MONITORING AND CONTROLLING MOULD MITES IN TISSUE CULTURE FACILITIES

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ABSTRACT

Mould mite numbers were successfully monitored in tissue culture laboratories using black tape where numbers of mites were high. Natural diamite and Parafilm "M" were the best barriers for preventing mould mite contamination. Treating surfaces with a miticide-containing paint (Artilin 3A) or bendiocarp prevented mites from entering containers holding food suitable for mould mites. Mites were not killed when sprayed with aerosols and 70% ethanol.

Keywords: barrier, mould mite, tissue culture, *Tyrophagus putrescentiae*.

INTRODUCTION

Contamination of *in vitro* cultures during micropropagation is often the result of fungal infections introduced by mites and insects. Blake (1988) found that 50% of tissue culture laboratories were contaminated with mites or insects at some time. While mites and thrips cause minimal damage to plant material, they may travel between culture vessels and tubs, carrying fungal spores and bacteria in and on their bodies (Jong 1987). One commonly transferred fungus, *Penicillium islandicum*, known as yellow rice fungus, is a human health hazard as it produces mycotoxins that cause liver lesions, cirrhosis and primary carcinoma. The fungus produces pigments that colour the substrates upon which it grows yellow or orange (M. Dance, pers. comm.).

When mould growth is caused by mite or insect contamination it is generally found growing across the entire surface of agar-filled tubs whereas a single mould spot is more likely to be due to poor aseptic techniques by operators (J. Seelye, pers. comm.). Outbreaks of mould mites (*Tyrophagus* spp.) usually result from the introduction of infested material (e.g. tubers or bulbs) into the laboratory. Numbers can increase 2000 fold in a single month (Anon. undated). However, permanent populations of mould mites can also occur in buildings. They may be found on stored kiwifruit and tubers, such as kumara. Although found in stored produce and dried meat they do not thrive on these diets (C.W. van Epenhuijsen, unpubl. data).

Most mites are *Tyrophagus* spp., such as *T. perniciosus* (B. Strolka, pers. comm.), although cyclamen mites (*Phytonemus pallidus* spp.) (George 2000) and *Siteroptes avenae* (Staal 1989) have been found in cultures as well. The most common mites in New Zealand are *Tyrophagus putrescentiae,* although *T. neiswanderi* (M. O'Donnell, pers. comm.) and *Siteroptes avenae* (N. Martin, pers. comm.) have been found in culture rooms as well as a predatory mite (*Amblyseius* sp.) in the microtubers of calla (*Zantedeschia* spp.) (M. O'Donnell, pers. comm.).

Mite control is generally achieved by adopting good hygiene measures, using mechanical and chemical barriers, fumigation, and protected storage facilities (Onions 1990). Other preventative methods include freezing all lab coats regularly and using a chemically treated doormat. Heating equipment to 40ºC for 48 h completely killed mites (Pulpan & Verner 1959). Zdarkova & Voracek (1993) found 60 min at -15ºC produced 100% mite mortality while 30 min at 55ºC or higher gave 100% mortality. Low pressure (95 mm Hg) gave 100% mite mortality (Zdarkova & Voracek 1993), and a vacuum of 190 mm Hg provided some protection to food against mites when applied at -15ºC or lower. Smith & Onions (1999) found storage at 4–8ºC reduced the activity of mites.

However, the mites became active again as soon as the temperature increased. A temperature of -20ºC for at least 3 days gave better control (Smith & Onions 1999).

For long-term cultures it is sometimes advised to dip the cotton wool vent into a miticide. However, this technique does not work with wide-mouth plastic tissue culture tubs (290 ml), which have snap-on lids for ventilation. Another method is to place cultures on a platform that is sitting in a thin layer of mineral or cottonseed oil (Davidson & Lyon 1979). Vaseline and silicone grease can be used but normally they are too labour-intensive to apply to individual tubs and are unpleasant to use. Parafilm "M" and cigarette papers (Snyder & Hansen 1946) are sometimes recommended as a barrier (Remakanthan 2003). However, mites have been reported to go through the film (P.S. Stewart, pers. comm.). Dipping leaves in a sodium hypochlorite solution before culturing can disinfest them of two-spotted spider mites (*Tetranychus urticae*) (Hadi et al. 1998).

