The Cestode Echinococcus multilocularis in Foxes in North Dakota

Abstract. *Red foxes* (Vulpes fulva) *from Ward County, North Dakota, were found to be infected with the cestode* Echinococcus multilocularis, *a parasite which has not hitherto been reported in the United States.*

Echinococcus multilocularis was reported first from North America by Rausch (*1*) who found adult cestodes in red foxes (*Vulpes fulva*) from Point Barrow and in Arctic foxes (*Alopex lagopus*) at Icy Cape and Wainwright on the Arctic Coast and inland near the northern edge of the Brooks Range, all in Alaska. With the exception of the last locality, the brown lemming was the common microtine intermediate host present in the area where the foxes lived. Because of the wide distribution of *Echinococcus multilocularis* in Alaska, Rausch (*1*) believed that it would be introduced into holarctic hiboreal regions of southern Canada and the United States, if, indeed, it was not already there. An abundance of microtine rodents and foxes in these regions provides hosts for the parasite. Six years later Choquette, McPherson, and Cousineau (*2*) discovered *E. multilocularis* in Arctic foxes at Eskimo Point on the western coast of Hudson Bay in Northwest Territories, the first reported occurrence on the Canadian mainland.

One of us (P.D.L.) found six of nine red foxes in Ward County, North Dakota, infected with adult cestodes that appeared to correspond with the descriptions of *E. multilocularis* given by Rausch (*1*), Vogel (*3*), and Yamashita *et al.* (*4*). Examination of 19 stained preparations of these cestodes by us and ten by personnel of the Communicable Disease Center of the U.S. Public Health Service (*5*) definitely identified them as *E. multilocularis*.

This first record of isolation of *E. multilocularis* from the United States amply confirms Rausch's postulation of its widespread distribution. This isolation also emphasizes that *E. multilocularis* is far more common than has been heretofore recognized in North America.

While Rausch (*1*) emphasized that this parasite goes through a sylvatic cycle (fox to microtine rodent to fox), others have pointed out that various species of domestic animals can serve as hosts. For example, Vogel (*6*) suggested that in urban areas there could be a domestic cat, house mouse, cat cycle. Thus this cestode would appear to have a greater role in zoonotic public health. Vibe (*7*) reported having infected dogs with *E. multilocularis* from livers of sheep containing the hydatids. Petrov and Lukoshenko (*8*) infected five of eight cats with adult parasites by feeding them livers of white mice and cotton rats containing alveolar hydatids. The viability of the eggs of the parasites was demonstrated by successful infection of white mice.

With a better understanding of the morphological differences between adults of *E. granulosus* and *E. multilocularis*, as pointed out so clearly by Rausch (*1*), a reexamination of specimens already in collections and critical evaluation of those collected in the future should clarify the distribution of these two species of cestodes in North America.

PAUL D. LEIBY
Department of Biology, Minot State College, Minot, North Dakota
O. WILFORD OLSEN
Department of Zoology, Colorado State University, Fort Collins

References and Notes

1. R. Rausch, *Am. J. Trop. Med. Hyg.* **5**, 1086 (1956).
2. L. P. E. Choquette, A. H. McPherson, J. G. Cousineau, *Can. J. Zool.* **40**, 1167 (1962).
3. H. Vogel, *Z. Tropenmed. Parasitol.* **8**, 404 (1957).
4. J. Yamashita, M. Ohbayashi, Y. Kitamura, *Nippon Juigaku Zasshi* **6**, 226 (1958).
5. I. G. Kagan, Communicable Disease Center, USPHS, Atlanta, Ga., personal communication.
6. H. Vogel, *Z. Tropenmed. Parasitol.* **11**, 41 (1960).
7. P. P. Vibe, *Dokl. Akad. Nauk SSSR* **129**, 471 (1959).
8. A. M. Petrov and N. P. Lukoshenko, *Med. Parazitol. i Parazitarn. Bolezni* **31**, 223 (1962).

