WHEN WATER RELATIONS GO BAD: NAVIGATING UNCHARTED WATERS

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BACKGROUND

There are some symptoms which appear on a mushroom that are NOT caused by pests or pathogens but are a result of the reaction of the mushroom to the environment in which it is growing. Some of these environmental factors include water, temperature, carbon dioxide and relative humidity/evaporation. The environmental factors interact with the mushroom crop as a single factor, or in combination, and deviations from the optimum can result in symptoms such as poor mycelium growth and/or poor cropping, distortions, scaling, stem disorders and pale gills. What can be confusing is that some of these same symptoms could also be attributed to the presence of a disease.

The purpose of this article is to explore the biological indicators the mushroom can express and to use some specific case studies to analyse multiple and complex symptoms to pinpoint contributory factors. The acute attention to detail to symptoms expressed underpins the level of surveillance necessary to monitor for the presence of new and/or emerging disease.

INTRODUCTION

Mushrooms comprise 90-95% water so supplying the mushroom with just the right amount of water during its growth and development is crucial to maximising quality, yield and ultimately, income. But getting the water balance 'right' can be difficult. Water relations within a mushroom bed is a complex interplay of compost moisture, casing moisture and atmospheric relative humidity (RH). Mushrooms do not have a waxy layer or protective skin like fruits and other vegetables, so achieving the correct water balance is difficult as water is easily lost and absorbed through the cap and stem tissues.

When a water imbalance does occur, affected mushrooms express a physiological response as a water stress symptom. Water stress symptoms may affect up to 100% of a crop, resulting in a significant drop in yield and quality. However, by looking closely and carefully at the expressed symptom, the grower will often be able to isolate where the imbalance is and when the problem occurred.

Mushrooms obtain water from both casing and compost. Unfortunately, there is no single industry-wide formula to determine the optimal moisture content of each component as farms run different cropping systems with different compost and casing compositions of different water holding capacities and different water availabilities. However, what is consistent is that when there is a breakdown in water relations, the mushrooms will tell you where it is.

This article introduces some common causes of water stress in the mushroom cropping system, illustrates the symptoms expressed by affected mushrooms and outlines the contributory factors which must be investigated and addressed in order to correct them. Look closely at what your mushrooms are trying to tell you, 'interpret the message' and act accordingly.

THE COMPOST AND CASING – MUSHROOMS' FOOD AND DRINK

In its vegetative stage, the growth of mushroom mycelium through the compost and into the casing is relatively slow. However, when it switches to reproductive growth within the casing, the mushroom enters a rapid expanding stage (RES) of pin development, when the mushroom may double its size every 24 hours. It is during the RES that the mushroom is extremely sensitive to any change in environment and nutrition and because it is during this growth phase that fruit quality is largely determined, an imbalance in water relations and nutrient availability at this stage will severely impact yield and quality.

The mycelium of *Agaricus bisporus* absorbs water from both the compost and casing to nourish the fruitbodies. Approximately 54-83% of water is derived from the compost while the casing layer contributes 17-46% depending on flush, casing thickness and harvesting time, but both layers are important in the water supply to the mushrooms (Kalberer 1990).

The transportation of dissolved nutrients and water occurs through a fine network of 'pipes' consisting of mushroom mycelium, which is laid down as the spawn runs through the compost. When the mycelium migrates into the casing, larger capacity 'pipes' or rhizomorphs (**Fig. 1**) are produced which supply the developing pins during the RES. It is therefore critical that the mushroom is provided a steady environment and a consistent and optimal watering regime to deliver the correct balance of compost nutrition, temperature and optimal compost and casing moistures. Initially, this will allow a healthy and robust 'pipe' network to establish but equally as important, it will keep the 'pipes', particularly the casing rhizomorphs, in good working order. But an under-supply or over-supply of water, or inconsistent watering will damage the network and prevent optimal amounts of nutrients and water reaching the developing mushrooms.

READ THE MUSHROOMS; INTERPRET THE PROBLEM

A particular water imbalance will generally cause similar symptoms to be expressed in affected mushrooms. **Table 1** introduces some of the common symptoms of water stress and the imbalance in the cropping system that causes them. Photographs are provided to assist in identifying the symptoms that may be seen on the bed. However, the list is by no means exhaustive. As demonstrated later, extreme symptoms do occur. But by carefully analysing even multiple and complex symptoms, the most likely root cause can be identified.

