



## NEMATODES

### INTRODUCTION

Nematodes are widespread microscopic eelworms which live in soil, decomposed organic matter, fresh water, marine environments, on and in living plants, fungi, insects and animals. Not only are they one of the most primitive animals on earth being the first to develop a body cavity, but they are also one of the most numerous animals on earth with 1m<sup>2</sup> of garden soil containing 2 - 4 million nematodes.

There are three broad groups of nematodes that can be found in *Agaricus* crops based on their feeding pattern

- predatory
- mycophagous or parasitic
- saprophagous

Predatory nematodes feed on other nematodes and fly larvae and have little to no effect on mushroom quality and production. In fact, predatory nematodes such as *Steinernema feltiae* are an accepted biological fly control agent available in Australia and can be added to casing to control sciarids in particular.

Mycophagous nematodes are significant pests of mushroom cultivation, feeding exclusively on fungi. They can rapidly destroy mushroom mycelium and breed very rapidly, up to 25,000 times in one week so populations increase extremely rapidly. However, due to modern composting techniques, mycophagous nematodes are rarely seen in mushroom beds.

The most common type of nematode found in mushroom cultivation is the saprophagous feeders. They are not primary pests of mushrooms, as they feed on bacteria and decaying organic material associated with mushroom cultivation and not the mycelium directly. Water is essential for feeding, movement and breeding making mushroom cultivation a favourable habitat and being so small,

they can survive in extremely thin films of water. Significant crop loss associated with high populations of saprophagous nematodes is most likely due to the nematodes fouling the substrate, making it unfavourable for mycelial growth.

Their presence in mushroom beds is an indicator of inefficient composting particularly in Phase II which results in a non-selective compost. This is primarily due to the compost not reaching sufficient temperature, often at the ends of a bulk Phase II tunnel where nematodes will also survive. The impact of saprophagous nematodes on mushroom yield and quality is worsened by poor hygiene and an incorrect growing environment.

In Australia, the most common saprophagous nematode is *Caenorhabditis elegans*.

Approximately 1mm in length, males are rarely produced but self-fertile females reach reproductive maturity in about 3 days and can each produce 250 to 350 eggs resulting in a 50 – 100-fold increase in the population each week. Due to rapid population expansion, early intervention is extremely important for effective nematode management.



**Figure 1** Kidney-shaped mushroom cap from a nematode-infested bed. *Image: Judy Allan*

## SYMPTOMS of SAPROPHAGOUS NEMATODE INFECTION

Because saprophytic nematodes are colourless creatures about 1mm long visible only with the aid of a microscope, they are difficult to detect. At low levels, they have little noticeable effect on the developing mushroom mycelium so early infestations often go unnoticed.

Their presence in spawned compost prior to casing is indicated by either fragmented *Agaricus* mycelium within wet compost or if high populations are present, by patches of black, barren and wet compost devoid of mushroom mycelium. After casing material is applied, the casing will be slow to colonize or, if colonized, the mushroom mycelium will fail to re-knit well in infected spots after the casing is ruffled.

As numbers increase, their influence on the mushroom crop becomes apparent. Pinning becomes poor and patchy resulting in fewer mushrooms and the demarcation between flushes becomes blurred with first flush often delayed by four to five days. In significant infections mushrooms can be directly affected. Caps from infested beds can become kidney-shaped with a characteristic notch in one side (Fig. 1) and they may be off-white compared to unaffected mushrooms and express overall reduced quality and poor shelf life.



**Figure 2** Patch of wet unproductive casing on a nematode-infested bed. *Image: Judy Allan*

In heavily infested trays and shelves toxins and waste products accumulate, 'poisoning' the mushroom bed.

The compost becomes wet and increasingly anaerobic, resulting in dark, sodden unproductive patches of pungent smelling compost unfavourable for mycelial growth (Fig. 2). In this case, total loss of the crop on the affected bed can occur. Any mushrooms that grow will be of poor quality and cap pitting and blotching may also express (Fig. 3).



**Figure 3** Example of a poor quality mushroom from a nematode-infested bed showing bacterial pitting and blotching. *Image: Judy Allan*

Patchy, barren casing is a symptom not confined to nematode infections. Virus dieback and some mould infections will also express in part as non-productive, dead casing. To confirm a nematode infection a sample will need to be taken from an affected area of mushroom bed that is cool – nematodes will migrate from heat – and be sent for diagnostic testing. Please contact the Project Team for assistance with sampling and sample submission as we have located a laboratory willing to identify nematodes occurring in mushroom cultivation.

