and petioles. With subsequent cropping and water runoff from rainfall or irrigation these small patches spread, coalesce and in three years the field may be completely infested with the stem nematodes.

Although *D. dipsaci* may cause death of alfalfa stems and leaves on occasion when infection is severe, very seldom is the whole plant killed. Instead the nematode attack predisposes the host to other pathogens and reduces its ability to compete with weeds and other non-agronomic plants found in conjunction with alfalfa in the field.

Damage to alfalfa in Arizona from stem nematodes is most severe in the years when the spring season is cool and wet, with decreased damage occurring again in the fall when temperatures start to drop. In Oregon and some parts of California stem nematode damage occurs nearly 12 months of the year. It is the author's speculation that the combination of mild temperature and high rainfall in these areas affords near optimum conditions for stem nematode development, reproduction, and infection throughout the year.

It is a common practice in Arizona to graze sheep on the alfalfa fields during January and February. It is not uncommon for a rancher to move his flocks from one alfalfa field to another over considerable distances during this time. If *D. dipsaci* could survive passage through the gastrointestinal tract of sheep this then would constitute an important means of spread of this pest. With these ideas in mind the author conducted an experiment to ascertain whether *D. dipsaci* could survive passage through the gastrointestinal tract of sheep.

In the past several years the alfalfa acreage in Arizona has been increasing, and in conjunction there has been a noticeable increase in stem nematode infestation of this crop. Previously *D. dipsaci* has been considered as a pest of minor importance and therefore given very little attention. At present the alfalfa stem nematode is restricted to Maricopa, Pinal, and Greenly Counties in Arizona. It is the author's opinion that as the acreage of alfalfa increases, *D. dipsaci* will assume a major role in limiting yields both directly and indirectly. Reasons for this opinion are based on the observations that _D. dipsaci_ can be spread by farm implements, runoff water, and feeding livestock; that present day alfalfa varieties adapted for use in Arizona are not stem nematode resistant; and that because of residue problems the use of chemical controls are severely limited.

Bearing in mind that damage caused by the alfalfa stem nematode is virtually nonexistent in alfalfa during the summer and winter in Arizona the author conducted experiments to develop an understanding of the bionomics of this alfalfa pest. These experiments included studies of the population dynamics, biology, and cultural effects on _D. dipsaci_.

Never before has the bionomics of _D. dipsaci_ been studied under conditions found in southern Arizona. It is hoped that the information gained from this study will make possible an understanding of the relationships that exist between the nematode, its host, and the environment. This knowledge will facilitate control measures as well as augment our present knowledge of this pest on other hosts under different environmental conditions.

LITERATURE REVIEW

The survival and perpetuation of plants and animals depends on their ability to withstand the most adverse conditions compounded by the environmental factors of their habitat (22). Many of the higher animals, such as birds, insects, and man have such high tolerance levels or such widespread optimums that they can temporarily abandon parts of their habitat when conditions become unfavorable. This is usually not the case with active nematodes. It has been reported (19, 29, 30, 33) that nematodes will migrate horizontally in soil to obtain temperature and moisture optimums, but this migration is within a micro-environment and must be considered insignificant as a means of escaping adverse conditions in view of the fact that nematode movement in the soil is seldom greater than 2 meters per year. Nematodes have developed various methods of adapting themselves to evade adverse changes in the environment of their habitat. The most notable examples of these adaptations in the plant parasitic nematodes are those that become quiescent or undergo cryptobiosis when conditions become so adverse that they cannot continue to live in an active state. Quiescence is a term generally used to describe dormancy in nematodes while cryptobiosis is a term used when no metabolism can be detected (28). Both of these terms are associated with a lowering of metabolic activity, but each describes a more or less distinct level of metabolism. Both of these metabolic levels can be induced by the same conditions such as loss of water, lowering of temperature, or increase in salts. They vary only in magnitude.

It is the author's opinion that nematodes that become inactive or dormant while in a living host or living host material are protected to some extent from excessive loss of moisture, increase in salts, and fatal temperature fluctuations, and are therefore quiescent rather than cryptobiotic. On the other hand, nematodes that can survive total dessication, and thus high salt concentrations, are thought to be cryptobiotic (28).

There are a few genera of plant parasitic nematodes that have exhibited some degree of quiescence or cyrptobiosis; such as Anguina, Heterodera, Tylenchus, Aphelenchoides, and Ditylenchus. It is possible that many other soil inhabiting plant parasitic nematodes exhibit some form of cryptobiosis or quiescence when conditions of moisture, temperature, oxygen, and food are below the optimum (11, 32). However, studies concerning this subject are too limited to support this hypothesis.

Various species of nematodes can live for varying lengths of time in a dormant state (32). Epps (9) found that 1 month was the maximum time after which he could recover viable *Heterodera glycines* Ichinohe larvae from cysts stored in seed bags, but the most well known examples of cryptobiosis or quiescence are found in the species *D. dipsaci*.

