Modelling mites, moulds and mushroom yields in the Australian Mushroom Industry

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Abstract: Efficient sustainable mushroom production is dependent on a consistent production of compost that needs to be of a high quality. Unwanted mites and soil fungi, especially *Trichoderma* species, can reduce yields so it is important to be able to manage this situation. The relationship between mites, fungi and mushroom yields are complex and a Bayesian Belief Network (Norsys Pty Ltd) (BBN) was used to try to resolve these complexities. Two BBN (Figure 1) models were developed using published information on the main species of mites, *Trichoderma*. Two independent datasets were used:

1. Data from growing experiments; and
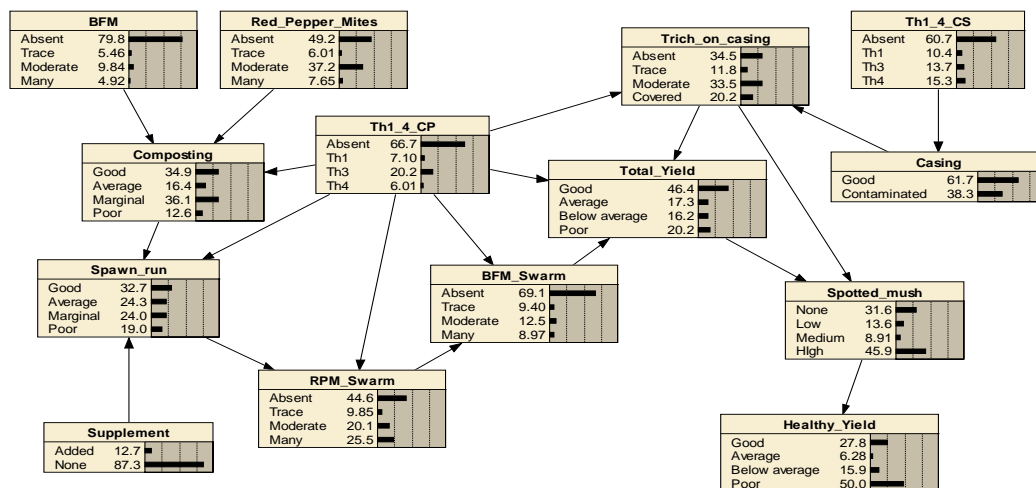2. Data from farm surveys

Figure 1. Bayesian Belief Network with 14 nodes describing the influence of mites and *Trichoderma* species on mushroom yields in Australia, based on published data from growing experiments.

A sensitivity analysis module from the BBN software was used to determine the relative importance of each of the 14 BBN nodes in relation to healthy yields (Healthy_Yield) and spotted mush (Spotted_mush). Results for Healty_Yield were similar for the two datasets (growing experiments and farm surveys); the six nodes (Spotted_mush, Trich_on_casing, Total_yield, Casing, Th1_4_CS, Th1_4_CP) accounted for 85% of the variance in Healthy_Yield for the growing experiment data and 55% of the variance for the farm survey data. The results for Spotted_mush were similar for the two datasets; the six nodes (Healthy_Yield, Trich_on_casing, Total_Yield, Casing, Th1_4_CS, Th1_4_CP) accounted for 89% of the variance in Healthy_Yield and the same six nodes accounted for 68% of the variance in Spotted_mush.

Both Spotted_mush and Trich_on_casing reflect how well the *Trichoderma* species were established. The results are discussed in relation to pest and disease management in the Australian mushroom industry.

Keywords: Mushroom mites, mushroom yields, sustainable management of pests and pathogens

1. INTRODUCTION

Worldwide mushrooms are grown in specially constructed rooms. Efficient mushroom production is dependent on consistent production of a high quality, selective substrate, termed mushroom compost. Despite this, the industry still has pest and disease problems, some of which are brought into the system in the compost of raw materials (Clift & Terras 1993, 1995, Nair et al. 1993, 1994).

