Can Two Different Models Help Improve Management of Dry Bubble Disease of Mushrooms?

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EXTENDED ABSTRACT

Commercially produced mushrooms experience pest and disease problems in spite of being grown in specially constructed rooms. These usually gain entry to the system at the weak points, such as application of the casing layer.

The main disease problem is dry bubble, caused by *Verticillium fungicola*. There has been substantial work over the last 30 years defining potential sources of the diseases, including flies, pickers hands, farm surfaces, dust and casing materials.

We are developing epidemiology models for this pathogen to improve the practical outcomes from the industry having access to a DNA polymerase chain reaction (PCR) probe to detect the pathogen. This is especially important in determining priorities as to where to look around the farm to identify potential sources of the pathogen and to reduce the spread of existing infections.

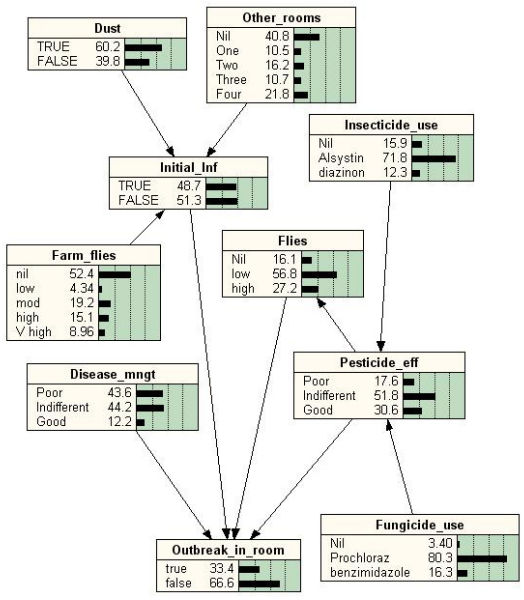

Fig 1 Netica Bayesian Belief Network using the Sydney basin survey data

Two modelling approaches were used: the first approach developed a Bayesian Belief Network (BBN) (Figure 1) to rank the known sources of the pathogen in both the initial infection and subsequent development of the disease. The data was from a pest and disease survey done on commercial farms in the Sydney basin, 1989 to 1991. A second set, derived from a pest and disease management service from 2002 to present, was included and the results compared.

The second approach was to use the Cox Proportional Hazard Model to determine the importance of various factors in development of disease outbreaks within a growing room. The data used was from disease incidence in three experimental crops grown in a research facility at Sydney University.

Most of the nodes in the BBN are self-explanatory, except FLIES, an assessment of mushroom fly populations inside each growing room and FARM FLIES, an assessment of fly populations in other growing rooms cropping in the same month and with infection by the pathogen. The BBN accounted for nearly 40% of the Belief Variance for the node INITIAL_INF, but less than 20% Belief Variance of the node OUTBREAK_IN ROOM. Similar results were obtained when the second set of data was read into the same BBN.

A Cox Proportional Hazard Regression model indicated that pathogen virulence and fungicide use were highly significant factors in development and spread of the disease, $p < 0.001$, but that fungicide resistance was not significant, $p > 0.4$.

The implications of these results for disease management in the mushroom industry are discussed. This modelling project has set up provisional epidemiology models for the mushroom disease caused by *V fungicola.* As more information becomes available during the evaluation of the PCR probe, these models will be refined and assist in the development of better disease management practices.

1. INTRODUCTION

Worldwide mushrooms are usually grown in specially constructed rooms. Despite this, the industry still has pest and disease problems, most of which gain entry to the system at the weak links, such as when the rooms are filled or the casing layer applied.

The main disease problem is dry bubble, caused by *Verticillium fungicola* (Figure 2). There has been substantial work over the last 30 years defining potential sources of the diseases, including cultural practices (Gandy 1973), flies (White 1981), peat moss, farm surfaces, pickers' hands (Wong and Preece 1987, Nair *et al* 1993), dust (Gandy 1973, Grogan 2002).