The stem and bulb nematode, *D. dipsaci* has been known to agriculturists, horticulturists, and plant pathologists for over 100 years. During this time it has been reported on over 300 hosts representing over 40 genera of both monocotyledenous and dicotyledonous plants (1, 5, 20, 22). Some of the more agronomically and horticulturally important plants known to be attacked by various races of the bulb and stem nematode are, red and white clovers, alfalfa, oats, onions, rye, narcissi, and tulips.

The nematodes that attack the previously mentioned hosts constitute several biological races which even though they are morphologically indistinguishable, are usually very host specific.

Those races attacking bulbous plants such as onion, narcissi, and tulips cause damage both above ground in the form of tillering, stunting, and swelling or puffiness of the leaves and stems, and below ground in the form of brown ring rot of the bulb and partial decomposition of the bulb scales (23). Plants such as red and white clovers and alfalfa are attacked above the ground at the crown and stem. Above ground symptoms are stunting, swelling of the crown buds, and a noticeable shortening of the internodes.

Fielding (11) found that he could recover viable D. dipsaci larvae from infected alfalfa material after five years of storage at room temperature. He also observed that viable D. dipsaci larvae could be revived from 23 year old herbarium specimens of teasel (Dipsacus fullonum L.). McBeth (20) and Corder (7) continued the observations made by Fielding (11) and reported the same results. Corder (7) also noted that practically all the nematodes that could be revived were pre-adults. Brosher and McKeen (3) found that a high percentage of D. dipsaci in a dessicated state could survive freezing for 20 minutes at a temperature of -80 C followed by vacuum dehydration and storage in vacuo for a period of 28 days when moistened with beef serum or concentrated sucrose solution. In water the nematodes were injured or killed by freezing to -80 C, which resulted in the formation of large vacuoles within their bodies. Brosher and McKeen suggest that soil organic matter may act like a solution of high osmotic pressure and protect nematodes in the soil from very low temperatures. High osmotic pressure would cause the water in the nematode body to be in a bound state and prevent large vacuoles from forming and rupturing the nematode cuticle. Thorne (27) states that D. dipsaci can survive the winter in Utah where the infected crowns of alfalfa plants are subjected to a temperature range of from 5 to 20 F below zero for periods of up to 4 months.

Cryptobiosis and quiescence not only protect nematodes from very low temperatures, it increases their resistance to high temperatures as well. Hastings et al. (16) observed that nematodes in the dry "nematode wool" stage were highly resistant to heat treatment of 110 F and made maximum recovery on the second day after treatment even when the duration of the treatment was for 5 hours.

Although temperature must play an important role in the complex of events that bring about cryptobiosis or quiescence, there is no evidence that either can be induced by temperature alone. Hodson (18) working on the biology of the stem and bulb nematode states that temperature affects these nematodes only indirectly. These nematodes may leave the infected plants in search of fresh hosts, but there is no apparent effort to avoid dessication in dead tissue. Nolte (21) reported that temperatures during the two years 1949 and 1956, when stem nematode damage was greatest in onion fields in Central Germany, showed no similarity, but that in both years rainfall was above average during the spring. Henderson and Williams (17) state that alfalfa plants infected with stem nematodes made a remarkable recovery during the hot, dry summer months when the nematodes were present but inactive. However, following a fall rain they could be found in some of the new shoots. Work done by Smith (25), in areas of the West where alfalfa is grown under irrigation practices, shows that the greatest injury occurs during the early spring when moisture conditions are favorable for nematode infection. It is interesting to note that stem nematode damage occurs during those times when there is an abundance of moisture and a transition between suboptimal and supraoptimal temperatures. Further evidence that there is a relationship between temperature and moisture and infection and reproduction is indicated by Barker (1). He took two populations of *D. dipsaci* larvae from North Carolina and inoculated 36 plant species and varieties with them. From this study he found that both populations of the nematodes reproduced to a greater extent at 65 F than at 55 or 75 F and that high moisture increased infection and reproduction at all 3 temperatures.

The survival of *D. dipsaci* for long periods of time under adverse conditions does not depend entirely on its ability to enter a cryptobiotic state, but also on its ability to store food in some form in its body. Food storage is necessary because even though metabolism is greatly reduced during periods of quiescence and cryptobiosis some physiologic activity continues and must therefore require food. Goodey (12, 13) believes the food is in the form of fat droplets scattered throughout the bodies of the quiescent pre-adult larvae.