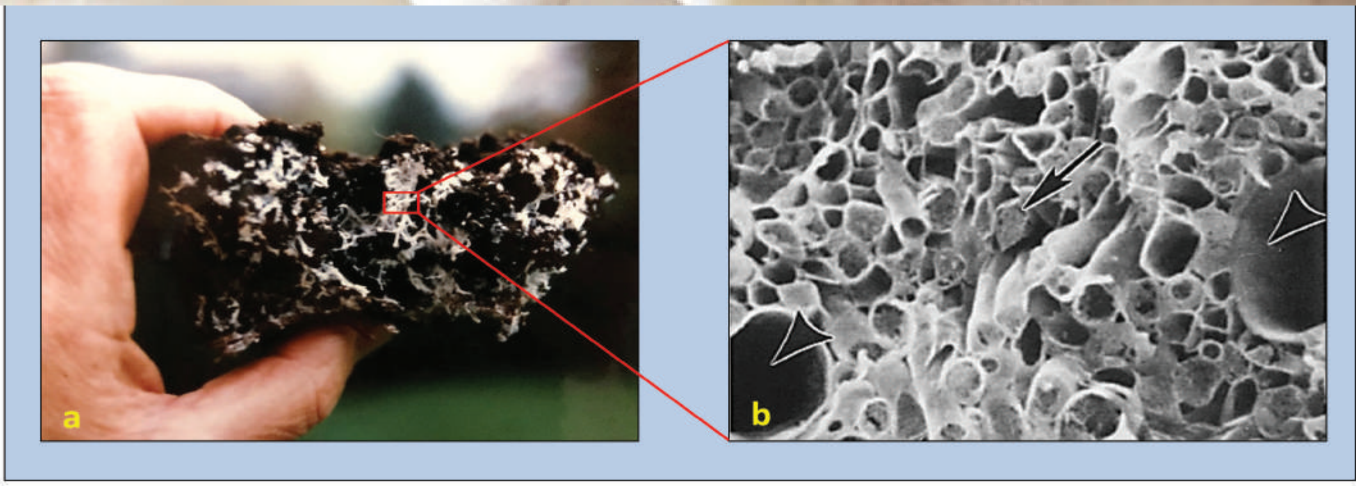


Figure 1 *Agaricus* rhizomorphs in casing **a)** the network of thick white rhizomorphs within the casing layer **b)** in cross section, the rhizomorphs are seen to be composed of regular hyphae (arrow) and large 'vascular' hyphae (arrowheads) which transport water and nutrients. *Photo credits: a) courtesy of Hans Tschierpe; b) Heckman et al (1989) courtesy of JSTOR*

EXTREME WATER STRESS SYMPTOMS

Case 1

In December 2018, the Project Team received an email from a grower describing unusual symptoms expressing on brown *Agaricus* mushrooms. In the email, the grower forwarded some excellent photographs illustrating the problem very clearly [**Fig. 2a-c**] and sent some affected mushrooms to the Tasmanian Institute of Agriculture for further investigation. From the photographs, the most characteristic symptom appeared to involve disruption of the gill tissue, so the disease 'drippy gill,' caused by *Pseudomonas agarici*, was the first consideration. However, after closer analysis of the photographs and studying symptomatic mushrooms, 'drippy gill' was eliminated as a possibility by the logical reasoning presented in **Table 2**. The symptoms were determined to be caused by water stress.

In this case, the occurrence of symptomatic mushrooms was widespread, affecting a significant proportion of the crop and became more severe as the crop matured. Furthermore, the problem had been expressing for three weeks, so was not an isolated case confined to a substrate microclimate. By referring to **Table 1**, the major symptoms – weeping, water-logged stems and watery flesh – appear to be consistent with mushrooms growing in wet casing over a dry compost. Water stress and an imbalance in water relations was diagnosed from these unusual and extreme symptoms.

In the affected mushrooms, symptoms were expressed in tissues in close proximity to the path of water transport [**Fig. 3a**] which was identified by Schütte in 1956 [**Fig. 3b**]. Both the stem and cap immediately above the gills were heavily waterlogged and the nearby cap tissue became discoloured. Water droplets issued from the edge of the cap, which is at the end of the water transport pathway and would be consistent with the 'overloading' of this system. An unusual 'jelly'-like material also formed close to the path of water transport and appeared to be caused by the swelling of the sub-hymenium, the tissue which gives rise to the gills. From light microscope examination, the 'jelly' was in fact seen to be composed of water-soaked, swollen, non-differentiated hyphal cells and was not the amorphous jelly that it initially appeared to be. Water-logging of the sub-hymenium prevented normal gill development and the turgid sub-hymenium became visible because it was swollen and the normally overlying differentiated gill tissue was absent.