## NEMATODE DISPERSAL and SURVIVAL

Nematodes are essentially small aquatic animals incapable of long-distance dispersal. But once their food supply has been exhausted or their environment becomes untenable due to accumulated waste products, they must relocate to more favourable conditions to survive. Nematodes have developed different methods of dispersal that maximise their chances of finding a suitable environment, ensuring survival of the population.

Nematode eggs and individuals can be spread around the farm on workers' footwear from infested casing and mushroom debris dropped onto the floor during harvest or splashed onto the floor during watering. Nematodes and eggs can also be picked up in infested organic matter by harvesters' tools, gloves and clothing and transported to adjacent



grow rooms and new crops. Nematodes can also dry out to form a resistant cyst state which enables them to tolerate harsh conditions and survive normally lethal temperatures. The nematodes can remain viable as a cyst for many years until they 'hatch' and colonize their new, fresh environment. The cysts are dispersed in dust and by wind but like dispersal in organic material, the chances of relocating to a desirable environment are not guaranteed. In these instances, survival of the population is assured by weight of numbers.

Saprophytic nematodes found in mushroom cultivation have developed a specific association with sciarid flies (*Lycoriella* spp.) called 'phoresy' to ensure their survival. When under environmental stress, the nematode enters a non-feeding, non-ageing, developmentally arrested resistant survival stage, called the 'dauer' larva which has a sticky outer skin. Individual dauer larvae swarm together in columns of hundreds of individuals at a high point

on the casing surface. The columns of larvae rhythmically curl and flex in a process called 'nictating' or 'winking' (Fig. 4) which acts as an attractant to flies. The columns of nictating nematodes can be seen by eye with the assistance of a flashlight held at a 45° angle to the affected bed surface. The nictating columns adhere to a passing fly and are transported to a different area of the mushroom bed or to a different crop in an adjacent room. Once exposed to favourable conditions, the nematode emerges from the dauer stage and resumes normal development to reproductive maturity. Because flies inhabit the same environment as nematodes, are highly mobile over relatively long distances and can access all parts of the mushroom farm, the chances of being transported to a favourable environment containing an abundant food source are very much higher than for other methods of dispersal.



**Figure 4** Nictating ('winking') columns of saprophagous nematodes at the surface of nematode-infested casing. Some of the nictating columns are indicated (yellow arrows). The glistening casing surface is evidence of free water and very wet conditions. Note how far above the casing surface some of the columns extend (white arrow) to adhere to flies and other vectors. *Image: Judy Allan*

## SOURCES of INFECTION

Nematodes are introduced to the mushroom cultivation system primarily in compost raw ingredients as they provide a habitat rich in food, water and oxygen. Nematode populations rapidly increase under the favourable conditions imposed during pre-wet and in the early stages of Phase I. As Phase I progresses, nematodes migrate from areas of high temperatures and survive in the cooler shoulders of the compost ricks and within clumps of

compost to be carried over into Phase II. The majority of nematodes will be killed in Phase II but isolated populations may survive in dry areas, excessively wet areas, clumped compost and cool areas, particularly if Phase II is inefficient and be carried over into spawning.

Once in spawning where environmental conditions are more favourable, surviving nematodes are distributed throughout the compost during mixing

of spawn and compost. The spawning machine may then contaminate subsequent trays and beds after processing a single batch of infested Phase II compost.

Peat is not considered a primary source of nematodes as it is relatively dry and has a low pH. Although modern deep-dug peat has a higher moisture content than blonde peat, its anaerobic environment does not support nematode growth. However, during preparation of the casing materials and the casing process itself, environmental dust which can carry encysted nematodes and flies carrying nematode dauer larvae can contaminate the casing. Once contaminated casing is introduced onto the mushroom bed, it presents very favourable conditions for growth and colonization by nematodes.

The most significant source of nematode infestation is cross-infection from contaminated crops on-farm. Nematodes infect new beds when they are introduced to the casing surface in dust and organic material carried on workers' boots, tools and hands. Nematodes may also adhere to flies, mites and farm personnel when the nematodes migrate to high points of the mushroom bed seeking a carrier to transport them to a fresh, favourable environment. Nematodes can also inhabit insulation and cracks and crevices in woodwork within a grow room where they will survive cookout. They can then be dispersed onto mushroom beds by water splash and condensation.

