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Technical Information Sheet No. 1

Prevention of mites in cultures

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Mites can be a problem in fungal culture collections. These small animals of the genera *Tyroglyphus* and *Tarsonemus* occur naturally in soil and almost any organic material and fungi are particularly susceptible to their attack. They can be seen by the naked eye as tiny white dots almost at the limit of vision, often about 0.25 mm in length. As they tend to bury themselves in the mycelium, they multiply rapidly and the first indication may be the deteriorated (moth-eaten) look of the cultures, with wandering trails of mini-colonies on uninoculated agar.

Mites can be brought into the laboratory on fresh plant material, decaying mouldy products, shoes, bodies of insects or even cultures from other laboratories. They thrive in moist warm conditions, so may often first appear in Europe in early summer or in new laboratories with new equipment, damp walls and new wood. Not only do they eat the cultures, but they also carry fungal spores and bacteria on their hairy bodies as they move from one culture to another.

Prevention

Prevention is preferable to having to control an outbreak. This can be summed up as general hygiene and screening of all cultures and material coming into the laboratory and destroying or isolating all infested material. Even so, separate handling, storage and quarantine of fresh material is desirable, as slightly infested cultures can develop heavy infestation if undetected at first examination. If it is necessary to handle infested material, the culture collection should be well isolated.

Control

Different workers have varying views on control. Some who are handling a quick turnover of infected material may not even regard a few mites as serious, but in a culture collection they spell disaster. A combination of prevention and action seems preferable. These can best be classed in several categories: (1) Hygiene, (2) Fumigation, (3) Mechanical and chemical barriers and (4) Protected storage.

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Hygiene

Hygiene coupled with quarantine is perhaps the best protection.

All work surfaces must be kept clean.

Cultures should be protected from airborne contamination.

Mites can be carried on workers' hands and clothing.

Cramped laboratory conditions and crowded arrangement of cultures increase the risk of infestation.

Work surfaces and benches should be washed regularly with acaricide. The acaricide is left for sufficient time to act (overnight) and washed off, preferably with alcohol. As some acaricides are toxic to man, protective gloves should be worn.

Acaricides used at the CMI have included Kelthane (Murphy Chemical Co.), Tedion V-18 (Middox Ltd), Chlorocides (Boots Farm Sales Ltd) and Actellic (ICI Ltd). Other acaricides available for agricultural and grain storage purposes are Murfit, Reldan and Dursban (Murphy Chemicals Ltd) and Satisfar (Sandoz Ltd).

As mites appear to become resistant, the acaricide should be changed from time to time.

Infected cultures should be removed immediately and sterilised if possible. All cultures in the immediate area should be checked and isolated.

Fumigation

Cultures may be stored in cupboards or boxes with acaricides either as preventative or short-term treatment. Camphor and paradichlorbenzene (PDB) have been used for this, but are now regarded as toxic, PDB also has some effect on fungal growth.

Drops of Kelthane and Crypo on culture plugs (Smith 1967) were effective.

Current safety practice would suggest that fumigation is no longer desirable.

Mechanical and Chemical Barriers

Many physical methods of prevention of infestation and spread have been tried.

Cultures are placed on a platform or tray surrounded by water, oil, petroleum jelly or other sticky material. Handling of cultures becomes unpleasant and protection is only from crawling mites.

Culture bottles or plates may be sealed, but it is necessary to allow growing cultures free respiration, so a means of sealing which is permeable to air is desirable.

Snyder and Hansen (1946) sealed bottles below screw caps or above the cotton wool plugs (well pushed down) with sterile cigarette papers using copper sulphate glue (20 g gelatine, 2 g copper sulphate, 100 ml water). The pores of the paper allowed respiration but prevented movement of mites, thus protecting clean cultures and isolating infested ones. Care is necessary to ensure the seal is effective.

Smith (1971) recommends the use of disposable plastic bottles with plastic caps which, when screwed down, still allow respiration but exclude mites.

Smith (1978) described a screw-lid closure with a hole sealed with Metricel.

Sealing Petri dishes and bottles with various modern plastic tapes often reduces spread but, by means of cracks or wrinkles, mites can eventually penetrate cultures stored for a long time.

Tight cotton wool plugs present a considerable barrier but are not completely effective, though a mite that has passed through cotton wool is often much cleaner.

Some workers treat plugs with mercuric chloride solution. This kills the mites but is poisonous and dangerous to handle even if a red dye is included to indicate its presence. It is also toxic to fungi.

Protected Storage

Many methods used for long-term storage of cultures in culture collections prevent infestation.

Mites do not infest cultures stored under mineral oil.

Cold storage at 4–8°C greatly reduces movement of mites but does not kill them, so they continue to multiply when the cultures are removed from the refrigerator.

Deep-freeze storage at approximately −20°C usually kills any mites present. Storage in a deep freeze for three or four days can be used prior to cleaning to treat infested material that is too valuable to discard.

Freeze-dried ampoules are sealed and totally protected.

Storage at ultra-low temperatures, for example in liquid nitrogen, gives total protection.

Cultures stored in silica gel are in vials or bottles with screwed-down caps, so are totally sealed.

References

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