Project title:	**Crook root disease of watercress**
Report:	**Final Report (October 1999) (Grower Summary)**
Previous Reports:	**Annual Reports (October 1997, October 1998)**
Project Number:	**FV 55f**
Project Leader:	**Dr. John Clarkson School of Biology and Biochemistry University of Bath Claverton Down Bath. BA2 7AY.**
Location:	**University of Bath**
Project Co-ordinators:	**Dr. Steve Rothwell Vitacress Salads Ltd Lower Link Farm St. Mary Bourne Andover, Hants. SP11 6DB. Mr. Charles Barter The Watercress Association The Nythe Alresford, Hants.**
Project Start Date:	**1st October 1996**
Project End Date:	**1st October 1999**

Crook Root Disease of Watercress

Objectives and Background

Crook root disease was first spotted in a watercress bed in Wiltshire in 1947. Since then, it has spread to all watercress growing areas in the U.K. Affected plants show characteristic 'crooked', swollen roots, the damage of which results in yellowing of leaves, and a general loss of vigour. The disease tends to first appear as temperatures drop in October, and spreads from the bed outlet back towards the water source. Symptoms persist into the following April, and severe yield losses can occur.

The organism responsible is a plasmodial organism, classified as *Spongospora subterranea* f. sp. *nasturtii*. It can survive for long periods, as tough resting spores in bed debris, until unknown environmental triggers cause it to germinate. Microscopic zoospores are then released, which initiate infection by swimming to host roots, and attaching. Once within the roots, the spores multiply, and release fresh zoospores into the water within seven days in ideal conditions. Levels of inoculum can thus increase very rapidly. In addition to causing crook root, *Spongospora* is also known to vector two damaging virus diseases of watercress (Watercress Yellow Spot, and Watercress Chlorotic Leaf Spot).

Almost by chance, in the 1950's, zinc was discovered to be able to control crookroot, and since then, it has been added to beds, often as zinc sulphate drip feed, over the winter months. However, total control is rarely achieved, and due to environmental concerns over its use, the levels that can be added to the water are tightly regulated (maximum levels of 0.075ppm zinc at the outflow of beds). It is therefore desirable to both optimise the use of zinc, and to investigate other potential control measures.

One aim of this project was to investigate how zoospores find watercress roots. If a chemical attractant specific to watercress was found, it could possibly be exploited in disease control (for instance, by supplying these chemicals to the beds to confuse and disorientate zoospores). As well as attempting to identify potential control measures based on host-pathogen interactions, the possibility of replacing or supplementing zinc with other metals (such as cobalt) was investigated.

In order to rationalise the current use of zinc, this project set out to develop a DNA based diagnostic test for *Spongospora*. This was based on a technique known as the Polymerase Chain Reaction (PCR), which can generate millions of copies of a specific DNA sequence, from a very small amount of starting material. The DNA region used in this study was the ITS ribosomal DNA. This varies between even closely related species, and hence is useful for designing a *specific* test for crookroot.

Summary of results

To investigate chemoattraction of zoospores to plant roots, a microscopical observation test was set up. Chemical extracts were prepared from watercress. and a variety of other plant roots. These were then investigated in a model system for their potential in attracting *Spongospora* zoospores. Several concentrations of extracts were tested, but at no level was watercress seen to be more attractive than other plant species (Figure. 1). These results were confirmed by examining attraction to intact roots of watercress and tobacco, where zoospores were seen to migrate to both species.

