NY234
Control of *Legionella spp* particularly *legionella longbeachae* in potting mixes

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RESEARCH FINDINGS
EXECUTIVE SUMMARY

In response to community concern over possible links between Legionnaire's Disease and bagged potting mix, the Nursery Industry of Australia and the Horticultural Research and Development Corporation initiated a four year research program to investigate the ecology, infection process and control of Legionella species in composted organic matter.

Between 1992 and 1995, at a cost to the industry and HRDC of over $240,000, Dr Trevor Steele of the Institute of Medical and Veterinary Science in Adelaide, SA conducted an extensive investigation into the bacterium and its possible links with composted organic material.

This Executive Summary provides the recommendations and main finding of that research.

RECOMMENDATIONS

Gardening is a healthy activity (Bell Digham, 1996). However, like all outdoor activities there are some potential risks and it is always advisable to wash your hands with soap and water after contact with organic materials.

In this context, the following recommendations are made:

- State Health Departments, the National Health and Medical Research Council and the Nursery Industry Association of Australia continue to provide the public with information about the possible hazards of handling all organic matter, including potting mixes and composted products.

- With the support of the Nursery Industry Association of Australia, the relevant health authorities ensure that all bagged organic matter carry an appropriate label with product handling advice.

- With the support of the Nursery Industry Association of Australia, the relevant health authorities ensure that appropriate information on product handling is displayed wherever bulk organic materials are processed or sold.

- The Nursery Industry Association of Australia maintain a Working Party to provide ongoing liaison and management of these issues.

SUMMARY OF MAJOR FINDINGS

Legionella longbeachae serogroup 1 is one of 16 species of *Legionella* that cause infection in humans. *L. longbeachae* infection is not limited to gardeners but they have a much higher incidence of infection than non-gardeners.

There is no known way of preventing *L. longbeachae* from multiplying in composting bark, sawdusts, finished potting mixes or in other plant or vegetable materials composted by home gardeners as determined by laboratory or field experiments. This study did provide much information about the ecology of legionellas in composted materials and will allow compost users and the Nursery industry to make informed decisions about potting mixes and other marketed products found to contain legionellas.

Although not all batches of potting mix made from composted waste wood products or composted manures contain detectable numbers of legionellas, it should be assumed that they are present in all composts. At least 15 species or distinct serogroups of species of *Legionella* have been found in bark based potting mixes. Of the 16 species known to cause infection in humans, 5 are very common in potting mixes and in other composts. These composted materials now from the largest known reservoir of *L. longbeachae* serogroup 1 in the Australian environment. Composted products have not been investigated as a possible source of infection by *Legionella* species other than *L. longbeachae* or by other microorganisms.

Many *Legionella* species are ubiquitous in the environment but *L. longbeachae* is only common in composted materials. It was not found in leaf litter or in ordinary soils. *Legionella* species other than *L. longbeachae* were isolated from 21% of tilled farm or garden soils. Of 27 freshly milled pine bark or sawdust samples examined since 1991, 12 grew a *Legionella* species of some

type. In this study, commenced in 1992, we examined 13 samples of milled bark or sawdust, all except one of which were sent by potting mix manufacturers. Of these, 5 of 13 (38%) contained *L. longbeachae* and an additional one contained an unidentified *Legionella* species. Positive samples came from major potting mix producers in South Australia, Victoria and Queensland. DNA amplification studies have identified gene sequences characteristic of members of the Genus *Legionella* in sawdust obtained from pine trees at the time of felling but legionellas were not grown from this sawdust on laboratory media. However, due to the difficulty of detection, isolation and identification of *Legionella* species, particularly *L. longbeachae,* these results cannot be considered conclusive.

Genetic typing studies of *L. longbeachae* strains from humans and potting mixes using fragments of DNA as probes and alloenzyme analysis have shown that these are closely related but small differences in the genetic make-up of strains can be detected using appropriate probes. The strains found in some humans with infection and in the soilless potting mixes they used, when examined with highly discriminatory probes and by pulse field gel electrophoresis, have appeared to be identical.