The widespread secondary occurrence of *V fungicola* spores throughout infected farms often masks the epidemiologically significant sources (Wong and Preece 1987). Fletcher *et al* (2004) considered that a combination of fungicide resistance, farm hygiene and cultural practices were responsible for an outbreak of cobweb disease, caused by *Cladobotryum* species. They commented that identification of sources was only part of the solution, with farm management, fungicide selection and application being more important in restricting existing outbreaks.

Clift *et al* (2004) related disease incidence on commercial farms and a mushroom research facility to both fungicide use and pest fly populations: however, other studies have been laboratory or research facility based. Therefore, there has been little effort to rank the various sources in terms of either starting a disease outbreak or in containing an existing outbreak in commercial farms.

The Australian Mushroom industry, in collaboration with Horticulture Australia and NZ Crop and Food Institute are funding work to develop commercial DNA polymerase chain reaction (PCR) probes to detect a range of mushroom pathogens, including *V fungicola.* (Romaine *et al* 2002). This project, termed the PCR Project, includes growing experiments to calibrate the probes by relating spore load and fungicide use to disease incidence and yield losses. There will also be field evaluation involving co-operating growers.

As part of this, we are developing epidemiology models for this pathogen to assist in interpreting outcomes from the industry having access to the probe. This is especially important in prioritising where to look around the farm to identify potential problems and to select farm management options to reduce the spread of existing infections.

2. DATASETS USED

There are three independent sets of data.

1. pest and disease survey, 1989 to 1992 in the Sydney basin, data on 160 crops, termed Survey, reported originally in Nair and Clift (1993).

2. pest and disease management project, 2002 to present in the Sydney basin, data on 120 crops, termed PDMS.

3. detailed disease incidence in three research crops grown at the Marsh Lawson Mushroom Research Unit, (MLMRU) University of Sydney, data from 180 growing trays.

Datasets 1 and 2 consisted of assessments of the identity and relative incidence of the major mushroom pests and diseases present on five commercial farms in the Sydney basin. All assessments were on a per crop basis. Dataset 3 is a subset of the records of pesticide treatments yields, pest and disease incidence maintained for the Marsh Lawson Mushroom Research Unit, The University of Sydney.

Each growing experiment involved up to 70 growing crates. Specifically the time infection by *V fungicola* was first noticed on each growing crate was recorded. The source of the pathogen is also recorded. Benzimidazole resistance is often present (Nair and Macauley 1987) and may be a factor in control problems. Mushroom flies were present in each of the three experiments.

Fig 2 Diseased mushroom between healthy mushrooms

3. MODELS EVALUATED

Two approaches were used: the first used a Bayesian Belief Network, (BBN) (Netica® Ver 3.24, 2007 from Norsys) the second Cox Proportional Hazard Regression (Statistica® Rel 7, 2006). The BBN was selected as one of the programs evaluated as it allows qualitative data to be used. The Cox Proportional Hazard Regression model was selected to quantify the importance of factors determining the development of disease outbreaks. This method makes very few assumptions about the underlying parameters.

3.1. Bayesian Belief Network

The BBN developed has ten nodes (Figures 1, 3) defined as:

Dust: This is a binary node with states True, dust does get into the "clean" parts of the farm and False, dust does not gain entry.

Other_rooms: This node indicates how many other rooms on the farm at the same time have *V fungicola* infection.

Farm_flies: An assessment of the level of mushroom fly activity in the other rooms on the farm that are diseased.

Initial_Inf: This is also a binary node with the states True, indicating the disease has been found, usually early in the growing cycle and False, there is no indication of disease present.

Disease_mngt: An assessment, applying to that growing room, of how effectively the disease was suppressed, especially regarding farm hygiene.

Insecticide_use and Fungicide_use: These two nodes input which pesticides are used in that room. It only records what was used, not how effectively they were used.

Pesticide_eff: This node contains the assessment of how effectively the grower/manager is using pesticides. This is a separate issue from the earlier node Disease_mngt.

Flies: This node contains information on the development of mushroom fly populations inside the growing room.

Outbreak_in_room: This node records the final outcome of all the preceding nodes. It is a binary node with states True, the disease has spread to at least 10% of the growing room and False, if the disease was contained to less than 10% of the room.