9 July 1964

Preservation of Chlorophyll in Leaf Sections by Substances Obtained from Root Exudate

Abstract. *Root exudate of sunflower plants was purified by means of paper chromatography. Two zones of the chromatogram were active in retarding senescence of barley leaf sections.*

The protein content of excised leaves decreases rapidly. A visible sign of this decrease is yellowing of the blade due to chlorophyll degradation. The breakdown of protein and chlorophyll can be retarded when the cut leaves are supplied with kinetin (6-furfurylaminopurine). This observation was first made in detached primary leaves of *Xanthium pennsylvanicum* by Richmond and Lang (*1*). Mothes and co-workers showed that a kinetin-treated spot of an isolated tobacco leaf remained green and attracted metabolites from the yellowing parts of the blade (*2*). Kinetin sustains RNA synthesis in cut leaves and thus affects protein metabolism (*3*). Although kinetin is active in a wide variety of plants (*4*), it has no effect on cut autumn leaves of cherry where, however, certain auxins retard senescence (*5*).

Aging of excised leaves is prevented or even reversed by the formation of new roots on the petiole. This fact led to the hypothesis that certain factors supplied by the root are responsible for regulating protein metabolism in the leaves (*6*). Recently Kulaeva tested root exudate for its capacity to retard chlorophyll breakdown by applying it directly to detached tobacco leaves (*4*). The response obtained was comparable to that induced by a low kinetin concentration, and it disappeared within a relatively short time.

This report describes evidence for the presence of two factors in root exudate which are active in delaying chlorophyll degradation in leaf sections. Sunflower plants (*Helianthus annuus*) were grown in natural greenhouse conditions for 8 weeks to a height of 50 to 60 cm. They were topped just below the cotyledonary node, and exudate was collected from the stump three to four times daily for three consecutive days.

The sap was frozen immediately after collection and dried in a lyophilization apparatus. The residue was chromatographed in an ascending system on Whatman No. 3 paper with a mixture of *n*-butanol, acetic acid, and water (4:1:1 by volume) as solvent. After the solvent front had moved 20 cm the paper was dried and cut into 2-cm strips; the fractions were eluted with 80 percent ethanol and tested for their capacity to retard chlorophyll degradation on sections of barley leaves. The barley was grown at 20°C under continuous illumination for 13 days. At this time the first leaf was fully expanded and the second had just begun to appear.

Plants were selected for uniformity, and a piece of each leaf was cut

between the 3rd and 4th cm from the tip of the blade. The sections were aged for 24 hours by floating them on distilled water at 25°C in darkness. After this period they were blotted and transferred to screwcap vials containing 1 ml of test solution and 250 units of penicillin G. Four sections were floated in each vial, and each fraction was tested in duplicate or triplicate samples. After 48 hours of incubation at 25°C in darkness the sections of each vial were extracted with 80 percent ethanol; chlorophyll retention was expressed by measuring the optical density of the extracts at 665 mμ. In this assay the kinetin response at concentrations between 0.003 and 3.0 μg/ml appears to be linear if plotted on a logarithmic scale. The advantage of this test over the one described by Osborne (*7*) lies both in the great uniformity of the tissue which is always taken from the same portions of leaves of identical physiological age and in the higher sensitivity of the assay to kinetin.

Eluates from two regions of the chromatogram retarded chlorophyll degradation in barley leaf sections (Fig. 1). The response of the first fraction exceeded the response obtained by the optimum kinetin concentration of 30 mg/liter. This experiment was repeated five times with different batches of sunflower plants, and the same results were obtained. A number of nutritional factors were tested for their possbile interference with the bioassay. Sugars, inorganic nitrogen (ammonium nitrate, potassium nitrate, and ammonium sulfate), organic nitrogen sources (amino acids, casein hydrolyzate), and a complete modified White's medium gave negative results. Therefore the activity of the two fractions of root exudate is attributed to specific factors which are translocated from the root to the shoot and which play a regulatory role in the metabolism of the leaves. Experiments have shown already that one of the two fractions (R_F 0.5 to 0.8) very actively induces cell division in tissue cultures of soybean callus. This test is considered specific for the detection of kinetin-like substances (*8*).