Spread of *D. dipsaci* in the field depends on a number of factors. Webster (34) indicates that infestation and spread of *D. dipsaci* in the field is due to intrinsic mobility and translocation by artificial and natural agencies. He also believes that extrinsic agencies such as drainage and cultivation cause a rapid spread of the nematode infection. Other authors (4, 31) agree with these observations. Cobb (6) notes an estimate made by Dr. G. H. Godfrey which showed as many as 400 *D. dipsaci* per gallon in irrigation water in areas where the crops were infected with the nematode. Thorne (27) made a similar observation in Utah where he found 11,910 *D. dipsaci* in 100 gallons of tail-water from an infected alfalfa field. He proposes that another method of spread is in infected plant fragments in alfalfa seed. He attributes the world wide distribution of the alfalfa stem nematode to this method of spread.

It is thought that *D. dipsaci* has long been a resident in Arizona alfalfa fields. It was probably introduced with the first alfalfa seed brought into the state or with livestock that were brought into Arizona for winter grazing. Today *D. dipsaci* is well established in Arizona and its spread is causing more damage to alfalfa fields each year. It is generally accepted that stem nematodes can be spread by farm machinery, natural agencies (wind and water) and the transport of infested hay to previously uninfested areas. However, it is not known whether D. *dipsaci* will survive passage through the gastrointestinal tract of farm animals. Smart (24) studies this problem with the soybean cyst nematode and found that of the 1000 encysted eggs and larvae of *Heterodera glycines* Ichinohe fed to swine, no encysted eggs or larvae survive passage through the intestine. Although he was able to recover almost 18% of the cysts, only six viable larvae emerged after incubation. These gave negative results in bioassy. Likewise, Faulkner et al. (10) showed that *Meloidogyne hapla* Chitwood, eggs and larvae were killed when placed in the rumen of a fistulated steer for 24, 48 or 72 hours.

MATERIALS AND METHODS

Sources of Stem Nematodes

A four year old field of Northrup King 919 Alfalfa in the Chandler, Arizona area that exhibited symptoms of moderate to heavy stem nematode damage was selected as the source for all plant specimens, soil samples and nematodes except for one experiment. The study of survival of nematodes after passage through the gastrointestinal tract of sheep was conducted by collecting fresh fecal samples from sheep grazing on a field heavily infected with stem nematodes. This field was located in the Mesa, Arizona area.

Population Dynamics

To investigate the population trend over an 11 month period, plant and soil samples were collected from the infested field at approximately 180 foot intervals across a distance of about 1080 feet. The total area of the field was approximately 1440 feet long and 90 feet wide. The first and last 180 feet of the field were left unsampled because the field was bordered by an irrigation and drainage ditch which could influence moisture conditions around them. A total of 6 plant samples each one consisting of 3 plants including about 6-8 inches of the root systems (mainly the tap root) were taken every 30 days for 11 months. Soil samples consisted of soil taken from around the roots of the plant samples. Both plant and soil samples were collected in plastic bags and those containing plant samples were refrigerated until analyzed for nematodes.

Each plant sample, consisting of three plants was further divided into leaves and petioles, stems, crowns, and roots. These samples were cut up into small pieces, placed in cheesecloth, and suspended in distilled water for 48 hours. At the end of 48 hours the distilled water-nematode suspension was poured through a 250 mesh screen backwashed and the total volume brought to 40 cc. All nematode samples were stored in the refrigerator at 4 C until nematode counts were made. Counting consisted of decanting the samples to 10-15 ml. and placing this aliquot in a ruled 50 x 15 mm plastic petri plate and counting the total number of nematodes or, in samples where the number of nematodes was very large the total was calculated by counting the number of nematodes in 1 ml. and then multiplying by the number of ml in the sample. The 50 x 15 mm petri dish was engraved on the outside bottom with a grid consisting of 80 square 4 mm on a side arranged in the form of a cross. Calculations for large samples were made by counting the number of nematodes in 40 of these squares, finding the mean number of nematodes per square and multiplying the total number of squares in the whole dish, 122, by the mean.

The nematodes were recorded as adults or pre-adults on the basis of size and sexual maturity.

Soil samples from the root area of the infected plants were treated in the following manner. Each soil sample consisting of a measured amount of soil was thoroughly mixed and 100 cc portions removed, placed in facial tissue, and the nematodes extracted on a Baremann funnel for 48 hours. After 48 hours 10-15 ml. aliquots were drawn off each sample and stored at 4 C until counted. Counting was accomplished by placing an aliquot in a ruled 50 x 15 mm plastic petri dish, previously described, and counting the total number of nematodes. These nematodes were recorded as adults or as pre-adults larvae as before.

Time and Temperature Studies of Dessicated Nematodes

This experiment was initiated in order to study effects of time and temperature on dessicated pre-adult *D. dipsaci*. The temperatures of 4 C, 25 C, and 45 C, were chosen as representative of the wide range of temperatures that would be encountered under natural conditions. The times chosen are representative of actual lengths of duration of environmental conditions severe enough to cause the nematodes to become dessicated.