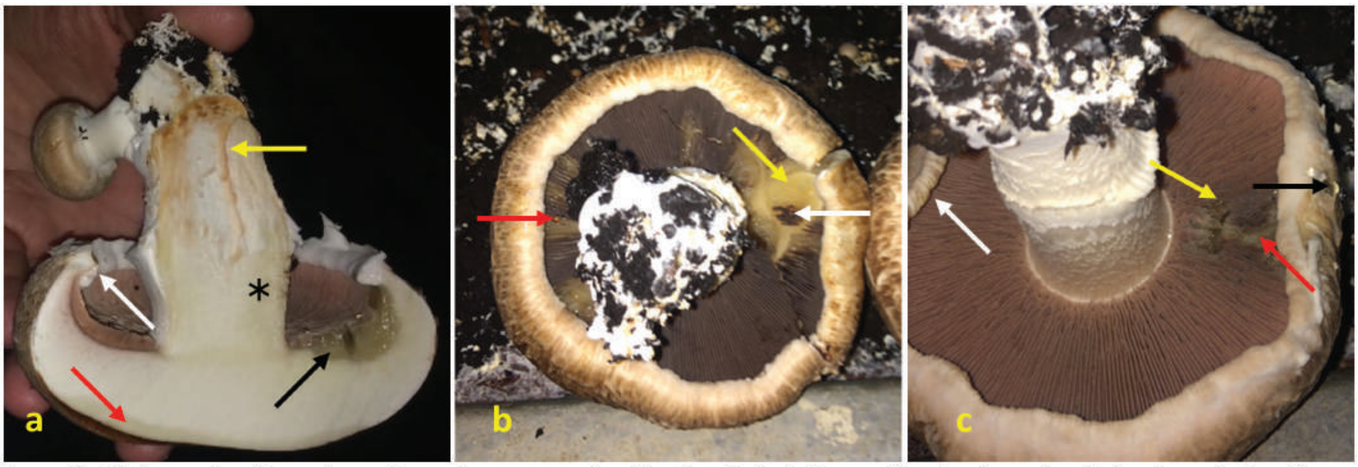


Figure 2 Photographs of symptomatic mushrooms received by the Project Team **a)** water-logged and discoloured stem tissue (yellow arrow) with free water sitting on the cut surface (*), a 'window' of water-soaked cap tissue (red arrow), yellow 'jelly' (black arrow), in-rolled margin (white arrow) **b)** clear brown water droplets on the gills (red arrow), water-soaked but otherwise healthy gill tissue (white arrow) and yellow 'jelly' (yellow arrow) which displaces the gills in places **c)** in-rolled margin (white arrow), issuing droplets from the healthy-looking gills (yellow arrow) despite being close to the 'jelly' (red arrow), clear water droplets issuing from the outer cap (black arrow). The stipe is outwardly asymptomatic. *Photo credit: Supplied by farm*

Table 1 Summary table of water-related disorders

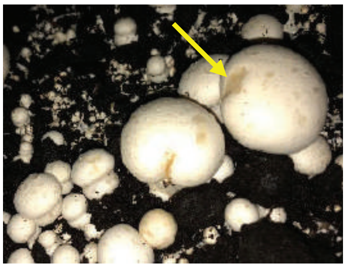
Symptom	Contributory factor(s) and comments	Example
1. Premature opening	Casing and compost moisture and nutrition • *Can express after heavy 1st flush results in a dry casing* • *Can express when high CO_2 levels occur in the microclimate. Too many pins are formed and picking management is not able to provide room for the mushrooms to grow and the compost heat to escape*	
2. Leggy mushrooms	CO_2 levels and casing moisture • *Common in dry casing. Can express when mushrooms grow in a crowded microclimate and CO_2 builds up and metabolic heat from the compost and mushrooms can't escape* • *When caps show a 'helmet' symptom, presence of virus disease should be explored*	
3. Split stems	Casing and compost moisture • *Can express in mushrooms grown in wet casing on wet compost* • *Can also be triggered by the watering regime between flushes* • *Mushroom cap and stem both high in moisture content*	
4. Saggy socks	Casing and compost moisture and evaporation • *Commonly triggered in mushrooms growing in between 1st and 2nd flush when a large amount of water is applied and the mushrooms grow unevenly when growth and evaporation rate (water out) can't keep up to water in the bed*	
5. Susceptibility to bruising	Casing and compost moisture and evaporation • *Mushrooms that are damp to touch and NOT white indicate lack of evaporation or water stress from a dry casing* • *Bruising occurs due to mechanical damage during harvesting, packing and transport*	
6. Internal watery blemishes ('windows')	Casing and compost moisture and evaporation • *Prevalent in wet casing on dry compost* • *A symptom of lack of evaporation*	
7. Wet stems	Casing and compost moisture and evaporation • *Result of an imbalance between excessive water uptake of the mushroom fruitbody over water loss* • *Mushrooms watered late in their development susceptible to uneven water distribution and excessive water in the stem* • *Increased compost temperature, e.g. as a result of compost activity when 2nd flush is pinning, can lead to wet stems expressing*	