Fungicides, such as fenbutatin oxide, or chemicals from the imidazol family can be included in the culture medium for some plants (Pype 1996; Werbrouck et al. 1999), but this does not prevent fungal contamination being introduced by mites. The insecticide acephate, Orthene®, when added to the medium, killed *Allothrips* spp. (Klocke & Myers 1984). A miticide with active ingredient pirimiphos-methyl is advised for use on workbenches (Smith & Onions 1999).

This paper describes a series of experiments aimed at monitoring mite populations in tissue culture laboratories and testing methods for excluding or eliminating mould mites (*Tetranychys putrescentiae*) from these areas.

MATERIALS AND METHODS

Rearing mites

A mould mite population was started using mites collected from contaminated tissue culture tubs of calla plantlets. The mites were reared in a 50:50 mix of yeast and rat food (Sharpes Animal Foods Diet 86). A pinch (approximately 0.02 g of diet infested with mould mites) was placed with approximately 10 g clean diet in 70 ml jars with 3 mm vents covered in fine mesh. The jars were cultured in an airtight container with moist paper towels at 27ºC and 70-80% relative humidity (RH).

The mite-infested diet was stirred with a needle and poured through a kitchen sieve (approximately 850 μm). The sieved material (a fine dust) was stuck on 50 mm wide black polyethylene self-adhesive tape (Sealed Air Insultape) by rolling a 200 g metal piece of pipe over it. All live mites in a number of randomly chosen 7 mm circles (cut out of a thin aluminium sheet overlay) were counted.

All trials were assessed 24 h after treatment for numbers of live mites as dead mites shrivel up within a day and cannot be distinguished in the diet.

Trapping and monitoring

A mould mite trap, consisting of black-painted plastic strips (300 x 100 mm) that were coated with Tangle-Trap® brush-on formula (The Tanglefoot Company, Grand Rapids, Michigan), was tested. The strip traps were placed on the floor with the sticky side up and in the racks of two tissue culture rooms, in a storage room and in the mite rearing unit. A mite lure, consisting of a 5 g sample of mould mite diet placed in a 15 ml glass container, was also tested. Two traps and lures per room were checked every 3 weeks under the microscope for more than 12 months for the presence of mites. The position of the traps was changed each time. Window traps (AgriSense-BCS Ltd) were tested for their ability to catch mites.

Barrier tests

Potential barriers for mites were tested using two covered acrylic cages (690 x 400 x 410 mm) held at 27ºC and 70% RH. The cages contained glass jars (50 ml, diameter 50 mm and filled with 6 ml diet) that had been given one of ten treatments. The cages had a Tangle-Trap® band around the circumference of the cage to prevent mites reaching the lid and dropping into the jars from above. There were 6 replicate jars of each treatment except the untreated control (7 jars), fluon (5 jars) and diamite (5 jars), which were randomly placed throughout the two cages.

The treatments were:

- untreated control
- Tangle-Trap®
- Vaseline (Petroleum jelly BP, Amcam Chemists Marketing Ltd) applied as a 15 mm band brushed on to the bottom of the jar
- Fluon (Fluon AD-1, an aqueous dispersion of polytetrafluoethylene) applied as a 15 mm band brushed on to the bottom of the jar
- Glycerol BP poured into an 85 mm Petri dish in a thin layer and the jar placed in the Petri dish
- kerosene (100%) poured into an 85 mm Petri dish in a thin layer and the jar placed in the Petri dish
- machinery oil (3-in-one, Home Products NZ Ltd) poured into an 85 mm Petri dish in a thin layer and the jar placed in the Petri dish
- natural diamite (DPL 13, ex New Zealand), a diatomaceous earth sieved through 500 μm and spread as a thin layer into an 85 mm Petri dish, and the jar placed in the Petri dish
- Parafilm "M" (American National Can™), a laboratory plastic wrap, stretched over the top of loosely screwed Bakelite lids on top of the glass jar
- Artilin 3A, a miticide-containing paint, brushed on to undercoated wooden disks (diameter 80 mm).

