Heterodera schachtii, Hatching Properties of Field Importance[1]

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Biological studies with the sugar beet nematode would be greatly facilitated by a continuing large supply of cysts of known origin and predictable character. For the development of large scale rearing of *Heterodera schachtii* it was of importance to have some knowledge of what events took place during the increase in population. In the course of experiments to elucidate the behavior of the plant parasites, knowledge was obtained which was useful in the explanation of some field observations and in the design of applied experiments.

Materials and Methods

For the study of population increase, sugar beets growing in sand in 6-inch pots were infected by adding 100 larvae per pot. The plants were divided into 4 lots of 10 plants and one lot was harvested every 6 weeks; cysts and white females were separated from roots and sand, screened, and collected for counting. Full and empty cysts were separated by picking before counting.

For studies with white females, young sugar beet plants growing vigorously in sand were infested with freshly hatched second stage larvae of *Heterodera schachtii*. After 36 days the plants were harvested and the roots and sand in which they were grown were thoroughly washed to collect the white females and cysts. White females without egg masses were selected from this source. Ivory or darker-colored females as well as cysts were discarded. The white females as well as other batches of cysts were tested for hatch response to a lyophilized material containing hatching factor. The cysts were used to confirm the activity of the hatch solution. The cysts and females were suspended at the surface of the solution by stainless steel screens in embryo dishes containing 2.5 ml. active solution. Each series containing six replicates of 75 white females or cysts was incubated in a humidity box at 25° C.[3] After collection of the larvae, the active solution was replaced.

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[3] For practical considerations more of the brown cysts were used but the curves in Figure 2 have been corrected to 75. The white females used for hatch tests became ivory colored by the end of the experiment.

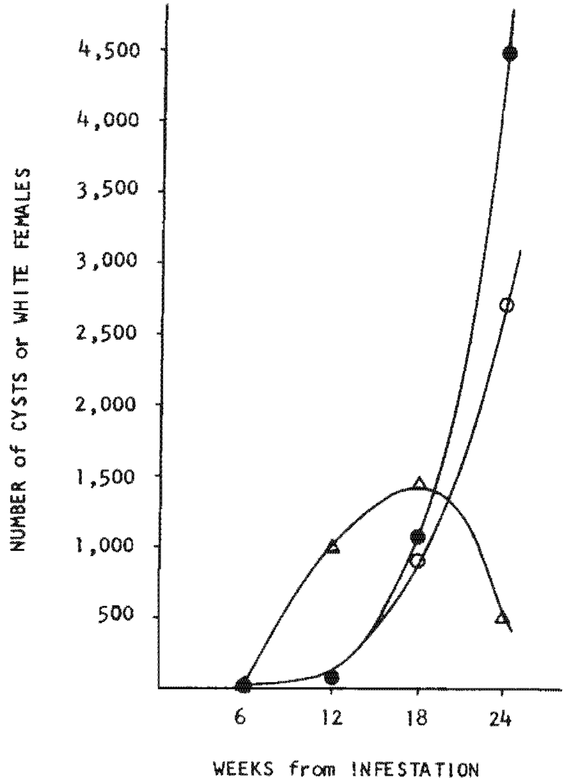

Figure 1.—Population increase of *Heterodera schachtii* Schm. from an initial infestation of 100 larvae. The solid circle represents the average of the total number of cysts; the open circle represents the average number of fuller cysts; the triangle represents the average number of white females.

Results and Discussion

Population increase

The curve described by cyst numbers is exponential as one would expect (Figure 1). With less than a hundred larvae which infected each plant and good growing conditions, a high population (white females plus cysts) developed in about the time period of two generations. This then explains how, with low populations in areas where the growing season permits less than two generations, it may be possible to employ shorter rotation periods safely, provided the nematode attrition rate is high enough. Effective control by rotation is dependent upon initial infestation as well as the rates of increase and decrease of the population. (For example, a similar experiment with about twice the initial infestation was found to give an additional fifty-fold increase in cysts in 24 weeks.)

In 24 weeks there are an appreciable number of cysts which are not full; viz., the difference between the two curves -dots, circles. Since cyst walls do not decompose readily, there is an inherent danger in the use of cyst counts as a population density indicator. In a heavily infested field on the order of 90% of the cysts can be empty (13); in addition there is the confounding problem of full cysts with non-viable contents resulting, for example, from fumigation, drying, etc. A simple unqualified cyst count is likely to be meaningless as an estimate of population potential.

