EFFECT OF SOIL-BORNE INOCULUM ON INCIDENCE OF ONION BLACK MOULD (*ASPERGILLUS NIGER*)

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ABSTRACT

Aspergillus niger is the cause of black mould of onions, which is primarily a postharvest disease. Systematic sampling of soil from onion fields in the Pukekohe/Waikato regions of New Zealand in 2002 and 2003 showed that the highest levels of soil-borne *A. niger* occurred in fields that had not been recently rotated out of onions. Levels of soil-borne *A. niger* were correlated with black mould incidence in bulbs stored at high temperature and high humidity, but not in bulbs stored at ambient temperature and humidity. It is likely that soil-borne spores are a principal source of inoculum for black mould of onions in Pukekohe, New Zealand.
Keywords: *Aspergillus niger*, black mould, onions, postharvest disease, inoculum.

INTRODUCTION

Black mould of onions is caused by the fungus *Aspergillus niger* van Tieghem. It is a high-temperature fungus, with an optimum temperature range for growth of 28-34°C. Warmth and moisture favour development of the disease (Maude et al. 1984). Although the disease can occasionally be seen in the field at harvest, black mould is primarily a postharvest disorder and can cause extensive losses in storage under tropical conditions (Thamizharasi & Narasimham 1992).

Black mould was first recorded causing a storage rot of onions in New Zealand in 1959 (Brien et al. 1959) and at that time it was thought to be of little economic importance (Dingley 1969). Over the past decade black mould has become of increasing importance to New Zealand onion growers. Although New Zealand is a temperate country, onions can experience tropical conditions in ships en route to markets in the Northern Hemisphere where a high incidence of black mould in some consignments has caused major marketing problems. Not only is the value of the product reduced, but also some importing countries regard black mould as a human health hazard.

Aspergillus niger is common as a saprophyte in soil and on decaying plant material (Commonwealth Mycological Institute 1966). The fungus can be seed-borne in onions, and up to 100% of seed lots in Sudan were found to be naturally infested (El-Nagerabi & Ahmed 2001). However, this method of dispersal has not been reported in New Zealand.

This study aimed to increase understanding of the biology of *A. niger* on winter-planted onions in New Zealand. The first objective was to detect and quantify inoculum of *A. niger* in the soil of onion fields at harvest, and to relate soil inoculum density to previous cropping history. The second objective was to determine the relationship between soil inoculum density and incidence of black mould in storage. To the authors' knowledge this is the first report to relate soil inoculum to onion black mould in storage.

METHODS

Inoculum density in soil

Over the period January-March 2002, soil samples were collected from nine onion fields in the Pukekohe and Waikato regions of the North Island of New Zealand. Each field was subdivided into 16 plots. Soil samples (5 ml each) were collected from the top

5 cm of soil at four evenly spaced positions along the centre two beds of each plot. The four samples from each plot were combined, resulting in a composite soil sample for each plot. Samples were collected from each field immediately prior to lifting and again when bulbs were removed from the field (harvest). Onions were left to cure in the field for at least 14 days before harvest. In January 2003, two fields were sampled and soil was collected from each field in the 1-2 months prior to harvest.

Soil suspensions were made from each of the 16 composite samples per field by adding 2.5 ml of dried, pulverised soil to 10 ml sterile reverse-osmosis (RO) water and agitating for 10 s with a vortex mixer. Aliquots of 0.1 ml of each soil suspension were spread across four plates of Difco Czapek-Dox agar amended with 100 μg/l streptomycin sulphate and 100 μg/l tetracycline hydroxide. The plates were incubated at 37°C in the dark for 3 days. Inoculum density of *A. niger* was calculated as colony forming units (cfu) per ml of soil.

Harvesting and storage of onions

In 2002, each field was divided into eight equal plots for sampling of onions. Prior to lifting, 24 onion plants were collected from each plot (192 bulbs from each field) to give a pre-lift sample. At harvest, single bins of onions were collected from the centre rows of each plot and stored in the growers' storage sheds. Samples of 25 onions were collected at random from each of the experimental bins after 2-3, 15, 30 and 60 days of storage and visually assessed for black mould. After 30 days in the ambient temperature storage of the growers' sheds, 400 onions from each field were removed from the bins and placed in a controlled environment room at high temperature (30°C) and high humidity (>90%) (HT/HRH) for a further 30 days, before being visually assessed for the presence of black mould. In 2003, onions were sampled from one field only, at random and after pickup and grading.

Data analysis

Data were analysed using a one-way analysis of variance (ANOVA) to distinguish means. Regression lines were fitted to the data plots.

