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Title: BIONOMICS OF THE HOP CYST NEMATODE, HETERODERA HUMULI FILIPJEV, 1934 (NEMATODA: HETERODERIDAE)

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Dr. Harold J. Jensen

The hop cyst nematode, Heterodera humuli Filipjev, 1934 was first reported from Europe in 1894, however, very little is known with respect to the biology and economic importance of this pest. Abundant material from Oregon hop yards provided an excellent opportunity to add additional details concerning the bionomics of this pest, and from the taxonomic viewpoint, to amend the existing descriptions of larvae and males.

Second-stage larvae (larvae that hatch from eggs) were reared at various temperatures on hop plants (Humulus lupulus L.) to determine the length of time needed to complete a life-cycle. As the temperature increased, time required to complete a life-cycle decreased and vice versa. No development took place beyond 80°F.

Thirty different kinds of plants were tested to determine their susceptibility to H. humuli. Host range of this nematode was noted to be predominently in the Urticaceae family. H. humuli also will

infect plants in Cruciferae, Cucurbitaceae, Leguminosae and Moraceae families.

Tests to determine the effect of temperature, moisture and soil upon the survival of the nematode indicated that H. humuli stored in wet soil withstood the longest storage period. Nematodes stored at 15°C survived longer than storage in other temperatures.

Twenty-three chemicals, consisting of acids, alcohols, amino acids, esters and lactones, heterocycles, phenols, miscellaneous organic and inorganic compounds were tested to investigate hatching stimuli. Highest hatch was obtained by urea (1840 larvae were liberated) and lowest by n-butyl alcohol (25 larvae were liberated). Ascorbic acid, citric acid, pyruvic acid and thiourea also had an efficient hatching effect. Inorganic compounds were poor hatching stimulators. It also was observed that a pH range of 5 to 7.5 was suitable for H. humuli larval emergence.

Tests to determine the relation between growth of hop seedlings and density of H. humuli indicated that the tolerance limit of hop seedlings to H. humuli is between 50 and 100 eggs per gram of soil. The effect of different population levels of H. humuli on cyst production indicated that the number of cysts increased at every inoculum level. Ratio of increase at highest inoculum level (256 cysts), however, was only 1.47, while lowest inoculum level (1 cyst) was 4. Highest ratio of increase (13.2) was obtained at the inoculum level

of 16 cysts.

Initial egg numbers (empty eggs) appeared to be directly correlated with the number of larvae that hatched over a period of time, and that had taken part in plant invasion. Approximately 34 empty eggs per cyst disappeared during the eight month storage period.

Bionomical investigation conducted in controlled environments indicate that the hop cyst nematode is well adapted to the climatic and edaphic conditions of Western Oregon hop yards. Serious injury to Oregon hops does not appear imminent because the population density in the hops does not approach the level required to produce the symptoms observed in the greenhouse. Since hop crowns lie dormant for several weeks each year and weed control measures destroy alternate hosts, destruction by H. humuli is impeded.

Bionomics of the Hop Cyst Nematode, *Heterodera humuli* Filipjev, 1934 (Nematoda: Heteroderidae)

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BIONOMICS OF THE HOP CYST NEMATODE, HETERODERA HUMULI FILIPJEV, 1934 (NEMATODA: HETERODERIDAE)

INTRODUCTION

The Hop Cyst Nematode, Heterodera humuli Filipjev, 1934 belongs to the genus Heterodera Schmidt, 1871, an important cosmopolitan group of plant parasitic nematodes. Although the hop cyst nematode was described in Europe about 70 years ago (1894), it was only recently found (1962) in the United States (Oregon and Washington). The economic importance of this nematode as a pest of hops has not been fully evaluated in terms of cone (female flowers) yield. Consequently only a few workers have been interested in the biology and taxonomy of this species. Thus there remains a wide fertile field for investigation.

The name Heterodera indicates the main characteristics of the genus, marked sexual dimorphism. Another outstanding character of Heterodera is enlargement of the female to a lemon or sub-spherical shape. Tanning of the body wall after death produces a horny covering or "cyst" which encloses all or most of the eggs. An individual Heterodera passes through six stages in its development from the unsegmented egg to mature adults, molting four times.

Heterodera eggs are typically jelly-bean shaped, and about three times as long as wide. The first-stage and the first molt occur

within the egg and is difficult to observe. Second-stage larvae are infective and hatch from the eggs. The second-stage larvae proceed through the succeeding stages after penetration in a host root. Evidence of the second molt appears shortly after the larvae have entered the roots. The total length including the cast skin is slightly shorter than the free-living second-stage. Larvae start increasing in width after completing the second molt. The larvae develop through the third-stage, and molt a third time. Body girth continues to expand and eventually the larvae become flask-shaped. The sexes usually can be distinguished now, by the well developed reproductive system. After the fourth and final molt, the larvae become adult males or females. There are some species, like Heterodera trifolii Goffart, 1932, which are not likely to develop males. The males regain the vermiform shape whereas the females become ovate or lemon shaped. Females contain eggs inside their body, also some eggs are deposited in a gelatinous matrix.

This investigator has sought to learn more of the biology of hop cyst nematode through studies of (i) life-cycle, (ii) host range, (iii) longevity, (iv) effect of pH and synthetic hatching agents, (v) host damage level, (vi) population trends and (vii) disappearance of empty eggs.

LITERATURE REVIEW

Because Heterodera humuli Filipjev, 1934 has not been the object of many publications, this review will include pertinent literature on other related species of Heterodera Schmidt, 1871. Where there is no relevent information on Heterodera, the review will include reports on other nematodes.

Taxonomic Status in the Genus. The earliest description of the hop cyst nematode was recorded by Voigt in 1894, but he and others (Percival 1895, Duffield 1927 and Triffitt 1929) considered this pest to be a strain of Heterodera schachtii Schmidt, 1871. The original specimens of Heterodera on hops came from Kent (England) and were sent to Voigt for identification by Percival in 1894. Percival (1895a) later associated this nematode with diseased hops in southern England. The taxonomic position of the "hop strain" remained unchanged until 1934 when Filipjev gave it a specific status as Heterodera humuli. Subsequently it was reported from Belgium (Gillard and van den Brande 1957), Germany (Simon 1957), Holland (Oostenbrink and den Ouden 1953), Italy (Goffart 1951), Russia (Ukraine) (Dmitriev 1954, Filipjev 1941), U. S. A. (Oregon and Washington) (Jensen et al. 1962, Cobb 1962), Canada (Andison 1962), New Zealand (Clark 1963) and probably in the Union of South Africa (Goffart 1951).

Dimensions and Description of Various Stages. Females/Cysts. In shape they are ovoid with a prominent vulva which placed them in the lemon-shaped class (Franklin, 1951). De Grisse and Gillard (1963) reported that H. humuli cysts were lemon-shaped and their coloring darkened with age. Cyst dimensions (average 0.48 × 0.37 mm) were appreciably smaller than those of all other Heterodera species. Duffield (1927) who examined a great number of cysts, stated that average length varied from 0.45-0.75 mm. Triffitt (1929) reported the average size as 0.534 × 0.402 mm. and Voigt (1894) reported the size to be 0.564 × 0.377 mm. Jones (1950) reported the smallest length, being 0.48 mm.

Eggs. Franklin (1951) found the dimensions of eggs to be 93μ × 37.1μ. Triffitt (1929) reported 89μ × 42μ and Duffield (1927) reported them to be 93.5μ × 38μ in size. De Grisse and Gillard (1963) observed 98μ × 38μ as the size of normal eggs and 139μ × 39μ as the size of giant eggs. Normal eggs are about the same size and shape as those of Heterodera cruciferae Franklin, 1945.

Larvae. Larvae of H. humuli are comparatively small. Duffield (1927) reported their length as 380μ, Triffitt (1929) as 390μ, Franklin (1951) as 399.7μ and Fenwick and Franklin (1951) as 405μ. De Grisse and Gillard (1963) described the length of normal and giant larvae as 425μ and 560μ respectively.

Males. Franklin (1951) found mean length of 11 males to be 1.032 mm. Males of the hop strain had an average length of 0.85 mm. according to Triffitt (1929).

Host Range and Host Parasite Relationship

The following list of host plants was compiled from the literature:

Humulus lupulus L. (Hop)
Urtica urens L. (Annual nettle)
U. dioica L. (Common nettle)
Cannabis sativa L. (Hemp)

Although Kirjanova (cited by De Grisse and Gillard, 1963) described the cyst nematode found on Ficus sp. as a new species (H. fici), Sher and Raski (1956) and Nuber (1958) considered H. humuli and H. fici to be the same. Cooper (1955) could not differenciate between the two strains using perineal patterns. He also mentioned another species (H. "urticae") on Urtica dioica roots. De Grisse and Gillard (1963) explored the host range of H. humuli in the field and found cysts on hops, also a few cysts were found on U. dioica roots while they could not detect any cysts on U. urens roots. Also they could not infect hemp with H. humuli cysts. Filipjev (1941) assumed that since U. urens and U. dioica are closely related to hop, the nematode found on them was probably H. humuli in all cases. Petherbridge and Jones (1944) also recorded cysts of the "sugar-beet" type on U. urens

growing on land infected with beet nematode.

Franklin (1951) did succeed in infecting U. dioica with cysts of H. humuli removed from hops. Triffitt (1929) found both U. dioica and U. urens infected in a field where H. schachtii was present on mangolds, but it could not be assumed that the nematodes were either H. humuli or H. schachtii.

Wallace (1963) reported that the reaction of plants to attack varied greatly with different nematodes and plant species. He found enough descriptive information to emphasize the importance of the study of host-parasite relations. This forms the basis for recognition and diagnosis of diseases in the field and for control by crop rotation and breeding for resistance.

Franklin (1945) reported that in some cases the brassica crops were a complete failure, in others damage was patchy - due to H. cruciferae. Very often cysts have been found where little damage was in evidence. It seemed likely that if brassica crops were carefully examined, the brassica nematode would be found more frequently than is supposed.

Since Duffield (1925) proved that "nettlehead" disease of hops was not caused by Heterodera, and that healthy appearing hops might have at least one cyst per 2.4 inch of root, he concluded that there is no evidence of harm caused by this parasite of hops. Sveshnikova (1956), Rademacher, Weil and Nuber (1958) and Simon (1957) were

unable to correlate the presence of H. humuli with disease symptoms. Because crop rotation was not followed in hop gardens, there seemed every likelihood that these nematodes should influence hop production, but so far there is no experimental evidence.

Longevity

Temperature. There are numerous observations of nematode mortality at low and high temperatures, tolerance to low and high temperatures, and optimum temperatures for development. Jones (1959) reported that temperature controls activity and for each species, upper and lower thermal points exist. Of greater ecological value is the temperature limits within which activity, and especially reproductive activity is possible. The optimum temperature for development or hatching of plant parasitic nematodes is 30°-40°C. (Franklin 1937, Triffitt 1929, Wallace 1963) and the minimum temperature is 5°-20°C. (Triffitt and Hurst 1935, Wallace 1963). Although there are examples of nematodes surviving in temperature extremes for long periods (Ichinoche, 1939), reproduction usually does not occur in these conditions.