Plants from the alfalfa field described previously were placed on a mister after the method described by Goodey (12). Forty-eight hours after initiation of the extraction process the nematode suspension that had been collected from the infected alfalfa tissue was passed through a 250 mesh screen. The nematodes were collected and suspended in distilled water. This nematode suspension was then filtered through No. 50 Whatman filter paper under very slight suction on a Buchner funnel. The filter papers containing the nematodes were air-dried at ambient temperature for 24 hours. After 24 hours the nematodes were resuspended in distilled water by placing the filter papers containing the nematodes, nematode side down, on facial tissue in a Baermann funnel. The nematodes collected by this method were all pre-adult D. dipsaci. Adult and early larvae forms of D. dipsaci including any free living nematodes were killed by the 24 hour dessication period.

One milliliter aliquots of the pre-adult nematode suspension containing 270 nematodes each were pipeted onto 5.5 cm discs of No. 50 Whatman filter paper. These discs were allowed to air-dry for 24 hours then they were placed in glass petri dishes and stored in incubators at 4 C, 25 C, and 45 C. Each petri dish contained four discs. There were sufficient petri dishes so that each temperature could be sampled at 1 day, 3 day, 5 day, 1 week, 3 week, 5 week, 7 week, and 9 week intervals. At the end of each time period the discs were placed, nematode side down, on facial tissue in Baermann funnels. After 48 hours aliquots averaging 12 ml. were drawn off each funnel and the nematodes counted.

Time and Temperature Studies with Nematodes in Distilled Water

The most probable extrinsic method of spread of *D. dipsaci* in Arizona is in waste water from irrigated alfalfa fields infested with this pest. Since irrigation is practiced throughout the year, and such water is handled in a variety of ways, this experiment was performed in an effort to study the effects of time and temperature on *D. dipsaci* suspended in water. The temperatures and times were chosen as representative of the wide range of conditions found under natural conditions.

Nematodes were collected from infected alfalfa plants by placing the infected tissue on a mister for 48 hours. After the extraction process the nematode suspension that had been collected from the infected alfalfa tissue was passed through a 350 mesh screen. The nematodes were collected and suspended in distilled water. The number of nematodes per ml of suspension was determined by placing 1 ml. of suspension in a ruled 50 x 15 mm counting dish (described previously) and counting the total number of nematodes. Each 1 ml. aliquot of suspension contained 415 D. dipsaci in all stages of development. Three ml aliquots of the nematode suspension were placed in 50 x 15 mm plastic petri dishes and enough distilled water added to bring the total volume to 10 ml. Enough petri dishes were treated in this manner so that nematodes could be stored at 4 C, 16 C, 25 C, 35 C, and 45 C. Each temperature was replicated 4 times. At 2, 4, 6, 8, 10, 12, 14, 16, 18, 30, and 60 day intervals, the number of nematodes surviving storage at the above temperatures was recorded.

Survival of Nematodes Passing Through the Gastrointestinal Tract of Sheep

It is a common practice for Arizona ranchers in the Salt River Valley area to pasture sheep on their alfalfa fields during the latter part of December, January, and the first part of February. Many times these sheep are moved from one field to another over considerable distances. If sheep grazing on a stem nematode infested field are moved to a field not infested with the pest, it may be possible for nematodes surviving passage through the gastrointestinal tract to start an infestation in this field. To investigate this possibility the following experiment was performed.

Fresh fecal samples were collected from sheep that had been grazed on a heavily infested alfalfa field for a period of approximately 2 weeks. Forty gm of feces were ground in a Waring Blender with 1 liter of distilled water for 1 min at low speed. The resulting suspension was washed through a 100, 200, and 350 mesh screen with distilled water. The debris on the 200 and 350 mesh screens was then back washed with more distilled water and placed on a Baermann funnel for 24 hours. After 24 hours, aliquots of approximately 12 ml. were drawn and the nematode population determined by the methods described earlier in this paper.

RESULTS

Population Dynamics

For 11 months nematode population densities were determined from the alfalfa field being studied. During this time the average daily temperatures ranged from a maximum of 101 F to a minimum of 33 F. The highest temperatures were recorded in June, July, August, and September. During these months the average daily maximum was 95 F or above and the average daily minimum was between 65 F and 75 F. The remaining seven months had average daily maximum temperatures of 85 F or lower, the lowest being 65 F in December. The average daily minimum temperatures for those seven months ranged from 53 F to 33 F (Fig. 1).

