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Armillaria Control at the Royal Tasmanian Botanical Gardens

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In recent years the Royal Tasmanian Botanical Gardens has suffered from a massive infection of *Armillaria* that threatened the survival of a large proportion of the Gardens. The background situation and containment including the eradication and prevention processes either considered, trialled, or used to attack the disease, are described. The success of the work is attributed to proactive fundraising and the courage to take drastic action.

INTRODUCTION

Fungal root rot disease caused by members of the genus *Armillaria* is a phenomenon that has a global distribution (Shaw and Kile, 1991). *Armillaria luteobubalina,* a species endemic to Australia, has been a major problem in the Royal Tasmanian Botanical Gardens (RTBG). Some background to the situation and the methods to treat the disease are covered below.

The fungus can be either saprophytic or pathogenic. It commonly exists in dead wood but in appropriate conditions can also infect living plants. The saprophytic phase, typically in a buried tree stump, can last several decades (Kile, 1981; Summerell, pers.comm.), or even centuries (Anderson et al., 1979) which makes its control problematic. The species does not produce rhizomorphs. Spread of infection can take place in one of two ways: either by windblown spore dispersal or by subterranean contact, usually across root systems. Local spread by root contact is believed to be the most common.

Not all plant species have equal susceptibility to the disease, but at present it is not possible to identify with any certainty species that are wholly resistant. The prognosis for plants that do become infected is bleak: younger trees tend to die quickly but older individuals may reach an apparent equilibrium for some years before eventually succumbing.

The pathogenicity of *Armillaria* seems to be a result of the hyphal invasion of the hosts' cambial layer, eventually spreading into, and obstructing the vascular system, though damage from fungal exotoxins has also been suggested (Kile et al., 1982). Visible signs of infection tend to reflect the extent of underlying vascular damage: sudden death in young trees and gradual foliage loss in the earlier stages in mature plants. Larger trees exhibit bole necrosis, reduction of height and diameter growth, with death occurring as vascular occlusion produces a ring-barking effect.

The picture that emerges is of an aggressive disease whose dominant features are rapid underground spread and a long list of potential hosts.

ARMILLARIA IN THE RTBG

Although the first confirmed diagnosis of *Armillaria luteobubalina* was made in early 1994 it is highly probable that the organism has been active for much longer than that, and may indeed have been present in the original native forest that occupied the site until nearly 200 years ago (Mohammed and Wardlaw, 1995). Until quite recently it was normal practice that if a tree was removed for any reason, the stump was cut close to the ground and either left or grassed over: except for small trees it was unusual for the root mass to be removed. One result of this has been to provide a large food resource for potential *Armillaria* inocula in sites which are mostly unrecorded.

The site of the first confirmed diagnosis was a mature cape chestnut almost in the centre of the rectangular main lawn. One year later a *Betula papyrifera* was affected, from a bed below the cork oak, and infected roots of the cork oak itself were subsequently identified.

Prior to about 1995 there is little accurately resourced data. However, it seems likely that the fungus has been active *in situ* for many years. Why there was a sudden apparent explosive extension of the disease from 1994 onward is explicable if it is postulated that the disease had been present but largely unrecognised. Proper recognition of the problem was quickly followed by the initiation of a program of systematic investigation and treatment which is continuing today. When the disease had been accurately identified and definitive action decided upon, the affected area occupied an elongated polygon, roughly 250 × 60 m, some 15,000 m^2.

Long before any comprehensive plan had been evolved it was clear that the size and complexity of any solution was going to be expensive. There were at that time no provisions in the budget for an outlay as large as this was likely to be, and so external fund-raising options were canvassed. Applications to government for funding provided some immediate assistance, but it was clear that it would not be sufficient. Therefore, a major public appeal was organised. Aided by *The Mercury* newspaper and other sponsors, *The Mercury* Save the Gardens Appeal 1996 raised over $220,000 to fund the work required.