Until 1985, *Trichoderma* species in mushroom beds were considered a minor, composting problem (Grogan 2006, Samuels et al. 2002), but in that year a major epidemic of green mould associated with severe yield losses started throughout Ireland (Seaby 1996), spreading to England and Europe. Seaby (1996) divided the *Trichoderma harzianum* isolates he was collecting into three, TH1, Th2 and Th3, termed the *T harzianum* complex. In 1993 a similar pathogen developed in North America and both pathogens were described as *T aggressivum europaeum* (Th2) and *T aggressivum aggressivum* (Th4) respectively (Samuels et al. 2002). The latter subspecies has been found on one farm in Australia in 2006 (Khan et al. 2008), where it was also associated with yield losses. Grogan (2006) considers *T atroviride* (Th3) to be the second most serious compost colonizing green mould, but Samuels et al. (2002) believe it to be a minor problem. Both authors consider *T harzianum* (Th1) to be a minor problem in compost. Results presented by Nair et al. (1993b) are consistent with Grogan (2006) that high incidence of Th3 is associated with high yield losses, but low incidence of Th1 is not of concern.

The study of Clift and Terras (1995) reported the occurrence of two species of mushroom mite, red pepper mite, *Siteroptes mesembrinae* (**RPM**) and bacteria feeding mite, *Histiostoma feroniarum* (**BFM**) in compost raw materials and also in experimental crops grown in a research facility. They proposed that the occurrence of both mite species was associated with major yield losses. Terras and White (1995) described the close association between RPM and the green mould fungus, *Trichoderma* species. Nair et al. (1993a, b) presented findings from a survey of pests and diseases on commercial mushroom farms near Sydney, including incidence of RPM, BFM and *Trichoderma harzianum* complex. They reported that RPM was invariably found with *Trichoderma*, but the reverse was not true. Reference samples of *T. harzianum* complex were freeze dried and subsequently identified using DNA sequencing (Khan et al. 2008), indicating the main species were Th3 and Th1. Fletcher (1994) in an independent survey reported these two species as the most common *Trichoderma* species in the Australian mushroom industry.

Clift and Terras (1995) found the occurrence of RPM and BFM in compost raw materials was very variable and was usually associated with damp of straw. The proportion of damp straw bales as delivered to compost yards was variable, ranging from nil to 20% (Clift and Terras 1995). Damp straw bales are more difficult to compost (Nair et al. 1994) and are more likely to result in poorly prepared compost. Grogan (2006) and Seaby (1996) both comment that Th1 and Th3 can be readily found in compost raw materials, especially straw, but Th2 and Th4 have only been found inside mushroom farms. This implies that the presence of Th1 or Th3 indicate composting problems, whereas the presence of Th2 or Th4 indicates a hygiene or contamination problem. The presence of swarming RPM or BFM indicates a composting problem (Clift and Terras 1995).

The effects on yield depend on which species of *Trichoderma* is present, but RPM appear to reproduce on all of these, so may not be an indicator of which species of green mould is present . The presence of swarming mites and incidence of Th1, Th3 or Th4 in relation to both compost preparation and mushroom yield is complex and we decided to use a Bayesian Belief Network (BBN) (Clift and Shamshad 2007) to assist in understanding this relationship. We present the BBN developed, describe the data used to validate the BBN and discuss the results in the Australian context.

2. DATASETS

Two independent datasets were used to test the model:

- Details of experimental crops grown using commercial prepared compost in a research unit at either NSW Agriculture facilities at Rydalmere, NSW, 1988 to 1995 or University of Sydney, 2002-2009, termed growing experiments.

- Pest and disease survey 1989 to 1992 in the Sydney Basin, reported in Nair et al. (1993a, b), a pest and disease management project, 2002 to 2008, plus farm visits, all termed farm survey data

Both datasets include incidence of RPM, BFM, Th1, Th3, Th4, plus comments on compost quality, condition of compost raw materials, conduct of composting process, estimates of infected mushrooms and farm

hygiene. In the field data, the comments are usually inferred, based on independent assessment of the farm, compost yard for Phase I and the facility undertaking Phase II. The growing experiments often specifically involved infection by Th1, Th3 or Th4 of either the mushroom inoculum or the casing layer. Incidence of RPM and BFM were closely monitored in these experiments. Figure 2 shows swarming RPM and Th3 sporulating on the casing.

Figure 2. Swarming RPM and sporulating Th3 in a growing experiment.

3. BAYESIAN BELIEF NETWORK

3.1.   Overview of Network

The package Netica® was selected to construct the BBN (Norsys Software Corp 1997). The BBN was developed using published information regarding mushroom composting, the occurrence of mites and green moulds, the growth and colonisation of compost by both green mould and mushroom mycelium and the effects on mushroom yields. Comments by Samuels et al. (2002) suggested that as TH1 and Th3 were common in straw, whereas Th2 and Th4 were present within the farm. Further, that infection could occur in different parts of the process and hence different parts of the network. Grogan (2006) reported that Th2, Th3 and Th4 directly reduced yield, as well as establish on the casing, then causing cap spotting. Grogan (2006) also reported Th1 as occurring mainly on the casing. It was therefore decided to have separate separate nodes for infection during composting and casing and for extent of *Trichoderma* growth on the casing.