Ferris (1956) reported that larvae of H. rostochiensis Woll., 1923 entered the roots at 65°, 75°and 80°F., but most rapid development was at 65°F. Epps (1958) observed that H. glycines Ichinohe, 1952 cysts stored at 70°-90°F for one month without soil did not

liberate any larvae when exposed to soybean plants. In another case (at 70°-90°F) larvae from cysts stored with soil for one month survived, while those stored an additional 2-3 months failed to survive.

Water/Moisture. Nematodes are well adapted for the inhabitation of soil pores. Their small size permits them to move through most soils, because their undulatory type of locomotion is along a sinusoidal path of the same width as the nematode's body. Excessive moisture does not favor nematode development. Wallace (1963) found that water-logged soil had an inhibiting effect upon growth and reproduction of nematodes. He thought this could be primarily an aeration effect. Cairns (1953) reported that death of nematodes, in water, was possibly caused by the melting of a body constituent. A loss of viability of encysted larvae subjected to some treatments suggests that combination of different temperatures and different levels of relative humidity over a relatively long period of time plays an important role in influencing survival (Sen, 1963).

If the water film enclosing the nematode evaporates, movement ceases and the nematode is subject to desiccation. According to Kämpfe (1959), larvae of Heterodera spp. are more susceptible to desiccation than are the eggs. Kämpfe demonstrated that the mortality of larvae of H. schachtii and H. rostochiensis decreased as the relative humidity increased.

Effect of Synthetic Agents and pH on Hatching

Hatching can be regarded as the starting point of Heterodera host infection and of the life-cycle. If a specific stimulus in the form of a hatching factor from a root-diffusate is required, then this substance must first enter the cyst and penetrate the egg-shell, after which the larvae hatch. The greater the host specificity shown by a species, the greater the difficulty in finding a source of food and the more the larvae depend on the protection of the cyst for survival.

Baunacke (1922) first realized the influence of host plant roots on eggs inside cysts. The hatching of several species of Heterodera was studied by Jones and Winslow (1953) and Winslow (1959). They concluded that mostly hatching was stimulated by leaching from host plants and that leachings from non-host plants (with few exceptions), was not effective. H. cruciferae was activated by brassicas, but not by other cruciferous hosts, i.e. Coronopus squamatus (Shepherd, 1962). Opinions on the nature of the hatching effect by root-diffusate on cyst-forming nematodes have differed widely according to Shepherd (1962). Dropkin, Martin and Johnson (1958) summarize that root-diffusates alter the permeability of egg or larval membrane permitting the passage of water, other molecules and ions.

Synthetic Agents (Chemicals). Many substances other than root-diffusates from plants have been tested for their stimulatory

properties on various species of Heterodera larvae. Rademacher and Schmidt (in Clarke and Shepherd, 1964) tested more than 300 compounds, but found only a few active ones. Clarke and Shepherd (1964) tested 283 compounds on H. schachtii, only 31 gave a hatch equal to or greater than that obtained with a beet-root diffusate. They suggested that many hatching agents functioned by acting as electron acceptors. Clarke and Shepherd suggest that irrespective of the immediate means by which larvae are released from the eggs (i. e. whether by mechanical, enzymic, physical or chemical action) the mechanics of hatching were almost certainly accomplished by a changed metabolism of the larvae. Winslow (1959) could not draw any conclusions on H. humuli hatching with anhydrotetronic acid.

pH Effects. Evidence of influence by pH on plant nematodes is contradictory. Ellenby (1946) reported that there was an apparent optimum for emergence of H. rostochiensis larvae at pH 6 and concluded that marked disturbance of pH on the alkaline as well as the acid side of neutrality might inhibit emergence. Robinson and Neal (1956) observed that emergence of H. rostochiensis cysts reached a maximum at pH 2. 5 in a range of 1 to 6. Simon (in Wallace, 1963) found a close correlation between soil pH and the population level of H. schachtii. Sugarbeets grow best in alkaline soils thus nematode infestation might be correlated with host plant growth rather than pH. Sen (1963) reported that range of pH for larval emergence in

H. trifolii was between 5 and 10 at 25°C. Optimum pH for larval emergence was at pH 8. There was no emergence above and below this range. Emergence in this range also was limited to 20°and 30°C in a temperature range of 15°, 20°, 25°and 30°C.

Host Nutrition

The invasion of a rootlet by large numbers of Heterodera larvae may result in death of the rootlet, either from direct injury or from secondary invasion of the damaged tissue. On the other hand, new lateral roots quickly develop near the sites where larvae settle, as a result of stimulation caused by larvae entering the roots. Franklin (1951) stated that certain cells in contact with the head of the feeding nematode become greatly enlarged and develop granular contents; these are usually referred to as "giant cells." Invading larvae take up a position parallel to the center cylinder in small roots; in larger roots they remain near the cortex. Thorne (1961) reported that giant cells are produced at the feeding point by a coalescence of cells near the head of the larvae. Thorne suggests the products of these cells nourish the nematodes during their growth to maturity. The greater effect of these changes within the root disrupt the translocation system which interferes with the nutrition of the plant. Franklin (1951) assumes damage eventually leads to an unhealthy, stunted plant appearance.

Host Damage Level

Wardojo, Hijink and Oostenbrink (1963) and Hiddink, Hijink and Oostenbrink (1963) noted that white clover was not appreciably damaged during the first 4-8 weeks after planting at densities of H. trifolii of up to 50 larvae per gram of soil. There was damage, however, after the nematodes had multiplied. Seinhorst (1965) reported that injury could occur only when nematode density exceeded the tolerance limit of the attacked plant. Seinhorst and Sen (1966) concluded from their experiment that H. trifolii will considerably damage well established white clover plants in pastures at densities in the order of 200 eggs per gram of soil or higher.

Population Trends

While describing the relationship between golden nematode (H. rostochiensis) eggs in soil and the crop yield, Jones (1965) stated that when there are few eggs, yield is unaffected because the plant fully compensates for the trivial injury to the root system. In the absence of a host crop, Jones reported that, the number of nematodes seemed to decrease at a steady rate regardless of the population unit in soil. In contrast to the population decrease, Wallace (1963) reported that when susceptible crops are grown, increase was greatly influenced by the density of the initial population.

Rapid increase was possible only with small initial population densities. As the density increased, the multiplication rate slowed and decreased when the maintainance line (tolerance limit) was crossed.

Kort (1962) suggested that a positive density dependent factor operates for low population levels in addition to the negative density dependent factor for higher population levels. The negative density factor might arise from competition for space or food, from weakened host roots or a shift in the sex ratio in favor of males (Ellenby, 1954). Ellenby (1957), den Ouden (1960) and Fassuliotis (1957) reported that a low initial population composed of a large number of males, would result in many fertilized females (i. e. well-filled cysts), which would eventually lead to a high population level.

Disappearance of Empty Eggs

Shepherd (1960) reported that the decline of cyst egg contents in soil under fallow or non-host crops might be partly due to the decay of eggs within cysts. She provided little evidence that many eggs normally decay in cysts in soil. Brown (1958), Hijner (in Shepherd, 1960) and Jones (1959) calculated the rate of decay of populations of Heterodera species in terms of eggs per gram of soil by recovering cysts from soil and estimating their egg content. Hesling (1958) and Wallace (1956) used cyst contents to measure the rate at which larvae emerged from cysts under various conditions in

the soil. These workers did not state as to how much of the reduction was attributed to emergence and how much to decay of eggs in cysts. Seinhorst and Sen (1966) reported that empty eggs remain recognizable a long time after the larvae have hatched, but eventually the empty eggs decay and disappear. The number of empty eggs in the cyst gives an impression of the number of larvae actually taking part in the invasion.

MATERIALS AND METHODS

General Procedures

Obtaining and Maintaining a Stock Culture of Cysts. Cysts were obtained from the soil by the gravity screening technique described by Cobb (1918) which required the following equipment, (i) two large dish pans, (ii) two sieves of 40 and 100 mesh and, (iii) a small glass jar. Soil from an infested hop yard was mixed thoroughly with tap water in one large dish pan, and the supernatant (soil wash water) was poured through the 40 mesh screen into the second pan. The supernatant (soil wash water) in the second pan was then poured through the 100 mesh screen. The first screen retains the larger particles of soil and organic matter, but allows the cysts to pass through. The second screen allows the smaller soil particles and organic matter to pass through but retains the cysts. Cysts retained on the second screen (100 mesh) were washed free of debris and collected in the jar with water and stored at 34° F. Accumulation of bacteria and fungi which might destroy the cysts was reduced by changing the water each day.

Increasing Inoculum. In order to have a continuous source of inoculum, several #10 cans were filled with non-infested soil and sown with hop seeds. Inoculation was accomplished by placing 3-4

cysts near each seed. A small amount of sand was sprinkled over the soil surface to facilitate proper drainage. The encysted eggs hatched, and the nematodes matured, reproduced, multiplied and established a large population in each can.

Obtaining Infective Larvae. The Baermann funnel technique was used to obtain infective larvae from the soil. This equipment consisted of a 6" plastic funnel with a piece of rubber tubing and a pinch clamp attached to the funnel to support the tissue paper and sample. The Baermann funnel technique was utilized as follows, (1) the funnel was filled with water to the wire screen, (2) a single layer of tissue paper was then placed on the screen and, (3) 60-80 grams of soil was placed on the paper and (4) excess paper was folded over the sample. Later enough additional water was added to the funnel to cover the soil sample. After a two-day lapse, a water sample was drawn from the funnel daily to collect the infective larvae.

TAXONOMICAL STUDIES OF H. HUMULI

The specimens were fixed in 4% formalin and preserved in a solution containing 4% glycerol in 30% ethanol. Metal slides, as described by Thorne (1961), were used for mounting. Camera lucida measurements were taken after mounting the specimens in dehydrated glycerine.

Eggs. Mature eggs range from 89 - 97 μ in length and 43 - 49 μ in width, with an average of 93 × 46 μ. First cleavage produces two equal cells within the egg and second cleavage is followed by mitotic division. As the embryo increases in length it becomes slender and eventually, through progressive increase, flexures are formed within the egg.

First-stage/First Molt. The first-stage larva is coiled inside the egg and very little can be seen in the head region. First molt occurs within the egg and is evidenced by the loose skin in the head region of the larva. The spear looks like an inverted "V," without any knobs (Fig. 4 A). The second-stage larva hatches either in the egg-sac or inside the cyst body, in which case the larva emerges through the vulval slit and begins its search for a host plant.

Second-stage Larva. L-400μ (370-420); a-19.5 (18-21); b-2.9 (2.7-3.1); c-10 (9-11); spear-23μ (21-25).