Table 1 Summary table of water-related disorders (cont…)

Symptom	Contributory factor(s) and comments	Example
8. Brown core	Casing moisture and evaporation • *Lack of evaporation when the pinheads are developing into mushrooms* • *This particular photo is of an early formed 1st flush mushroom so the room conditions were understandably being managed for the majority of the flush and therefore the humidity and evaporation were not ideal for early mushrooms*	
9. Weepers	Casing and compost moisture • *Often occurs in wet casing on dry compost* • *Water droplets can also be seen on the stem of affected mushrooms* • *Interpreted as a stress symptom when more water is pumped into the mushroom than is evaporated out*	
10. Hollow stem	Casing moisture • *Occurs in wet casing on dry compost* • *The whole mushroom is very wet* • *May occur following period of excessive water uptake followed by a period of rapid evaporation*	
11. Scaly caps	Air flow and humidity • *Occurs when humidity is low, or air velocity is excessive* • *Beware that too low air flow may result in bacterial blotch expressing* • *If a 'harsh' drying phase is initiated, mushrooms can express symptoms of both scaling from a lot of evaporation over a short period of time and blotch from not enough evaporation at other times*	
12. Misshapen, irregular or asymmetrical cap development	Casing moisture and evaporation • *Commonly triggered in mushrooms growing between 1st and 2nd flush when a large amount of water is applied and the mushrooms grow unevenly when growth and evaporation rate (water out) can't keep up to water in the bed* • *Small pins can be damaged by the picking process or if beds are 'cleaned' by the pickers* • *Asymmetrical cap development may indicate presence of high numbers of nematodes*	
13. Internal stipe necrosis	Casing moisture • *Occurs chiefly in very wet casing* • *Often occurs with a corky stem core tissue* • *Characterized by browning of water conductive stem tissue* • *Generally thought that the bacterium* Ewingella americana *is responsible for the browning but this may be due to physiological deterioration of water-soaked tissue* • *This disorder needs more investigation*	
14. Mycelium overgrowth	Casing moisture and evaporation • *Surface growth of the mycelium is important to ensure water can penetrate into the compost* • *Where the casing surface is 'sealed' by mycelium, water will collect on the top so total water applied will be reduced, leading to water stress* • *Overgrowth will reduce pinning and cause pin death*	

Photo credits: J. Allan 2, 4, 5, 6, 7, 8, 11, 12; 14; W. Gill 9, 13; Fletcher & Gaze (2008) 1, 3, 10