Nair et al. (1993a) reported that farms with poor compost preparation and used supplement in the compost often experience Th1 or Th3, as well as swarming RPM, with associated yield problems. Hence use of supplement was included in the model. Seaby (1996), Samuels et al. (2002) and Grogan (2006) all considered contamination of the casing layer with any of the Th species to be important, so a node for casing contamination was included. Nodes for RPM and BFM in compost materials and for swarming mites were included.

Initially all aspects of composting and mushroom cultivation as well as each species of *Trichoderma* were incorporated into the BBN, but the outcomes were not satisfactory in that no major factors were indicated and overall about six nodes accounted for less than 20% of the variance. Phase I and Phase II operations were condensed into a single node, spawning and spawnrun condensed to one node and all *Trichoderma* species were condensed into one node. However, two times of infection, the first in compost materials or during spawning were retained as two nodes and a node for *Trichoderma* growth on the casing was included.

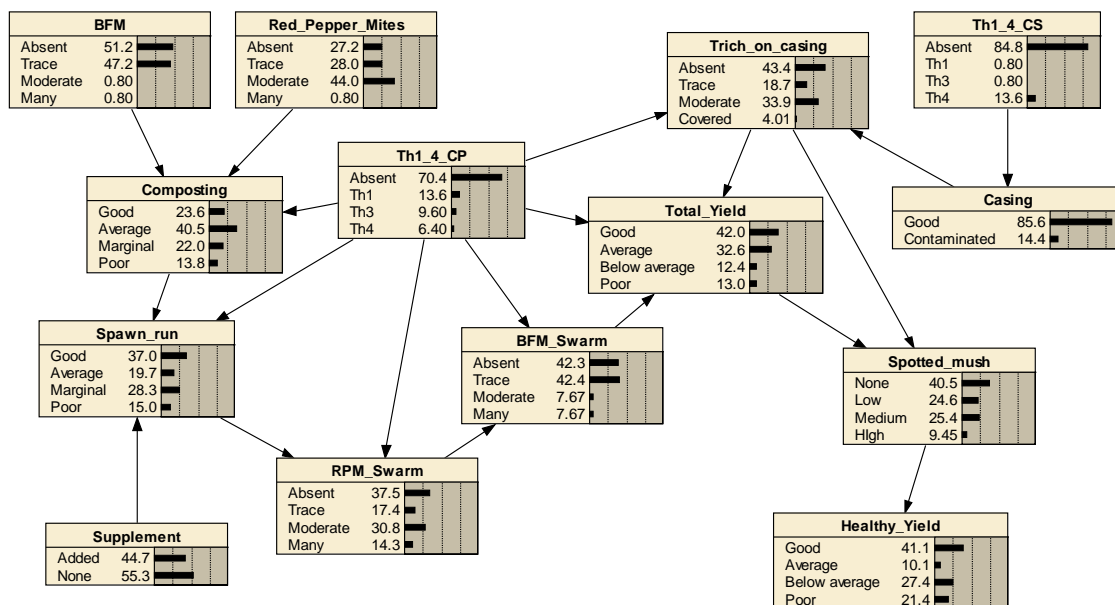The 14 node BBN finally developed is shown in Figures 1 and 3.