Larval body is striated (average 1.2 μ between striations),

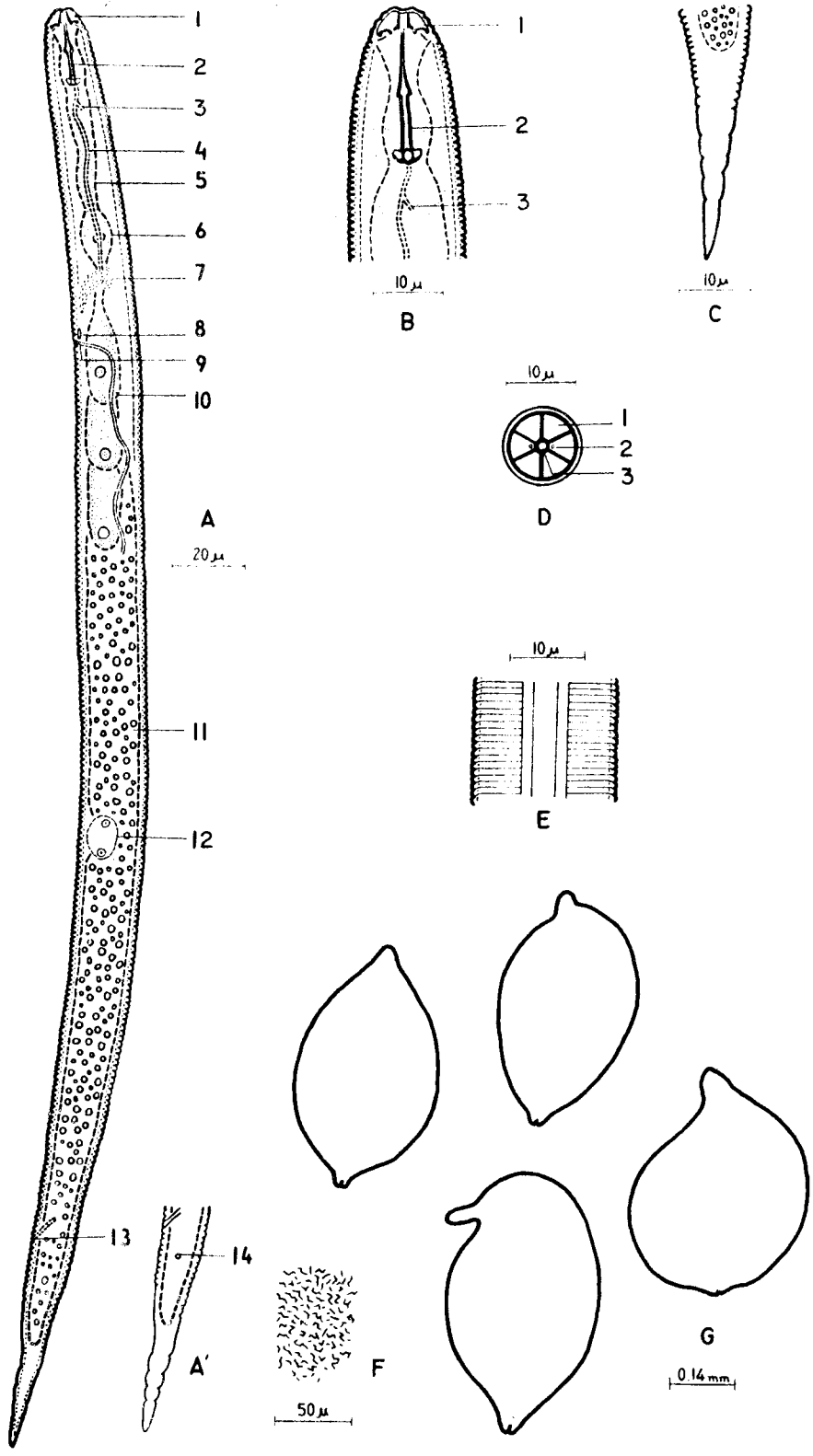
cylindrical with blunt anterior end and conoid posterior region tapering from terminal fourth of body length. Lateral fields are marked by four incisures beginning in esophageal region and terminating near anus. Lip region is divided into six equal sectors with elliptical amphid apertures located in the oral aperture, bearing three annules. Labial framework is heavily cuticularized, supporting prominent spear which bears three forward pointing knobs.

Dorsal esophageal gland duct opens at 5 μ from base of the spear. Median bulb is elliptical; isthmus is elongate; esophageal glands are ventrally located, distinctly lobed bearing three prominent nuclei. Excretory pore opens immediately behind elliptical hemizonid; excretory duct can often be traced just beyond esophageal gland region. Nerve ring is strongly developed and encircles isthmus adjacent to median bulb.

Genital primordium is ovoid (10-14 μ long and 8-10 μ wide), and is located near ventral surface, slightly posterior to middle of body. Primordium consists of two or four nuclei, though not distinctly separated by cell walls. Rectum is prominent, extending across one-half of body width, leading to anus which is located in terminal tenth of body. Clear tip of tail is half of tail length. Phasmids are difficult to observe in lateral view although openings occur about 1/2 - 3/4 of distance between anus and end of intestinal protoplasm. Tail is elongate conoid with blunt terminus.

Fig. 1. Heterodera humuli Filipiev 1934, Larva and Cysts

Larva: A, A', B, C. 1- lip, 2- spear, 3- dorsal esophageal gland opening, 4- lumen of esophagus, 5- corpus, 6- median bulb with valve, 7- nerve ring, 8- hemizonid, 9- excretory pore, 10- dorsal gland with gland nuclei, 11- intestine, 12- genital primordium, 13- anus, 14- phasmid.
Face view: D. 1- lip, 2- oral aperture, 3- amphid
Lateral lines: E
Cyst wall pattern: F. Cysts: G.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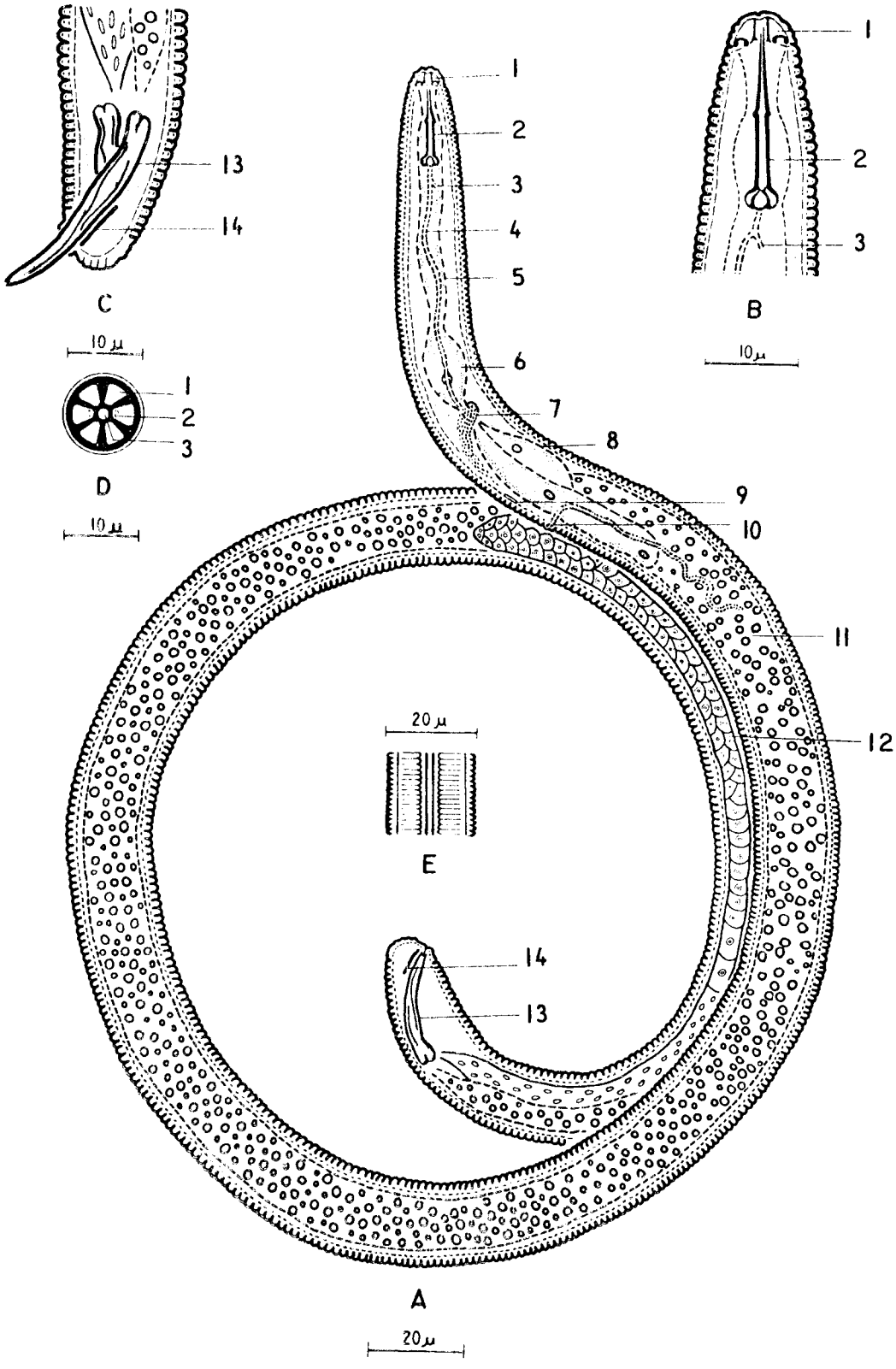
11
12
13
14
A'
1
2
3
10μ
B
10μ
C
10μ
1
2
3
D
10μ
E
F
50μ
G
0.14mm

Fig. 2. Heterodera humuli Adult male

A, B, C. 1- lip, 2- spear, 3- dorsal esophageal gland opening, 4- lumen of esophagus, 5- corpus, 6- median bulb with valve, 7- nerve ring, 8- dorsal gland with gland nuclei, 9- hemizonid, 10- excretory pore, 11- intestine, 12- testis, 13- spicule, 14- gubernaculum.
Face view: D. 1- lip, 2- amphid, 3- oral aperture.
Lateral lines: E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20μ
B
10μ
C
10μ
D
10μ
E
20μ

Adult male: L-800μ (670-1000); a-31 (30-32); b-7.7 (7.6-7.8); c-55 (50-61); T-42.5 (35-50); spear-24μ (20-28); spicule-30.5μ (29-33); gubernaculum-7.5μ (7-8).