A range of configurations were evaluated using the Sensitivity to Findings function of Netica®. The, point of influence of the various nodes on Initial_Inf and/or Outbreak_in_room was varied and the amount of variation accounted for was determined. The configuration accounting for the highest proportion of Belief Variance in the node Initial_Inf was selected as the BBN to use.

3.2. Cox Proportional Hazard Regression

The start date for each of the three experiments was used as the first date in the Proportional Hazard Model. The second date was when *V fungicola* infection was first observed in each growing crate. If no disease was found on any crate, this line of data was CENSORED, otherwise it was COMPLETE.

Other factors included were **Virulence**, which was a numerical value between 0 and 2, determined by the aggressiveness of the isolate on the original farm and also in the MLMRU; **Resistance**, which indicated if the isolate was susceptible to benzimidazole fungicides on the farm of origin; **Fungicide**, which took the values 0, 0.5 if fungicide incorporated into the system at MLMRU, or 1 if the fungicide was applied as a split application over two drenches, each at half rate. Grogan *et al* (2000) had established that the split application is more effective than a single application at the start of the experiment. The experiment ID was used as the Grouping variable.

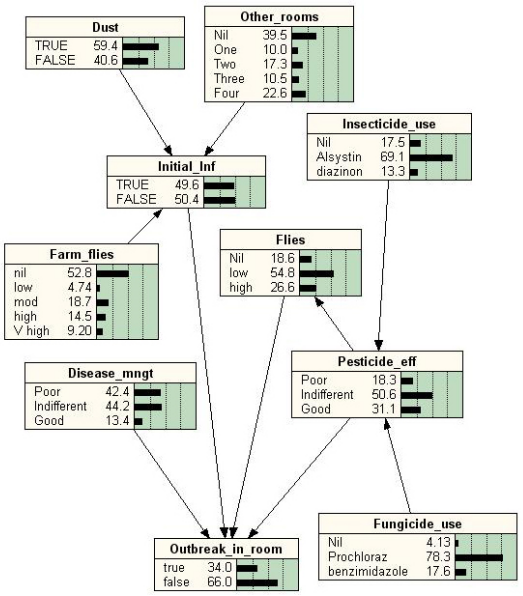

Fig 3 Netica Bayesian Belief Network using the PDMS data

4. RESULTS

4.1. BBN Sensitivity Analysis

The results of the sensitivity analyses are given in Tables 1 and 2. The Sensitivity function lists all the nodes in the BBN in order from most influential to least on the nodes Initial_Inf and Outbreak_in_room. The Belief Variance accounted for by each node is also given.

For either dataset nearly 40 percent of the Belief Variance for the node Initial_Inf was accounted for by the nodes Farm_flies, Other_rooms and Dust. However, for the node Outbreak_in_room, the BBN accounted for less than 20 percent of the Belief Variance.

Table 1 Sensitivity of Initial_Inf to a finding at another node. Number in brackets is Belief Variance as a percentage.

Node	Variance	
	Survey	PDMS
Initial_Inf	0.249 (100)	0.250 (100)
Farm_flies	0.059 (23.7)	0.058 (23.3)
Other_rooms	0.035 (14.1)	14.1 (14.5)
Dust	0.003 (1.14)	1.14 (1.3)

Table 2 Sensitivity of Outbreak_in_room to a finding at another node. Number in brackets is Belief Variance as a percentage.

Node	Variance	
	Survey	PDMS
Outbreak	0.224 (100)	0.225 (100)
Pesticide-eff	0.029 (12.7)	0.029 (13.0)
Flies	0.008 (3.66)	0.009 (3.89)
Initial_inf	0.006 (2.78)	0.006 (2.75)

The BBN does indicate that disease sources within the farm, Farm_flies and Other_rooms are major factors in starting the Initial_Inf. Dust was of relatively low importance. Pesticide_eff was the most important factor in developing a disease outbreak within the room. Other factors clearly need to be considered.

4.2. Cox Proportional Hazard Regression

A Kaplan-Meir plot showing the combined data from the three experiments is presented as Fig 4. The regression coefficients for the Cox Proportional Hazard Regression model are presented in Table 3.