The remaining curve clearly indicates the danger of incautious use of a white female count as a population indicator. It can be seen, for example, that a count of 500 can either comprise over 95 percent of the potential (inclining portion of curve) or less than 16 percent (declining portion of curve).[3] The steepness of the gradient also shows that the growth period is an important factor. The evidence here presented serves as a reminder of the wide variability of which the animal is capable.

With respect to field problems, it can be said with certainty that, if sugar beet nematode cyst counts can be made from field samples, then at some time *Heterodera schachtii* was present and may still be present; if white female counts can be made, *H. schachtii* is present. Any estimates of population potential based on such counts must be interpreted with extreme caution since they can be misleading. A proper population estimate (neglecting the problems in effective sampling for the purposes of this discussion) would require the determination of the viable contents of cysts and females. The criteria for a laboratory determination of viability are as yet poorly defined but a reasonable evaluation can be made by an emergence assay followed by cyst rupture and estimate of the viable remainder after the method of Feldmesser and Feder (4). The caution noted previously of the influence of environmental history (which, for practical considerations includes the environment from the time the sample leaves the field and the assay is made) upon results of emergence assays and "egg" determinations must be stressed. The use of this approach in Europe, however, testifies to its practicality (6).

Hatch response of white females

It had been observed that fresh cysts obtained from a growing bed gave very high water hatches when nearly all such cysts could

[3] Potential can be considered a summation of full cysts and females since viability is assured.

be at most 2 to 3 generations old (initial infestation from larvae). In view of the population increase curve, it was of interest to investigate the hatch response of white females. The literature on this point appears to contain conflicting views. According to Goodey (8), Chatin (1) concluded that "if the eggs were destined to be set free immediately the wall broke down and liberated them. If, on the other hand, they were to remain within the body, the wall of the latter became changed to a brown cyst . . ."

Fuchs (7) concluded that throughout the summer months the majority of females remained white and after dropping off from the roots of the host, liberated larvae at once which then infected fresh roots and only towards autumn did brown cysts form in quantity and retain their eggs. Triffitt (12) reported that larvae are not set free from cysts until the latter have turned brown. The first larvae issued about one month after the removal of the parent worms from the roots. A similar conclusion had been suggested earlier by Sengbusch (10) according to Filipjev and Schuurmans Stekhoven (5).

Presumably, the report of Thorne (11), that the eggs deposited in the gelatinous matrix at the posterior end of the female hatch readily, was taken into account. More recently, Franklin (6) and Christie (2) have suggested that though many larvae in eggs can remain dormant within the cysts for many years, some are capable of hatching as soon as they are fully developed. This remark introduces a further complication as to the meaning of "fully developed," since the term was neither qualified nor explained.

It is obvious that there is considerable hatch of larvae from the white females used in this experiment (Figure 2). It should also be apparent that there is a significant increase in hatch due to an active component in the hatch factor material. Though the percent increase in emergence is the same or less than that of the cyst controls, the increase in number of larvae emerging is greater than the total hatch of the best cyst trial.

The use of trap cropping as a control procedure has been considered risky. The hatching obtained with white females serves to confirm the hazards of the practice. In the use of any but immune crops the period of plant growth may be too short to make the trap crop useful.

Of equal importance for assay purposes is that in the interval examined, emergence is a linear function of time. Practically, it is far simpler to relate linear functions than complex functions. The linear curve indicates that emergence is independent of potential, i.e., not a function of hatchable larvae; either there is an excess of hatchable larvae initially or they mature to this state during the experiment at a rate greater than emergence so

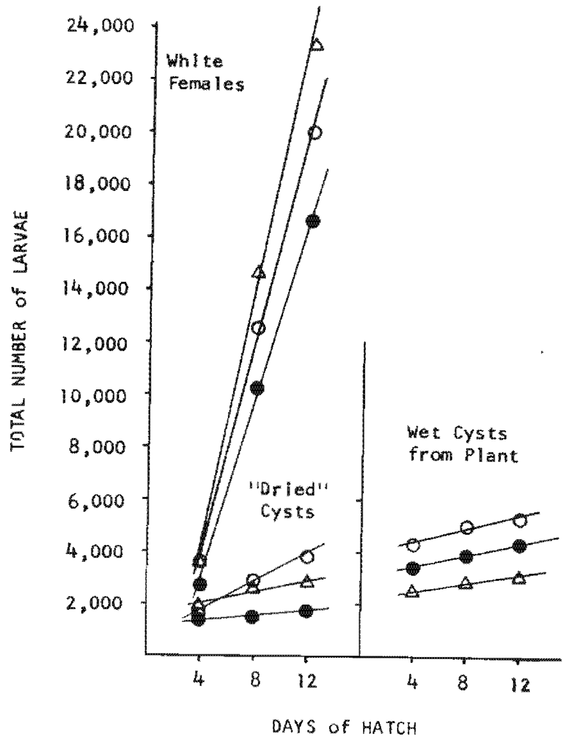

Figure 2.—The hatch response of 75 *Heterodera schachtii* Schm. white females and cysts from different sources. The solid circle represents the average water hatch; the open circle represents the average response to lyophilized sugar beet leachings at 1.0 mg/ml; the triangle represents the average response to leached material at 0.04 mg/ml.

