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This Week's Citation Classic

Krusberg L R. Studies on the culturing and parasitism of plant-parasitic nematodes, in particular *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* on alfalfa tissues. *Nematologica* **6**:181-200, 1961.
[Rothamsted Experimental Station, Harpenden, Hertfordshire, England]

A method was developed for monoxenic laboratory maintenance and propagation of certain phytoparasitic nematodes on 'callus' produced from sterile alfalfa seedlings plated onto plant tissue culture agar medium containing 2,4-dichlorophenoxyacetic acid. [The *SCI*® indicates that this paper has been cited in over 100 publications since 1961, making it the 2nd most-cited paper published in this journal.]

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"In the mid-1950s, maintenance of live cultures of many phytoparasitic nematodes was often a precarious business. Root-feeding nematodes were then, and are now, routinely maintained on intact plants growing in pots or beds in a greenhouse, although a report over 20 years earlier (1933) had demonstrated that root-knot nematodes could be propagated on root cultures.[1] However, shoot-feeding nematodes were often more difficult to maintain on intact plants, and it seemed like there should be a better way. Interest was mounting in a good laboratory method for culturing those plant-feeding nematodes which refused to be cultured on fungi. W.B. Mountain in 1955 had reported a method for propagating a lesion nematode on root cultures of several plants, but the reproductive rate was often not that great and frequent subculturing was required as the roots rapidly outgrew the culture containers.[2] It seemed to me, a naive graduate student, that some kind of plant callus culture with its compact and slower growth might be a better substrate for these nematodes and could be stored for longer periods between subcultures.

"As a graduate student at North Carolina State University, Raleigh, I got interested in 1957 in seeing if plant callus in culture might be a good substrate for nematode propagation. I decided that propagation of true plant callus by subculturing was too slow and time-consuming for what nematologists needed, and thus experimented with various plant tissues that could be made to 'callus' directly, such as sterile carrot, soybean cotyledons, alfalfa seedlings, and other plant tissues. Callusing was induced by adding 2,4-dichlorophenoxyacetic acid (2,4-D) to the plant tissue culture medium.[3] It turned out that the 'callus' produced by sterile alfalfa seedlings planted on this agar medium came closest to an ideal substrate for nematode propagation; relatively large quantities of 'callus' could be produced quickly and several species of nematodes reproduced to develop large populations on the plant tissue. Nematodes of several species were laboriously surface sterilized and inoculated into the plant tissue cultures, and results were awaited—sometimes two months elapsed before success or failure was known to be the result!

"Then dissertation research required my full attention and nematode culturing was placed on a back burner. When I went to Rothamsted Experimental Station, England, on a National Science Foundation postdoctoral fellowship I took the successful nematode-plant tissue culture combinations with me, and added a nematode while there to the others which I had successfully cultured, and still maintained. By the time I completed my research at Rothamsted, nematode culturing had become an integral part of my program and it was a prominent feature of the publication resulting from my postdoctoral research. There must have been many interested and frustrated nematologists around who had had problems or interest in laboratory culturing of phytoparasitic nematodes when this paper was published in 1961 because my stock of reprints was quickly exhausted.

"I think this technique filled a need of many nematologists for a fairly simple, reliable method for maintaining and propagating certain phytoparasitic nematodes in the laboratory. Unfortunately, it has not been successful with some of the most important plant parasites, such as the root-knot and cyst nematodes, but it has worked very well with a growing number of nutritionally less demanding species. I have recently published work in this field."[4]

1. **Tyler J.** Reproduction without males in aseptic root cultures of the root-knot nematode. *Hilgardia* 7:373-88, 1933.
2. **Mountain W B.** A method of culturing plant parasitic nematodes under sterile conditions. *Proc. Helminthol. Soc. Wash.* **22**:49-52, 1955.
3. **Hildebrandt A C, Riker A J & Duggar B M.** The influence of the composition of the medium on growth in vitro of excised tobacco and sunflower tissue cultures. *Amer. J. Bot.* 33:591-7, 1946.
4. **Krusberg L R & Babineau D E.** Application of plant tissue culture to plant nematology. (Sharp W R, Larsen P O, Paddock E F & Raghavan V, eds.) *Plant cell and tissue culture principles and applications.* Columbus, OH: Ohio State University Press, 1979. p. 401-19.