Both Virulence and Fungicide are highly significant coefficients, $p < 0.001$, but Resistance was not. This suggests that, even in a research facility, it is how the fungicide is applied, more than fungicide susceptibility, that determines disease spread. Virulence of the pathogen is equally significant.

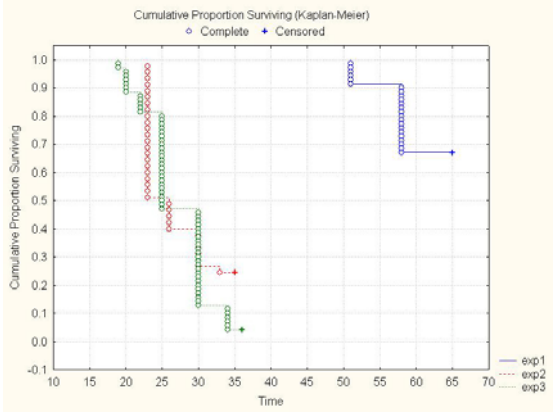

Fig 4 Kaplan-Meir plot showing the disease incidence from three growing experiments

Table 3 Cox proportional Hazard Regression Model regression coefficients and probabilities

Variable	Beta ± SE	*t* value	p
virulence	1.116 ± 0.243	4.60	< 0.001
resistance	0.241 ± 0.299	0.81	0.42
fungicide	-1.021 ± 0.296	-3.45	< 0.001

5. DISCUSSION

The BBN developed in this report supports the conclusion that the inoculum produce within the farm is the main source of problems. The most significant factor in the BBN in relation to Initial_Inf was Farn_flies, the second most important was Other_rooms (Table 1). These two nodes explained approximately 38 percent of the variation. This conclusion is supported by other workers (Nair and Clift 1993, Grogan *et al* 2000, Fletcher *et al* 2004).

Dust was a significant factor in Initial_Inf, but not as major as was thought during development of the BBN. The role of dust in disease introduction is consistent with observations made by Gandy (1973) and Grogan (2002).

The BBN was less conclusive regarding the subsequent development of an outbreak within the room. Pesticide_eff and Flies explained less than 17 percent of variation (Table 2). Disease_mngt was not significant.

Gandy (1973) and Fletcher *et al* (2004) both emphasise the importance of cultural practices, so the BBN developed here has a node for disease management. The data used had relatively little information for this node (Disease_mngt) because of the absence of detailed information on disease management on the farms concerned.

The Cox Proportional Hazard model, using different data, indicated fungicide application and pathogen virulence were significant factors (Table 3). Fungicide resistance was not significant, but the growing experiments that provided the infection data were not balanced in terms of type of fungicide used. Fletcher *et al* (2004) relied heavily on detailed information on both fungicide use and susceptibility of the pathogen they were working with for each of the farms.

Benzimidazole resistance is widespread in many mushroom pathogens, including *V fungicola* (Nair and Macauley 1987, Grogan *et al* 2000, Beyer and Kremser 2004). Although fungicide resistance was present in Experiment 2, this effect was not distinguishable from the Virulence and Fungicide application.

The information available to develop the models is clearly limited. The intention behind developing these models was to establish a foundation based on information that had been collected previous to starting the PCR project. The preliminary models presented here indicate the type of information required to develop better epidemiological models for this pathogen.

The PCR project is generating data on: fungicide susceptibility of various isolates of *V funcicola*, some of which are also used in the growing experiments, effectiveness of various fungicides under different use patterns against pathogen isolates of known resistance status and more detailed information on disease management practices used by co-operating growers. The data will be underpinned by the independent assessment of pathogen incidence by the PCR probe, especially in relation to dust, pest flies and farm equipment.

Using two modelling approaches to the one situation has enabled limitations in one to be handled by the other. The BBN was more successful in predicting initial infection and the Cox Proportional Hazard in determining factors important in subsequent development of the disease outbreak. The data described above will be used to expand and refine both the BBN and the Cox Proportional Hazard models.

