Occurrence of *Asellus* (Crustacea. Isopoda) in Esthwaite and Ullswater, English Lake District

In a previous paper[1] *Asellus aquaticus* was recorded for Esthwaite from one station only, and it was also stated that *Asellus* was absent from Ullswater. Since then the situation has changed, as *Asellus aquaticus* is now established throughout Esthwaite and *Asellus meridianus* has been found at the south end of Ullswater.

The implications of this are of sufficient interest to warrant full publication, but the following points may be made now. In the case of Esthwaite, it seems from old records that *Asellus* was definitely recorded from the boat house and reed bed area at the north end in 1950, although it is possible that it may have been seen as early as 1944. Despite my own collections in Esthwaite in 1948 and 1951 and those of the Freshwater Biological Association supply department in intervening years, *Asellus* was not reported again until 1955—again around the boat house, one of the places where I collected in 1948. This raises the point as to whether it is more prudent to record a species 'as not found' rather than 'absent'. One has on several occasions, after prolonged and vigorous collections in the Midlands and in the Lake District, found a single specimen of *Asellus*. How often on other occasions must the odd specimen have been overlooked despite every care ?

Esthwaite is 1·5 miles long and less than 0·5 miles across at its widest, and after 14 years *Asellus* can be found around the whole lake and to a depth of 3–4 m. Whether this represents a genuine 'spread' of *Asellus* from a single centre, or the build-up from a population so diffuse that the collecting methods used failed to detect it, is a matter for consideration. It would be extremely interesting to re-examine the other lakes in the district where I previously failed to find *Asellus*. So far as the Ullswater district is concerned the only other known occurrence of *Asellus*, after many years collecting, is in three garden pools above the lake. One of these pools contains *A. aquaticus*, the other two *A. meridianus*, and *Asellus* is known to have been introduced with weeds. The pools are a mile away in direct line from the *Asellus* localities in Ullswater, and apart from these the nearest known occurrence is in Windermere, ten miles away over Kirkstone Pass.

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[1] Moon, H. P., *J. Anim. Ecol.*, **26**, 403 (1957).

Antifungal Activity in Orange Tissue infected with *Aspergillus niger*

Orange fruits that had been wounded and inoculated at fixed points with conidia of *Aspergillus niger* became infected with *Penicillium digitatum*, and it was observed that growth and sporulation of the latter were halted some distance from the regions of the fruit colonized by *A. niger*. Cylindrical portions of the pulp from regions supporting the *A. niger* growth placed on plates of potato–dextrose agar seeded with the spores of various fungi inhibited the growth of several, but no such inhibition was obtained with pulp from healthy fruits (Table 1).

Table 1. Diameter of Zone of Inhibition (mm) caused by Healthy or *A. niger*-infected Fruit (5 mm³ Portions)

Test organism	Healthy	Infected
Alternaria brassicicola	0	43
Aspergillus niger	0	0
Botrytis cinerea	0	0
Cladosporium cucumerinum	0	26
Glomerella cingulata	0	0
Penicillium digitatum	0	31

Furthermore, macerated orange pulp when inoculated with *A. niger* developed similar antifungal activity although potato–dextrose agar, which was supporting *A. niger*, did not. Therefore, in further investigations, peeled oranges were macerated in a Waring blender and the homogenate was distributed in 11-cm diameter Petri dishes in layers approximately 0·7 cm deep. After inoculating the surface of the fruit pulp at several points with a suspension of *A. niger* conidia in water, the dishes were incubated at 25° C for four days, by which time the surface was covered with a thick mycelial mat on which conidial heads were just developing. This was lifted, the adhering pulp brushed from its underside into the residual liquid and the mat discarded. Sufficient alcohol was added to bring the final concentration in the treated pulp to 80 per cent ethanol and the mixture was left at 4° C for 24 h to precipitate proteins. It was then filtered and the residue, which had no antifungal activity, was discarded. The alcohol was removed from the filtrate at 30° C under reduced pressure when lipid material separated. This was filtered off; it was non-fungitoxic and was discarded. The remaining clear aqueous solution was highly acidic, strongly antifungal to *A. brassicicola*, less so to *B. cinerea* and inactive against *A. niger*.

Laboratory investigations indicated that spores of *A. brassicicola* were acid-sensitive, and when samples of deproteinized aqueous extracts of both healthy and *A. niger*-inoculated pulp were passed through columns of ion-exchange resin ('Amberlite *IR*-4*B*(OH)') to remove acidic constituents fungitoxicity was lost. The adsorbed acids were eluted from the columns with 6 N formic acid which was then removed under reduced pressure. Two-dimensional chromatography of the eluted acids showed that citric acid was the predominant acid in both extracts. Titration against standard alkali indicated an equivalent of approximately 4,000 μg/ml. of citric acid in healthy pulp and 28,000 μg/ml. in infected pulp. The degree and range of antifungal activity exerted by citric acid solution at the above concentrations correlate closely with that of the supernatant from centrifuged healthy and *A. niger*-infected pulp (Table 2).

Table 2. Diameter of Zone of Inhibition (mm)* caused by 0·1 ml. of Juice of Healthy or Infected Orange and of Two Levels of Citric Acid Solution

Test organism	Healthy	Infected	Citric acid (28 mg/ml.)	Citric acid (4 mg/ml.)
Alternaria brassicicola	0	41	45	15
Aspergillus niger	0	0	0	0
Botrytis cinerea	0	20†	20†	0
Penicillium italicum	0	0	0	0
Penicillium digitatum	0	27	26	0

* Diameter of filter paper disk, 13 mm. † Retarded growth.

Thus it would seem that much of the antifungal activity shown by *A. niger*-metabolized orange tissue towards certain fungi is due to citric acid production, though further experiments have indicated that other antifungal materials are present. The —OH resin-treated aqueous extract from infected pulp was of low activity, but an ethyl acetate extract was toxic to the spores of *A. brassicicola* at 250 μg/ml., though less active against *Glomerella cingulata* and *Botrytis cinerea* and inactive against *A. niger*. Similar extracts from healthy pulp were inactive below 5,000 μg/ml. against all the test fungi. Furthermore, some other fungi, for example, *Glomerella cingulata*, when inoculated into orange pulp, produce ethyl acetate-soluble antifungal materials without the large production of citric acid characteristic of *A. niger*.

The development of antifungal activity in plant tissues as a result of infection by pathogens has recently received considerable attention[1]. The resistance to further infection by orange tissue already infected with *A. niger* can therefore be considered in relation to the production of phytoalexins[2], substances defined as "antibiotics which