

## Raising humidity to increase mushroom yield must be balanced with the increased risk of bacterial blotch

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The populations of pseudomonad bacteria in mushroom casing are known to be essential in stimulating the initiation of mushrooms as well as detrimental in causing bacterial blotch disease. Diagnostic tests which could be used to screen for mushroom initiation stimulatory and/or blotch causing pseudomonads would provide information on the likely effects of the casing material on initiation and incidence of blotch disease.

The aims of this work were to:

- develop screening tests for *Pseudomonas* species in mushroom casing materials to assist in understanding their role in the stimulation of mushroom initiation and disease development
- screen casing materials used in the UK for *Pseudomonas* species using the above test and relate the results to mushroom pinning and blotch incidence
- establish the independent effects of the casing material, *Pseudomonas* species and the growing room humidity on mushroom cropping and the development of blotch disease.

Mushrooms were grown using several peat + sugar beet lime casing materials, obtained from the UK, Ireland and the Netherlands, at two relative humidities: 88% and  $\geq 92\%$ . Controlled studies on bacterial blotch development showed that natural infections occurred at 18 °C only when the relative humidity (RH) was maintained at 92% and above. At 88% RH, no blotch disease developed at all. Under the higher humidity conditions, the number and yield of mushrooms increased, by up to 20 and 30% respectively, compared with the same experimental production conducted at 88% RH. However, bacterial blotch symptoms developed under the high RH conditions in an average of around 8% of the mushrooms produced. New molecular PCR tests (developed in conjunction with the University of Wageningen, Netherlands) did not detect what is thought to be the major cause of bacterial blotch (*Pseudomonas tolaasii*) on any of the blotched mushrooms grown above 92% RH on several casing sources. However, other fluorescent *Pseudomonas* species were isolated from these diseased mushrooms. When inoculated onto fresh casing materials at high RH, these isolates induced 'mild' blotch symptoms (Fig 1), compared with severe symptoms observed following inoculation with reference strains of the known blotch causing bacteria *P. tolaasii* (Fig 2) and *P. gingeri* (Fig 3). Although differences in the severity of blotched mushrooms were observed, even the mild symptoms were sufficient to make the mushrooms unmarketable. The source of casing material had no apparent effect on the incidence or severity of blotch disease following inoculations. Although casing materials appeared not to be a source of *P. tolaasii* in this study, it remained unclear

whether they could be sources of other mild blotch-forming bacteria. Further characterization of these bacteria, similar to that conducted for *P. tolaasii*, will be required before test methods can be adapted for their detection. Potential increase in mushroom numbers and yield in response to increased humidity or longer and more frequent watering regimes needs to be carefully balanced with the increased risk of reducing quality and marketability due to bacterial blotch disease. The main conclusions from this work were:

- mushroom yields and numbers were greater at higher humidity ( $\geq 92\%$  RH) than at 88% RH
- at the higher humidity, blotch symptoms were caused by an unknown *Pseudomonas* species
- although the symptoms were milder than those caused by the known blotch causing pseudomonads (*P. tolaasii* or *P. gingeri*), they rendered the mushrooms unmarketable
- no natural infections of *P. tolaasii* or *P. gingeri* were observed on several casing materials
- characterisation of the mild blotch causing pseudomonads is needed before they can be detected in different casing materials, and further work is needed to determine if these populations relate to the occurrence of blotch on different casing materials.



Figure 1. Mild blotch symptoms caused by unknown fluorescent *Pseudomonas* species.



Figure 2. Severe blotch symptoms after inoculation with *Pseudomonas tolaasii*



Figure 3. Ginger blotch symptoms caused by *Pseudomonas gingeri* (right) and cap pitting caused by *P. costantinii* (right)

A current project with Fera Science Ltd (Dr Joana Vicente) and the University of Cambridge (Prof George Salmond) funded by AHDB Horticulture is examining the use of antagonist Pseudomonads and bacteriophages for the control of blotch disease. New molecular PCR tests have been developed for two other blotch causing bacteria: *Pseudomonas gingeri* and *P. costantinii* (Fig. 4). Combined with an enrichment technique, this should enable the sources of blotch causing bacteria on mushroom farms to be identified.

### References

- Elphinstone J., **Noble R.**, (2018) AHDB Horticulture Report M 63. Mushrooms: Identification, detection and control of *Pseudomonas* species causing different types of bacterial blotch symptoms
- Taparia T., Krijger M., Haynes E., Elphinstone J.G., **Noble R.** & van der Wolf J. (2020) Molecular characterization of pseudomonads from *Agaricus bisporus* reveal novel blotch pathogens in Western Europe. *BMC Genomics* 21:505; doi.org/10.1186/s12864-020-06905-3