The issue of fungicide application is one that has been raised previously (Nair and Clift 1993, Grogan *et al* 2000, Beyer and Kremser 2004). Clearly, this has directly impacted on the growing experiments and also on the data used in the BBN. Factors to be considered, include timing and application volume, as well as the active ingredient used.

The Cox proportional Hazard Model indicated the importance of fungicide application method. This was possible because detailed records had been kept of all treatments and these were all replicated experiments. Pathogen virulence is another a significant aspect and needs to be included in future modelling.

6. CONCLUSION

Neither model could predict the first infection on a farm, but the BBN clearly highlighted the role of existing infections as the source for the new rooms on the farm. There are clearly issues in terms of determining that first source, but the PCR probe would be expected to provide additional information.

A better BBN can be developed once there is a means to reliably detect the pathogen at low levels. It will be essential to include the cultural practices and disease management protocols if an effective epidemiological BBN is to be developed.

The Cox Proportional Hazard model may provide better information on factors determining the development of an outbreak from an initial infection. Experimental work can provide the data to develop such a model. However, the conclusions need to be compared to those from a model based on disease outbreaks on commercial farms.

More work at the farm level with the PCR probe will be required to develop a monitoring program. The existing BBN provides a starting point and framework to gather more detailed information on the presence of the pathogen from a range of locations.

7. REFERENCES

Beyer, D.M and J.J. Kremser, (2004). Evaluation of fungicide tolerance and control for three fungal diseases of mushrooms. In: *Proceedings of the XVIth International Congress on the Science and Cultivation of Edible and Medicinal Fungi*, pp 421-429, (Romaine, C. P., C. B. Keil, D. L. Rinker and D. J. Royse, editors), Miami, FL. U.S.A.

Clift, A.D., A. Shamshad, and M.A. Terras, (2004). Flies and dry bubble in cultivated mushrooms. In: *Proceedings of the XVIth International Congress on the Science and Cultivation of Edible and Medicinal Fungi*, pp 459-464, (Romaine, C. P., C. B. Keil, D. L. Rinker and D. J. Royse, editors), Miami, FL. U.S.A.

Fletcher, J.T., J. Allan and G.K. Seymour (2004). Managing cobweb disease in Australia. In: *Proceedings of the XVIth International Congress on the Science and Cultivation of Edible and Medicinal Fungi*, pp 711-715, (Romaine, C. P., C. B. Keil, D. L. Rinker and D. J. Royse, editors), Miami, FL. U.S.A.

Gandy, D.G. (1973). Observations on the development of *Verticillium malthousei* in mushroom crops and the role of cultural practices in its control. *Mushroom Science*, 8, 171-181.

Grogan, H.M., (2002). Verticillium – an interesting experiment, *Australian Mushroom Growers Association Journal Autumn 2002*, 25-27.

Grogan, H.M., C. Keeling and A.A. Jukes (2000), *In vivo* response of the mushroom pathogen *Verticillium fungicola* to Prochloraz manganese, *Proceedings of Brighton Crop Protection Council – Pests and Diseases, Brighton, UK, 2000*, 273-278.

Nair, N. G. and A.D. Clift (1993), Integrated pest and disease management in cultivated mushrooms, *Final Report Project Mu002 NSW Agriculture*, 25 pages.

Nair, N.G. and B.J. Macauley. (1987), Dry bubble disease of *Agaricus bisporus* and *A bitorquis* and its control by prochloraz manganese complex. *NZ Journal Agricultural Research*, 30, 107-116.

Romaine, C.P., B. Schlagnhaufer and M. Stone (2002), A polymerase chain reaction-based test for *Verticillium fungicola* causing dry bubble disease on the cultivated mushroom *Agaricus bisporus*, *Applied Mycological biotechnology*, 59, 695-699.

Norsys Software Corp. (1997), Netica Application Users Guide. Norsys Software Corp, www.norsys.com.

White, P.F. (1981). Spread of the mushroom disease *Verticillium fungicola* by *Megaselia halterata* (Diptera: Phoridae) *Protection Ecology*, 3, 17-24.

Wong, W.C. and T.F. Preece (1987). Sources of *Verticillium fungicola* on a commercial mushroom farm in England, *Plant Pathology*, 36, 577-582.