The greatest total number of adults and pre-adults occurred when the average daily maximum temperature was between 76 F and 64 F, and the average daily minimum temperature was between 44 F and 33 F. In those months when the maximum temperature was above 76 F or the minimum temperature was below 38 F the preponderance of the total population was pre-adult forms. In this study the temperature range between 76 F and 38 F appeared to be the most favorable for development of *D. dipsaci*. Using symptomology as the basis for determining nematode activity

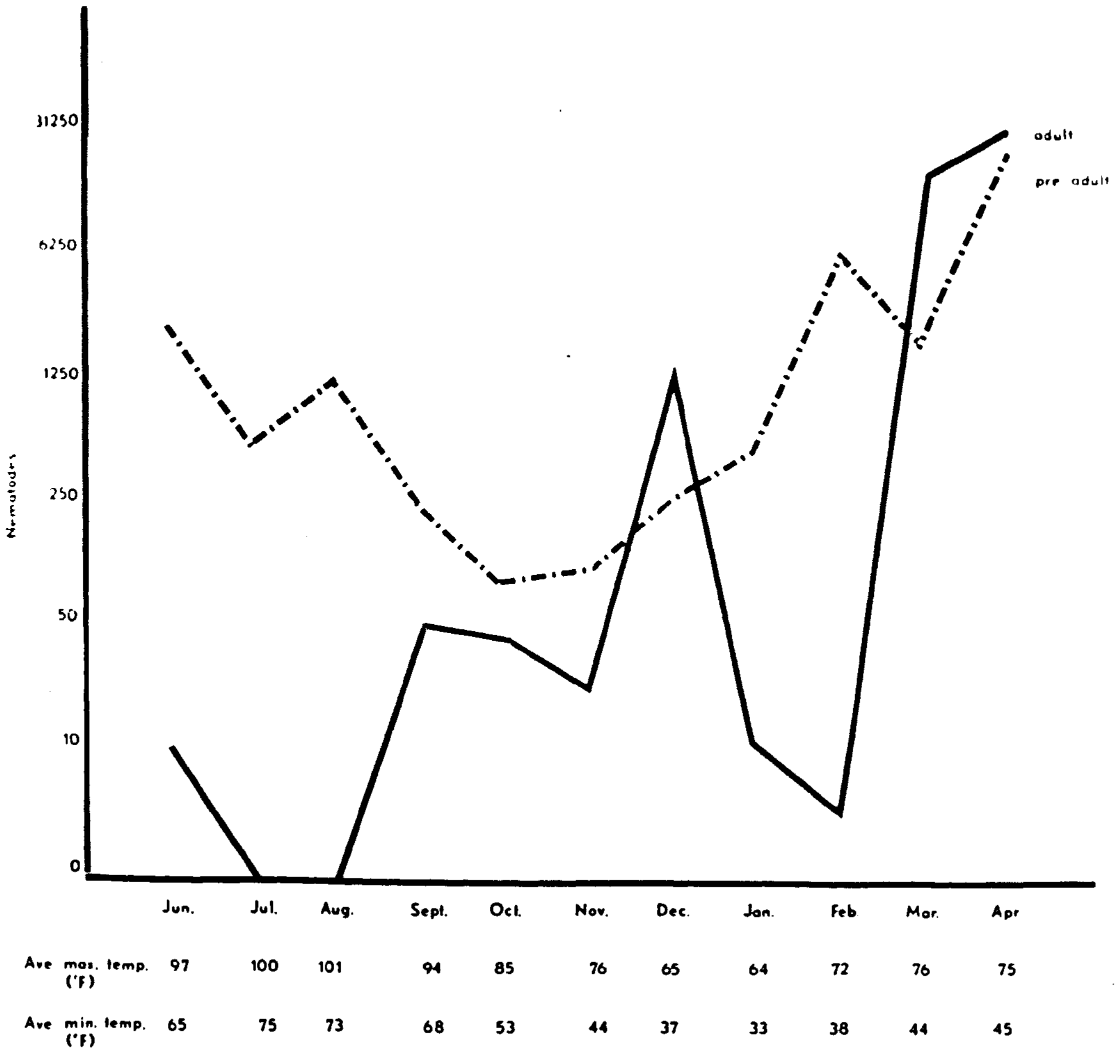

Figure 1. Total number of adult and pre-adult <u>D. dipsaci</u> in relation to month and temperature.

it can be stated that the temperature range between 76 F and 38 F also appeared to be the most favorable for nematode activity. During the months when the temperatures fell in the range given above (76 F to 38 F) both adults and pre-adults were recovered from the crowns, stems, and leaves of the alfalfa plants (Table 1) which showed moderate to heavy stem nematode damage.

Time vs. Temperature Studies of Dessicated Pre-adults

Pre-adult *D. dipsaci* that had been placed on filter discs, dessicated, and stored in incubators at 4 C, 25 C, and 45 C were rehydrated and the percentage of survival determined. These data are presented in Fig. 2.

The survival rate of pre-adult *D. dipsaci* attained a maximum after 5 days of dessication at both 4 C and 25 C. The data presented in Fig. 2 indicate that there is a trend toward an increase in survival from one to 5 days at both 4 C and 25 C. After 7 days this trend was reversed and survival decreased steadily, but not uniformly through the remainder of the test period. Survival at 4 C was greater at every time interval than at 25 C or 45 C. Survival at 45 C was very low and was never higher than .2% at any time interval.