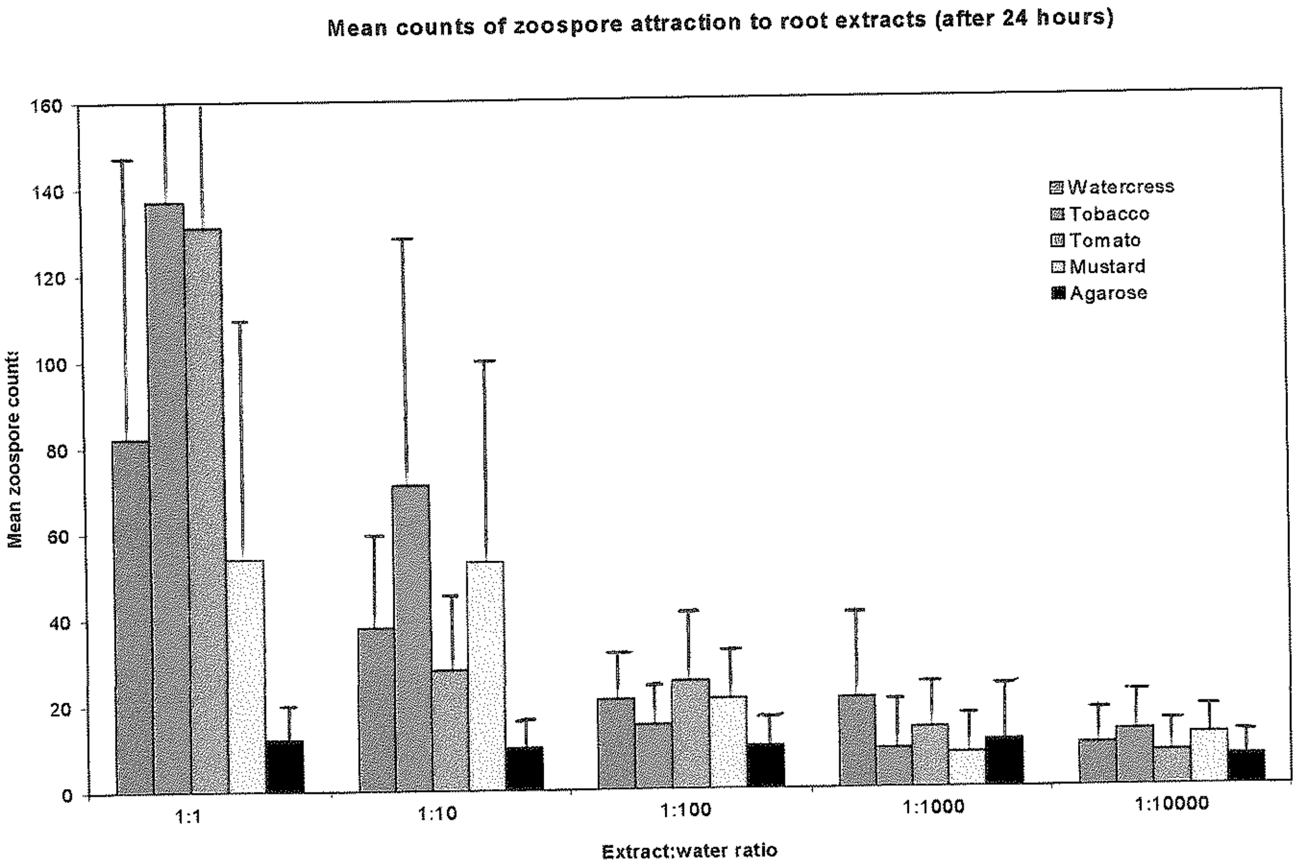

Figure 1. Zoospore attraction to plant root extracts is non-specific at a range of concentrations tested.
(1:x = ratio of root mass (g) to water (ml) used)
Agarose is the agent used to fix the extracts in a gel, and is tested as a control. Results are means of 15-20 replicates.

These results suggest that a general plant exudate is acting as a signal to zoospores, guiding them to watercress roots.

Although in laboratory based tests, zinc did not seem to be effective at levels used in the field, it was still the most effective cation tested against *Spongospora*. In the field, zinc is apparently effective at 0.075ppm, and this was previously demonstrated in the 1950's by Tomlinson, under laboratory conditions. During this project, however, zoospores were often able to survive at levels up to 2ppm in significant numbers. Cobalt seemed the only other metal to affect zoospore viability, but it was not as effective as zinc control (Figure 2)

