

Spotlight on Agriculture

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THE INVOLVEMENT OF PLANT PATHOGENIC FUNGI IN THE NATURAL DIEBACK OF BLACKTHORN IN NAMIBIA: PART 4

INTRODUCTION

An organism's *pathogenicity* is its ability to induce a disease. If an organism is suspected of being a pathogen, it will show the identical disease-causing symptoms under laboratory conditions.

It is often the case that people do not have adequate knowledge of what is required to treat an infection by an opportunistic plant pathogen. In the case of the previously unrecorded disease evident in the dieback of Blackthorn (*Acacia mellifera*), a set of rules known as the Koch's postulate can be followed in determining whether a particular pathogen is causal. Using this approach entails the following: (a) the micro-organism needs to be associated constantly with the disease; (b) the micro-organism needs to be isolated from the contaminated tissue and established in a pure culture; (c) if a healthy plant is inoculated with the micro-organism, it has to result in the corresponding symptoms of the disease; and (d) the relevant micro-organism needs to be isolated from the contaminated tissue of the inoculated plant.

The previous papers (*Spotlight on Agriculture* No.'s 82 and 83) reviewed the disease symptoms and confirmed that plant pathogenic fungi were involved in the dieback of Blackthorn bushes. In the studies undertaken in this regard, fungi were constantly isolated from all disease-infested plant material and, therefore, were associated with the disease. The objective of this study in general was to determine which of the isolated fungi were pathogenic in Blackthorn, i.e. causal for the dieback in these bushes.

METHODOLOGY

A range of infection studies was conducted to determine the pathogenicity of the fungus isolated from affected plant parts. This research consisted of field trials in Namibia and glasshouse studies at the University of Stellenbosch in South Africa. The field trials were conducted at the Uitkomst Research Station and the Neudamm Experimental Farm. In the field trials, individual bushes were inoculated with contaminated toothpicks. In these inoculations, Isolates of *Phoma glomerata*, *P. cava*, *P. eupyrena*, *Cytospora chrysosperma* and seven other species of fungus, not consistently recovered from bushes with dieback, were used. In the glasshouse studies, spore suspensions and contaminated toothpicks with the same organisms were used to inoculate seedlings that were germinated from Blackthorn seeds disinfected through boiling.

Blackthorn is usually a many-stemmed shrub with a short, compact main trunk branching prolifically at or above soil level. Occasionally, a single-stemmed tree can occur. To overcome the problem of a large variation in trunk size, shoots instead of trunk bases were inoculated. At the end of the three-year trial period, bushes were dug out and rated for basal rot.

RESULTS

Glasshouse studies

Seedlings inoculated by means of spore suspensions and toothpicks were examined after a few weeks for lesion formation, growth of fruit structures (reproductive cycle), and internal discoloration at the stem base and upper taproot. Isolations were then made from discoloured wood. The leaves of seedlings inoculated with *P. glomerata* became chlorotic, the petioles eventually turned necrotic and dropped off. Fruit structures were observed on the stem base and upper taproot of all seedlings inoculated with *P. glomerata*. The latter fungus also developed on the stem base and upper taproot.

A common feature of the seedlings inoculated with *P. glomerata* by means of toothpicks was the greenish-yellow



Multi-stemmed Blackthorn stump

to blackish-green internal discoloration of the wood. *P. glomerata* was also recovered from pure culture in Petri dishes.

The other fungi that were inoculated were not recovered from any plant part, and did not reveal any of aforementioned symptoms.

Field inoculations

In order to determine which of the fungi were involved with the dieback, inoculated shoots or branches were removed at intervals and examined for external lesions or cankers. The extent of internal wood discoloration was also determined. The results showed that all four of the fungi in question did not cause external lesions or cankers. *P. glomerata* caused a distinct internal greenish-yellow to blackish-green discoloration of the wood. The discoloration advanced above and below the original infection site and was primarily confined to the old wood.

Although care was taken to choose disease-free sites for inoculation studies, natural dieback was observed later on at both the sites. Therefore, inoculated bushes were evaluated for twig and branch dieback, and were excavated and rated for basal rot. Of the bushes treated at Uitkomst, 77% showed no basal rot; at Neudamm, 91.2% of the treated bushes showed light to severe basal rot.

P. glomerata was isolated as the predominant wood-discolouring organism and was regularly isolated from the discoloured wood in all treatments. Isolations of the target fungi were primarily confined to the area adjacent to the area inoculated by means of the toothpick.

