Life Cycle, Host Range, and Reproduction of *Heterodera betulae*

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Abstract: *Heterodera betulae*, a cyst-forming nematode, originally recovered from roots of river birch in Arkansas, appears to have a limited host range. Of 80 plant species in 20 families tested, only five species of *Betula* and *Cleome spinosa* supported reproduction. The minimum time for a complete life cycle was 52 days at 28 C. No reproduction occurred at 31 C or above, and development was very slow below 20 C. Successful population propagations from single larvae demonstrated that males were not necessary for reproduction.

The birch cyst nematode, *Heterodera betulae* Hirschmann and Riggs, from the bank of Middle Fork of White River, east of Fayetteville, Arkansas (6), has been described (2) as a parasite of river birch, *Betula nigra* L. This is a report of studies of certain aspects of the biology of this nematode.

MATERIALS AND METHODS

H. betulae was maintained in the greenhouse on river birch seedlings grown in infested soil either in 15-cm clay pots or in a bed 30 cm × 1.2 m × 20 cm deep. Birch seedlings were usually grown from seeds germinated in sterilized sand in the greenhouse. When the seedlings were 2.5–5.0 cm tall, they were transplanted to either 250 ml Beacups® (polypropylene plastic) or 7.5-cm clay pots filled with sterilized fine sand from the Arkansas River Valley.

Nematode inoculum was prepared using the method described for the soybean-cyst nematode (4). Briefly, cyst-infested soil was suspended in water and poured through a 60-mesh sieve. The residue on the sieve was blended 3–5 min in a Waring blender. The resulting mixture was poured through a 60-mesh sieve and the liquid portion containing eggs and larvae was retained. The residue on the sieve was again washed into the blender and the process repeated until most cysts and mature females were broken. The liquid portions were combined to make up the inoculum.

Studies of the life cycle of the nematode were conducted in constant temperature tanks at 14, 16, 20, 22, 24, 28, 31, 33, 34 and 35 C. River birch seedlings were transplanted into sand in Beacups. Shortly after the seedlings had become established, they were inoculated each with a suspension of 500–1000 eggs and larvae. To insure equal opportunity for the nematodes to penetrate and initiate infection, inoculated seedlings were held on a greenhouse bench for 2 days before being exposed to the temperature treatments. To determine the degree of nematode development, three seedlings were examined from each tank at 2–3 day intervals from the 10th to the 30th day after inoculation, and at 7–8 day intervals thereafter. The sand of each pot was screened for females and larvae. The roots were stained using the bromphenol blue method of Kirkpatrick and Mai (3) for observation of stages of nematode development in tissues.

Host studies were conducted in a greenhouse at 23–30 C except during short periods on summer days when the temperature reached 38 C. Test plants were seeded in

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sand in 7.5-cm clay pots, and when the seedlings were approximately 2 weeks old, three pots of each plant species to be tested were inoculated each with a suspension of 500–1000 eggs and larvae. River birch was included as known host to check viability of the inoculum. About 8 weeks later, the sand from each pot was screened to recover any mature females which developed.

Mode of reproduction was determined by single larva inoculations. River birch seedlings were transplanted into sand in 7.5-cm clay pots without holes. When the seedling roots had penetrated throughout the sand mass, a single larva was placed in each pot. Twelve weeks later the sand was screened to recover mature females or cysts. When females were found they were placed in B.P.I. watch glasses and broken to determine egg production and larval development. The contents of each female was introduced into the sand around the roots of another birch seedling and 10 weeks later the sand was screened to recover mature females and cysts.

RESULTS

LIFE CYCLE STUDIES: Development of *H. betulae* occurred over a wide temperature range (Table 1). Maturation was recorded from 14 to 31 C. Rate of development was slower at the lower temperatures. The shortest period recorded from second-stage larvae to second-stage larvae was 52 days at a temperature of 28 C. At 24 C a complete cycle required 60 days. A few males were recovered after 28 days at 22 and 28 C.

MODE OF REPRODUCTION: Of 50 seedlings inoculated with a single larva each, a single white female or cyst was recovered from each of 13 seedlings after 12 weeks These females and cysts were of normal size and contained eggs in various stages of development and viable larvae. When the contents of each female were reinoculated individually to new birch seedlings, infection was established and 10 weeks later several females were recovered from each of the inoculated plants.

TABLE 1. Effect of temperature on rate of development of *Heterodera betulae* on river birch.

Temp. C	Time from inoculation to occurrence of: Mature Females	Second-stage larvae
14	82 days	None found
16	82 days[1]	137 days
20	82 days[1]	82 days
22	26 days[2]	60 days
24	26 days	60 days
28	19 days[2]	52 days
31	33 days	None found
33	None found	None found
34	None found	None found
35	None found	None found

[1] No check was made between 60 and 82 days.
[2] A few males were recovered after 28 days.

HOST STUDIES: Host studies were undertaken with two different plant groups: (i) *Betula* spp. and closely related genera; (ii) species of other plants occurring in the type locality or recorded as hosts of other *Heterodera* species. In addition to *Betula nigra,* four other species, *B. lenta* L., *B. pendula* Roth, *B. populifolia* Marsh., and an unidentified species of *Betula* were good hosts for the nematode and supported good reproduction. A member of a related genus, *Alnus glutinosa* Gaertn., supported meager reproduction. The nematode did not reproduce on hazelnut, *Corylus americana* Marsh.