BFM
Absent	51.2	
Trace	47.2	
Moderate	0.80	
Many	0.80	

Red_Pepper_Mites
Absent	27.2	
Trace	28.0	
Moderate	44.0	
Many	0.80	

Trich_on_casing
Absent	43.4	
Trace	18.7	
Moderate	33.9	
Covered	4.01	

Th1_4_CS
Absent	84.8	
Th1	0.80	
Th3	0.80	
Th4	13.6	

Th1_4_CP
Absent	70.4	
Th1	13.6	
Th3	9.60	
Th4	6.40	

Composting
Good	23.6	
Average	40.5	
Marginal	22.0	
Poor	13.8	

Total_Yield
Good	42.0	
Average	32.6	
Below average	12.4	
Poor	13.0	

Casing
| Good | 85.6 | |
| Contaminated | 14.4 | |

Spawn_run
Good	37.0	
Average	19.7	
Marginal	28.3	
Poor	15.0	

BFM_Swarm
Absent	42.3	
Trace	42.4	
Moderate	7.67	
Many	7.67	

Spotted_mush
None	40.5	
Low	24.6	
Medium	25.4	
High	9.45	

RPM_Swarm
Absent	37.5	
Trace	17.4	
Moderate	30.8	
Many	14.3	

Supplement
| Added | 44.7 | |
| None | 55.3 | |

Healthy_Yield
Good	41.1	
Average	10.1	
Below average	27.4	
Poor	21.4	

Figure 3. Bayesian Belief Network showing the proposed relationship for two species of mites, three species of *Trichoderma* species and mushroom yields in Australia, based on farm survey data.

3.2. Description of the Nodes

The 14 Nodes are described below.

- **BFM**: this indicates the incidence of BFM in the compost raw materials, mainly straw.

- **Red_Pepper_Mites**: similar to above for the other mite species.

- **Composting**: Phase I is the first stage of composting where the ingredients are mixed and initial fermentation allowed to occur. This operation is done on an open concrete slab, covered slab or roofed bunkers. This is when Th1 or Th3 normally enter the process. Phase II, the second stage of composting where the fermentation is more tightly controlled and includes a 60° C kill phase to supposedly eliminate mites and unwanted fungi, including Th1 and Th3. This operation is done in specially constructed rooms or tunnels, usually holding between 40 and 100 tonnes compost material. Any mites present have survived the composting process.

- **Spawn-run**: The mushroom inoculum, termed spawn, is added to the compost after it has cooled to 35°C. This should be done in an enclosed space with filtered air, but sometimes doors are left open, spillages occur and contamination can occur. This is the first occasion Th4 can enter the process. Spawn_run, is the colonisation of the compost by the mushroom mycelium. If Th3 or Th4 is present, the colonisation by the mushroom fungus is restricted.

- **Supplement**: this is an additional nutrient supply added to the compost after Phase II, often during spawning. If Phase II has not been done correctly, the supplement can readily encourage growth of Th1, Th3, and RPM.

- **RPM_Swarm**: this provides an estimate of how well the RPM have established and reproduced, usually occurring within the first 4 weeks after spawning.

- **BFM_Swarm**: as for RPM, except this mite usually swarms over the last 2 weeks.

- **Casing**: the application of a layer of moist, neutralized peat moss onto the beds after colonisation of the compost. This operation is usually done inside an enclosed space, but does provide an opportunity for Th1, Th3 or Th4 to infect.

- **Trichoderma_on_casing**: , provides an indication of the established of each *Trichoderma* species

- **Th1_4_CP**: indicates which *Trichoderma* species present in compost materials or as contamination during spawn-run

- **Th1_4_CS**: indicates which *Trichoderma* species present in as contamination during casing

- **Spotted_mush**: level of infection of the mushrooms by *Trichoderma* species – this was known more accurately for the growing experiments

- **Total_Yield**: the complete yield produced – this was known more accurately for the growing experiments

- **Healthy_Yield**: this is basically Total_Yield minus Spotted_mush – this was known more accurately for the growing experiments

4. RESULTS

4.1. Differences between field and experimental data files

The BBN for the growing experiment data is shown in Figure 1 and the field survey data is shown in Figure 3. The main differences were in the proportion of either high or low extremes in mites swarming, *Trichodema* on the casing, spotted mushrooms, and healthy yield. The data from commercial farms from the farm survey data tended to have fewer instances of high pest/disease levels or poor yields as such crops would be terminated early. The growing experimental data provided more detail because crops were continued until completion, usually three weeks after the start of harvesting.

4.2. Sensitivity analysis

The results of the sensitivity analysis for six nodes (Spotted_mush, Trich_On_casing, Total_Yield, Casing, Th1_4_CS, Th1_4_CP) in relationship to Healthy_Yield and for six nodes (Healthy_Yield, Trich_on_casing, Total_Yield, Casing, TH1_4_CS, Th1_4_CS) in relationship to Spotted_mush are presented in Tables 1 and 2. Values for the first six nodes are given. All values are expressed as percentage of the total Entropy or total variance respectively.

Table **1.** Influence (%) of entropy reduction and variance of indicated nodes on Healthy_Yield for the growing experiment and farm survey datasets.

