ISOLATION OF A SEED STORAGE FUNGUS FROM SUGARCANE SEEDS

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Abstract

The ability of seed storage microflora to survive at the low moisture content of 12 to 18% in stored seeds makes their control difficult. At Mount Edgecombe a *Penicillium* species was isolated from sugarcane seeds which had a moisture content of about 13%. It appeared that where the seed coat had been damaged, it was likely that the micro-organism would invade the seed. The ultrastructural changes caused by the fungal hyphae suggest that the viability of stored seeds could be greatly diminished. It is therefore essential that seed be kept free of storage fungi to ensure that as many seedlings as possible can be used in the breeding programme.

Introduction

The production of true seed is an essential step in the sugarcane variety improvement programme. Sugarcane is a polyploid plant which is normally propagated vegetatively. The number of seedlings giving rise to outstanding varieties is very small, therefore it is essential to minimize their loss so that as many as possible are included in the selection programme for evaluation as commercial varieties.

At the South African Sugar Association Experiment Station approximately 1 000 crosses are made annually and of these, about 600 are planted. The period for which seed is stored, (ie from the time the seed matures physiologically on the plant until it is sown (Harrington[6])), may be a few months or a few years. They should therefore retain their viability throughout this period.

It has been shown that during storage, microflora reduce the ability of seeds to germinate or cause disease in the seedling (Christensen[5]; Neegaard[9]). The classification of these fungi is not taxonomic, but is based on their ecology; primarily on their moisture requirements (Christensen[5]). Field fungi are those species commonly associated with seeds which are on the plant before harvesting and which have a moisture content high enough for fungi to grow (25 to 30%). The genera found in sugarcane caryopses include *Cladosporium, Curvularia, Fusarium, Helminthosporium, Pestalotia* and *Phoma* (Sanguino & Tokeski[14]).

The storage fungi are those species which are able to tolerate the low moisture content of stored seeds and include *Aspergilli* and *Penicillia.* Moisture contents of between 12 and 18% (no free water available) and temperatures between 25 and 33°C, provide optimum conditions for the growth of these fungi but they can withstand extremes in temperature as high as 50 to 55° C and as low as 0 to 5°C (Christensen[5]).

In sugarcane breeding, seed is prepared for storage in conditions which favour infection by storage fungi. The seed is allowed to mature on the plant and is then dried in ovens at 30°C for 36 hours, until the moisture content is between 12 and 14%. The seed is packed in polyethylene bags with sachets of silica gel and stored in a deepfreeze at -10°C.

The purpose of this study was to establish which fungi were present in the stored seeds, to characterize them and to ascertain the effects they might have on stored seed.

Materials and Methods

Sugarcane seed was manually separated from the glumes and then stored in sealed pill phials in a refrigerator at 6 ± 2°C. The surface of the seeds was sterilized in a sodium hypochlorite solution (1% available chlorine) for five minutes and rinsed three times in sterile distilled water. Aseptic techniques were used when the seeds were cut either longitudinally or transversely as close to the embryo as possible without damaging it. The section containing the embryo was placed with the cut surface down, onto high-salt potato dextrose agar (PDA) plates and cultured at 25°C. This PDA has been found to be favourable for storage fungi which grow under conditions of osmotic stress. The fungus that was isolated from the seeds was characterized using the light, scanning electron and transmission electron microscopes so that it could be included in the dossier on seed storage fungi (Berjak, Dini & Evers[3]) which is being compiled by the International Seed Testing Association.

Seeds were also artificially infected with fungus which had been isolated from the seeds on the agar. The infected seeds were put into a phial with others and incubated at 25°C. The fungus was transferred to the previously uninfected seeds which were then fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin (Cazalet[4]).

A germination test was carried out on 100 seeds which had been stored for either six or 18 months. The seeds of the crosses were placed on wet filter paper in a Petri dish and incubated at 25°C. After five days their germination was assessed (using radicle emergence as the criterion) and expressed as the percentage viability.

Results and discussion

In this germination trial, seeds that had been stored for six months (seed from crosses made during the present crossing season) had a viability of 90% while those which had been stored for 18 months had a viability of 70%. In Mauritius, Peerun, Hermelin and Lalouette[10] estimated that stored sugarcane seed had a viability of 35% and that about 5% of the seeds were damaged when they were mechanically separated from the glumes. Harrington[6] found that for each 1% reduction in moisture content, or for each 5°C reduction in temperature, the life of the seed was doubled.

