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Notes on the Longevity of *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936, and Its Ability to Invade Wheat Seedlings after Thirty-Two Years of Dormancy

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ABSTRACT: *Anguina tritici*, second-stage larvae, in wheat galls which were stored in sealed glass tubes under conditions of low, constant humidity, and others stored in a refrigerator at about 5 C, were able to resume activity after 32 years. Larvae from the refrigerated galls were able to invade wheat seedlings readily. Larvae stored at low constant humidity were not tested. The reactivated larvae in tap water retained ability to move for as long as 408 days.

This work is a continuation of the dormancy test reported by Limber (1962) on the wheat nematode, *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936. In addition, through the kindness of Dr. A. Morgan Golden (Plant Science Research Division, ARS—USDA, Beltsville, Md. 20705), the writer received more galls of the same collection from which Dr. G. Steiner had provided the material for the earlier experiments. These galls had been stored in a refrigerator since 1939 and 1948, respectively, at a temperature of about 5 C.

The wheat galls sealed in glass tubes, which remained from the earlier work, had been stored from 1961 until 1970 at room temperatures, as previously, except for 3 days in September 1970 when they were in an attic where the temperature reached 107 F for a few hours each day.

The percentage of viable larvae was determined as follows. The galls were wrapped in wet paper toweling and placed in a 25-ml plastic vial for 48 hr. The softened galls were opened with dissecting needles without injury to the larvae and placed, individually, in vials containing 2 ml of water. After shaking, the suspension was diluted until 10 or 12 drops contained from 100 to 150 larvae. Drops were then placed in rows on a microscope slide and the larvae counted, using a 14× hand lens or a microscope with the 25× lens combination.

Since thousands of larvae were to be counted, criteria which would permit a rapid count were necessary. Therefore activity was chosen. But since Fielding (1951) has shown that some inactive larvae may still be alive, some larvae were judged by appearance. Inactive larvae which lie in coils or in smooth curves will nearly always be seen to move if observed long enough. Any errors in judgment are not believed to be significant for our purposes.

Distilled water, tap water, rainwater, and leachings from pots of growing wheat seedlings were tested for reactivation of larvae. Since all gave about the same results, distilled water and tap water were used for most of the tests because there was less difficulty from infusorians and fungi.

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Table 1. Revival of *Anguina tritici* (second-stage larvae) stored for 32 years under constant humidity, and 32 and 23 years under refrigeration at about 5 C.

Treatment	Time	Maximum activity	Populations	Average
Constant humidity, undried galls	32 yr	26%	3	26%[1]
Constant humidity, galls dried 5 min at 76 C	32 yr	96%	6	91%[2]
Refrigerated galls	32 yr	100%[3]	48	74%
Refrigerated galls	23 yr	100%[3]	8	97%

[1] Average of two living populations.
[2] Average of two living populations.
[3] Since only a 100-larvae sample was examined each day, the presence of a few dead larvae in the population is not excluded.

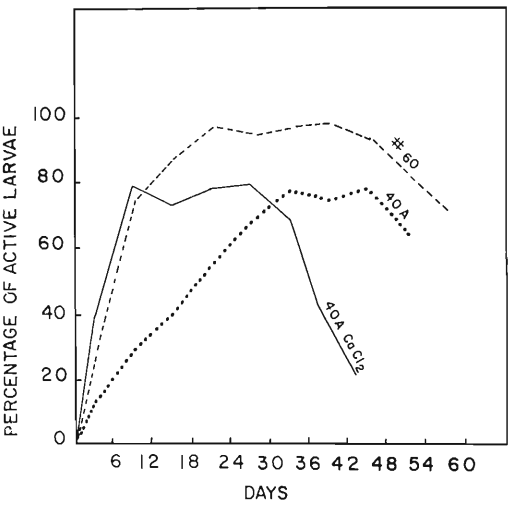

Figure 1. The rise and decline of activity in *A. tritici* populations, each stored differently, over a period of time. The dotted line, 40 A, shows the percentage of active larvae of a population stored under constant humidity for 32 years; the solid line, 40 A $CaCl_2$, represents the pattern of activity of a population dried for 5 min at 76 C then stored in a constant humidity tube which contained a few flakes of $CaCl_2$ separated from the galls by cotton; the broken line, #60, shows the pattern of activity of a population refrigerated for 32 years at about 5 C.