The occurrence of *L. longbeachae* in composted products is not necessarily due to contamination of products by legionellas present on manufacturing sites although it is probable that cross contamination of varying degrees does occur on most manufacturing sites. It would be virtually impossible to prevent cross contamination on even the most hygienic site. *L. longbeachae* and several other species developed to readily detectable levels in pine bark purchased from a remote mill and composted for 12 weeks on a recently levelled suburban home site that had never been used to make composted products.

In longitudinal studies of composting bark, *L. longbeachae* grew rapidly in the outer layers of an experimental batch of composting bark while high temperatures in the centre of the pile killed them. Maximum populations of *L. longbeachae* were reached within 2 weeks in one closely followed batch. Water content and temperature were two important factors in determining the numbers of legionellas present at completion of composting and in determining if multiplication occurred in bagged mix. In studies of three batches of potting mixes made in February, June and September, multiplication of legionellas including *L. longbeachae* occurred within bags of mix stored for 6 weeks at room temperature made from two batches that did not contain detectable legionellas during the composting process or at the time of bagging. The third batch contained 2.8 x 10 *L. longbeachae* per gram at the time of bagging. Legionella numbers remained stable in the bags from the third batch for several months before declining to low levels. We have observed similar increases and decreases in Legionella populations in serially examined bags of mix from other batches. These findings indicate that negative results on laboratory testing before or after bagging cannot be used to exclude the possibility of legionellas being present in potting mixes. Storage in bags of wetted potting mixfor several weeks at room temperature frequently resulted in substantial multiplication of L. longbeachae even when initial levels were very low. Wetted potting mix should be used immediately and not stored in a wetted state.

While studies of long term survival of legionellas were conducted on bagged composted organic matter, *L. longbeachae* may be widespread in the environment and care needs to be taken when handling or working with any organic matter.

L. longbeachae and other species appear to multiply entirely or predominantly within susceptible free-living amoebas present in composting bark or plant wastes. However most composts we examined contained some bacteria, fungi and predatory protozoa that inhibited or killed legionellas. These other organisms, which occur in populations of 10^5 - 10^9 per gram, may have controlled the final numbers of legionellas that grew in composted bark but did not prevent legionellas from multiplying to levels of between 10^3 and 10^5 CFU per gram. Protozoa require their food bacteria to be present in high numbers, > 10^7 per gram, and in the natural environment seldom reduce food bacteria to less than 10^6 CFU per gram.

The reason why *L. longbeachae* occurs mainly in composted bark and sawdusts and *L. pneumophila* in bulk composted vegetable matter is unknown. It may be related to the prevalence of the various *Legionella* species in the material being composted or to the environment in which the composting is done. The nature of the material being composted and its microbial content may also be important. In laboratory conditions *L. pneumophila* and *L. bozemanii* are able to grow in amoebae at temperatures 2 to 3°C higher than *L. longbeachae* can. However it is unlikely that temperature was a major determinant of the predominant species in composts. *L. pneumophila* multiplied in composted bark and *L. longbeachae* in home made composts provided these were steamed before being seeded with small numbers of these organisms.

More than 200 inhibitory organisms, a range of composts, soils and several antimicrobial agents (gentamicin, polymyxin, actidione and amphotericin) were tested to see if they inhibited growth of *L. longbeachae* in potting mix but none prevented it from growing to normal levels in laboratory experiments (unpublished observations, TW Steele).

Ferrous, copper and zinc sulfates in low to high concentrations did not reliably promote or inhibit the multiplication of legionellas in iron deficient or iron sufficient composted bark. Copper at 500 micrograms (μg) per gram, a phytotoxic level, promoted growth of *L. longbeachae* in some experiments.