Sugarcane seed is stored in a deepfreeze at -10°C and it is important that the moisture content be kept below 15% to avoid damaging the membranes (Roberts[11]). At Mount Edgecombe, stored sugarcane seed has a moisture content of about 13% (wet weight).

The range in moisture content suitable for a particular species of fungus is sharply defined and is also influenced by the type of seed. Murray[8] found that the storage of seeds at low temperatures changed the spectrum of fungi, certain species being excluded while others were able to grow. Competitive exclusion has also been shown in the seed storage fungi (Neegaard[9]).

The fungus that was isolated from the seeds was found to be a *Penicillium* species. Christensen[5] noted that some of the common seed-invading species of *Penicillium* were able to grow slowly at temperatures below freezing.

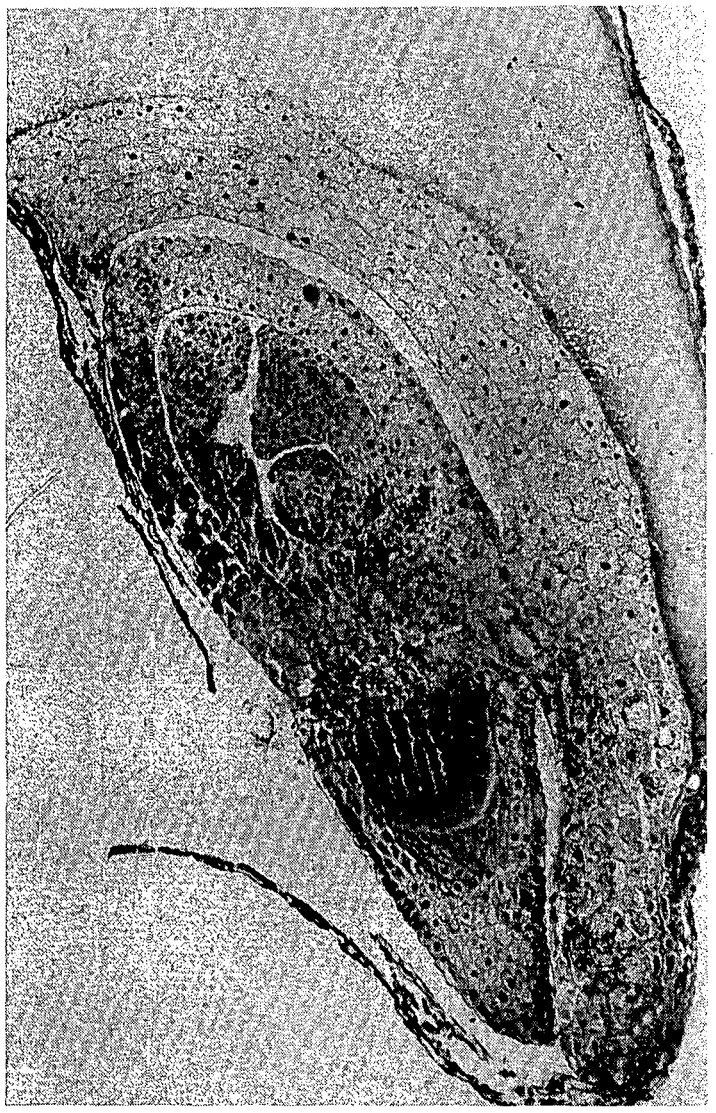

FIGURE 1 Cross section of a seed

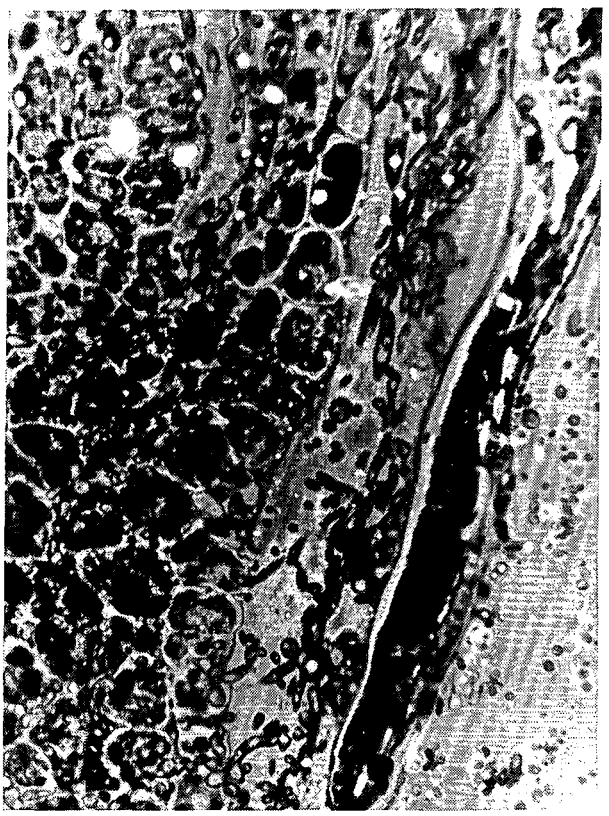

FIGURE 2 Hyphae under pericarp

Infection of seeds by the fungus

The embryo is situated close to the seed coat which, if slightly damaged, would probably enable the fungus to invade the seed (see Figure 1). There are many spaces between the dried embryonic structures which are ideal sites in which the fungus could grow and establish itself before invading the surrounding tissue.

Figures 2 and 3 show how the fungus moved from these spaces under the pericarp, between the embryonic leaf and coleoptile, and between the coleoptile and scutellum into the surrounding tissues.

FIGURE 3 Hyphae in scutellum