## **NEMATODE MANAGEMENT and CONTROL**

Because they are so widespread, nematodes will inevitably be introduced into the compost yard at some point in raw materials. It is impractical to try to eradicate nematodes in raw materials, so the most effective way of preventing nematode infestation of mushroom beds is to carefully manage the composting process to ensure that Phase II compost is selective for *Agaricus* mycelium. Nematodes cannot colonise and reproduce in well-prepared, effectively pasteurized, spawned compost as the free water and the bacterial food source that they require are not available and they cannot compete with rapidly growing mushroom mycelium.

There are specific nematode control measures for each step of the composting and growing processes, but it is critical that strict general hygiene protocols are followed throughout the farm to support these control measures. Strongly consider increasing hygiene if a nematode infestation is suspected.

### **Phase I**

In Phase I it is important to regularly turn the cold shoulders of the ricks into the centre to maintain temperatures and to ensure all ingredients are thoroughly mixed. Moisture content is critical to nematode survival so monitor the compost constantly and add water as required. Avoid over-wetting the compost as this promotes bacterial growth which is the main food source for nematodes. Similarly, do not allow the compost to become dry as these areas will not reach a sufficient kill temperature. Stringent Phase I conditions are important to minimise the number of viable nematodes from passing into Phase II.

### **Phase II**

Phase II is the critical stage in compost preparation for nematode control because nematodes surviving Phase II will carry into spawning and into the mushroom beds at fill. The ability of surviving nematodes carried over from Phase I to establish populations in the mushroom bed will be due to failures in Phase II. Areas of dry and/or cooled compost will protect nematodes from thermal death in Phase II and must be avoided. Compost should be pasteurized at 60°C for a minimum of 2 hours to kill nematodes under ideal conditions but if the compost is dry or the compost is clumped due to inadequate mixing, temperatures up to 71°C will be required to kill. Calibrate temperature probes to ensure temperature readings are true and that target kill temperatures are maintained. As in Phase I, Phase II compost must not be allowed to become dry, nor must it allowed to become soggy as bacteria will grow in excessively wet conditions.

### **Spawning**

After Phase II, there are no further opportunities to control the growth of nematodes on the compost by heat treatment and nematodes that carry into spawning from Phase II will eventually pass into the mushroom beds at fill. Phase II compost batches suspected of being infected with nematodes should be processed last and the spawning machine sanitized between infected batches. Mixing of the compost at spawning will distribute nematodes throughout the Phase III compost and introduce them to all mushroom beds filled from the infected batches.

### **Casing**

Casing is very susceptible to nematode infection so it is important that casing ingredients are stored and mixed in a clean area free of runoff water and dust which may contain nematode cysts. Pre-wet casing must not become waterlogged or be held over from

one week to the next and the casing depth must be consistent across the beds.

### Growing

Grow room conditions should be set to favour growth of the mushroom mycelium with evaporative conditions set to prevent free water collecting on the bed after watering and to reduce bacterial growth. Because infested crops are the main source of inoculum, fly control is extremely important and traffic must be managed to prevent workers, particularly harvesters, from transferring nematodes to 'clean' beds. Saprophagous nematodes thrive on beds where harvesting practices are bad, so the beds must be cleared of stumps and other mushroom debris immediately after harvesting to prevent decaying mushroom tissue and rot bacteria from developing which the nematodes feed on. The floors ought to be cleaned of dropped organic matter to prevent infested organic material being tracked into unaffected rooms.

**Table 1** APVMA-registered Abamectin products

Product Name	APVMA No
ACCENSI ABAMECTIN INSECTICIDE	53080
BARMAC ABAMECTIN INSECTICIDE	83173
BINTON 18 INSECTICIDE	90098
CROPRO STEALTH MITICIDE AND INSECTICIDE	53511
CROPSURE ABACUS 36EC INSECTICIDE	91188
EUROCHEM ABAMECTIN 18EC INSECTICIDE	89149
GENFARM ABA 18 MITICIDE / INSECTICIDE	59313
GENFARM ABAMECTIN 18EC INSECTICIDE / MITICIDE	89282
IMTRADE ABACHEM 18 MITICIDE / INSECTICIDE	53325
RAINBOW ABAMECTIN INSECTICIDE / MITICIDE	65750
RELYON ABAMECTIN 18 EC INSECTICIDE / MITICIDE	83210
SORCERER 36 MITICIDE / INSECTICIDE	84720
TITAN ABAMECTIN 18 INSECTICIDE / MITICIDE	65889
VANTAL UPGRADE MITICIDE / INSECTICIDE	67524
VERTIMEC PRO INSECTICIDE / MITICIDE	69685