that the potential pool of hatchable larvae in white females is not limiting. In the case of cysts the larvae presumably have had ample time for maturation so that the hatchable larvae potential could not be limiting. The hatching from white females is reflected as a maximum with increasing concentration of active component. The response reflected by the wet cysts from plants is a minimum, whereas the response of the "dried" cysts is still different.

It appears difficult to reconcile these observations with the report of Jones (9) that "A period of maturation appears to be necessary before eggs are fully sensitive to hatching stimuli," and Duggan (3) that a period of four weeks for maturation was necessary for cysts of *H. schachtii* before they were capable of hatching. The conclusions of Jones or Duggan are based on indirect evidence perhaps interpretable in other ways. The hatch tests reported here were conducted under artificial conditions but it seems unlikely that an egg able to hatch from a white female detached from a root would be unable to hatch from a female attached to a root. More likely the sequence of environmental conditions during the development of the animals preceding emergence effect the observed responses. The apparent disagreement is an indication of our poor understanding of the factors governing hatching.

In this report the hatch-active factor in all cases is of the same batch and at the same concentration and the cysts and females are of common ancestry; therefore, one must conclude that the environmental history of the animals previous to testing is in part responsible for the differences in emergence. This being the case, then caution need be exercised in the comparison of hatch data from cysts of different origins or in the interpretation of occasional hatch tests with an unstandardized batch of cysts.

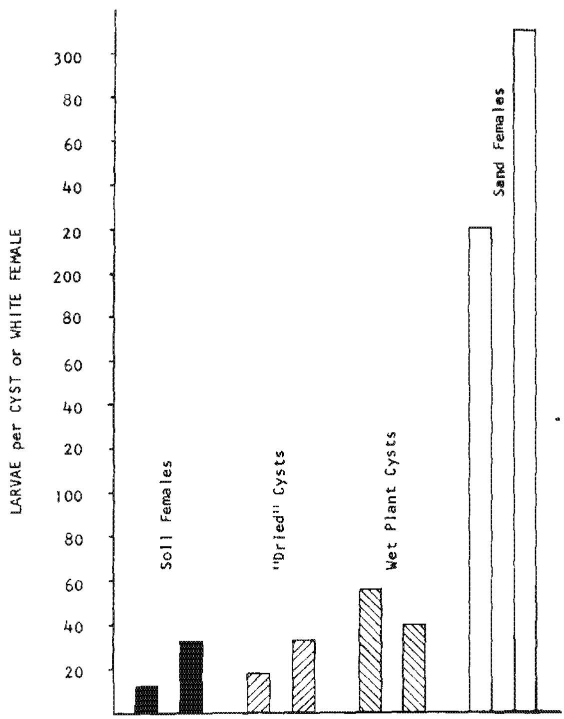

Figure 3.—The total larval hatch response per white female from two sources and per cyst from two sources after 12 days. Left bar, the response with water; right bar, the response to lyophilized leaching at 0.04 mg/ml.

In fact, there is no definite assurance that the reaction resulting in an increased hatch response is the same in white females as in wet cysts from a plant, i.e., the agent that is limiting may not be the same. In such a case the use of animals whose environmental history is uncertain could lead only to disaster. Figure 3 illustrates these points. The total number of larvae emerging in 12 days with two concentrations of active factor, namely 0.0 and 0.04 mg/ml. The hatch from another batch of white females of undetermined age obtained from soil is also shown. The results are quite different which, in view of the previous discussion, could be expected.

Summary

The population increase of *Heterodera schachtii* beginning with the addition of 100 infective second stage larvae to each pot of 4 lots of growing sugar beets is followed by harvesting a lot at 6-week intervals over a 24 week period. Sand and roots were washed, screened, and counts were made of total cysts, full cysts and white females. Cyst numbers increase exponentially and empty cysts begin to appear about the 15th week. The numbers of white females increase to a maximum then decrease during the 24 week interval.

White females less than 36 days old and with no visible egg masses were found to hatch profusely in distilled water. Immersion of the white females in lyophilized sugar beet leachings was observed to stimulate increased hatching over water controls.

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