Adult male is slender and cylindrical with obtuse ends; posterior part of body is commonly twisted. Cuticle is finely striated (1.4μ between striations) bearing four lateral lines, which begin in the esophageal region and extend around the tail terminus from one side to the other. Amphid openings are elliptical, which appear on lateral lips near oral openings when observed in face view. Lip region is continuous with neck region, bearing four annules and slight depression at oral aperture. Cephalic framework and spear are heavily cuticularized. Spear length is 20-28μ, prorhabdions equal in length to remainder of spear. Prominent knobs (three in number) are slightly flanged gradually blending into shaft (mesometarhabdions).

Dorsal esophageal gland opening is near base of the spear; median bulb is elliptical with oval valvular apparatus located in the posterior third. Esophagus terminates in lobed esophageal glands bearing three conspicuous gland nuclei. Nerve ring encircles isthmus and terminates near elliptical hemizonid. Excretory pore is 6-8 annules behind hemizonid. Intestine is darkly granulated and dominates the remainder of the body except for the well developed testis which is prominent in the posterior third of the body. Spicules measure 29-33μ in length (measured along curved median line), are

arcuate, robust and gradually tapers posteriorly toward bi-dented distal ends. Gubernaculum is simple, slightly sinuate in shape and measures 7-8μ. Male tail is usually twisted upon killing, hence phasmids could not be seen.

Morphological Characters Separating H. humuli from its Most Closely Related Species

Males and Cysts offer the best stages for separation from closely related species.

Male. Accepted criteria for separating Heterodera males are measurements (body, spear, spicule), form of spicule and gubernaculum, and number of annules in the lip region. Hesling (1965) characterized the males of all known species of Heterodera in a chart form. According to these data H. cruciferae Franklin, 1945, seems most closely related to H. humuli because of similarities in measurements. They can be separated, however on some points. H. humuli has four annules in the lip region while H. cruciferae has 5 or 6. The lip region appears to be distinctly set-off by a depression in H. cruciferae while it is continuous with the body in H. humuli. The hemizonid appears to be 4 annules anterior to the excretory pore in H. cruciferae and 7-8 annules in H. humuli. The tip of the spicule is bidentate in H. humuli and tridentate in H. cruciferae. The average lengths of males of H. cruciferae and H. humuli are 1.179

and 0.800 mm. respectively.

Cysts. Cysts of H. humuli are 0.54 mm long (including neck) and bifenestrate whereas H. cruciferae cysts are ambifenestrate and 0.55 mm long (excluding neck).

Larvae and Eggs. Very little difference in size exists between larvae and eggs of H. humuli and of H. cruciferae. Larvae of H. humuli are 400μ long and of H. cruciferae are 418μ long. Dimension of H. humuli and H. cruciferae eggs are 93 × 46μ and 93.5 × 46.75μ respectively.

LIFE-CYCLE

Penetration of Host Plant. Hop seedlings established in sterilized soil were inoculated by adding water containing newly hatched second-stage larvae to the root zone. After inoculation, one or two seedlings were removed every hour, washed, stained with acid fuchsin in a lacto-phenol solution (Jensen, 1962) and examined for the presence of larvae within the roots.

Modified Goodey's (1937) staining technique was used for examining the nematodes in root tissues. The staining solution, containing 0.1 gram of acid fuchsin in 100 cc of lacto-phenol (a solution containing lactic acid, phenol, glycerine and water in equal parts) was brought to a near boiling point, and the roots were placed in this solution for a couple of minutes. Excess stain was then washed off the roots by placing them in a beaker of tap water. The roots were then allowed to destain in a clear lacto-phenol solution, for a period of about two days. During this time, the root tissues destained while the nematodes retained the stain.

Larvae were observed inside the roots after a period of 48-50 hours from the time of inoculation. All larvae entered just behind the growing point of roots, which seemed to be the zone of greatest attraction. After entering the root most larvae remained parallel to the central vascular bundles. Often more than one larva invaded

the same area.

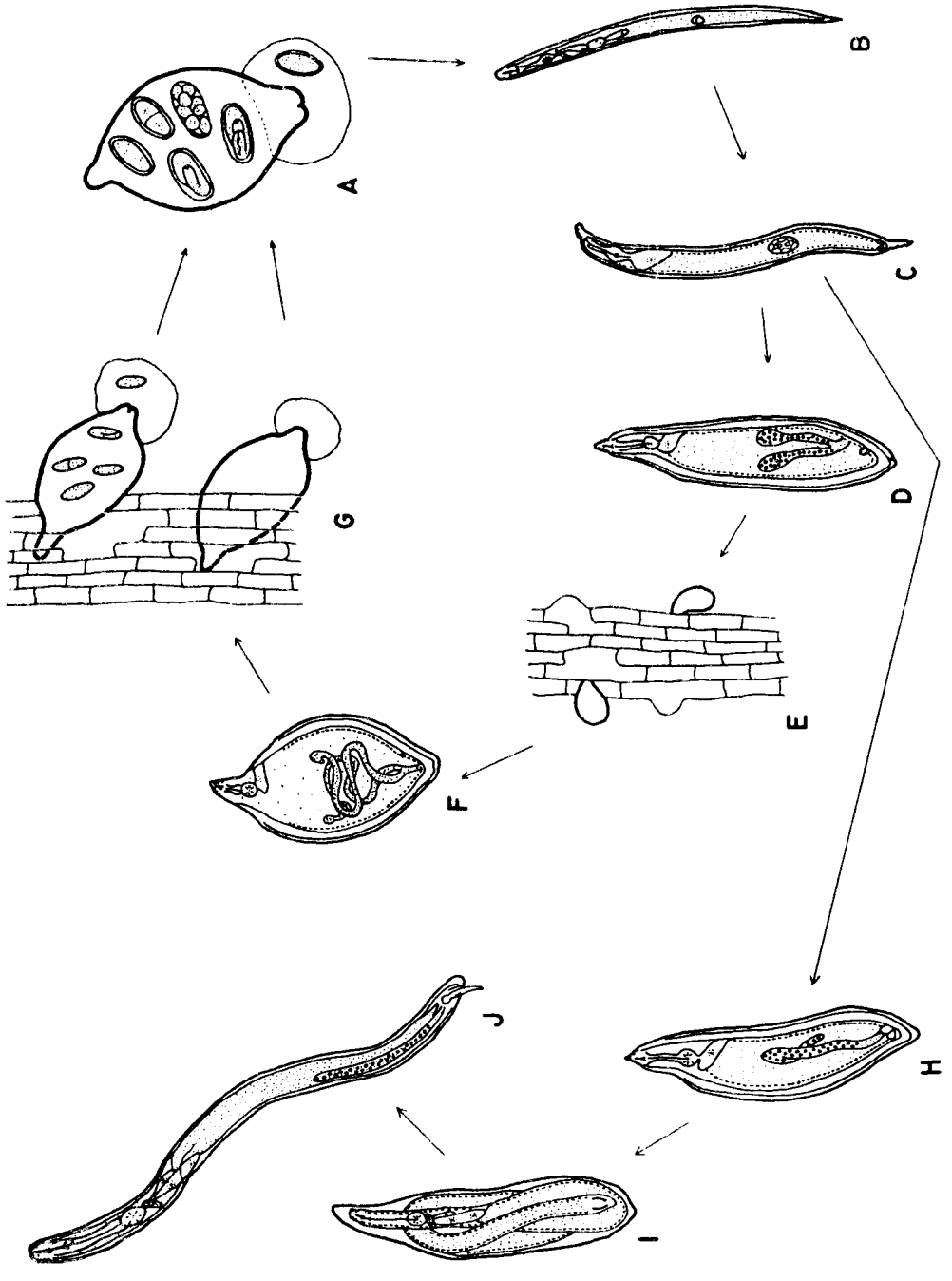
Temperature Effects Upon the Life-cycle. Hop seedlings were planted in flats and inoculated with larvae as described previously. After allowing 50 hours for larvae to penetrate their host, the plants were removed and roots were washed to remove any nematodes that had not entered the roots. These plants were then transplanted in #10 cans which contained sterilized soil. The cans were then placed in constant temperature tanks.

Seven thermostatically controlled tanks were used for this experiment. The tanks were adjusted to maintain constant temperatures of 60° (15.5°C), 65° (18.5°C), 70° (21°C), 75° (23.5°C), 80° (26.5°C), 85° (28.5°C) and 90°F (32°C). The cans were watered every day to maintain the optimum moisture for plant growth. The water level in the tanks was also kept constant to minimize temperature fluctuations. Every two days, two seedlings from each temperature tank were removed. The roots were washed, stained and examined with a dissecting microscope. Differential staining (previously described) facilitated observation of nematode development within the roots. Time required to develop from one stage to another was noted.

Stages of Development (Fig. 3). The first-stage larva develops and molts once while still inside the egg. Following the first molt, the second-stage larva hatches from the egg and begins searching for

Fig. 3. Diagrammatic life-cycle of Heterodera humuli

A. Embryonic development of eggs within cyst or egg-sac
B. Second-stage larva (infective stage)
C. Second molt (inside root)
D. Third molt, female (inside root)
E. Fourth-stage, female (protruding through root)
F. Fourth molt, female (outside root)
G. Cysts with developing eggs (in or outside root)
H. Third molt, male (inside root)
I. Fourth molt, male (inside root)
J. Adult male (outside root)


A
B
C
D
E
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J

a host plant. This study begins after the second-stage larva had penetrated the host plant. The second-stage larva takes between 48-50 hours to penetrate a host plant. Soon after penetration, the larva passes through a short quiescent period before continuing its development.

Second Molt. Evidence of the second molt was observed in 45, 34, 26, 24 and 16 days after penetration at 60°, 65°, 70°, 75°, and 80°F respectively (Table 1). Molting starts in the posterior part of the body and then begins in the anterior region. The larva decreases slightly in length and measures 330-347μ. The larva increases in width and the digestive system appears. The esophageal gland lobe become shortened and the nuclei are greatly enlarged. The genital primordium dimensions are approximately 15 × 12μ and it occupies almost the entire width of the body cavity. Vertical development of the primordium starts at this time, and later the cells start forming near the external margin of the genital primordium. The rectum is well developed. The newly formed larvae still retain a worm-like appearance. All larvae completed the second molt in 57-28 days after root penetration (Fig. 4 B).

Third-stage. Evidence of the third-stage was found 56-28 days after penetration at temperatures reported earlier. Body annulations could only be distinguished in the head region. The larva increases in body width (beginning of the flask-shaped appearance), but the total

Table 1. Development time for various stages of H. humuli at different temperatures beginning with host penetration.