The fungal hyphae penetrated the scutellum to a greater extent than the embryo and this is thought to be related to the location of the food source. Van Dillewijn[16] noted that diastase (amylase), which is capable of mobilizing the food reserves of the nearby endosperm was present in the scutellum tissue. This could mean that the hyphae were able to obtain the nutrients required for growth more easily in the scutellum than in the embryo. The cells of the embryo have very small reserves of immediately available nutrients which could be utilized by the invading hyphae (Cazalet[4]).

Influence of hyphae at cellular level

In Figure 4 it is shown how the hyphae moved from the spaces between the structures to the embryonic axis and invaded the surrounding tissue. The confluence of the lipid bodies of the cells in the vicinity of the hyphae, is also shown. In the cells of the control seeds the lipid deposits remained small and compact (Figure 5).

Observations of the hyphae with a transmission electron microscope showed that they were active and in the dense cytoplasm contained ribosomes, glycogen and numerous mitochondria. The hyphae were found in the extraprotoplasmic space between the cells and they had also penetrated the cells.

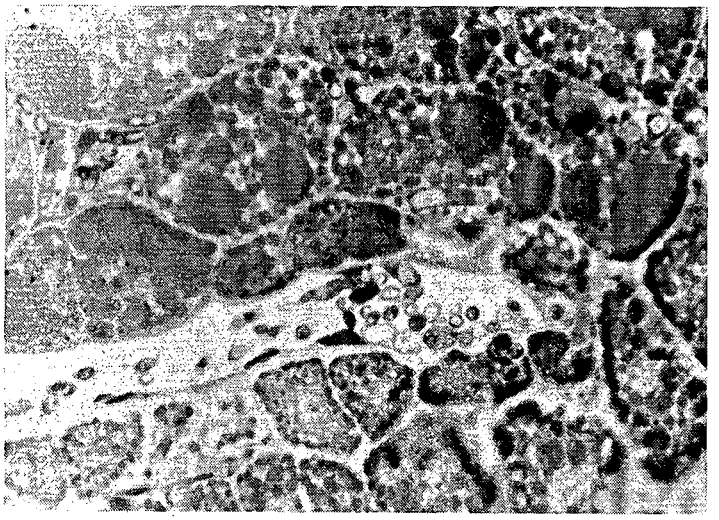

FIGURE 4 Hyphae at embryonic axis

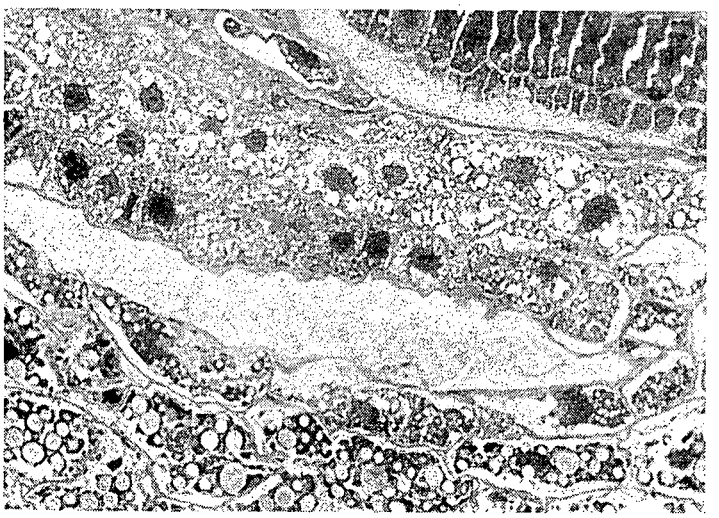

FIGURE 5 Control embryo - lipid bodies small

The ultrastructural changes noted in the seeds which had been infected with the fungus were attributed largely to the presence of the fungus and not to their ageing. Russell, Murray and Berjak[13] have listed deteriorative changes caused by the fungus as opposed to those which were age-related. Berjak[2] showed that deterioration due to physiological ageing of seeds in storage was dependent on temperature. Such deterioration was unlikely to have occurred in this case since the sugarcane seeds had been stored at -10°C for 15 months.