Eighteen small lids (diameter 45 mm) with infested diet were placed at random between the treated jars in each cage. A glass jar with a paper wick filled with water kept the humidity in the cages higher than 78%. All mites were counted in 6 randomly chosen circles of 7 mm diameter of the diet per jar after 5 weeks.

Tunnel trial

Plastic pipes, 350 mm long with an internal diameter of 17 mm, were cut open longitudinally and covered on the inside with the pesticides artilin, fentabutin (Ficam® W) or left untreated. Ficam® W is a carbamate containing 800 g/kg bendiocarb. It is a registered public health miticide in New Zealand. There were 6 pipes for each pesticide and the control. The slits in the pipes were closed with tape.

One end of each pipe was attached with a sealant (No More Gaps, Selleys Pty Ltd) at random into holes cut in the side of a 5 litre plastic bucket. Pipes were 40 mm from each other and 50 mm from the bottom radiating out horizontally. At the other end of the pipe, outside of the bucket, a 70 ml specimen jar was attached with the sealant, filled with 15 ml of clean diet and placed at 27ºC. A heavily infested diet with mites was placed in the bucket with some damp paper towel and the bucket was closed. The diet in the specimen jar was sieved onto a black tape 18 days later and the number of mites in four circles per specimen jar was counted. The number of live mites on the total area of the black tape (approximately 50 cm^2) was counted if no mites were found in the four randomly chosen circles.

Overhead spraying

A highly infested mite diet (approximately 1.5 g) was sieved through a small kitchen sieve into a Petri dish and sprayed with one of three aerosols, 70% ethanol or a distilled water mist control for 4 seconds. Ethanol and water were sprayed with a low-pressure household sprayer. The three aerosols were:

- CheckMite, a herbal aerosol for the control of indoor allergens from *Dermatophagoides* spp.
- 'Raid fast kill odourless hypoallergenic' containing 1.54 g/kg tetramethrin, 0.34 g/kg d-phenothrin and 0.34 g/kg d-allethrin
- 'Raid multipurpose insect killer' containing 4.0 g/kg tetramethrin and 1.0 g/kg permethrin

Water-sensitive paper (Spraying Systems Co., USA) laid between the Petri dishes was used to verify that the cover applied over the treatments was the same. Petri dishes were covered for 15 min after treatment; thereafter the diet was poured into a 70 ml jar, closed and kept at 27ºC for one day. The diet was then sieved onto a black tape as

described previously. Four replicates of four areas of randomly chosen circles on the black tape were assessed for live mites.

RESULTS

Trapping and monitoring

When mould mites escaped from the containers in the rearing unit they were caught on the Tangle-Trap® coated strips or lured into baits. No mites were found in the traps in the cell culture rooms. Dried bodies of mites and other insects were found on the strips in the cell culture rooms. Mites were caught on tape stuck on the bench where a contaminated tuber had been laid. Tape samples taken at random and assessed under a microscope gave a good indication of whether mites (or dried skins) were present in the rooms. Window traps (AgriSense-BCS Ltd) also successfully detected mites, but the brown background made it too difficult to locate them.

Barriers

There were significantly ($P<0.05$) fewer mites in jars that were 'protected' with Parafilm "M", natural diamite, machinery oil and Tangle-Trap® compared to levels found in the control jars (Fig. 1). Dead mites were found on the treated disks of artilin but the barrier was probably not wide enough to kill all mites. The layer of kerosene crystallised quickly and mites were able to walk over it to the diets.