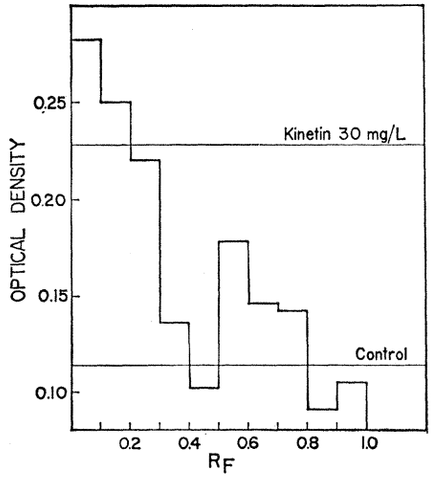

Fig. 1. Paper chromatogram of root exudate obtained from seven plants (44 ml of sap).

H. KENDE

Department of Plant Physiology, Negev Institute for Arid Zone Research, Beersheva, Israel

References and Notes

1. A. E. Richmond and A. Lang, *Science* **125**, 650 (1957).
2. K. Mothes, *Naturwissenschaften* **47**, 337 (1960).
3. R. Wollgiehn, *Flora* **151**, 411 (1961); O. N. Kulaeva and I. P. Vorob'yeva, *Soviet Plant Physiol.* (English trans.) **9**, 77 (1962); D. J. Osborne, *Plant Physiol.* **37**, 595 (1962); B. E. S. Gunning and W. K. Barkley, *Nature* **199**, 262 (1963).
4. O. N. Kulaeva, *Soviet Plant Physiol.* (English trans.) **9**, 182 (1962).
5. D. J. Osborne and M. Hallaway, *Nature* **188**, 240 (1960).
6. A. C. Chibnall, *Protein Metabolism in the Plant* (Yale Univ. Press, New Haven, 1939).
7. D. J. Osborne and D. R. McCalla, *Plant Physiol.* **36**, 219 (1961).
8. C. O. Miller, in *Modern Methods of Plant Analysis*, H. F. Linskens and M. V. Tracey, Eds. (Springer, Berlin, 1963), vol. 6, pp. 194–202.
9. The technical assistance of Mrs. Viviane Vacknine is thankfully acknowledged.

2 June 1964

Self-Sterile Auxotrophs and Their Relation to Heterothallism in Sordaria fimicola

Abstract. *Eighty morphological mutants in the homothallic fungus* Sordaria fimicola *were tested on liquid minimal medium for nutritional requirements. Five had nutritional requirements, one for adenine, three for arginine, and one for lysine. All five were from among the eighty single gene mutants that were also partially or completely self-sterile. Nutritional requirements and centromere-locus intervals provide better criteria than morphological characters for selecting self-sterile mutants at complex loci governing heterothallism.*

Although extensive genetic investigations have been carried out on the homothallic ascomycete *Sordaria fimicola* in the past decade, attempts to obtain mutants with specific nutritional requirements have all met with failure. In all these past investigations tests were carried out on minimal agar medium in plates, and the failure was attributed largely to the lack of conidia (*1*). In another homothallic species *S. macrospora* studied by Esser (*2*) and Heslot (*3*), no auxotrophic mutants have been reported.

The ascospores of *S. fimicola* (Fig. 1*a*) have been shown (*4*) to be as good a tool as conidia for obtaining mutants of various morphological characters. Bearing in mind that inheritable morphological characters are the phenotypic expression of physiological processes under gene control, I set out to test a large number of morphological mutants for nutritional deficiencies. With the exception of a few ascospore color mutants, all of those tested were self-sterile as a result of the same mutation that was expressed morphologically.

The minimal medium of Beadle and Tatum (*5*) adopted in this study was modified in that sucrose was replaced by glucose as the only carbon source and the ingredients were dissolved in water distilled in glass twice. In order to eliminate any carry-over in the inoculum, the mutants were grown on water agar medium for 2 to 3 days, after which small inocula were cut out at the edge of the colony and transferred to 125-ml erlenmeyer flasks containing 15 ml of the minimal medium. The flasks were placed on a gyratory shaker for 5 to 10 days before the results were recorded. The use of liquid rather than agar minimal medium

Table 1. Auxotrophic mutants of *S. fimicola*.

Characteristics	Requirements
st-59	
Nonautonomous spore color mutant producing hyaline inviable ascospores, slow growth	Arginine
a-3	
Abortive asci, slow growth	Arginine (partial)
st-412	
Only mycelium produced, slow growth	Arginine (partial)
st-401	
Only mycelium produced, slow growth	Adenine
st-64	
Irregular ascospore maturation, normal and abortive ascospores	Lysine