Female	Temperatures in °F													
	60° (Days)		65° (Days)		70° (Days)		75° (Days)		80° (Days)		85° (Days)		90° (Days)	
Stage/Molt	1*	2*	1*	2*	1*	2*	1*	2*	1*	2*	1*	2*	1*	2*
2nd Molt	45	57	34	46	26	38	24	36	16	28				
3rd Stage	56	69	44	58	36	50	34	48	28	40				
3rd Molt	67	81	56	70	49	62	46	61	39	52	NO DEVELOPMENT TOOK PLACE			
4th Stage	80	94	68	83	60	75	59	73	50	65				
4th Molt	90	105	78	94	70	86	69	84	60	76				
Adult	115		104		96		93		86					
Brown Cyst	177		165		158		156		148					
Male														
3rd Molt	70	87	59	76	52	68	48	66	42	58				
4th Stage	82	98	70	88	64	80	62	78	52	68	NO DEVELOPMENT TOOK PLACE			
4th Molt	90	112	80	101	74	93	72	91	62	79				
Adult	126		115		108		106		94					

1* - column indicates when first observed
2* - column indicates when completed

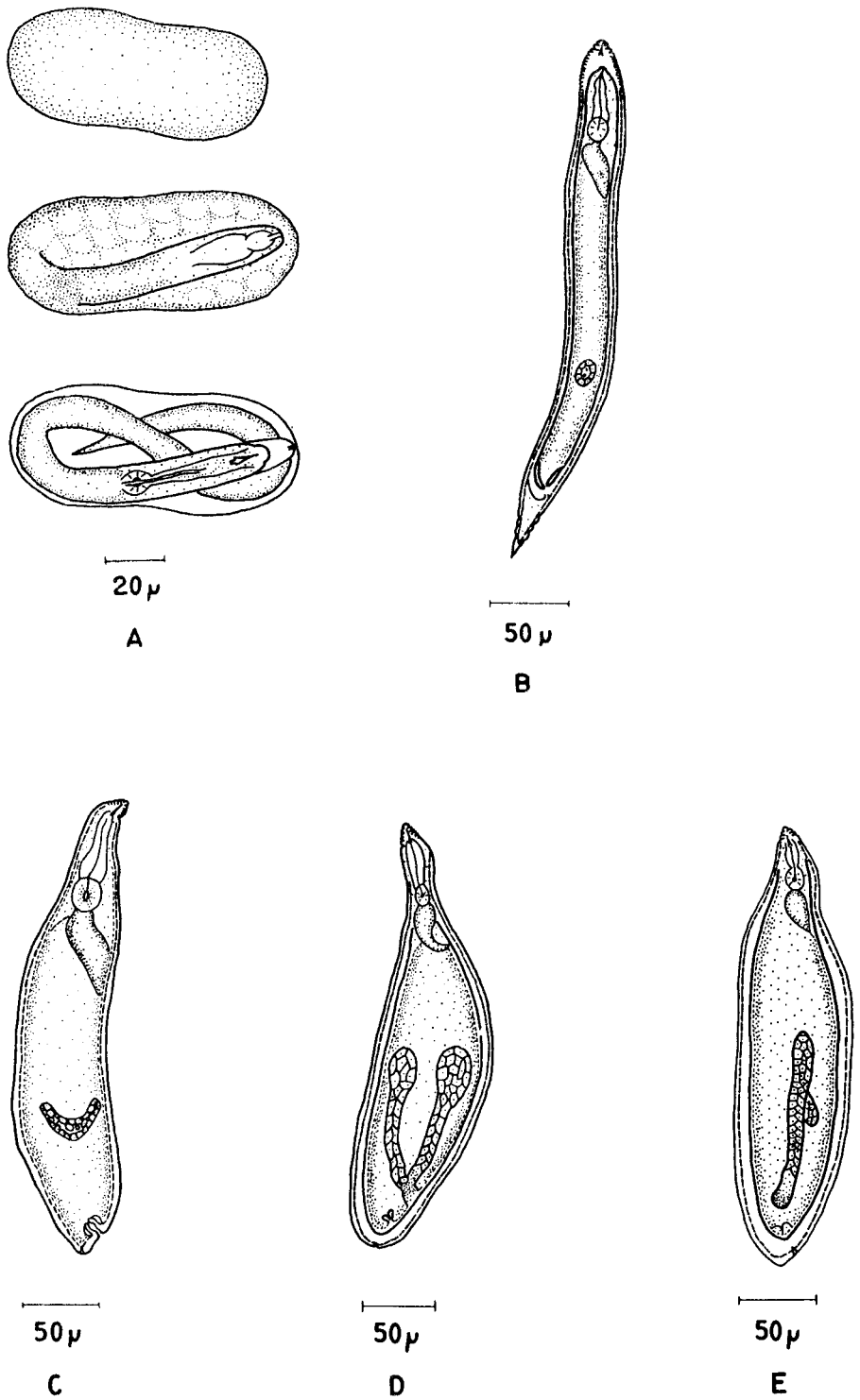


Fig. 4. Stages of development of Heterodera humuli.

A- first-stage larva and first molt; B- second molt; C- third-stage; D- third molt, female; E- third molt, male.

length (306-324μ) decreases. The esophagus is well developed. The genital primordium is twice the size of a second-stage primordium which later makes a 90° turn, becoming vertical to the body axis. Then it assumes a concave shape with the concave surface facing the anterior end of the body. Larvae, in which the primordium develops this way, become juvenile females. In young males, the genital primordium increases in length at both ends, without making a 90° turn, or becoming concaved in shape. During this time rapid cell division and growth take place as evidenced by the swollen appearance of the primordium termini (Fig. 4C).

Thus by the end of the third-stage, sex differentiation is evident. The ovaries which have assumed a "U" shaped appearance continue to elongate. In males, the anterior end of the primordium extends forward then reflexes; the posterior end extends to the rectum. All young females and males completed the third larval stage 12 days after second molt regardless of temperature.

Third Molt (Female). The larvae of this molt are flask-shaped. Length of body is 325-339μ. Formation of two ovaries was obvious as the genital primordium became "U" shaped with the ends pointed anteriorly. The two ovaries continue to elongate during this molt. The anterior ends of the ovaries are swollen and oblong in shape. The rectum and rectal glands also are prominent. All larvae completed the development of third molt 12-13 days after completing the

third-stage at all temperatures (Fig. 4 D).

Third Molt (Male). Larvae are still flask-shaped. Testis formation is complete. The rectum is visible but indistinct. Genital primordium elongates anteriorly and is reflexed; posterior elongation approaches the rectum. All specimens examined completed the third molt in 18 days after completing the third-stage at various temperatures (Fig. 4 E).

Fourth-stage (Female). The fourth-stage larvae are flask-shaped but wider than those of third molt. Evidence of the fourth-stage was first noticed in some larvae a few days before all larvae completed third molt. Six days after the third molt all larvae were in the fourth larval stage, and 13-14 days after the third molt development of this stage was complete. Length of a fourth-stage female is 351-368μ. The internal cuticular surface becomes granular while the external surface is marked by irregular lines. The ovaries undergo considerable development during this stage and become reflexed. The vagina is ventral to the anus and extends almost to the cuticle. The matrix glands begin to appear near the rectal area (Fig. 5 F).

Fourth-stage (Male). The developing male larva assumes a worm-like shape inside the old cuticle. Lip region starts to develop and the shape of an indistinct spear can be recognized. The testis connects to the rectal region. Full development of the fourth-stage

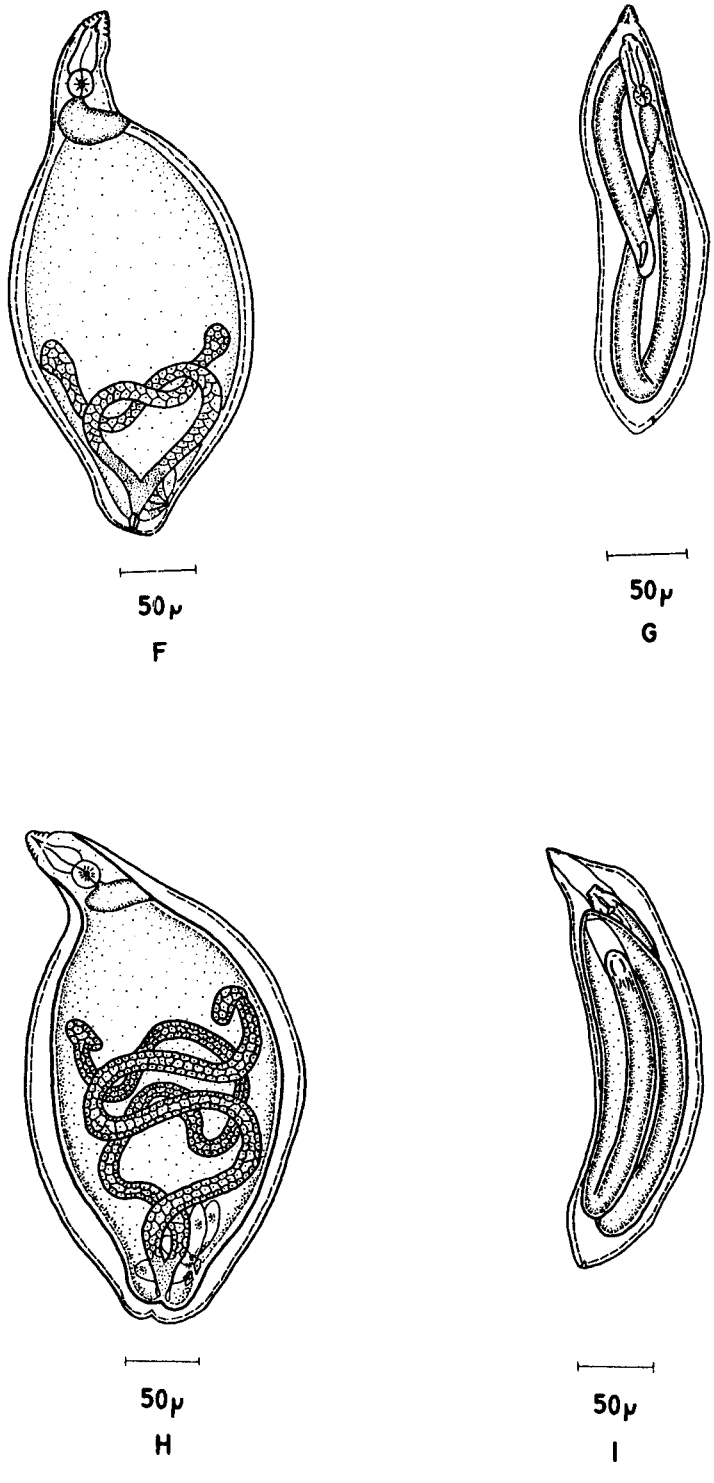


Fig. 5. Stages of development of Heterodera humuli.

F- fourth-stage, female; G- fourth-stage, male; H- fourth molt, female; I- fourth molt, male.

larvae is completed 10-12 days after third molt. The larvae measure 547-600μ in length within the cast skin (Fig. 5 G).

Fourth Molt (Female). Approximately 90-60 days after penetration, one out of four larvae examined from each temperature range were beginning the fourth molt; and 11 days after all larvae examined had completed the fourth molt. Body length varies between 360-385μ. The ovaries increase in length and almost fill the body cavity. The reproductive system becomes complete and extends to the vulva. After this molt, formation of a gelatinous matrix (egg sac) begins around the posterior part of the body. Ovaries continue to enlarge and the development of the ova to fully formed eggs occurs 10-15 days later (Fig. 5 H).