OPTIONS

There were two steps in dealing with the disease. First, it was realised that we needed to contain the spread of the fungus and eradicate it wherever possible, and second that we needed to implement a long-term strategy to prevent infection in new sites. Containment and eradication involved trialling mapping, chemical containment, and inoculum reduction. Prevention has involved attempts to limit spread by quarantining areas, by the use of competitive or antagonistic fungi, and by an ongoing program of stump removals.

CONTAINMENT AND ERADICATION

Mapping. Before you can contain or eradicate the disease you must know where it is. It rapidly became apparent that although sites of known infection had been identified satisfactorily, nothing was known about the extent of subterranean spread towards plants not yet affected. The difficulty here is that the fungus grows only inside wood, and it is not possible to make a diagnosis until plants are showing signs of infection, by which stage the disease is well established.

A trial using wooden stakes as bait to determine subterranean spread was therefore organised. It was hypothesised that, if brushwood stakes of a species susceptible to infection by *Armillaria* were inserted into the ground close enough to an inoculum, left in place for several months and then withdrawn, evidence of infection on them should be detectable. Using a large number of stakes in a pattern around the infective foci it was hoped that the true limits of the disease could be defined and then mapped.

Stakes were implanted to a depth of about half a metre in parallel rows with each stake half a metre apart. Stakes were withdrawn after they had been in the ground for more than 6 months. Those showing visible mycelia were retained for further examination, the remainder being discarded. The outcome revealed a disappointing result and a disparity in patterns between the two sites.

In the main lawn, *Armillaria* was grown from only 10 stakes, and from the bank above the gazebo only four, even though the disease was known to be present in both sites.

The low percentage of infected stakes placed in highly contaminated areas is probably due mainly to the absence of rhizomorphs formed by this species. Though *A. luteobubalina* is extremely infectious, to colonise a new host it must be in close contact with it and it is this lack of proximity which is the most likely factor in the low success rate. There are other possibilities such as the length of time spent in the soil, the wood used and even local environmental conditions, but a better strike rate could probably have been achieved simply by placing the stakes much closer together.

As a predictive tool, in its current form this technique is of little value: a negative culture does not reliably mean that the fungus is absent from the area being tested. Some improvement could be obtained by not only reducing the inter-stake distance but by using a concentric or spiral pattern of placement around the suspected focus.

Chemical Containment. Neither of the two known chemical treatment techniques of *Armillaria* infection has received universal approval for its effectiveness (Shaw and Roth, 1980) but there have been reported successes for some species of *Armillaria* in some specific situations.

We decided to use a few selected stumps for a limited trial with the fungicide sodium *N-methyldithiocarbamate* (Vapam), using stump injection as the delivery medium. Ten stumps of various ages, species, and diameters were chosen. Holes were drilled at appropriate intervals in the stump and around the root collar, a measured dose of fungicide was poured in, the holes were stoppered, and the stump and immediate environs were covered by plastic sheeting.

As with the stake-baiting trial, this was not set up as a controlled experiment: the pre-treatment criteria of infection was the presence of basidiomes or mycelia on the roots, but laboratory diagnosis was not always available. The post-treatment criterion became the reappearance of fruiting bodies, though some culture of stump material was carried out later.

About 9 months after treatment, in the autumn fruiting body season, most of the stumps produced vigorous clumps of basidiomes indicating the disease was flourishing; in those few that did not, there existed a possibility that the infection had been absent in the first place. Again, because of the design of the trial it would be misleading to draw firm conclusions, as the apparently universal failure may have more than one cause. What was clear was that chemical treatment had not worked for us.