Node	Growing experiment		Farm survey	
	Entropy reduction	Variance	Entropy reduction	Variance
Spotted_mush	62.4	55.1	44.2	28.6
Trich_on_casing	21.4	14.3	17.9	11.6
Total_Yield	11	7.4	18.8	12.4
Casing	5.0	3.8	1.6	0.6
Th1_4_CS	4.6	3.5	1.4	0.6
Th1_4_CP	3.1	1.0	3.8	1.8

The node Spotted_mush was the major factor in determining Health_Yield for both experimental growing experiments and the farm survey, but the proportion of variance attributed was higher for the experimental data (Table 1). Trich_on_casing was also an important factor on both Healthy_Yield (Table 1) and Spotted_mush (Table 2). The node Casing influenced both Healthy_Yield and Spotted_mush, and the nodes Th1_4_CS and Th1_4_CP indicated the role of both species of *Trichoderma* and point of infection. The two yield nodes were included in the six most important nodes in Spotted_mush, but this is an artifact from the way the model is constructed

Although there were nodes for the two mite species, the composting process and spawn run, none of these nodes occurred in the first six from the ranked list produced by the sensitivity analysis.

Overall, the data from the growing experiments were more accurate in terms of accounting for variance. The same six nodes were at the top of the ranked list produced by the Sensitivity analysis, suggesting the same factors were operating in both data sets. However, the ranked order of the six nodes differed between the two datasets.

Table **2.** Influence (%) of entropy reduction and variance of indicated nodes on Spotted_mush for the growing experiment and farm survey datasets.

Node	Growing experiment		Farm survey	
	Entropy reduction	Variance	Entropy reduction	Variance
Healthy_Yield	60.4	51.5	44.2	30.2
Trich_on_casing	26.4	19.6	24.6	16.4
Total_Yield	15.6	9.1	26.8	17.9
Casing	5.9	4.2	2.0	0.8
Th1_4_CS	5.4	3.8	1.7	0.7
Th1_4_CP	4.4	1.3	5.8	2.8

5. DISCUSSION AND CONCLUSIONS

The BBN has indicated that the node Spotted_mush was the major factor in determining the level of Spotted_mush and Healthy_Yield. In addition, the species of *Trichoderma,* level of colonization of the casing and the time of infection are also major factors in determining the severity of the infection as determined by Healthy_Yield and Spotted_mush. The absence of the nodes set up to include data on the mites present, the effectiveness of the composting process and the efficiency of the spawn run was not expected. However, the node Trich_on_casing incorporates information on the efficiency with which the *Trichoderma* has colonized the mushroom compost and/or casing, rendering the other nodes on compost effectiveness and spawnrun efficiency redundant.

Clift and Terras (1994, 1995) reported that RPM on its own was not associated with yield losses, which is consistent with the outcome from the BBN. However, the species of *Trichoderma* is a major factor and although the data used related mite swarming to the species of green mould involved, the BBN may have regarded the mite data as redundant. The use of supplement in the compost could not be related to the species of *Trichoderma* and to composting effectiveness.

Seaby (1996), Samuels et al. (2002) and Grogan (2006) all consider Th1 and Th3 to occur routinely in the straw used to produce compost. Marginal compost preparation can occur regardless of the presence of Th1, Th3 and mites, so if these organisms occur during spawnrun, it is a consequence of the compost problem, rather than the cause (Grogan 2006). Th2 and Th4 occur within the mushroom farms (Seaby 1996, Grogan, 2006) and while these species will colonise correctly in prepared compost, they will grow better in marginal compost (Seaby 1996).

All the Th species will grow on the mushroom casing and cause mushroom spotting, but Th2, Th3, and Th4 will also grow in the compost and directly reduce yields (Grogan 2006). The BBN, as constructed, ranks nodes that are involved in the establishment of the various green mould species as more important in determining the node Healthy_Yield. These nodes include Spotted_mush, Trich_on_casing, TH1_4_CP and Th1_4_CS. Although the nodes referring to the mites were not ranked highly, their occurrence would be expected to result in composting problems, but the BBN is accounting for this aspect in the nodes associated with establishment and growth of the green mould fungi. The BBN has indicated certain nodes as important, based on consequences of earlier events occurring and the model does not always reflect this, although 85% variance can be accounted for using the data set from the more detailed growing experiments.

In terms of managing green mould on commercial farms, the main issues are restricting access of the Th complex to the compost and casing and restricting their establishment within the beds. Use of clean, dry, un-infested straw would also be expected to enable efficient composting and spawnrun (Clift and Terras 1995, Nair et al. 1994).

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