Fourth Molt (Male). The developing male is reflexed about three times within the previous stage cuticle and measures 620-700μ in length. Spicule and gubernaculum are visible for the first time. The lip region is distinct and spear measures 15-20μ in length. Median bulb and esophageal glands are distinct. All larvae examined completed the fourth molt in 11-14 days after the fourth-stage (Fig. 5 I).

Adult Female. The cuticle retains a grayish color until the final molt. Following the gray color, the cuticle starts to turn yellow and becomes brown when the females die. Adults measure between 420-480μ in length and 21-32μ in width. The body shape

varies from ovoid to spheroid. The ovaries fill the entire body cavity and it becomes impossible to follow the tortuosity throughout their length. The vagina is enlarged anteriorly, then tapers slightly as it extends toward the vulva. The oocytes near the distal ends of the ovaries enlarge, forming egg. As the female matures, a few eggs are deposited in the gelatinous matrix (Fig. 1 F, G).

Adult Male. This has been described under Taxonomical studies of H. humuli, in page 17.

Brown Cysts. Brown cysts were obtained 148-177 days following root penetration by second-stage larvae. The cysts measure 480-600μ in length and 260-380μ in width. Numbers of eggs vary between 200-300, and about 1/6-1/8 of this number is deposited in the egg-sac. Cyst wall is thin with a zig-zag surface pattern showing irregular minute punctations. The vulval cone is without bullae and bifenestrate. Fenestrae is twice as long as wide and the vulval slit is as long as the wide bridge. Eggs in the cyst contain coiled and fully developed larva. Few hatched larvae are often found inside the cyst.

Number of Generations (Life-cycle) Per Year. A life-cycle or generation is determined by the time interval from egg stage--passing through all the stages of development--ultimately producing eggs again. H. humuli larvae take about 148-177 days, depending upon temperature, to complete one life-cycle (from egg to adult) in

the presence of abundant soil moisture, favorable temperature and a suitable food supply. It is evident that under the conditions described before, H. humuli will have two generations per year.

The number of eggs deposited in the egg-sac is 1/6-1/8 of the total egg content. The eggs deposited in the matrix are able to hatch and infect earlier than those retained inside the cyst. Therefore the new generation will commence earlier with the onset of egg deposition in the gelatinous egg-sac.

Time taken to complete a life-cycle varies according to the soil temperature (Table 1). The lower the temperature, the longer time required to complete a life-cycle and vice versa. At higher temperatures (85° and 90°F) larvae did not develop. Beginning in the third-stage, the larvae swell and assume a flask-shaped appearance. The adult females are ovoid to spheroid in shape. The developing male larvae retain their flask-shaped appearance until the third molt. After this period they assume a worm-like shape inside the old cuticle and continue their development. The adult males are vermiform in shape. The genital primordium continues to develop from the second-stage and ultimately form ovaries (in females) or testis (in males). In females, the primordium takes a 90° turn during the third-stage to form two ovaries, while in males the primordium develops parallel to the body axis. The hop cyst

nematode, H. humuli, completes only two generations a year, which is in accordance with other workers (Percival, 1895a; Duffield, 1927 and DeGriesse and Gillard, 1963).

HOST RANGE

Thirty kinds of plants (Table 2) were seeded into different #10 cans and were placed in the greenhouse. Four replications were made, each replicate consisted of one can each of the 30 different types of plants. Three replications were inoculated with cysts and the fourth replicate was treated as a control. The plants were randomized within each replicate. Water was added to insure adequate moisture for plant growth. Fertilizer (20-20-20) was applied twice, to maintain healthy and normal plants.

The plants were grown for approximately five months before removal from the cans, taking care not to dislodge the adhering cysts. The roots were placed in a large dish pan half-filled with water, to separate the roots from each other so that examination for cysts could be accomplished. When cysts attached to the roots were observed (or objects suspected to be cysts), that root portion was removed and examined with a dissecting microscope. When cysts were actually protruding from the root epidermis, the plant involved was regarded as a host.

Results of the host range studies (Table 2) point out that the host range of H. humuli was predominently the Urticaceae family. This nematode, however, will infect some plants in Cruciferae, Cucurbitaceae, Leguminosae and Moraceae families. A nematode is

Table 2. Sensitivity of plants to Heterodera humuli infection.

Family	Plant	Variety	Binomial name	Sensitivity rating
Leguminosae	pea	Perfection	Pisum sativum L.	Infected
	bean	Bush	Phaseolus vulgaris Var. humilis Alef	Infected
	clover	White	Trifolium repens L.	Infected
	alfalfa	Ranger	Medicago sativa L.	Non-infected
	vetch	Hairy	Vicia villosa Roth	Infected
	soybean	Capital	Glycine max (L) Merrill	Non-infected
Compositae	lettuce	Gt. Lake	Lactuca sativa var. capitata L.	Non-infected
	marigold	Fireglow	Tagetes erecta L.	Non-infected
Umbelliferae	carrot	Imperator	Daucus carota L.	Non-infected
Cruciferae	mustard	Black	Brassica nigra (L) Kock	Infected
	br. sprout	Catskill	Brassica oleracea var. gemmifera L.	Non-infected
Chenopodiaceae	sugar beet		Beta vulgaris L.	Non-infected
Solanaceae	tomato	Bonny best	Lycopersicon esculentum Mill.	Non-infected
	tobacco	Common	Nicotiana tabacum L.	Non-infected
	potato	Netted Gem	Solanum tuberosum L.	Non-infected
	pepper	Yellow wonder	Capsicum frutescens L.	Non-infected
	egg plant	White	Solanum melongena L.	Non-infected
Urticaceae	hop	Late cluster	Humulus lupulus L.	Infected
	nettle	Annual	Urtica urens L.	Infected
		Common	U. dioica L.	Infected
Moraceae	hemp	Common	Cannabis sativa L.	Infected
Graminae	oat	Atlas	Avena sativa L.	Non-infected
	barley	Hannchen	Hordeum vulgare L.	Non-infected
	rye	Perennial	Lolium perenne L.	Non-infected
	wheat	Elmar	Triticum vulgare Vill.	Non-infected
Cucurbitaceae	cucumber	Boston pickling	Cucumis sativus L.	Infected
	pumpkin	Gt. striped cushaw	C. pepo L.	Non-infected
	squash	Zucchini	Cucurbita moschata L.	Non-infected
	water melon	Shipper	Citrullus vulgaris Schrad	Non-infected
Polygonaceae	dock		Rumex acetosella L.	Non-infected

attracted to the plant because the plant produces root-diffusate. The fact that bean, clover, cucumber, hemp, hop, mustard, nettle, pea and vetch plants produce root-diffusates does not necessarily classify them as hosts. Plants may produce root-diffusates which stimulate the encysted eggs to hatch, but the larvae may fail to enter the plant roots. This may occur because the epidermal or cortical layers in the root resist larval penetration. Therefore a larva may hatch after stimulation of a root-diffusate, be attracted to the plant, and even then fail to infest that plant.

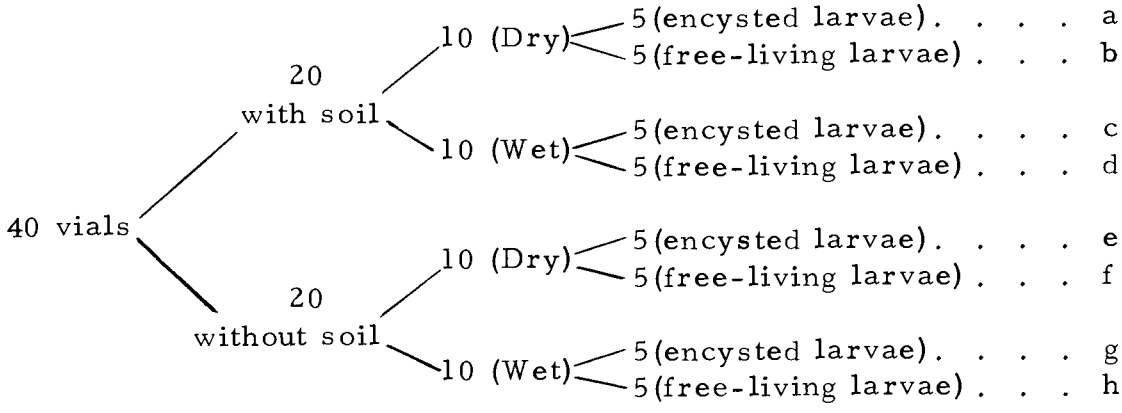
It is evident from Table 3, that bean, clover, cucumber and mustard plants had fewer cysts than hemp, hop, nettle, pea and vetch. It could be concluded here that more larvae entered hemp, hop, nettle, pea and vetch roots even though all nine plants produced active root-diffusates. Control plants, in general, were 2-3 times heavier in weight (root) than the susceptible ones. Severely infected rootlets, in most cases, were somewhat darker in color than the healthy, non-infested roots.

Table 3. Effect of H. humuli on susceptible plants as analysed by cyst count and root weight.

Plants	Number of cysts/plant	Weight of roots (gms)		Percent weight loss
		Susceptible	Control	
Bean	8	0.59	0.95	37
Clover	10	0.49	0.81	39
Cucumber	7	0.55	0.90	39
Hemp	29	0.24	0.65	63
Hop	45	0.24	0.75	68
Mustard	9	0.52	0.88	40
Nettle	30	0.36	0.72	50
Pea	19	0.28	0.60	53
Vetch	22	0.29	0.62	53

LONGEVITY

Forty glass vials (3.5 × 1.2 mm.) with screw caps were used as storage containers, and were equally divided into two sets of 20 each. One set was half-filled with soil, and the other set was left empty. They were then divided and sub-divided again according to the following diagram:



The final arrangement became:

a. Five vials each with 40 cysts in dry soil

b. Five vials each with 40 larvae in dry soil

c. Five vials each with 40 cysts in wet soil

d. Five vials each with 40 larvae in wet soil

e. Five vials each with 40 cysts in dry (without soil or water) condition

f. Five vials each with 40 larvae in dry (without soil or water) condition

g. Five vials each with 40 cysts in wet (1/2 filled with water) without soil

h. Five vials each with 40 larvae in wet (1/2 filled with water) without soil

One dry and one wet vial in each series were tied together and placed in different temperature chambers ranging from -10°to 5°, 15°, 25°and 30°C. All vials were stored at their respective temperatures for five months. After one month, a few cysts and free-living larvae were taken from each vial and inoculated to a can of sterilized soil containing 10 hop seedlings. When the hop seedlings were 3-4 inches in height, a few (2-3) were removed and stained to determine if the larvae had survived, hatched (in case of cysts) and entered the roots. The remaining plants were left in the can for additional five months. After which they were removed and searched for cysts. The temperature, time and storage media in which the nematodes survived, developed and reproduced were noted. The process of inoculation and leaving the remaining plants for additional five months, was repeated every month for five months to determine the length of time nematodes could survive in different moisture, temperature and media (dry and wet soil or submerged conditions).

