Journal of Nematology 17(2):165–168. 1985.

Responses of *Anguina agrostis* to Detergent and Anesthetic Treatment[1]

DONALD L. RIDDLE[2] AND ALAN F. BIRD[3]

Abstract: The infective dauer juvenile (DJ2) of *Anguina agrostis*, a stage capable of surviving desiccation, is up to sixfold more resistant to the detergent sodium dodecyl sulfate than are freshly hatched juveniles or adult males, and twofold more resistant to the anesthetic phenoxypropanol. Thus, the DJ2, like dauer stages of other species, may also be more resistant to various types of environmental stress in its natural habitat. In *A. agrostis*, however, resistance appears to be acquired gradually during development of the second juvenile stage, rather than during a molt.

Key words: Lolium rigidum, physiology, dauer juvenile, rye grass, seed galls, toxicity.

Growth and reproduction of *Anguina agrostis* in Australia occur during the spring in the developing inflorescence of its rye grass host, *Lolium*. The nematodes molt once in the egg, hatch, and grow into the infective dauer juvenile (DJ2) stage, feeding from cells that line the cavity of a gall developed in place of an ovule (10). Development of the DJ2 marks the transition from a feeding parasitic stage to a dispersal form. The DJ2s are able to enter an anhydrobiotic state in which they can survive the hot, dry summer season. With the advent of autumn rains the DJ2s become hydrated, emerge from the seed gall, and move through the soil. They are not thought to undergo further development until they reach rye grass inflorescences the following spring. Several males and females develop within a single seed, molting three times to the adult stage (8). Association of *Corynebacterium rathayi* with *A. agrostis* in the galls kills the nematodes and leads to a condition commonly referred to as annual rye grass toxicity, which can be lethal to grazing animals (3,9).

The freshly hatched juvenile (FHJ2), 500–600 μm long, develops into the DJ2, over 800 μm long, within the gall without molting (5). Morphological changes associated with the transition from FHJ2 to DJ2 include thickening of the cuticle, changes in the shape of the lateral alae, and the accumulation of lipid storage granules. The DJ2 can survive for years in the anhydrobiotic state, and hydration of dried galls provides a convenient source of active DJ2s for laboratory experiments. In con-

Received for publication 16 August 1984.

[1] Experiments done at the CSIRO Institute of Biological Resources, Division of Horticultural Research in September 1983. Supported by grant HD00367 from the National Institutes of Health, the University of Missouri Alumni Development Fund, and CSIRO.

[2] To whom correspondence should be addressed: Division of Biological Sciences, Tucker Hall, University of Missouri, Columbia, MO 65211.

[3] CSIRO Division of Horticultural Research, GPO Box 350, Adelaide, South Australia 5001.

We thank Ms. S. D. Harris for technical assistance.

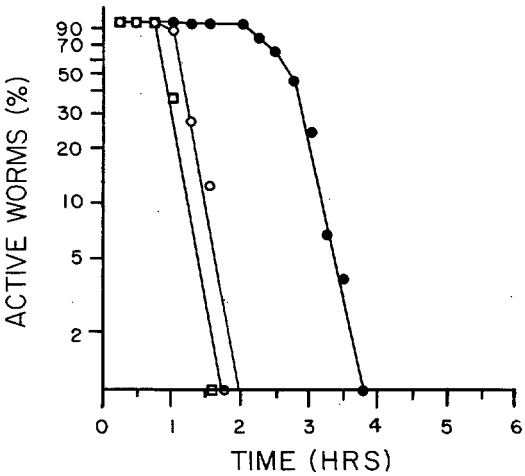

FIG. 1. Semi-logarithmic plot showing inactivation of *Anguina agrostis* in solutions of 1% phenoxypropanol. Adult males (□), freshly hatched juveniles (○), and dauer juveniles (●).

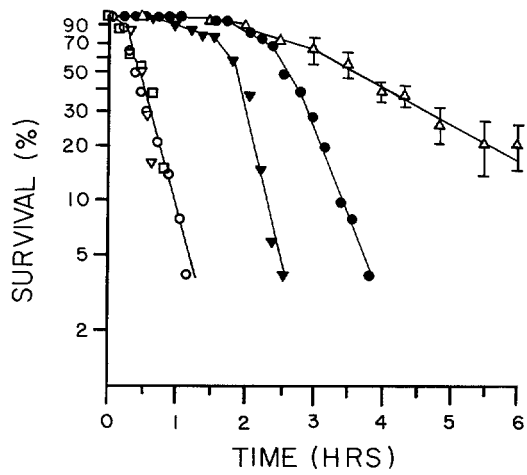

FIG. 3. Survival of juvenile and adult *Anguina agrostis* in 2% sodium dodecyl sulfate. Adult males (□), freshly hatched juveniles (FHJ2) from first (less developed) gall (▽), FHJ2 from second gall (○), dauer juveniles (DJ2) from first gall (▼), DJ2 from second gall (●), hydrated DJ2 from five galls collected in 1981 (△). Bars represent standard error of the mean.

trast, adults and FHJ2s are available only in the spring when they can be dissected from seed galls (4). Development on *Lolium multiflorum* callus tissue does not proceed beyond the DJ2 stage (5).