Survival definitely decreased over the 9 week period of the experiment but there was no direct

Table 1. Adult and pre-adult populations and their location within the host during the 11 month study period.

	Soil		Root		Crown		Stem		Leaf	
Month	Adult	Pre-adult	Adult	Pre-adult	Adult	Pre-adult	Adult	Pre-adult	Adult	Pre-adult
June	15	46	0	0	0	1492	0	0	0	0
July	0	0	0	7	0	983	0	0	0	0
August	0	0	0	0	0	1309	0	0	0	0
September	0	0	0	0	42	369	0	0	0	0
October	0	0	0	0	39	111	0	86	0	0
November	32	4	0	0	443	17	26	0	53	0
December	0	0	0	0	347	216	441	56	470	104
January	0	0	0	0	16	1673	0	2280	NA[a]	NA
February	0	5	5	0	0	5292	NA	NA	NA	NA
March	0	7	0	0	12376	2487	3204	784	3753	117
April	31	100	0	0	5148	2111	10960	22115	28930	28900

[a]Not Available.

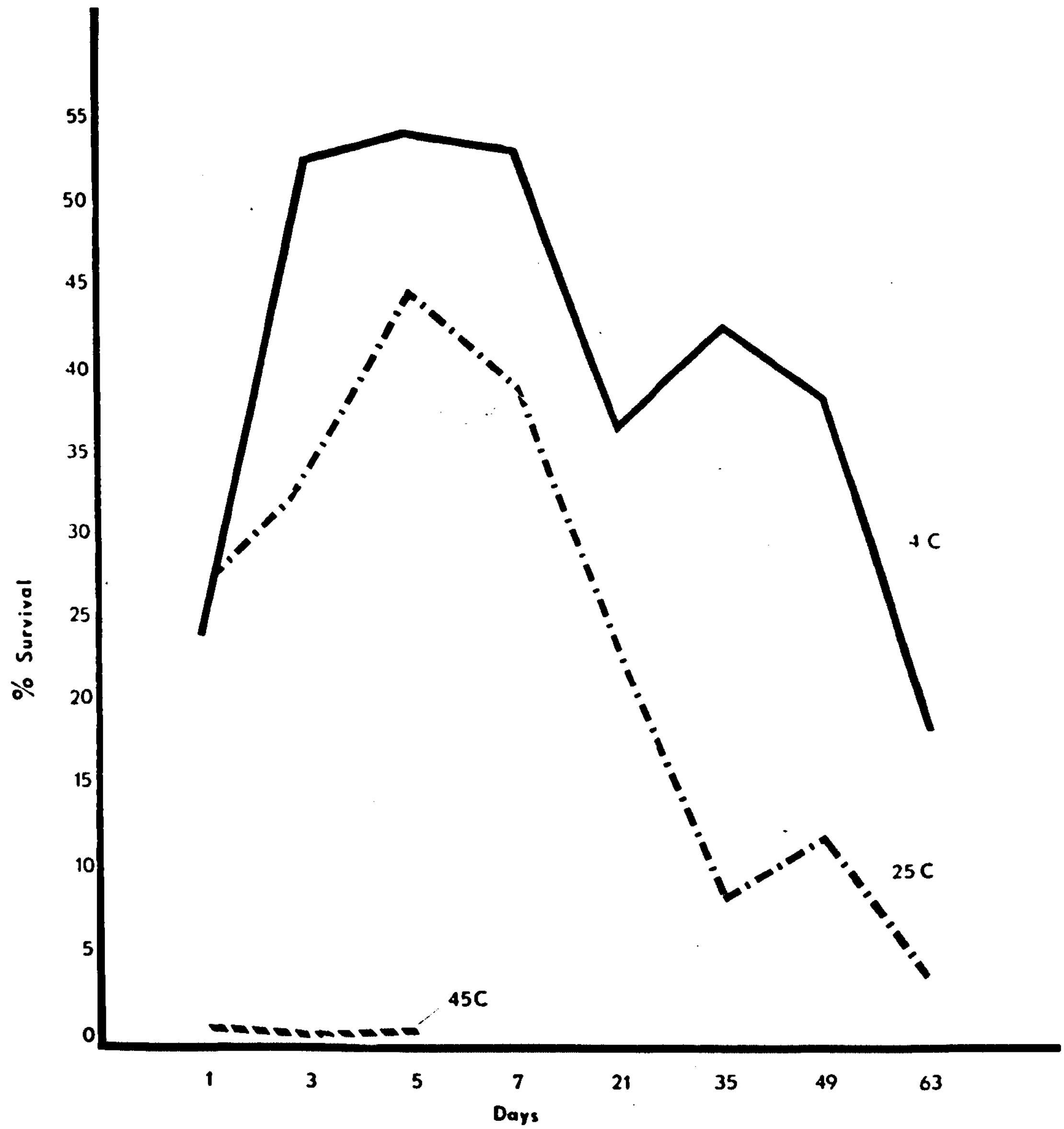

Figure 2. Percent of survival of dessicated pre-adults of D. dipsaci.

correlation between length of the dessication period and the percent of nematodes that survived.