RESULTS

In 2002, there were no significant differences ($P>0.05$) in numbers of *A. niger* colonies recovered from the soil at the two sampling times (lifting and harvest). There were large between-field differences ($P<0.05$) in the amount of *A. niger* recovered.

The relationship between the number of successive onion crops and the inoculum density of *A. niger* in soil from each onion field is shown in Figure 1. Inoculum density of *A. niger* was positively correlated (R^2 = 80%) with the number of years fields had been continuously cropped with onions.

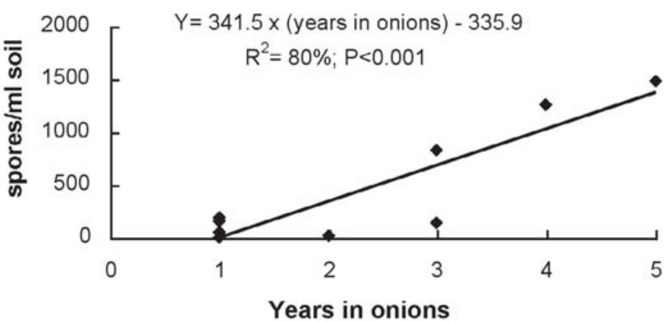

FIGURE 1: Relationship between cropping history and inoculum density of *Aspergillus niger* in the soil.

No black mould was found in any onion samples collected prior to lifting. Black mould was found in only one onion from the samples collected at harvest. The incidence of black mould was low in onions from all fields after ambient temperature storage (0% at 15 days, 0-2% at 30 days and 0-3.5% at 60 days). There was a large increase in the incidence of black mould from some fields under the HT/HRH conditions compared with onions held for the same period at ambient temperature and humidity (data not shown). The relationship between soil inoculum density and black mould after HT/HRH storage is shown in Figure 2. Those fields with highest incidence of black mould after HT/HRH storage were those that had the highest inoculum density in the soil.

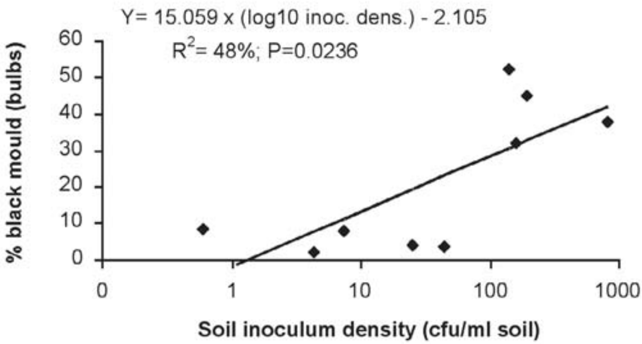

FIGURE 2: Relationship between soil inoculum density at harvest and percentage onion bulbs affected by black mould after high temperature/high relative humidity storage.

DISCUSSION

When stored under conventional commercial conditions (ambient temperature) in a number of different locations there was minimal development of black mould. When samples were removed from ambient storage to conditions of high temperature and humidity there was a dramatic increase in incidence of black mould in onions from some fields. This suggests that the incidence of black mould detected on onions after conventional storage is not a good indicator of their potential keeping quality if stored in conditions of high temperature and high relative humidity during shipping.

Several sources of inoculum have been suggested for black mould of onions including airborne spores, soil and contaminated seed (Hayden & Maude 1992). Hayden et al. (1994) proposed that one or more of these sources may contribute to the disease, and that the importance of each source may vary in different climatic regions. The present work has shown a strong positive correlation between numbers of spores in the soil and incidence of mould after storage in conditions conducive to mould development. Soil-borne spores are likely to be a principal source of inoculum for black mould of onions in Pukekohe, New Zealand.

Van Konijnenburg & Ardizzi (1997) found that in Argentina black mould incidence was related to cropping history, drying time previous to storage and the percentage of diseased bulbs at harvest. In this study, it was found that there was a correlation between the number of successive crops of onions in a field and the numbers of spores in that soil. Inoculum level in turn was correlated with postharvest black mould development in conducive environments. A greater knowledge of the relationship between soil inoculum density and the predisposition of bulbs to black mould would allow the potential black mould risk for any crop to be determined. That in turn would allow growers and marketers to make informed decisions on the most appropriate disposition of at-risk crops.

Given that no fungicide is registered in New Zealand for black mould of onions, control needs to focus on minimising inoculum levels in the soil through cultural control, i.e. rotations with non-*Allium* crops. This is an example of a minor pathogen that has become a major problem with changes to a more intensive onion-after-onion cropping system. The relationship between cropping history and soil spore load should be further investigated particularly with regard to the rate of decline of spore numbers during crop rotation. In the meantime, it is a valuable reminder of the risks associated with extended onion-after-onion cropping strategies.

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