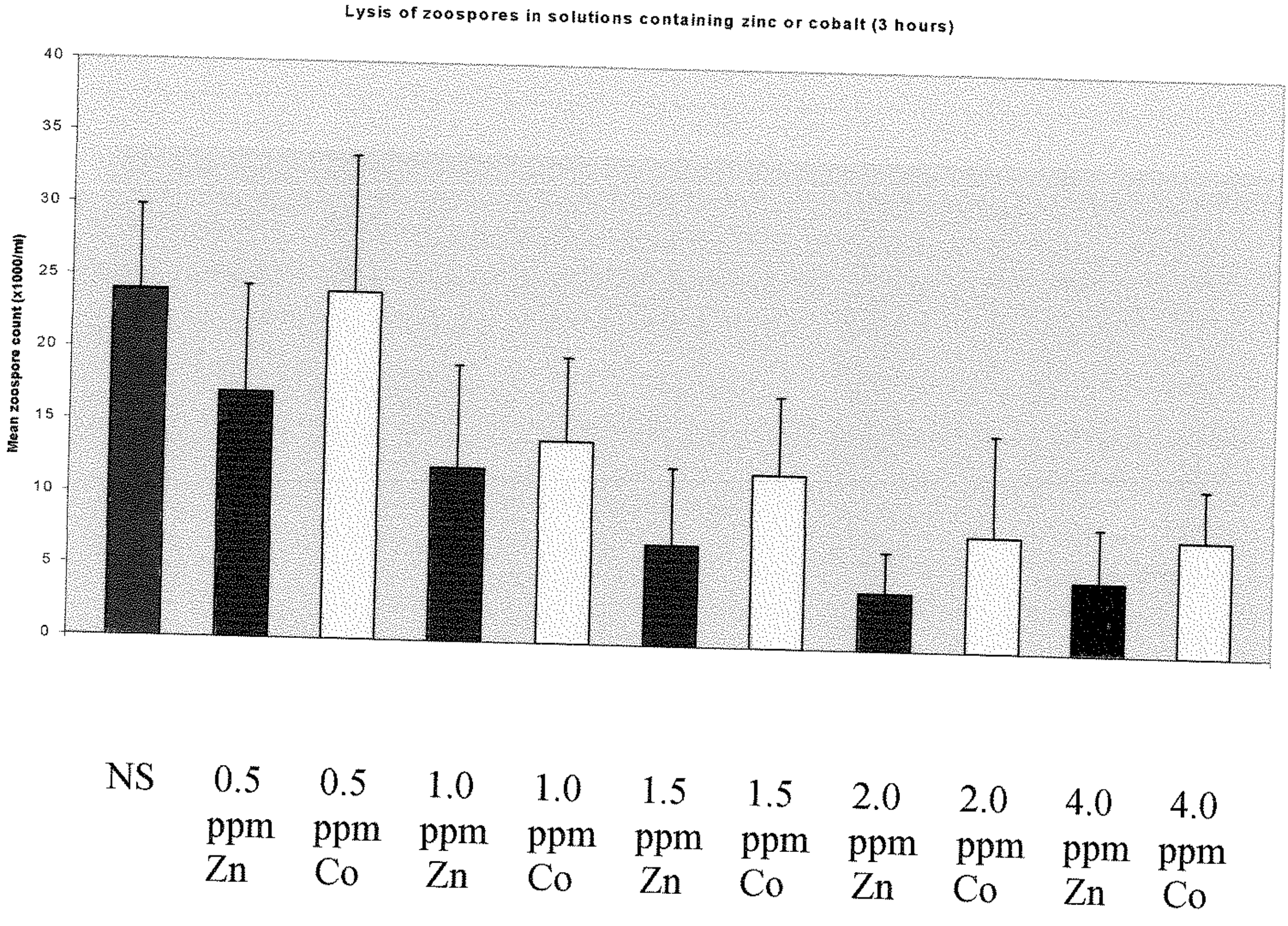

Figure 2. *Although zinc (as zinc sulphate) doesn't completely kill zoospores at The highest levels used, it is more effective than cobalt (as cobalt chloride).*
NS = nutrient solution (optimal for zoospores)
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Spongospora ITS ribosomal DNA was amplified, and its DNA sequence compared to similar organisms, such as *Plasmodiophora brassicae,* the causal agent of clubroot. As these organisms are closely related, it is likely that their DNA's will be quite similar. Therefore DNA sequences which *differ* between *Spongospora,* and these related organisms, are likely to be unique to *Spongospora.* Such sequences were identified, and exploited to design DNA probes specific to *Spongospora.* The probes were then tested for their ability to uniquely detect *Spongospora,* from a range of geographical locations.