Time vs. Temperature Studies of Nematodes in Distilled Water

The percent of survival of D. dipsaci in all stages of development except eggs that had been stored at 4 C, 16 C, 25 C, 35 C, and 45 C in distilled water is presented in Fig. 3.

Temperatures of 35 C and 45 C were lethal to 100% of the nematodes after 2 days storage at these temperatures. Survival was generally high after 2 days at 4 C, 16 C, and 25 C being 59%, 50%, and 41% respectively. After 2 days the percent of survival dropped sharply at 16 C and 25 C and continued this trend until the tenth day at 16 C when all nematodes were considered dead, and the eighth day at 25 C when less than 1% had survived. Survival at 4 C was not marked by a sharp decrease after 2 days, but rather decreased gradually and irregularly throughout the 60 day test period, the lowest point being 25% after 30 days.

Survival of Nematodes After Passage Through the Gastrointestinal Tract of Sheep

Fresh fecal material that had been collected from sheep pastured on an alfalfa field heavily infested with stem nematodes was tested for the presence of nematodes that may have survived passage through the gastrointestinal

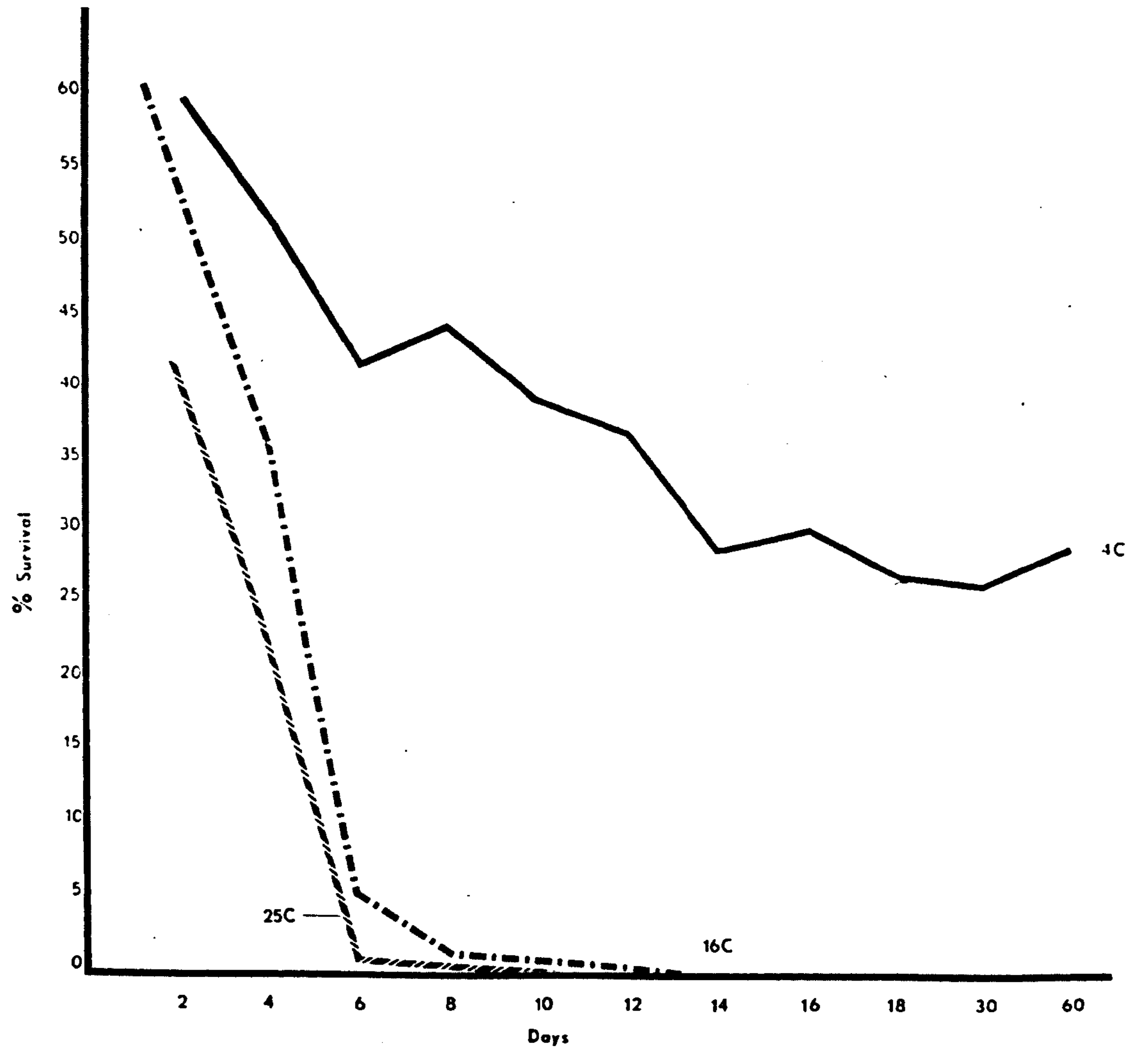

Figure 3. Percent of survival of mixed cultures of <u>D. dipsaci</u> in distilled water.

tract. No adults, larvae or eggs were recovered and it was concluded that this nematode could not survive digestive action.