Inoculum Reduction. Countries of the northern hemisphere in which *Armillaria* root disease is a long-standing and continuing problem have used removal of stumps and roots as a primary control measure for decades (Barss, 1913; Roth et al., 1980). The persistence of infectivity in buried stumps (vide supra) makes their removal an attractive curative treatment, but as with most aspects of the treatment of this problem there are limitations. Most obviously, unless there is an accurate horticultural history, or the stumps themselves are identifiable, removal is likely to be incomplete. The difficulty of tracking underground spread and the seasonal unreliability of the basidiomes has already been alluded to, which means that accurate excavation may not be possible and large additional volumes of soil have to be removed to ensure a reasonable safety margin.

Cost is possibly a more significant factor in this treatment than in the others, as is feasibility: in a small area with few infective foci the outlay may be reconcilable with the probability of a cure. In the situation in the RTBG however, where a large area was affected but the stump locations inaccurately known, excavation and refill is an expensive exercise.

The decision to use stump removal in the RTBG was taken when it was realised that there were few other viable options. The disease was extending its area and the loss of any more large trees in a setting as small as this would have had a disproportionally large effect.

There were four main excavation sites: adjacent to the cork oak (300 m^3); on the main lawn (1000 m^3); above the gazebo (1200 m^3); and north of the gazebo (400 m^3). Essentially, the areas to be excavated were determined empirically, using the infected trees and stumps as starting points, then digging outward to a point beyond the limit of that trees' rhizosphere. Where roots from adjoining trees were encountered and also found to be infected, then that tree too was sacrificed with its root system.

Excavation stopped when we ceased to find infected wood material or live roots in the ground. At that stage all roots at the edge of the excavation were sampled for *Armillaria*.

The results of this work have been in a dramatic improvement in the health of the RTBG. From a high of 1.5 ha infected with 98 identified sites in 1997, the incidence of the disease fell to zero positive identifications in 1998, and so far, one positive in 1999.

PREVENTION

We have tried three approaches to prevent the spread of *Armillaria*:

1) Quarantining areas of the RTBG that are believed not to have the disease;
2) The use of competitive and antagonistic fungi;
3) Removal of all stumps and roots of trees that die in the future.

Quarantine. With quarantining the technique is to cut trenches through the RTBG to below the level of root penetration to separate off areas. Root barriers are then installed in the trenches which are then filled with gravel, covered with soil and re-grassed. The first such trench was dug across the main lawn between the infection site and the large conifers on the upper side of the Arthur Wall. This technique is however, of limited application because of the need to minimise damage to drainage lines, natural aquifers and to tree roots.

Trials of Competitive Fungi. Following the work by Pearce and Malajzcuk (1990) the RTBG began trials inoculating stumps with a friendly nonpathogenic wood rotting fungus, *Phanerochaete filamentosa,* to test if this would out compete *Armillaria* and thus prevent its spread. Subsequent examination and culturing from treated stumps have failed to find any evidence that this process has been successful.

Antagonistic Fungi. Although excavation and the removal of infected trees and wood material has been found to be effective in eliminating the disease, it means that infected trees are sacrificed. There is no known cure for the disease in living trees. Considerable research is clearly needed into this subject. One option worthy of investigation is the proposal to inject another fungus, *Trichoderma*, into the trees. It is claimed that this procedure cures Kiwi fruit vines of *Armillaria* infection in New Zealand. Researchers Hank Cutler and Robert Hill (pers. comm.) have also proposed the injection of an extract of *Trichoderma* could be an effective fungicide that would translocate better than the fungus itself. However, although universally acknowledging the technique would be nonharmful, local plant pathologists were strongly opposed to the trialing of this technique and without their assistance research had to be abandoned.

SUMMARY

The RTBG has suffered from a significant and debilitating infection of *Armillaria.* Although many techniques have been trialled, and some are worthy of further investigation, to date excavation of infected wood material and removal of infected trees have proven the only effective way to deal with the disease. The latter have been spectacularly successful, with a massive reduction in the number of confirmed infected sites. The dramatic improvement is attributable to positive action in informing the public about the disease, proactive fundraising, and direct action to address the problem.

The future health of the RTBG, and other similar sites will depend on vigilance, adequate funding, and the courage to act.

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