STUDIES OF REPRODUCTION OF APHELENCHOIDES RITZEMABOSI (SCHWARTZ) ON PLANT TISSUES IN CULTURE

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*Aphelenchoides ritzemabosi* reproduced rapidly in culture with tobacco, carrot, periwinkle, or marigold callus tissues. Reproduction was inhibited by lowering the temperature, or by using a medium inadequate for best tissue growth. Addition of a chelating agent (ethylenediaminetetraacetic acid) caused severe inhibition of reproduction, suggesting the importance of cations in the reproductive processes. A low calcium medium also reduced the reproduction rate.

Plant parasitic nematodes have been maintained on both roots in tissue culture or seedling roots grown aseptically (see Dougherty 1960) and on callus tissue derived from higher plants (*Ditylenchus destructor* Thorne by Darling, Faulkner & Wallendal, 1957, *D. dipsaci* (Kühn) by Krusberg, 1960, *Meloidogyne incognita* (Kofoid & White) by Sayre, 1958). Use of tissue culture techniques for quantitative experimentation with nematodes may lead to a better understanding of their food requirements and reproduction than we have now.

This article describes cultivation of *Aphelenchoides ritzemabosi* (Schwartz) on callus tissue cultures derived from several higher plants, the influence of the composition of the nutritive media and of temperature on reproduction of the nematode, and inhibition of multiplication by ethylenediaminetetraacetic acid.

PREPARATION OF TISSUE CULTURES WITH APHELENCHOIDES RITZEMABOSI

Stock cultures of callus from higher plant tissues were maintained using methods and the medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) (D medium) of Hildebrandt & Riker (1958). The tissues were six single-cell clones of hybrid tobacco (*Nicotiana tabacum* L. × *N. glutinosa* L.) and one clone each from the Wisconsin T strain of *N. tabacum*, carrot (*Daucus carota* L.), marigold (*Tagetes erecta* L.), periwinkle (*Vinca rosea* L.), tomato (*Lycopersicon esculentum* Mill.), grape (*Vitis vinifera* L.), sunflower (*Helianthus annuus* L.), and Paris-daisy (*Chrysanthemum frutescens* L.). Iron was supplied as the ferric salt (12% Fe) of ethylenediaminetetraacetic acid (EDTA) at 25 mg/l. Tissue pieces 5 mm in

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diameter (about 100 mg fresh weight) from these stock cultures were placed on 1.5 ml of solid medium in 3 ml testtubes.

Individual adult females that emerged into distilled water from chrysanthemum leaves containing *A. ritzemabosi* were surface sterilized by immersing them in 100 ppm HgCl₂, 1 per cent streptomycin sulfate, and sterile distilled water for 2 minutes each using dental pulp-canal files dipped in 250 ppm HgCl₂ before use. A single active adult female was placed on each tissue piece. Placement of the nematode in agar near the tissue was also satisfactory. The cultures were incubated at 24° C.

After 4 weeks the tissues were removed from the testtubes and incubated at 26° C in sterile Petri dishes with sterile distilled water. After 1 to 3 days the nematodes were counted through the dish top. Dissection of tissue under the microscope showed that nearly all nematodes emerged under these conditions. Some cultures were retained unopened at 4° C to provide inocula descended from a single female for subsequent experiments which also eliminated need for repetition of disinfestation procedures. Tissue fresh weights after incubation in water were about one third the fresh weight of comparable unincubated tissues.

**APHELENCHOIDES RITZEMABOSI REPRODUCTION ON CALLUS TISSUE IN CULTURE**

Reproduction of *A. ritzemabosi* was obtained on all seven clones of tobacco callus, as well as on carrot, marigold, periwinkle, tomato, grape, sunflower, and Paris-daisy callus. Maximum reproduction occurred with tobacco, where in one case 3880 progeny were obtained from one gravid female after 29 days at 24° C and 4 days (incubation) at 26° C. High reproduction (over 400 progeny from a single female in 1 month) was also obtained with carrot, periwinkle, and marigold callus cultures. Ability of these nematodes to reproduce was not affected by the tissue from which they were obtained nor by successive selection through several generations from single females.

French & Barraclough (1961) obtained a maximum number of 3500 progeny from a single *A. ritzemabosi* female after 38 days at mean greenhouse temperatures of 17° to 25° C. The maximum reproduction rate on tobacco callus was slightly higher, probably because of the higher temperatures at which our cultures were maintained. On excised chrysanthemum and aster leaves population development from single *A. ritzemabosi* females was much slower (Sanwal, 1959).

French & Barraclough (1961) observed a sex ratio ranging from 1 male: 3 females to 1 : 8 with a mean of 1 : 4.6. We observed a range from 1 : 4 to 1 : 17 with a mean of 1 : 5.2. The ability of single isolated larvae to develop to maturity, the failure of isolated mature females that developed from these larvae to lay eggs, and the large variation in the rate of reproduction by small groups as noted by the above authors, were confirmed by us. Variation was not correlated with fresh weight of the tissues. The percentage of males did not correlate with the total number of progeny obtained in each culture.