Results in Table 4 indicate that encysted larvae and free-living larvae stored in wet soil survived longer than those stored in dry soil. Encysted larvae stored in dry soil died after a month's storage at 5°C, and two month's storage at 15° and 25°C. Free-living larvae did not survive a month's exposure at any of the above mentioned temperatures. In wet soil, encysted larvae survived two months at 5°C, five months at 15°C (died on sixth month), and three months at

25°C. Free-living larvae survived one month at 5°C and three months at 15°C, although a few were found dead after the second month, and two months at 25°C. Neither encysted larvae or free-living larvae survived a month's exposures at 30° and -10°C.

Data in Table 5 indicate that encysted larvae stored in a dry condition survived longer than free-living larvae. Encysted larvae in dry condition survived a storage period of one month at 5° and 25°C, and two months at 15°C (however, some of them had died before the first month). Free-living larvae did not survive a month's exposure at any of these above temperatures. At 30° and -10°C neither encysted larvae nor free-living larvae survived these storage conditions even for one month. This was probably due to the extreme temperatures. High temperature probably killed the encysted larvae by inactivating the enzymes or by protein coagulation. At very low temperatures (-10°C) death occurred by freezing and formation of intracellular ice-crystals.

Both encysted larvae and free-living larvae survived longer in wet storage conditions. Encysted larvae survived a storage period of two months at 5° and 25°C, and three months at 15°C; and free-living larvae only survived one month at 5° and 25°C, and a two months exposure at 15°C. At the two extreme temperatures (30° and -10°C) both encysted larvae and free-living larvae died.

It is evident from these data that H. humuli stored in wet soil

Table 4. Effect of temperature and storage conditions upon survival of H. humuli encysted larvae and free-living larvae in dry and wet soil.

		Period of storage in months									
		1		2		3		4		5	
Temperature	Condition	EL	FL	EL	FL	EL	FL	EL	FL	EL	FL
5°	Dry'	S	D	D							
	Wet"	S	S/D	S/D	D	D					
15°C	Dry'	S	D	S/D		D					
	Wet"	S	S	S	S	S	S/D	S	D	S/D	
25°C	Dry'	S	D	S/D		D					
	Wet"	S	S	S	S/D	S/D	D	D			
30°C	Dry'	D	D								
	Wet"	D	D								
-10°C	Dry'	D	D								
	Wet"	D	D								

Dry' -- vials with dry soil. Wet" -- vials with wet soil

Table 5. Effect of temperature and storage conditions upon survival of H. humuli encysted larvae and free-living larvae in dry or submerged condition without soil.

		Period of storage in months									
		1		2		3		4		5	
Temperature	Condition	EL	FL	EL	FL	EL	FL	EL	FL	EL	FL
5°C	Dry"	S	D	D							
	Wet'	S	S/D	S/D	D	D					
15°C	Dry"	S	D	S/D		D					
	Wet'	S	S	S	S/D	S/D	D	D			
25°C	Dry"	S	D	D							
	Wet'	S	S/D	S/D	D	D					
30°C	Dry"	D	D								
	Wet'	D	D								
-10°C	Dry"	D	D								
	Wet'	D	D								

Dry" -- vials kept dry and empty (no soil). Wet' -- vials half-filled with water (no soil)

S - survived; D - dead; S/D - few dead, few alive; EL - encysted larvae; FL - free-living larvae.

withstood a longer storage period than when stored in any other conditions. This is very clearly shown especially at 15°C, when both encysted larvae and free-living larvae survived a longer storage period in wet soil than in submerged condition. Few nematodes can survive in dry condition, but only for a short time. Death of nematodes in dry condition is probably due to lack of moisture, which resulted in dehydration. Death of nematodes in submerged conditions was probably caused by water-logging (lack of oxygen--anaerobic condition), melting of a body constituent or starvation. Thus nematodes in a submerged condition survived a shorter period than when stored in wet soil. The decline in viability of encysted larvae subjected to some of the treatments, indicates that combinations of temperature and moisture for a relatively long period of time is important in survival. Total absence or reduction in availability of oxygen and moisture is probably one of the few biological phenomenon which would cause a rapid death.

EFFECT OF SYNTHETIC AGENTS AND pH ON HATCHING

Synthetic Agents (Chemicals). Twenty-three chemicals, including acids, alcohols, amino acids, esters and lactones, heterocycles, phenols, miscellaneous organic and inorganic compounds were tested to investigate hatching stimuli. All compounds were tested at a 3 mM concentration in a distilled water solution; also a series of concentrations were tested for some compounds. The concentration in which maximum hatch occurred was recorded (Table 6).

Chemicals were weighed or measured and added to distilled water to obtain the desired test concentrations. Approximately 30 cysts were transferred to each petri-dish containing 50 cc of the test solution. Three replications were made and stored at room temperature (75°F), and the hatched larvae were counted every week for three weeks. The results are summarized and given in Table 6.

Results in Table 6 indicate that different compounds vary in effect upon hatching of H. humuli larvae from cysts. It is suggested that many hatching agents act as electron acceptors (oxidising agents, hydrogen acceptors). Not all oxidizing agents, however are hatching agents, because of the molecular specificity required by the organism.

Highest hatch was obtained by urea (1840) and lowest by n-butyl alcohol (25). Ascorbic acid, citric acid, pyruvic acid and

Table 6. Efficiency of synthetic agents for hatching H. humuli larvae from cysts.

Chemicals		Larval emergence in weeks 1	2	3	Total
Acid:	Citric acid	510	600	330	1443
	Pyruvic acid	450	540	300	1290
	Ethylenediaminetetraacetic acid(EDTA)	90	120	101	311
Alcohols:	n-Butyl alcohol	8	12	5	25
	Methanol	86	99	55	240
Phenols:	Picric acid	150	181	62	393
	Phenol	61	120	67	248
Amino acids:	Methionine	180	306	108	594
	Histidine monohydrochloride	61	81	52	194
	L-Cysteine hydrochloride hydrate	69	97	52	218
	Proline	61	77	51	189
	Glycine	86	206	101	393
Heterocycles:	Riboflavin	302	420	265	987
Esters and Lactones:	Ascorbic acid	510	600	328	1438
	Amyl acetate	128	184	99	411
	Anhydrotetronic acid	150	240	90	480
Misc. organic compounds:	Thiourea	480	595	315	1390
	Urea	594	831	415	1840
Inorganics:	Potassium permanganate	112	125	89	326
	Ammonium nitrate	48	61	35	144
	Ammonium sulphate	47	55	31	133
	Potassium iodate	67	88	52	207
	Sodium nitrate	30	45	10	85
CONTROL:	Water	21	27	15	63

thiourea have efficient hatching effects. These chemicals resulted in an emergence of 1438, 1443, 1290 and 1390 larvae respectively. Among the amino acids, methionine liberated 594 larvae, which was the highest number, and proline 189 larvae, the lowest number. As a group, inorganic compounds were poor hatching stimulators however, potassium permanganate produced a response which hatched 326 larvae; sodium nitrate, on the other hand, liberated only 85 larvae.

pH Effects. Distilled water was adjusted to the desired pH level with either 0.1N hydrochloric acid (for acidic range) or 0.1N sodium hydroxide (for basic range). The pH range was varied from 3 to 10. About 15 ml of each pH solution was poured in 13 different petri dishes containing 20 cysts. Four sets were arranged, with each set containing three replications (13 × 3 petri dishes), total number of petri dishes thus involved were 156 (39 × 4). Each set of petri dishes was incubated at different temperatures, 15°, 20°, 25° and 30°C for a period of 12 days. From the sixth day onwards, the emerging larvae was counted daily. The combined effect of pH and temperature upon emergence of larvae were recorded in Table 7. It is evident from the data in Table 7 that a pH range of 5 to 7.5 is suitable for H. humuli emergence. Emergence of larvae started at the pH 5 range in all temperatures, reaching a maximum at pH 6.5 then declining sharply with none at pH 8 (except only at 25°C).

Table 7. Effect of pH and temperature upon emergence of H. humuli larvae.

pH range	Temperature in °C			
	15°	20°	25°	30°
	(emergence per cyst: average of three replications)			
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
4.5	0	0	0	0
5	2	4	5	1
5.5	10	17	25	3
6	18	25	38	6
6.5	20	35	51	8
7	9	14	16	5
7.5	4	6	7	2
8	0	0	2	0
9	0	0	0	0
10	0	0	0	0

Incubation at 25°C seemed to be optimum for hatching. At this temperature level (25°C) maximum emergence was obtained at pH 6.5. At 30°C the rate of emergence declined considerably indicating that this temperature is not suited for larval emergence at any pH range. Routine checking of the pH for irregularities revealed that the pH increased. This increase was approximately 0.1 unit but never exceeded 0.2 unit. The difference was immediately corrected and the solutions were returned to the original pH level.

HOST DAMAGE LEVEL

Sterilized soil (about 25 lbs.) was inoculated with enough cysts to obtain a density of 400 eggs per gram of soil (a cyst contains approximately 200 eggs). Portions of inoculated soil were mixed with non-inoculated soil to obtain densities of 200, 100, 50, 25, 12, 6 and 3 eggs per gram of soil. Five #2 1/2 (401 × 411) cans were filled with each of these soil mixtures and five additional cans of non-infested soil were used as controls. Ten hop seedlings were transplanted in each can. The cans were then placed in randomized fashion in the greenhouse for three months. After this period, the soil was washed from the plants, and tops and roots from each can were separately stored for drying and weighing. The amounts of dry matter harvested from each inoculum density are given in Table 8.

Despite variations in dry weights among replicates, a noticeable reduction in growth did not occur at lower densities up to 50 eggs per gram of soil. The average weights, however, remained almost in a straight line up to the initial density of 50 eggs per gram of soil. Beyond this range, there was a sharp and continuous decrease in the dry weight of hops as nematode density increased (Fig. 6). The average dry matter harvested in control was 2.89 grams for shoots and 2.0 grams for roots. In contrast, average dry matter harvested

Table 8. The relation between growth of hop seedlings and population density of H. humuli.