### Pesticide

Pesticide application is only to be considered when nematode populations are extremely high. There are currently 15 products containing 18g/L Abamectin registered for use on Australian mushroom farms (Table 1). Be aware of the withholding period stipulated on the product label (Fig. 5) for mushroom crops: DO NOT HARVEST FOR 3 DAYS AFTER APPLICATION

### Crop termination

Cookout is the most effective way of eradicating a nematode infection but nematodes are able to inhabit cracks in timber trays and survive cookout and be carried over into the next crop. Maintaining compost temperatures in cookout at 60°C for 2 hrs

minimum should be sufficient to eradicate nematodes, but in dry compost, temperatures upward of 70°C may be required.

**A checklist of key action points is provided below (Table 2) to assist in managing and preventing nematode infestations.**

### KEY REFERENCES

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*Title header image credit: Bob Goldstein, UNC Chapel Hill*



Mushrooms	Red pepper mites (Siteroptes mesembrinae) Mushroom pygmy mites (Microdispus lambi) Soil borne nematodes of the family Rhabditidae	6 mL / 50 L of casing material  3mL in 1.5 L of water/m <sup>2</sup> of growing medium	3 days (H)	Apply when pests first appear using a water cart or knapsack spray. Repeat depending upon infestation. Apply as a casing drench or if in crop over beds. DO NOT apply more than 2 applications per crop with a minimum retreatment interval of 14 days. Application of abamectin should be made at casing material preparation stage or 2 applications watered onto casing layer as split applications. Include cultural control methods as part of an integrated pest management strategy in addition to chemical control.
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Figure 5 Excerpt from the label directions for Abamectin application to mushroom crops

Table 2 Checklist of key action points for prevention and control of nematode infestation

Location	✓	✗	?	Action point
Phase I	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Compost mixed uniformly – cold ‘shoulders’ of compost heap avoided
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Water content maintained to prevent dry spots where nematodes can survive high temperatures
Phase II and III operations	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Confirm temperatures during Phase II pasteurization and conditioning are in range to achieve a selective compost
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ensure there are no ‘cold’, ‘overly wet’ or ‘dry’ spots in the compost
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Confirm that spawn run is ‘even’ and complete before breaking up spawn-run compost
Spawning	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Suspected nematode-infested batches of Phase II compost processed last – spawning equipment sanitized between compost batches
Filling & casing	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Casing ingredients such as peat moss and CAC’ing (Compost-added-at-Casing) contain no nematodes or very low numbers of nematodes
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Casing ingredients are stored and mixed in a clean area
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Mixed casing is not held over from one week to the next
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Avoid ‘over-wetting’ the casing at pre-wet and / or mixing
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Casing depth is as even as possible
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Apply Abamectin if high numbers of nematodes are present: <ul style="list-style-type: none"> <li>incorporate 6mL product / 50L casing material during casing preparation; or</li> <li>apply 3mL product in 1.5 litre water / m<sup>2</sup> growing medium in two treatments, watered onto the casing layer as a split application 14 days apart</li> </ul>
Grow room	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Grow under evaporative conditions
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Over-watering onto anaerobic casing and compost avoided
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Flies controlled to reduce spread of nematodes
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Traffic managed to prevent transfer of nematodes between crops
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Stumps and other mushroom debris removed from the beds after harvest
Crop termination	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ensure an effective cookout temperature (55-60°C) is reached and maintained for sufficient time
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Temperature probes calibrated
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Grow room floor, particularly cracks and joins, is sanitized after cookout
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Ensure crop is well covered with an approved disinfectant before emptying if cookout not possible
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Early crop termination considered especially if there is heavy infection
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Spent mushroom compost removed immediately after cookout, not stockpiled on site
General	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Strict hygiene observed throughout the farm
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Farm dust is well-managed
	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Spilt compost or casing from infected beds is immediately cleaned up to prevent nematodes being transferred to other crops on footwear

Correlated from Fletcher & Gaze (2008) and Coles (2002)

## MU21007 – Pest and Disease Management for the Australian Mushroom Industry

UNIVERSITY of TASMANIA



Tasmanian Institute of Agriculture

**Hort  
Innovation**  
Strategic levy investment

**MUSHROOM  
FUND**

This project has been funded by Hort Innovation using the mushroom research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit [horticulture.com.au](http://horticulture.com.au)

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