Previous work has shown that the *Anguina tritici* DJ2 can survive temperature extremes and dryness (2). Our objectives were to determine if the *A. agrostis* DJ2 has resistance to chemical agents such as detergents and anesthetics, and to determine whether such resistance is acquired gradually or abruptly during the transition from FHJ2 to DJ2.

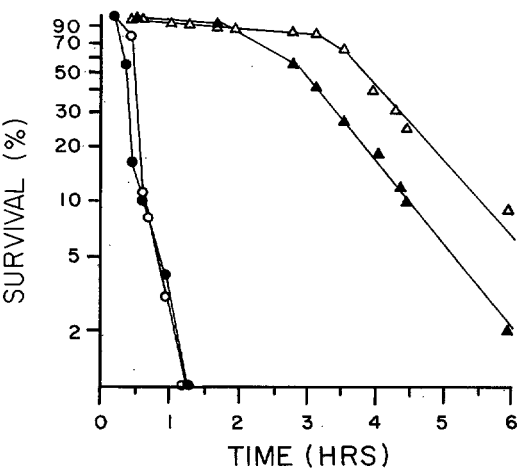

FIG. 2. Survival of *Anguina agrostis* in two different concentrations of sodium dodecyl sulfate (SDS). Freshly hatched juveniles (FHJ2) in 2% SDS (○), FHJ2 in 5% SDS (●), dauer juveniles (DJ2) in 2% SDS (△), DJ2 in 5% SDS (▲).

MATERIALS AND METHODS

Anesthesia of adults, FHJ2, and DJ2 suspended in 1% aqueous 1-phenoxy-2-propanol (Nipa Laboratories Ltd., N. Pontypridd, Glamorgan, Great Britain CF38 2SN) was tested in glass depression plates so that movement could be observed through a dissecting microscope. A nematode was judged to be anesthetized when it ceased movement and failed to respond to mechanical stimulation with an eyelash mounted on a stick.

Survival of FHJ2 and DJ2 stages suspended in 2% or 5% (w/w) aqueous solutions of SDS (sodium dodecyl sulfate, BDH Chemicals, Port Fairy, Victoria, Australia 3284) was also tested in glass depression plates so that viability could be assessed by direct observation through a dissecting microscope. Death was scored when the nematodes ceased movement and failed to respond to mechanical stimulation with a platinum wire. When unresponsive animals were removed from the SDS and placed in water, they invariably failed to recover. Killed nematodes became straight and transparent as internal tissues were progressively dissolved by the detergent.

In each experiment, 20 or 50 nematodes were used per test population.

Results

Comparative tests with phenoxypropanol were performed on DJ2s hydrated from dried galls collected in 1981 at Murray Bridge, South Australia, and on adults and their FHJ2 progeny dissected from galls collected in the same paddock in spring 1983. Adult females were not used in these tests because of their tendency to burst when handled. The anesthetic had different effects on FHJ2, DJ2, and adult males (Fig. 1). In test populations of 20–25 nematodes freshly dissected from galls, adult males and FHJ2 responded similarly. Fifty percent of the males were anesthetized after 50 minutes of exposure and all were inactivated within 90 minutes. The FHJ2 were perhaps marginally more resistant; 50% were anesthetized in 70 minutes, and all were anesthetized after 105 minutes. In contrast, none of the DJ2 hydrated from 1981 galls were anesthetized before 105 minutes, and it took 150 minutes to reach 50% inactivation. All the DJ2 were inactive after 210 minutes. Thus, the DJ2 in the test sample were about twofold more resistant to this anesthetic than were FHJ2 or adult males. Suspension in water for 24 hours without anesthetic did not inactivate the larvae or adults.

In the initial tests for sensitivity to detergent treatment, survival of DJ2 hydrated from a single dried gall collected at Murray Bridge in 1981 were again compared with FHJ2 from a gall collected in the spring of 1983 (Fig. 2). The FHJ2 were rapidly inactivated by treatment with either 2% or 5% SDS. Populations of 50 nematodes were 50% inactivated in 30 minutes by 5% SDS, or in 40 minutes by 2% SDS. Survival of FHJ2 in 1% SDS (not shown) was longer, with most juveniles killed in 2–3 hours. The hydrated DJ2 were fivefold to sixfold more resistant than the FHJ2, with 50% dead at 170 minutes in 5% SDS, or 225 minutes in 2% SDS (Fig. 2). Survival of DJ2 in SDS was concentration dependent, with mean survival time in 5% SDS 25% less than survival in 2% SDS. The relative resistance of the DJ2 in comparison with FHJ2, parallels its relative insensitivity to anesthetic.