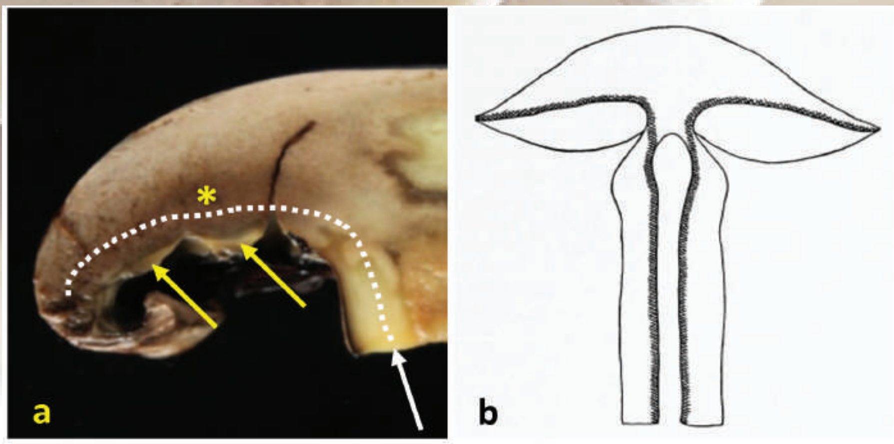


Figure 3 Water movement through mushrooms **a)** path of water movement through a water-soaked mushroom (white arrow and dotted line) surrounded by browning water-soaked cap tissue (*). Yellow 'jelly' has displaced gill tissue (yellow arrows) **b)** Cross-section of a typical *Agaricus*-type mushroom indicating the region of water transport (hatching). *Image credits: 3a, W. Gill; 3b, Schütte (1956) courtesy of JSTOR*

Figure 4 In-rolled margin and wavy gills often expressed by affected mushrooms. Note asymptomatic stem. *Photo credit:*

It is likely, too, that the wavy gills and in-rolled margin (**Fig. 4**) are also due to the swelling of the sub-hymenium as the increased cellular volume and consequent increased length would distort the overlying tissue to fit into the predetermined space between the stipe and cap margin.

Case 2

This 'bleeder' (**Fig. 5**) is a very extreme example of a water-stressed mushroom involving multiple bacterial infections, one of which gives the mushroom the appearance of bleeding. Bacteria producing red pigments such as *Serratia marscesens* are known to inhabit mushroom casing. In addition to the obvious weeping symptom, the white *Agaricus* mushroom showed slight scaling of the cap and was generally 'off-white' in colour. Referring to **Table 1**, the weeping symptom is due to mushrooms growing on a wet casing over a dry compost while the scaling and browning would be caused by low humidity or excessive air velocity. As this was a solitary example, this symptomology is indicative of an isolated and very localised microclimate such as an overly dry or overly wet patch of casing and/or compost and exposure to high air flow, perhaps near a vent or outlet. Weepers are more likely to be brown strains than white, so this is an unusual occurrence for that reason alone. Isolated examples like this are a curiosity and are not necessarily indicative of wholesale water imbalances across the cropping system.

Case 3

An extraordinary example of an *Agaricus* brown strain weeper (**Fig. 6**). The weeper has exuded water around the base of the stem where it has begun to form a foul smelling 'foam' and has become a 'stinker.' The cause of the foam is not known, but due to the odour produced, it is likely to be a biological fermentation. Alternatively, the mushroom 'sap' may have reacted with something in the casing such as lime, to create this reaction, but this does not account for the odour. Again, this was an isolated example and did not recur after the crop ran its course and the room was cooked out. Presumably this symptomology was elicited by a substrate microclimate of a patch of wet casing overlying a dry compost. There has been a recent report of this exact phenomenon occurring at another Australian farm. Wuest (1982) differentiates weepers into three distinct categories: the 'leaker,' where water droplets form and remain on the cap and stipe, the 'weeper' where the droplets form and then fall from the mushroom onto the casing and the 'stinker' which occurs when a weeper dissolves into a white, putrid foam or when the water from a 'weeper' collects on the casing and a malodorous foam forms such as in this example.

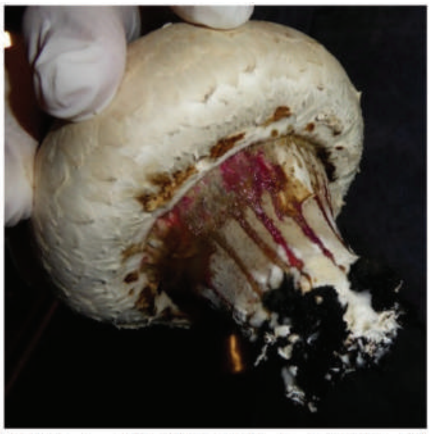


Figure 5 The 'bleeder', a complex of pathologies. *Photo credit: J. Allan*

Figure 6 A weeper that has become a 'stinker'. *Photo credit: W. Gill*

CONCLUSION

Ever since the widespread adoption of hybrid *Agaricus* strains and denser 'deep-dug' black peat as the primary casing component, water relations in mushroom growing have shifted. As the mushroom bed has become wetter and the growing environment more humid, more environmental-mediated abiotic disorders expressing novel symptoms have emerged.