In laboratory experiments the only successful methods of killing legionellas were:

1) by heating small bags (5L) of potting mixes at 60°C for more than 12 hours;
2) by steaming loose mix for 15-45 minutes; or 3) by microwaving 2.5 Kg bags for 5 minutes. The temperature of 60°C was chosen because this was the mean maximum temperature in our experimental composting bark and is a commonly used pasteurisation temperature. Temperatures of 70 to 80°C would be more effective but would be more costly to use. We did not have facilities to test at these temperatures. The commercial fumigation of potting mix with methyl bromide effectively killed legionellas but other organisms survived this treatment. Other fumigating agents were not examined.

Heated potting mixes were highly susceptible to recolonisation by *L. longbeachae* and *L. pneumophila* in laboratory experiments. *Legionella* species other than *L. longbeachae* recolonised 30% of pots containing steam heated mix placed outdoors for 12 weeks in a limited field study. Potting mix fumigated with methyl bromide did not regrow legionellas when stored in the laboratory. However, Australia is a signatory to an international agreement to phase out the use of methyl bromide by 2005. Some years ago regrowth of *L. longbeachae* to much higher levels than the control was observed in potting mix treated with disinfectant which had initially appeared to control the organism (personal communication, David Nichols, Debco).

Since recolonisation of heat treated and of disinfected composts can result in higher levels of Legionella species than are found in untreated controls and these treatments can lead to high populations of *L. longbeachae* over time, the value of these non-specific treatments in commercial practice needs to be carefully assessed.

L. longbeachae and other *Legionella* species survived for a year or more in closed 22 litre bags of potting mix stored at room temperature in the laboratory. Survival times were reduced by placing bags at 36°C or by using the potting mix to grow plants outdoors. However the mix stored at 36°C still contained *L. longbeachae* at 4 months as did that used in pot plants and 1 of 12 pots grew *L. longbeachae* at 1 year.

The way in which individuals acquire their infection with *L. longbeachae* is unknown. This is in contrast to the level of understanding for *Legionella pneumophila*. We found that this organism will survive on hands contaminated with potting mix for at least 30 minutes after handling the mix and therefore could be accidentally ingested. Air sampling studies did not detect *L. longbeachae* or other legionellas in the air when:

a) each of 14 bags of mix containing legionellas was first opened (50 litres of air sampled at each bag, an average adult breaths 0.5 litres of air per breath);

b) when suspended pots were watered over a concrete floor in a confined space (total sample 1125 litres); or

c) when a fan ventilated cool house in a commercial nursery was tested on 3 occasions (total sample 1600 litres), even though the bags or pots at all sites contained potting mix with high populations of *L. longbeachae*.

These tests suggest that airborne contamination, if it occurs, is probably very low.

CONCLUSION

Legionella species occur naturally and are widespread in the environment. In this study, we isolated Legionella species from garden soils, home made compost, plant and vegetable waste and from animal manures composted in bulk. It remains a strong possibility that some species of Legionella are closely associated with trees or plants. The reason why *L. longbeachae* occurs mainly in composted bark and sawdusts and *L. pneumophila* in bulk composted vegetable matter is unknown.

The findings of this report indicate that laboratory testing before or after bagging cannot be used to indicate that legionellas are definitely not present in a potting mix. If non-specific treatments such as heating or disinfection were applied to kill legionellas, it would be extremely difficult to prevent cross contamination of treated material even on the most hygienic sites. Cross contamination in laboratory experiments often led to higher populations of legionella than in untreated controls.

The way in which individuals acquire their infection with *L. longbeachae* is unknown. This is in contrast to the suggested mechanisms for *L. pneumophila.* We found that this organism will survive on hands contaminated with potting mix for at least 30 minutes after handling the mix but not for 1 hour. It was readily washed off with soap and water.

Studies have shown that gardening is a healthy activity (Bell Dignam, 1996). Yet like all outdoor activities there is a low risk of injury or infection. Hand

washing with soap and water after handling composts or any organic matter is a strongly recommended practice.

References:

Bell Dignam, 1996. A report from a national survey of 601 gardeners and non-gardeners who own detached or semi-detached dwellings, conducted in August 1996. Australian Horticultural Corporation, Sydney.