Preliminary trials indicated that as few as 1000 zoospores in a water sample could be detected under laboratory conditions. Field tests trapped zoospores either by filtration of water samples, or by baiting zoospores using root extracts. At present, it is possible to detect *Spongospora* using these methods, but not consistently as yet.

Using DNA fingerprinting techniques, *Spongospora* has been shown to belong to a diverse group of organisms known as protists (Figure 3). These occur in a range of habitats, including both soil and aquatic systems. Although there are few examples of economically important plant pathogens within this grouping, some do exist, such as the oomycetes (including *Phytophthora* species), and it is possible that measures used to control these pathogens, could be investigated to determine whether similar measures could be used on crook root.

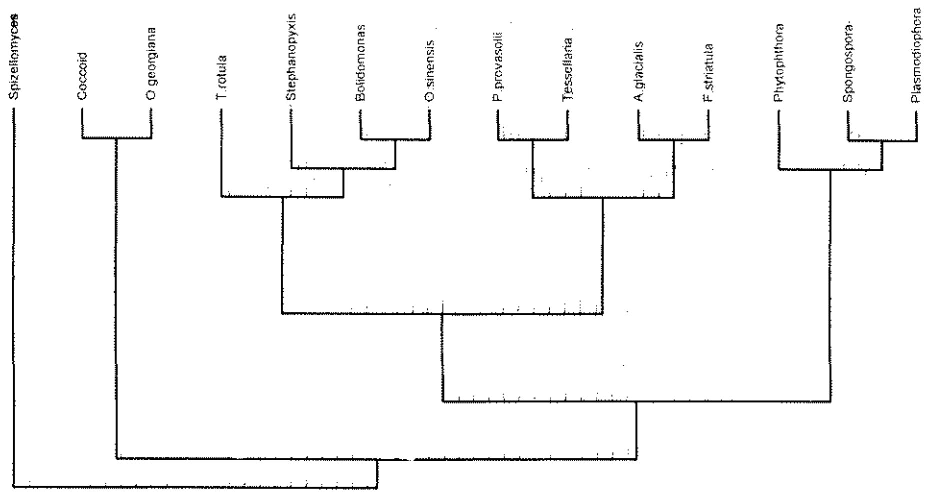

Figure 3. Alignment of DNA sequences produces a 'tree'. The above tree groups Spongospora and Plasmodiophora together, indicating a close relationship. All other organsims present are protists, except the most distant species, the chytridiomycete, a fungus. This implies that Spongospora is a protist.

Action points for growers

- The results of this study have confirmed that zinc remains the only economically viable control agent for crook root, and is more effective than other alternatives tested.

- Zinc should continue to be used prudently. Research at the University of Bath aims to provide a simple diagnostic test to allow more rational zinc application, targeted against a measured degree of bed infection.

Practical and Financial Benefits

- Field tests of the diagnostic test show good potential. Zoospores collected by filtration or baiting, have been detected by DNA probes. Additional R&D is needed to optimise field sampling techniques.

- Once field trials of the diagnostic test are completed, rational use of zinc should become possible. This would enable the industry to control crook root in a more effective manner, and also reduce potential environmental impacts of zinc.

