# Longevity *in vitro of Ditylenchus dipsaci* (Kühn) Filipjev from Narcissus

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Previous studies (Hasting, 1942) of survival of the pre-adult stage of *Ditylenchus dipsaci* in the form of nematode "wool" as found in the basal region of narcissus bulbs have shown 100% mortality after storage in glass vials at room temperature for a period of four years. Fielding (1951) records survival of *D. dipsaci* in dried plant tissues for a period of 23 years, indicating that maintenance of life in these nematodes is affected by obscure factors of environment. Further studies (Bosher and McKeen, 1954) showed that *D. dipsaci* in the dry state from narcissus "wool" and in certain media survived freezing to -80° C. followed by vacuum dehydration (lyophilization) and storage *in vacuo* in sealed tubes for a period of up to 28 days. It was postulated that lyophilization may prove to be of value as a method for the maintenance of stock cultures for laboratory investigations. Survival of these nematodes in relation to environment has been further investigated as follows.

## MATERIALS AND METHODS

Clusters of nematode "wool" from narcissus collected in 1951 were divided into two portions and placed in separate screw-cap glass vials. One portion was placed on a laboratory shelf at room temperature and one lot was stored in a household type refrigerator at 2°-4° C. Portions of the clusters were removed at 2-year intervals until 1958 and the percentage of motile nematodes recorded after 48 hours immersion in shallow tap water as shown in Table 1.

Sealed tubes of nematodes *in vacuo* prepared in 1953 by lyophilization at -80° C., held in storage at room temperature for 5 years, were opened and viability of the nematodes was determined by immersion in shallow tap water. Table 2 shows the percentage of nematodes that regained motility as compared with similar samples examined in 1953 shortly after lyophilization.

Table 1. Revival of *Ditylenchus dipsaci* from narcissus "wool" *in vitro* in relation to time and storage temperature.

| Storage | % revival after storage for: 1 yr. | 3 yrs. | 5 yrs. | 7 yrs. |
|---|---|---|---|---|
| Room temperature, approx. 21° C. | 86 | 38 | 3 | 0 |
| Refrigerator at 2°-4° C. | 89 | 86 | 81 | 78 |

## RESULTS

Nematodes that revived from the material stored at low temperature regained active motility within 24 hours after being placed in water. The small percentage that revived after five years at room temperature exhibited comparatively feeble movement between 24 and 48 hours after immersion.

Nematodes from tubes of the lyophilized series were poured into pots containing bulbs of narcissus var. King Alfred from nematode-free stock. Examination of the bulbs after one year's growth showed populations of *D. dipsaci* of all stages from eggs to adults in bulbs inoculated with nematodes from lyophilized dry wool and dry wool in beef serum. No nematodes were found in bulbs inoculated with *D. dipsaci* treated as dry wool in sucrose or in water.

Table 2. Revival of *Ditylenchus dipsaci* from narcissus "wool" subsequent to lyophilization at -80° C. in relation to time of storage *in vacuo* at room temperature.

| Nematode state | % revival after storage for: 28 days | 5 years |
|---|---|---|
| Dry wool | 80 | 20.4 |
| Dry wool in beef serum | 80 | 11.8 |
| Dry wool in 50% sucrose | 90# | 0 |
| Dry wool in water | 30 | 0 |

#12 days storage.

## DISCUSSION

The results presented herein are a further indication of the remarkable resistance to unfavorable environment of the pre-adult stage of *D. dipsaci* in the dry state.

Storage at low temperature is indicated as a more effective method for maintenance of visability of these nematodes than lyophilization at extreme cold followed by storage *in vacuo*.

## LITERATURE CITED

BOSHER, J. E. and W. E. MCKEEN. 1954. Lyophiliaztion and low temperature studies with the bulb and stem nematode *Ditylenchus dipsaci* (Kühn 1858) Filipjev. Proc. Helminth. Soc. Wash. 21:113-117.

FIELDING, M. J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proc. Helminth. Soc. Wash. 18:110-112.

HASTINGS, R. J. 1942. Longevity of congelations of bulb nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, from narcissus. Sci. Agr. 23:1-3.

# Isolation of *Trichomonas gallinae* from the White-winged Dove, *Zenaida a. asiatica*

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On September 13, 1959, a series of 17 throat swabbings were obtained from white-winged doves shot by hunters near Edinburg, Texas. The swabs were placed in tubes of Diamond's trichomonad medium (Jour. Parasit., 43: 488-490, 1957) and mailed to the Patuxent Research Refuge for examination. Upon arrival at the refuge, two and three days later, the tubes were placed in an incubator at 37.5° C. The following day the cultures were examined for trichomonads.

Six of the 17 were positive for *Trichomonas gallinae;* all six were swabbings from the 10 adult doves that on external examination were normal and fat. No *Trichomonas gallinae* was isolated from the 7 immature birds.

Although *T. gallinae* frequently has been isolated from mourning doves, this is, to our knowledge, the first report of its isolation from the white-winged dove.

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