The only plant outside the family Betulaceae, on which appreciable reproduction occurred, was *Cleome spinosa* L., in the family Capparidaceae. Two females were recovered from orchard grass, *Dactylis glomerata* L., Gramineae; three from sweet alyssum, *Lobularia maritima* Desv., Cruciferae; and one from 'butternut' squash, *Cucurbita moschata* Duchesne, Cucurbitaceae.

Plants tested on which reproduction was not detected include the following: Acan-

thaceae; *Thunbergia* Retz. sp.: Amaranthaceae; *Amaranthus tricolor* L., *Celosia* L. spp.: Cactaceae; *Opuntia basilaris* Engelm. & Bigel., *Opuntia* Mill. spp., *Selenicereus* Britt. & Rose sp., *Aporocactus flagelliformis* Lem.: Caryophyllaceae; *Dianthus Armeria* L.: Chenopodiaceae; *Beta vulgaris* L., *Chenopodium album* L., *C. amaranticolor* Coste & Reyn., *Spinacia oleracea* L.: Compositae; *Ambrosia artemisiifolia* L., *Helianthus annuus* L., *Lactuca sativa* L., *Vernonia* Schreber sp., *Xeranthemum annuum* L., *Zinnia* L. sp.: Cruciferae; *Brassica juncea* var. *crispifolia* Bailey, *B. oleracea* var. *acephala* DC., *B. oleracea* var. *capitata L., B. oleracea* var. *Napobrassica* Mill., *B. Rapa* L., *Raphanus sativus* L.: Cucurbitaceae; *Cucumis Melo* var. *inodorus* Naud., *C. sativus* L., *Cucurbita maxima* Duchesne ('Blue Hubbard' and 'Buttercup' squash), *C. Pepo* L., ('Caserta', 'Cocozelle', 'Early Straightneck', 'Early Crookneck', and 'Royal Acorn' squash and 'Connecticut Field' and 'Small Sugar' pumpkin): Euphorbiaceae; *Euphorbia* L. sp.; Geraniaceae: *Geranium maculatum* L.: Gramineae; *Avena sativa* L., *Briza maxima* L., *Bromus inermis* Leyss., *Coix Lacryma* L., *Festuca elatior* L., *Hordeum vulgare* L., *Paspalum dilatatum* Poir., *Poa pratensis* L., *Setaria* Beauv. sp., *Sorghum halepense* Pers., *S. vulgare* Pers., *Triticum aestivum* L., *Zea Mays* var. *everta* Bailey, *Zea Mays* var. *rugosa* Bonaf.: Leguminosae; *Albizzia Julibrissin* Durazz., *Canavalia ensiformis* DC., *Dolichos Lablab* L., *Glycine Max* Merr., *Lespedeza stipulacea* Maxim., *Lupinus albus* L., *L. luteus* L., *Melilotus officinalis* Lam., *Phaseolus coccineus* L., *P. vulgaris* L., *Robinia Pseudoacacia* L., *Trifolium pratense* L., *T. repens* L., *Vigna sinensis* Savi.: Malvaceae; *Hibiscus esculentus* L.: Onagraceae; *Oenothera laciniata* Hill: Rosaceae; *Geum* L. hybrids: Scrophulariaceae; *Antirrhinum majus* L., *Linaria canadensis* (L.) Dum.-Cours., *Penstemon* Mitch. sp., *Veronica spicata* L.: Solanaceae; *Capsicum frutescens* L., *Lycopersicon esculentum* Mill., *Nicandra Physalodes* Gaertn., *Nicotiana Tabacum* L., *Physalis Alkekengi* L., *Solanum Melongena* L.: *Umbelliferae*; *Apium graveolens* L., *Daucus Carota* L.: Valerianaceae; *Valerianella olitoria* Poll.

Discussion

The known host range for *H. betulae* differs from that of all except one other *Heterodera* species. No other member of this genus is known to reproduce on *Betula* spp. and only *H. glycines* has been reported to reproduce on *Cleome spinosa* (5). Many of the plants tested are known hosts of at least one *Heterodera* species. The narrow host range for *H. betulae* is not unusual; a high degree of host specificity is exhibited by other *Heterodera* species such as *H. rostochiensis, H. tabacum, H. fici,* and *H. carotae.* The main purpose of the present host testing was to establish possible host relationships of *H. betulae* with other species of *Heterodera.*

On river birch, the best host, the minimum time for the completion of the life cycle was 52 days at 28 C. This contrasts with *H. glycines* which requires only 21 days at 28 C(7). *Heterodera rostochiensis* reproduces at a lower temperature and is intermediate with respect to development time, taking 38 days for a complete cycle at temperatures fluctuating from 15 to 20 C (1).

Reproduction in the absence of males is not unusual among *Heterodera* spp.; *Heterodera trifolii, H. galeopsidis,* and *H. lespedezae* reproduce by mitotic parthenogenesis (8). Males, although occurring occasionally, were not necessary for reproduction in *H. betulae.* This indicates that reproduction is probably by parthenogenesis.

Results of limited surveys suggest this

species occurs in a limited area along White River and its tributaries in Northwest Arkansas. A wider range, however, may be anticipated due to natural spread, especially downstream from the type location.

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