DISCUSSION

It is difficult to define a sick plant comprehensively. The reason is that concepts such as *healthy*, *sick*, *normal* and *abnormal* are terms that are relative to one another. Thus, sick is generally accepted to signify a deviation from the normal condition of a living plant or its components, which results in a disruption or change in normal functions. This deviation may be induced by stress factors such as climatic conditions, attacks by insects or microbiological organisms. These factors result in a specific condition associated with sickness, such as defoliation, chlorosis of the leaves, wilting, and dieback of twigs and branches. If the presumption is that a micro-organism is the cause of the above-mentioned symptoms, then it is necessary to diagnose the causal organisms as well as determine the interactions between organism and plant.

From the results of the research in Namibia and South Africa, it can be concluded with certainty that a micro-organism is causal for the dieback of Blackthorn bushes. In the glasshouse trials, after inoculation with *P. glomerata*, leaves became chlorotic and defoliation occurred. The most conspicuous symptom of this disease, the internal greenish-yellow to blackish-green discoloration of the wood, occurred in the seedlings after inoculation. In addition, *P. glomerata* was recovered from the stem base and upper taproot of the young plants.

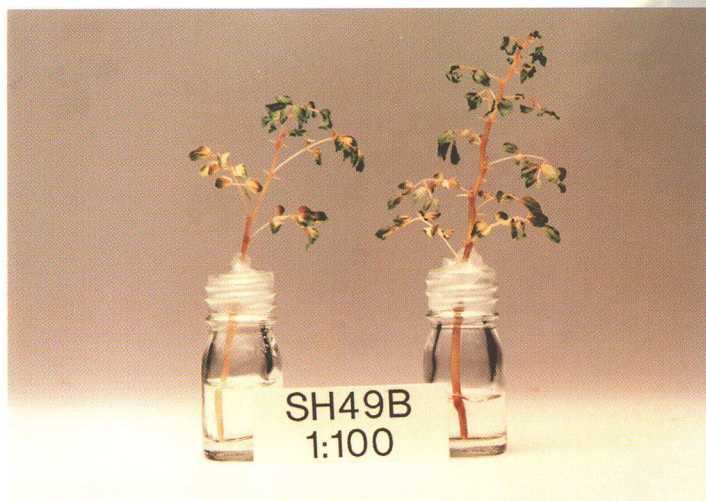
In the field inoculation trial, a distinct internal greenish-yellow to blackish-green discoloration of the wood was observed. This discoloration advanced above and below the original infection site and was primarily confined to the old wood. The consistency of *P. glomerata* in discoloured wood and in wounds made during this study indicates this fungus plays a primary role in the disease.

The symptoms revealed in this trial not only correspond with previous observations made, but the rules of Koch's postulate are also satisfied. It can be concluded with certainty, therefore, that a micro-organism is causal for the dieback of Blackthorn and that *P. glomerata* plays a major role in this disease.

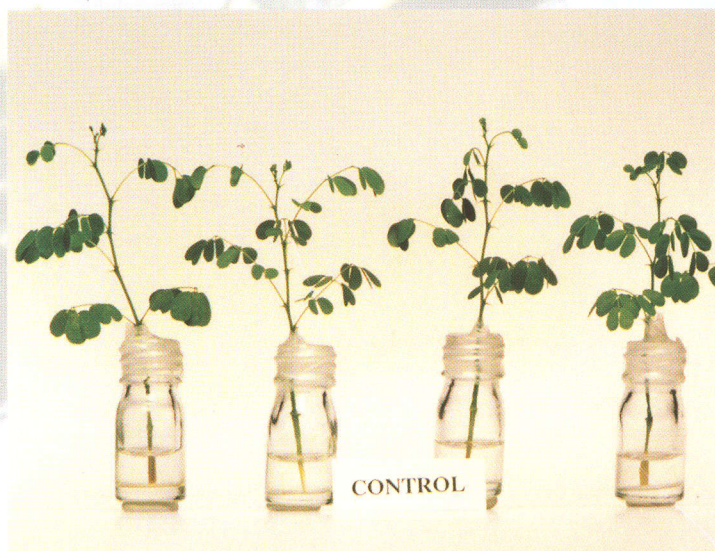
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Inoculated seedlings showing symptoms of chlorosis



Healthy seedlings that were not inoculated