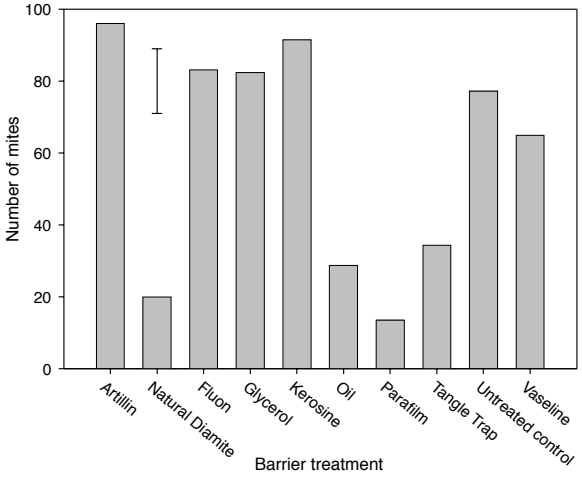

FIGURE 1: The number of mites entering glass jars given various barrier treatments. The bar indicating the LSD (P=0.05) is 17.98 units.

Tunnel trial

The number of live mites in the untreated control was very high (Table 1). No mites were found in the artilin treatment. A few mites were found in the Ficam®W when extra counts were made. Dead mites were found as brown clusters of dust in the treated pipes.

Overhead spraying

There was no evidence of a difference in efficacy between any of the three aerosols or ethanol treatments and the water mist control (data not shown).

TABLE 1: Number of mites found on 24 disk areas from samples taken from the jars at the end of plastic pipes.

Treatment	Number of mites
Untreated	88.17
Ficam®W (0.3%)	0.00 (0.67)[1]
Artilin 3A	0.00

[1]Mites found in the remaining diet on the tape.

DISCUSSION

Mites are a problem in tissue culture laboratories because they are a common source of contamination and their numbers can increase very rapidly. The trapping and monitoring techniques described in this paper worked well if mite numbers were high. Sticky black tape on the benches successfully monitored mite infestations of calla tubers, catching up to 10 mites on a 50 cm^2 tape. Bait (mite diet) and Tangle-Trap® covered strips can detect mites. The sprays tested were not effective. Results suggest that hygiene measures and establishing barriers to mite infestation are the most promising methods of control.

These trials suggest that coating laboratory floors with Ficam or painting walls with artilin (which kills both mites and larvae) may provide effective control for up to 3 years (C. Ricard, pers. comm.). Alternatively, putting the legs of benches in diatomite may provide some control. However, it is important that the ability to clean floors is retained because mites breed in dust. Our observations suggest that commonly recommended barriers, such as water, glycerol and eucalyptus paste, are not effective.

Barriers and other measures used to prevent mites from entering tubs were not 100% effective. Mould mite nymphs can pass through very small holes; early stages passed through 120/120 wire mesh and 3M Micropore™ Tape, cigarette and pipette filters, loose foam and cottonwool closures (C.W. van Epenhuijsen, unpubl. data). Vented vessels from Gaooze (Korean Scientific Technique Industry) using the 3M Micropore™ did not exclude mites. Mites also may traverse taped areas by passing through the channel where the tape overlaps. A similar problem can occur with plastic cling film as the surface of the flasks might be uneven allowing mites to enter.

Maintaining high hygiene standards is important because plant material is the main source of contamination in culture rooms. Hot water treatment of infested tubers has produced disinfested plant material, but microwaving is ineffective since the agar and plastic tubs melt before mites are killed (C.W. van Epenhuijsen, unpubl. data).

In conclusion, many barriers and control methods are ineffective. It is recommend that before plant material is placed in tissue culture rooms it is disinfested. In addition, walls in tissue culture rooms could be painted with artlilin paint.

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