Rep.		Eggs per gram of soil								
		Control 0	3	6	12	25	50	100	200	400
I	Shoot	3.25	2.70	2.28	3.19	1.30	1.20	1.13	0.49	0.40
	Root	2.27	1.90	1.58	1.53	1.58	1.10	0.57	0.30	0.21
II	Shoot	3.19	2.91	2.69	2.31	2.49	1.02	1.12	0.49	0.33
	Root	2.15	1.75	1.43	1.40	1.69	1.00	0.40	0.33	0.19
III	Shoot	3.11	3.04	2.20	2.18	2.15	2.28	0.95	0.45	0.25
	Root	1.99	2.10	1.43	2.00	1.49	1.32	0.37	0.24	0.11
IV	Shoot	2.70	2.32	2.20	1.89	2.12	1.70	1.20	0.40	0.29
	Root	1.97	1.64	1.80	1.78	1.40	1.25	0.57	0.35	0.15
V	Shoot	2.33	2.66	1.87	1.50	1.87	1.52	1.13	0.68	0.19
	Root	1.69	1.41	1.70	1.60	1.31	1.25	0.40	0.40	0.11
Av.	Shoot	2.89	2.72	2.24	2.21	1.98	1.56	1.10	0.50	0.29
	Root	2.01	1.76	1.58	1.66	1.45	1.18	0.44	0.30	0.15
Percent reduction			5.88	22.49	23.52	31.48	46.02	61.94	82.70	89.96
			12.43	21.39	17.41	27.86	41.29	78.10	85.07	92.53

Analysis of variance

Source of Variation	d.f.	S.S.	M.S.	F
Subtotals		60.6308		
Treatment	8	52.3200	6.5400	31.4574*
Block	1	6.6477	6.6477	31.9755*
Exp. error	8	1.6631	0.2079	
Sampling error	72	5.6934	0.079075	
Total	89			

* Significant at 1% level.

In comparing the means of shoots and roots, the following L.S.D. (at 5% and 1% level) is used:

L.S.D. at 5% is 0.4597
at 1% is 0.3470

Fig. 6. Effect of different population densities of H. humuli on hop seedlings (variety Late Cluster) after a five month growing period.

from the inoculum level of 400 eggs per gram of soil was 0.29 gram for shoots and 0.15 gram for roots.

It is concluded here, that the tolerance limit of hop seedlings to H. humuli is between 50 and 100 eggs per gram of soil. Hop seedlings could withstand injury at the inoculum densities of 3, 6, 12, 25 and 50 eggs per gram of soil, but not at 100 eggs per gram of soil.

As the larva enters the root and affects plant growth by disrupting vascular bundles, the plant produces new roots to compensate for the damage. In low inoculum levels, a plant can maintain average growth, but in high inoculum levels a plant can not develop normally. When the nematode population level is high, almost all roots are invaded. At the same time additional larvae still remain in the soil. Therefore, as soon as the plant produces a new root, it is almost immediately invaded. When this invasion continues for some time, plant resources become exhausted and fail to produce new roots. The plant then can not maintain a favorable growth balance which leads to reduction in growth rate.

Several Oregon hop yards were examined for this nematode, and for an evaluation of damage. It was observed that the density of eggs in the field during different seasons remained between 10 to 35 eggs per gram of soil. The hop yards apparently maintain a continuous low nematode population. The hop crowns are left in the

yards after harvesting the tops, hence the crowns become dormant and do not produce secondary replacement roots as the rhizomes do not need to support top growth. Therefore, even if a nematode population is always present in the soil, hops apparently are not substantially damaged by this nematode unless the population density exceeds 50 eggs per gram of soil as indicated by the greenhouse experiment.

POPULATION TRENDS

Hop seedlings were grown in sterilized soil prior to inoculation. Cysts were obtained by the screening method as previously described. Three series of nine cans were inoculated with a geometrically increasing cyst number, ranging from 1 to 256 cysts per can. Inoculation was achieved by placing cysts directly in the can for the lower inoculum levels. For higher inoculum levels, the cysts were mixed with the soil before filling the cans. One healthy hop seedling was transplanted into each can. The hops were grown in the greenhouse for approximately six months before the cysts on the roots were counted and the soil screened for cysts. Differences between final and initial cyst count were interpreted as an increase in cyst production.

Results (Table 9) indicate that the number of cysts increased at every inoculum level, although the rate of increase varied with each level. At the highest inoculum level (256 cysts), amount of increase was much less than at the lowest inoculum level (1 cyst). At inoculum levels of 4, 8 and 16, the multiplication rate was high, with the highest rate occurring at 16 which increased 13.2 times. This rate effect is attributed to a negative density dependent factor and also competition. The negative density dependent factor may arise from the competition for space and food, from debilitation of the host

roots or from a population shift in favor of males.

Table 9. Effect of different population levels of H. humuli on cyst production over a six month period.

Initial population (P_i)	Final population (P_f)	Difference ($P_f - P_i$)	Ratio of increase
1	4	3	4.00
2	12	10	6.00
4	34	30	8.50
8	93	85	11.62
16	211	195	13.20
32	286	254	8.93
64	189	125	2.95
128	253	125	1.97
256	377	211	1.47

DISAPPEARANCE OF EMPTY EGGS

Ten cysts of H. humuli were added to each of 20 plastic bags containing 500 grams of non-infested soil. One bag of soil was washed and screened every two weeks to recover the cysts. All 10 cysts were recovered, and opened to count the contents (full and empty eggs). This process was repeated for a period of eight months.

Data in Table 10 indicate that empty eggs disappeared slowly during the storage period. Initially cysts were found to contain an average of 133 eggs, which included 45 full eggs and 88 empty eggs. Approximately 34 empty eggs per cyst disappeared during the eight month storage period. The number of full eggs per cyst declined from 45 to 34, indicating that rate of spontaneous hatching is not very high for H. humuli.

Initial egg numbers (empty eggs) appear to be directly correlated with the number of larvae that hatched over a period of time. Also this correlation gives an indication of the number of larvae that had taken part in plant invasion (empty eggs), and the number of additional larvae that could take part if there is a potential host (full eggs).

An analysis of these data reveal that there was a decrease in both empty and full eggs (Table 10). Over a period of eight months 38.63% of the empty eggs disappeared, and full eggs decreased

Table 10. Disappearance of empty H. humuli eggs in relation to time.

Observation (at 2 week interval)	No. of cysts/bag	Eggs/Cyst			Ratio of	Ratio of
		Total	Empty	Full	empty/total	full/total
1	10	133	88	45	0.66	0.34
2	10	131	86	45	0.66	0.34
3	10	129	85	44	0.66	0.34
4	10	127	83	44	0.65	0.35
5	10	125	82	43	0.65	0.35
6	10	123	80	43	0.65	0.35
7	10	118	76	42	0.64	0.36
8	10	115	74	41	0.64	0.36
9	10	112	72	40	0.64	0.36
10	10	111	71	40	0.64	0.36
11	10	108	69	39	0.64	0.36
12	10	105	67	38	0.64	0.36
13	10	103	66	37	0.64	0.36
14	10	101	64	37	0.63	0.36
15	10	98	62	36	0.63	0.37
16	10	94	59	35	0.63	0.37
17	10	93	58	35	0.62	0.37
18	10	91	57	34	0.62	0.37
19	10	89	55	34	0.62	0.38
20	10	88	54	34	0.61	0.39

24.44%. Rate of disappearance of empty eggs per week was 1.86. The decrease of full eggs occurred because of spontaneous hatching and there was no increase in hatching because host plants were not available. The disappearance of empty eggs from the cysts made the ratio of full eggs over total eggs increase from 0.34 to 0.39 at the end of the experiment.

It could be predicted on this basis that if a continual decrease (38.63% in eight months) of empty eggs is maintained, all empty eggs will disappear from the cyst in 21 months. In the same way full eggs decreases 24% (spontaneous hatching) in eight months, therefore in approximately 33 additional months all full eggs will hatch. Considering all of these possibilities, based on the data available, it could be predicted that if the land is kept fallow for a period of 41 months (33 + 8) or approximately 3 1/2 years, all larvae will hatch and emerge from the cysts.

SUMMARY

Taxonomical descriptions of males and second-stage larvae have been given and compared with the most closely related species, H. cruciferae.

Second-stage larvae of H. humuli were reared on hop seedlings at 60°, 65°, 70°, 75°, 80°, 85° and 90°F temperatures to determine the length of time required to complete a life-cycle. Time taken to complete a life-cycle varied according to the soil temperature. The cooler the temperature, the longer the time required to complete a life-cycle and vice versa. At higher temperatures (85° and 90°F) larvae did not survive. The females formed an ovoid to spheroid shape at maturity, whereas males regained the vermiform appearance. During sexual maturation the genital primordium took a 90° turn during the third-stage to form two ovaries (in females), in contrast, the primordium developed parallel to the body axis to form a testis (in males). H. humuli completed only two generations in a year.

Thirty different kinds of plants were tested to determine their susceptibility to H. humuli. It was determined that the host range of this nematode is predominently the Urticaceae family. H. humuli will also infect some plants in Cruciferae (mustard), Cucurbitaceae (cucumber), Leguminosae (bean, clover, pea) and Moraceae (hemp)

families.

Tests to determine the effects of temperature, moisture and soil upon the survival of nematode indicated that H. humuli stored in wet soil (at 15°C) withstood longer storage period than when stored in any other condition. The nematodes stored in any type of wet condition (with or without soil) survived longer than the dry conditions.

Twenty-three chemicals, consisting of acids, alcohols, amino acids, esters and lactones, heterocycles, phenols and miscellaneous organic and inorganic compounds were tested to investigate hatching stimuli. Greatest hatch was obtained by urea (1840 larvae) and least by n- butyl alcohol (25 larvae). Ascorbic acid, citric acid, pyruvic acid and thiourea had an efficient hatching effect producing 1438, 1443, 1290 and 1390 larvae respectively. Inorganic compounds were poor hatching stimulators. Best hatching effect (326 larvae) was produced by potassium permanganate and least by sodium nitrate (85 larvae).

The effects of pH on the hatching of encysted eggs were determined by placing the cysts in different pH solutions. It was observed that a pH range of 5 to 7.5 is suitable for H. humuli emergence, with the best effect at 25°C.

Tests to determine the relation between growth of hop seedlings and the population density of H. humuli indicated that the tolerance limit of hop seedlings to H. humuli is between 50 and 100 eggs per

gram of soil. The hop seedlings could withstand the damage at the inoculum densities of 3, 6, 12, 25 and 50 eggs per gram of soil. Average weight of control plant roots (no eggs) was 2.01 grams as compared to 0.15 gram for plants at 400 eggs per gram of soil. Reduction percentage in root weight at 400 eggs per gram level was 92.53 as compared to 12.43 at three eggs per gram level.

The effect of different population levels of H. humuli on cyst production indicated that the number of cysts increased at every inoculum level but the rate of increase varied. Ratio of increase at highest inoculum level (256 cysts) was 1.47, whereas at lowest inoculum level (1 cyst) was 4. Highest ratio of increase (13.2) was obtained at the inoculum level of 16.

Empty eggs disappeared slowly during the storage period. Approximately 34 empty eggs per cyst disappeared during the eight month storage period. The decrease of only 11 mature eggs during the experiment indicated that the rate of spontaneous hatching of H. humuli is low.

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