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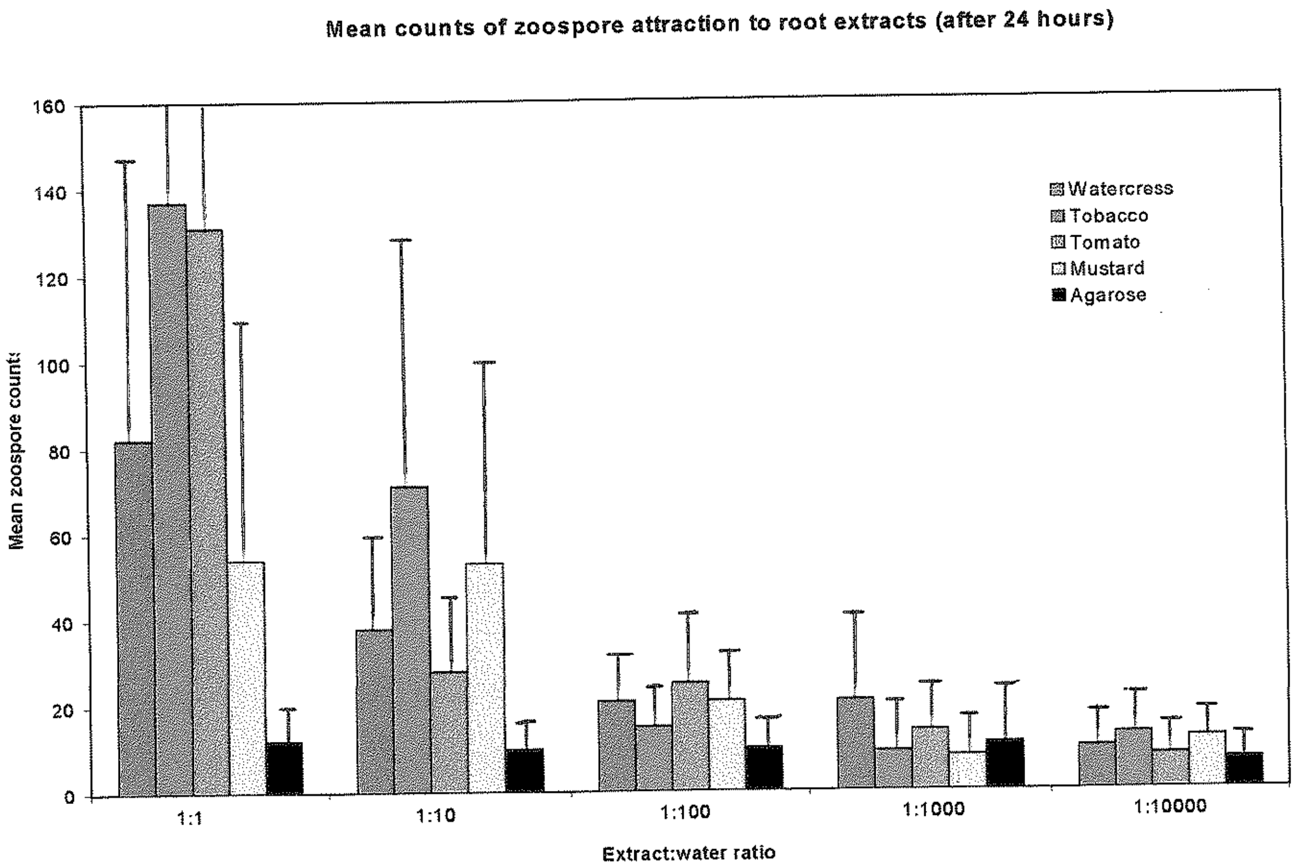