Furthermore, the wetter conditions also favour some pathogenic fungi such as *Cladobotryum* (Cobweb disease) and the appearance of new and emerging bacterial diseases, such as the soft rots and the internal stipe necrosis bacterium, are closely correlated with the wetter growing regime. In addition to the new and emerging pathogens, previously known pathogens are 're-emerging' and

Table 2 Symptomatic mushroom and drippy gill symptoms compared

Symptom	Symptomatic mushroom	'Drippy gill'-affected mushroom
Water-logged stem Fig. 2a; Fig. 3a	Heavily water-logged, free water observed on cut surface of stem	Not water-logged. Stem is dry and splits longitudinally, exposing central stem core tissue. Splits may exude bacteria as infection develops
Water-logged cap tissue Fig. 2a; Fig. 3a	Water-logging forms 'windows' in the cap tissue	Cap tissue shows no signs of water-logging
'Jelly-like' material in gills Fig. 2a-c; Fig. 3a	Develops in the sub-hymenium and protrudes through hymenium. Replaces gill tissue in affected areas, or locally prevents formation of the gill tissue	'Jelly-like' material not observed in 'drippy gill'-affected mushrooms
In-rolled margin Fig. 2a,c; Fig. 4	Margin heavily in-rolled	Margin not affected
Water droplets on gills Fig. 2b,c	Clear water droplets were exuded close to the yellow 'jelly'	Droplets exude from the gills and splits in the stem. The droplets are opaque and composed of bacteria and they coalesce and form 'ribbons' of slime when they touch
Water droplets at cap periphery Fig. 2c	Leaking/weeping. More frequent on brown strains than white. Droplets on the outside edge of the caps are transparent, indicating no bacterial infection. Not seen on stipe or central cap	Droplets do not form on the exterior of the cap
Wavy gills Fig. 4	Gills wavy, no evidence of tissue collapse or necrosis	Gills are disrupted, become necrotic and collapse. Deteriorate and rot, do not become wavy
Time of symptom expression	Express while mushrooms are still closed	'Drippy gill' symptoms may be expressed before veil break[1] while the mushroom is still closed

[1]Early workers assumed that *P. agarici* bacteria existed within the mushroom hyphae because symptoms were expressed before veil break, before the hymenium was exposed to compost bacteria. In *A. bisporus*, the veil is a double membrane, which makes it even more unlikely that pathogenic bacteria from the compost or casing would be able to access the hymenium prior to veil break. It has since been demonstrated (Gill & Cole 2000) that 'drippy gill' bacteria migrate through the cap tissue to the hymenium and fall from the gills in droplets. They also migrate through the cap tissue to the stipe and exit through the split stipe. Bacterial migration is driven by gravity

expressing symptomology not previously attributed to them as they adapt over time to the wetter growing environment. Such an example is *P. agarici*, the causal organism of drippy gill disease. Recent reports of this bacterium from Serbia indicate that the primary symptom is now not the classic bacterial droplets issuing from necrotic gill tissue and longitudinally split stipes, but a browning symptom which is considered to be a greater problem than that attributed to brown blotch caused by *Pseudomonas tolaasii* (Milijaevi-Mari *et al* 2016).

Don't wait for a major problem to arise before reviewing your watering regime. If left unchecked, an imbalance in water relations within the mushroom cropping system can have a significant detrimental impact on yield, quality and ultimately farm profitability. By looking carefully and looking closely at the symptoms expressed and referring back to the symptom summary table (**Table 1**), the problem can be identified, isolated and rectified.

And lastly, a quote which concisely sums up the theme of this article:

"Finally, growers must read the mushrooms, it's growth, its stress symptoms and react to what they are telling you".

David Beyer (2001)

GROWERS' SUMMARY

- Not all symptoms expressed on mushrooms are caused by pathogens. Learn to distinguish common water stress-induced symptoms from disease symptoms.
- Many symptoms are biological indicators. They are reactions to a sub-optimal environment and are often water related.
- Multiple or complex symptomologies may have multiple roots. Identify the separate symptoms. Refer to Table 1 and determine the cause of one symptom at a time.
- Look very carefully and accurately interpret what the mushrooms are telling you. Act accordingly.
- If you observe a symptom you cannot identify or explain, photograph it in situ and contain or remove the symptom. Contact the Project Team with the photographs to develop a plan of action.
- Analysing symptoms very closely and paying acute attention to detail are fundamental to effective monitoring for new and/or emerging diseases.

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