Larvae under constant humidity in sealed tubes

Three galls of a lot sealed in a glass tube without drying [second item listed in Table 1 (Limber, 1962)] were soaked on 19 December 1970, and were opened on 22 December. The highest percentage of revival, 26%, was reached on 26 January, 31 days after the first observed movement.

Six galls of a lot dried for 5 min at 76 C, then sealed in a glass tube containing a few flakes of $CaCl_2$ covered by a loose cotton plug, were soaked and opened after 48 hr. Four of these galls contained no living larvae. The larvae in the fifth gall showed a maximum revival of 96% after 6 days and activity remained high for 40 days. The population of the sixth gall showed maximum activity of 84% after 35 days. The last active larvae were found on the 167th day.

Thus, in both of these constant humidity experiments, many larvae were alive after 32 years.

Refrigerated galls stored for 32 years

These galls, collected in 1939, were stored in a refrigerator at a temperature of about 5 C. Forty-eight gall populations were examined by the same methods as used for the galls stored in sealed glass tubes.

Of these 48, two populations contained no living larvae, but 20 populations gave counts of 100% active larvae in from 1 to 20 days. The other populations varied greatly, from 1 to 98%; but most of them were above 69%. One population reached its maximum in 48 hr. The others required from 7 to 41 days.

Refrigerated galls stored for 23 years

These galls were from the North Carolina collection of 1948 and were stored in the same manner as those of the 1939 collection above. Eight gall populations were studied by the same methods. These larvae revived quite uniformly in contrast to those stored for 32 years. They averaged 97% revival (average of the 10 best counts of each population).

Summary of Revival Tests

The summary is given in tabular form in Table 1.

The graph (Fig. 1) shows three revival patterns. Due to the great variation in the revival of the larvae in different galls after long stor-

age, these are only examples and cannot be considered as representative of every population in their groups.

Inoculation Tests

Since so many populations of the refrigerated galls were strongly active after 32 years, the populations of two galls were combined and tested for ability to invade wheat seedlings. Eighteen wheat seeds were moistened for 48 hr in wet paper and placed on unsterilized soil in a clay pot. They were inoculated by placing several thousand (estimate) active larvae directly on the seeds with a dropper before the seeds were covered. The larvae used had been active for 19 days; 95% of the larvae were active.

Examinations were begun after 18 days and were continued for 20 days. Four infested plants were found but in only one were there more than two larvae. That plant contained four.

The experiment was repeated using freshly activated larvae and soil partly sterilized with boiling water. Fifteen plants were examined. Nine were infested. Infestation ranged from six to 500 larvae but usually was less than 50 per plant. The larvae were nearly all in the basal inch of the stem in the period of examination which was less than 20 days.

Since these tests differ in two ways, six more tests were run using freshly activated larvae in unsterile soil and in partly sterile soil in order to determine the effect of the different soil treatment. When the results for these six tests were averaged there was no significant difference in the amount of infestation. This seems to eliminate the difference in soil as a cause. The variation is probably the result of chance, or that after 19 days the larvae lose some of their ability to invade seedlings.

Discussion

The experiments on low constant humidity storage were suggested by the writer's observation, in 1938, that the galls of *Anguina tritici* varied in weight, from day to day, directly with the atmospheric humidity.

Revival of second-stage larvae of *A. tritici* occurred after 32 years in storage by each method, i.e., under low constant humidity and under refrigeration at about 5 C. This is an interval of 4 years longer than Fielding (1951) reported for this nematode.

Low respiration and low metabolism, with resulting low use of stored fats in larvae, have been reported by von Brand (1960) to vary directly with the temperature. Von Brand (1960) stated that Pigon and Weglarska found the respiration rate of dehydrated *Macrobiotus hufelandii* 600 times slower than active ones. Thus it seems probable that the long life of *A. tritici* in both types of storage is the result of reduced respiration and metabolism which either low humidity or low temperature can produce.

Golden and Shafer (1960) found that the larvae of *Heterodera schactii* remained active in plain tap water for 6 months, but all were apparently dead at the end of 7 months (approximately 214 days). Larvae of many populations of *A. tritici*, stored for 32 years, contained a few active larvae after 240 days. The longest observed period of activity among the populations studied is 414 days. This population still contains a few larvae which make slow movements separated by relatively long intervals of inactivity.

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