DISCUSSION

Under the conditions stated previously in this paper the results obtained from the study of the population dynamics indicate that the most favorable temperature range for reproduction, development, and activity of D. dipsaci is between 76 F and 38 F. There are two periods in the year when the temperatures fall within these limits. One period is in the spring and the other one is in the fall. Stem nematode infestation occurring during the spring causes moderate to severe damage, but infestation during the fall is usually less severe. Both of these periods are characterized by temperatures that fall within the range given above. During July and August, and again in January and February stem nematode damage is not observed and the majority of the nematodes recovered are pre-adult forms. These observations lead to the conclusion that the nematodes are inactive during these periods.

It was also observed that during the periods when the nematodes were inactive plant growth was severely retarded, average daily temperatures were at a maximum or minimum, and irrigation frequency at a minimum. These periods constitute adverse environmental conditions to the nematodes, and it is conceivable that they could react to these conditions. It was during these periods of nematode inactivity that the preponderance of the nematodes were pre-adults collected exclusively from the crowns of the plants. Because of these observations the author concludes that *D. dipsaci* reacts to adverse environmental conditions by concentrating itself in the crowns of the infected alfalfa plants where it becomes quiescent.

On first observation it might be concluded that temperature is the determining factor in the summer and winter quiescent periods. Wallace (32) and Van Gundy (28) state that quiescence can be induced by low temperatures, but Wallace (32) also states that it is yet to be proven that high temperatures alone can induce quiescence.

It is generally accepted that temperature alone is only indirectly related to periods of activity and inactivity of *D. dipsaci* (1, 3, 18, 20, 21, 26). It was stated earlier that stem nematode infestation during the fall in Arizona was usually of less severity than the spring infestation but that the temperatures were within the range found to be most favorable for nematode activity and development in both cases. It is the author's opinion that the severity of the spring infestation is due to a combination of environment and cultural practices unique to that time of the year. Prior to the spring infestation the weather is mild and the irrigation frequency is at a maximum. This coupled with the fact that more nematodes survive the mild winter to provide inoculum for the spring infestation, than survive the severe summer to provide inoculum for the fall infestation, accounts for the difference in severity of attack.

Data obtained from studies of time temperature relationships of dessicated nematodes or nematodes in distilled water indicate that the alfalfa race of the stem nematode in Arizona behaves quite similarly to other races of the stem nematode subjected to similar treatment (3, 8, 15). One observation made by the author that was not found in the literature is that there was a trend for the survival rate of dessicated pre-adults kept at 4 C and 25 C to rise steadily for 5 days and then begin to decrease.

It was found that after 5 days more nematodes survived, at comparable temperatures, in the dessicated state than in distilled water especially at high temperatures. It is possible to interpret these observations in three different ways: (a) there is a decrease in activity of dessicated nematodes and normal activity of nematodes in water; (b) there is an increase in activity of nematodes in water especially at high temperatures, resulting in a decrease in longevity; and (c) there is some survival mechanism that is affected in a detrimental way by storage in water.

Mixed populations of *D. dipsaci* (adults and larvae) could be kept alive for long periods of time in distilled water. Even though no field observations were made as to the number of *D. dipsaci* present in irrigation water it is possible to conclude from the data obtained in the laboratory that sufficient numbers of nematodes could survive in irrigation water long enough to constitute a means of spread of the stem nematode. In Arizona alfalfa is grown under irrigation and tail water from infested fields used to irrigate non-infested fields can spread the stem nematode rapidly over large alfalfa growing areas.

A well established cultural practice in Arizona is for ranchers and farmers to graze sheep on their alfalfa fields during the winter months. These animals are brought in from various parts of Arizona and from states where the stem nematode is a serious pest. Since flocks are often moved from field to field during this time the possibility of spread of stem nematodes from infected to non-infected fields in the fecal material of these animals was questioned. It was found that no nematodes or eggs survived passage through the gastro-intestinal tract of these animals and it was concluded that this possible method of spread was not of importance. However, it is possible and quite probable that infected plant material clinging to the hooves and wool of the sheep would constitute a very efficient means of dissemination of the nematode and probably accounts for the increased number of fields infected with this nematode in the Salt River Area of Arizona. It is in this particular area of the state where winter grazing is a common practice.

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