The acquisition of increased resistance to detergent by the DJ2 was studied further because the response to SDS revealed the greatest differences between FHJ2 and DJ2. Solutions of 2% SDS were used in tests of adult males, FHJ2, newly developed (pre-anhydrobiotic) DJ2 from freshly collected 1983 galls, and additional hydrated DJ2 from galls collected in 1981 (Fig. 3). Hydrated DJ2 from five different 1981 galls were tested separately and the data averaged. Fifty percent of these DJ2 were killed in 220 minutes, in agreement with the previous results obtained with a population from a single gall (Fig. 2). The similar response of DJ2 populations from different hydrated galls suggests that the degree of resistance exhibited by these populations of fully developed DJ2 is quite reproducible. However, the slope of the survival curve is not as steep as that obtained from other samples. This indicates that although populations from different galls are similar to each other overall, there is a greater variation in the degree of resistance among individual hydrated DJ2 within each gall than occurred in the other samples.

Newly developed DJ2 and FHJ2 were taken from two different 1983 galls, each containing mixed populations of juveniles. The two galls contained differing proportions of unhatched eggs, FHJ2, and DJ2 in the growing population. Fifty juveniles of each type were picked on the basis of their appearance and tested for sensitivity to 2% SDS (Fig. 3). Fifty percent of the FHJ2 were killed in 30 minutes. Both populations of pre-anhydrobiotic DJ2, on the other hand, exhibited degrees of resistance to SDS intermediate between the FHJ2 and the rehydrated DJ2. The DJ2 sample from the gall containing the higher proportion of eggs and FHJ2 was 50% killed in less than 2 hours, whereas 50% of the DJ2 from the second gall survived 160 minutes. Finally, a pooled population of adult males dissected from approximately 20 galls exhibited a degree of SDS sensitivity indistinguishable from that of FHJ2 (50% killed in 30 minutes).

Discussion

Many nematodes form dispersal stages which are specialized to survive periods between host infections (6). In *Anguina agrostis*, the pronounced morphological and physiological changes associated with the

transition from the FHJ2 to the DJ2 occur without molting (5). Similar morphological changes without molting have been documented in *Meloidogyne* (1). This process differs from the development of the dauer juvenile dispersal form in *Caenorhabditis elegans,* a free-living soil nematode which feeds on bacteria (7). The *C. elegans* dauer juvenile is formed under conditions of overcrowding and limited food. Development is arrested at the second molt, feeding stops, and a specialized, resistant cuticle is synthesized. About 6 hours after entering the molt, water is excreted and the nematode body shrinks radially, producing a more slender juvenile of greater density. About 1 hour after radial shrinkage of the body, the dauer juvenile acquires resistance to treatment with detergents such as 1% SDS (11). Thus, the acquisition of dauer juvenile characteristics is rapid in *C. elegans* once the process has been initiated.

The gradual acquisition of detergent resistance by the developing J2 of *A. agrostis* follows the previously characterized changes in morphology. The resistance of the fully developed DJ2 to detergent may be related to its modified cuticle and to the fact that it does not feed. The DJ2 is thought to be the only stage of *A. agrostis* normally found outside the seed gall. The relative resistance of the DJ2 in laboratory experiments suggests that it may also be more resistant than other stages to other types of environmental stress in its natural habitat. Thus, *A. agrostis* may provide an example of a nematode which significantly alters its susceptibility to environmental insult without molting.

Literature Cited

1. Bird, A. F. 1978. Root-knot nematodes in Australia. Division of Horticultural Research Technical Paper No. 2. CSIRO, Australia.

2. Bird, A. F., and M. S. Buttrose. 1974. Ultrastructural changes in the nematode *Anguina tritici* associated with anhydrobiosis. Journal of Ultrastructure Research 48:177–189.

3. Bird, A. F., and D. L. Riddle. 1984. Effect of attachment of *Corynebacterium rathayi* on movement of *Anguina agrostis* larvae. International Journal for Parasitology, in press.

4. Bird, A. F., and B. A. Stynes. 1981. The life cycle of *Anguina agrostis*: Embryogenesis. International Journal for Parasitology 11:23–33.

5. Bird, A. F., and B. A. Stynes. 1981. The life cycle of *Anguina agrostis*: Post-embryonic growth of the second-stage larva. International Journal for Parasitology 11:243–250.

6. Evans, A. A. F., and R. M. Perry. 1976. Survival strategies in nematodes. Pp. 383–424 *in* N. A. Croll, ed. Organization of nematodes. New York: Academic Press.

7. Golden, J. W., and D. L. Riddle. 1984. The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food, and temperature. Developmental Biology 102:368–378.

8. Price, P. C., J. M. Fisher, and A. Kerr. 1979. Annual rye-grass toxicity: Parasitism of *Lolium rigidum* by a seed-gall forming nematode (*Anguina* sp.). Annals of Applied Biology 91:359–369.

9. Stynes, B. A., and A. F. Bird. 1980. *Anguina agrostis,* the vector of annual rye grass toxicity in Australia. Nematologica 26:475–490.

10. Stynes, B. A., and A. F. Bird. 1982. Development of galls induced in *Lolium rigidum* by *Anguina agrostis.* Phytopathology 72:336–346.

11. Swanson, M. M., and D. L. Riddle. 1981. Critical periods in the development of the *Caenorhabditis elegans* dauer larva. Developmental Biology 84:27–40.