Some of the ultrastructural observations that were made and which were consistent with those reported by Anderson, Baker and Worthington[1], Murray[8] and Russell[12] were:

- the hyphae caused the confluence of the lipid bodies;
- the cell walls were disintegrating, giving them a patchy appearance;
- the plasmalemma pulled inwards from the cell wall; and
- large coalesced lipid bodies were extruded via the plasmalemma into the extraprotoplasmic space.

A large lipid body in the cell of the scutellum is visible in Figure 6, while in Figure 7 the patchy cell wall, the plasmalemma that was pulled back and the extruded lipid body are evident.

Possible control measures

The presence of a seed storage fungus (in this case a species of *Penicillium*) can be expected to have a deleterious effect on the viability of the stored seeds. Fungicides have little effect on the fungi of caryopses (Sanguino & Tokeski[15]) probably because of the low moisture content of the stored seeds. Externally applied fungicides would not be expected to have any effect on seed storage fungi already within the tissues of the dry seed. The only time that a fungicide might be beneficial would be at the time of harvesting when the moisture content is about 35%. However, it would have to be applied under a slight vacuum so that it penetrated the seed (Laing[7]) but this could prove to be deleterious to the seed.

The mechanical removal of the glumes surrounding the seeds is being considered. This is so that the plant breeder is able to count the seeds from each cross more easily and because the

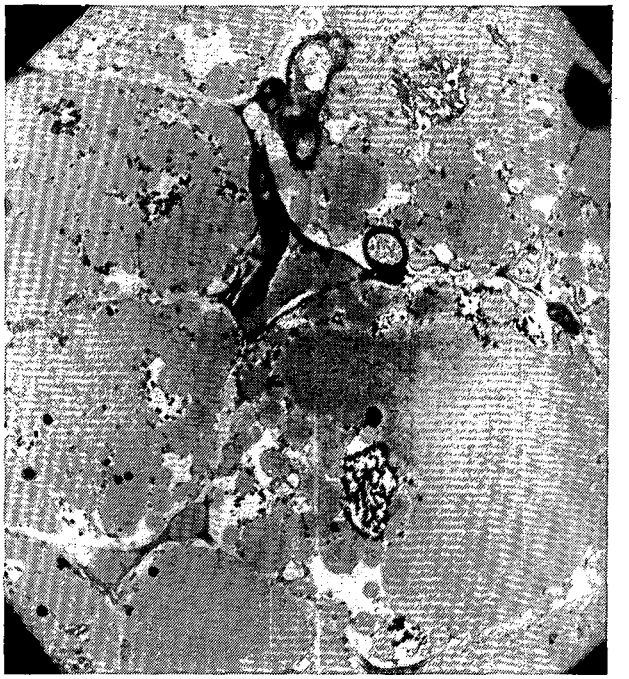

FIGURE 6 Hyphae in scutellum cells - large lipids

FIGURE 7 Hyphae between cells - 1. Large lipid
2. Patchy cell wall
3. Plasmalemma pulled away
4. Lipid extruded

glumes are probably ideal sites in which seed storage microflora can be harboured. The seed and embryo must not be damaged during this process because damage increases the rate of infestation by these fungi and seed viability is reduced (Roberts[11]).

Conclusion

The process of preparing seed for storage creates favourable conditions in which seed storage fungi can establish themselves. Seed hygiene is important so that the fungi are prevented from having a reservoir from which to invade the seeds. The mechanical removal of the glumes must therefore be carefully assessed because the slightest damage to the seed makes it susceptible to invasion by seed storage fungi. The position of the embryo also makes seeds vulnerable to damage.

The isolation of a seed storage fungus from stored sugarcane seeds is additional evidence of the universal occurrence of these micro-organisms in various types of air-dried seed. The effect that they have on the viability of stored sugarcane seeds can be seen from their penetration of the seeds and the ultrastructural changes which result.

Acknowledgements

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