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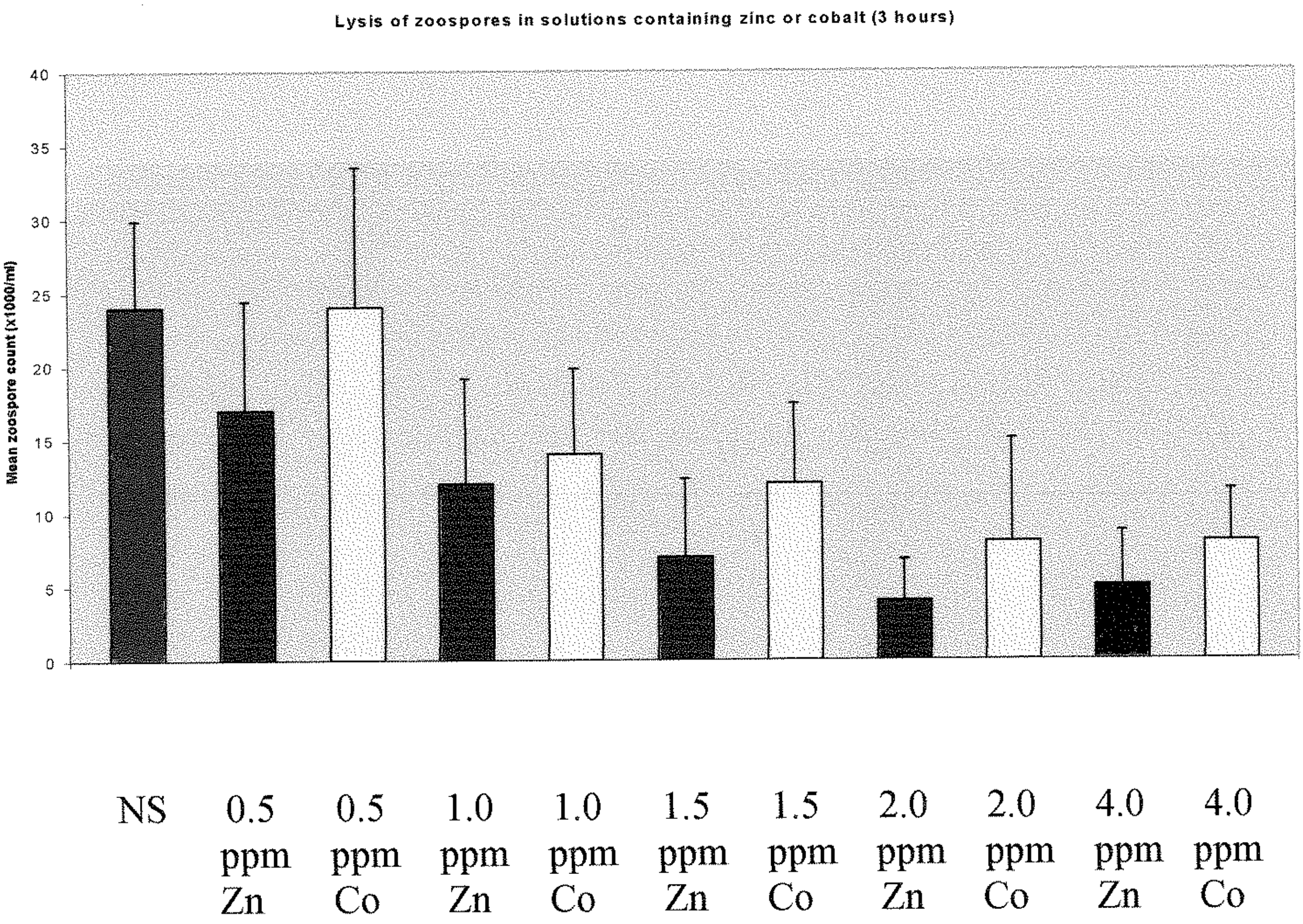

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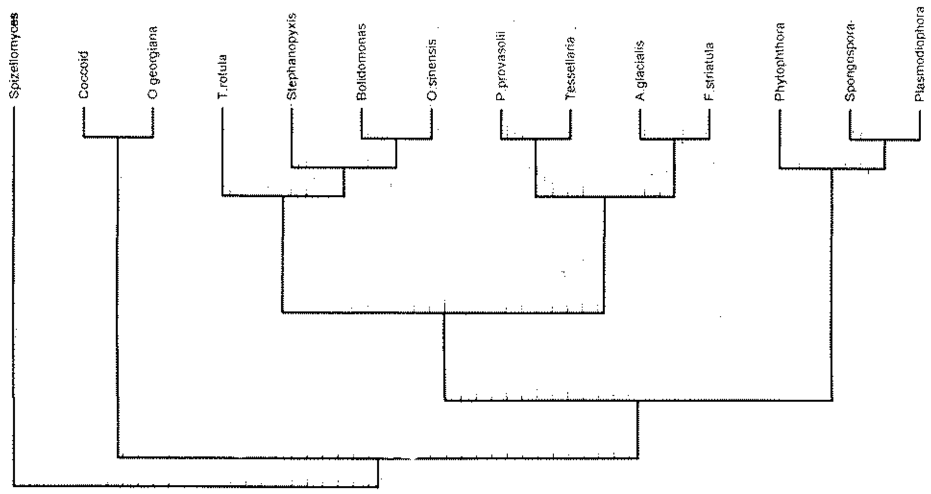

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