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ROOT COLLAR EXCAVATION WITH TRICHODERMA INOCULATIONS AS A POTENTIAL MANAGEMENT STRATEGY FOR HONEY FUNGUS (*ARMILLARIA MELLEA*)

Glynn C Percival[1], E. Thomas Smiley[2] & Roland T.V. Fox[3]*

Summary

Honey fungus (*Armillaria mellea*) is an important pathogen that can cause severe damage to infected trees and other plants. In this study we investigated the effect of air-spading and/or inoculation with a bio-control fungus as controls for honey fungus. Air-spading uses compressed air to de-compact soil while causing minimal disturbance or damage to the root system. Air-spading can also use compressed air to permanently remove soil from the base of the tree trunk to the depth at which main roots originate, a technique known as root collar excavation. The bio-control fungus *Trichoderma harzianum* strain (Trade name Trianum) is a root symbiont that is known to protect host plants from a range of pathogenic fungi. Raised beds were constructed and artificially inoculated with *A. mellea* while non-*A. mellea* infected beds acted as controls. One year later *A. mellea* infected raised beds were subjected to one of the following treatments i) no treatment ii) air-spading, iii) air-spading + *T. harzianum* or iv) *T. harzianum* only. The effectiveness and longevity of air-spading and *T. harzianum* was determined by potting up strawberry plants cv Cambridge favourite (highly susceptible to honey fungus attack) at month 6, 12, 18 and 24 after treatment. *A. mellea* infection was then quantified at day 90 after potting up by assessing visual by plant condition, leaf chlorophyll fluorescence Fv/Fm, leaf chlorophyll content (SPAD) values and fruit yield per plant. Air-spading *A. mellea* infested soil with and without *T. harzianum* resulted in a two year protective period in which *A. mellea* failed to re-infect strawberry plants. In addition, failure to re-isolate *A. mellea* from media after air-spading indicates the air-

[1] Dr Glynn Percival, Plant Physiologist, Bartlett Tree Research Laboratory, Harborne Building, School of Biological Sciences, Whiteknights, University of Reading, Reading, Berkshire, RG6 6AS, United Kingdom (gpercival@bartlettuk.com). *Author for correspondence.

[2] Dr Tom Smiley, Arboriculture Researcher, Bartlett Tree Research Laboratory, Adjunct Professor Clemson University, 13768 Hamilton Rd, Charlotte NC 28278, United States of America (tsmiley@bartlett.com).

[3] Dr Roland T.V. Fox, Honorary Fellow, School of Biological Sciences, University of Reading, Reading, Berkshire RG6 6AF (r.t.v.fox@reading.ac.uk).

spading process may have eradicated *A. mellea* from the infected raised bed. Some degree of protective properties against *A. mellea* was confirmed when applying *T. harzianum* alone. For example, based on visual plant condition, application of *T. harzianum* reduced *A. mellea* severity by 12.5–65.7% over the two year study. Findings of this study strongly indicate that air-spading followed by inoculation with *T. harzianum* appears to offer promise as a joint cultural/bio-control strategy for the management of *A. mellea*. Results however, should be interpreted with some degree of caution as experiments were conducted under controlled conditions where soil conditions may vary from that in a woodland, forest or urban environment.

Key words: Chlorophylls, chlorophyll fluorescence, plant health care, soil borne pathogens, biotic stress, soil excavation.

Introduction

Honey fungus caused by the soil borne pathogen *Armillaria mellea* is worldwide in distribution attacking many species of fruiting, ornamental and forest trees and shrubs (FOX, 2001). Severe losses can occur in orchards or vineyards if planting occurs in infected soils (BAUMGARTNER, 2004). Most commonly, however, losses from this disease are steady, but inconspicuous, appearing as a slow decline and death of the occasional tree. Death is also greater when trees suffer from moisture stress or defoliation (RAZIQ, 2000). Infected trees demonstrate symptoms that include; reduced growth, smaller yellowish leaves, dieback of twigs and branches followed by gradual or sudden death. Affected trees appear scattered at first, but soon circular areas of diseased trees appear because of the spread of the pathogen from its initial infection point (MORRISON, 1976).

Symptoms of root attack can be expressed in several ways. A tree, particularly large trees, may die after a period of increasing ill health or show a general deterioration in crown condition indicating root or root collar problems. Other trees exhibit no outward symptoms, but blow over due to rotted roots. Such is typically the case with oak that, while rarely killed by the fungus, is subject to windthrow from rotted roots. Resin, gum or watery liquid may bleed from the lower stem of affect plants. In the autumn, clumps of honey-brown toadstools, similar in appearance to the cultivated mushroom, appear near or at the base of infected plants. A fan-like, whitish mat of fungal tissue (mycelium) is often found under the bark of *Armillaria* infected trees. Rhizomorphs, which resemble black bootlaces, may also be present. These rhizomorphs function to carry water and nutrients to the cambium under the bark of roots being attacked from existing rotted roots and stumps, leaves or even the soil to aid in their decay (FOX, 2001; WEST and FOX, 2002; RAZIQ and FOX, 2004a).

With the withdrawl of the phenolic emulsion Armillatox for plant protection purposes, control of honey fungus was forced to rely primarily on cultural methodology (WEST and FOX, 2002). In landscape situations all sources of infection such as infected stumps and major roots are removed from site. Where stumps are not dug out, chipping or grinding is used as an option followed by digging out of large un-chipped pieces. If chippings are left in the soil, replanting is generally delayed for at very least a season. If infected stumps and roots cannot be removed, the use of a physical barrier to prevent the spread of the fungus has sometimes been successful providing it is possible to separate the source of infection from healthy plants. Soil sterilization with steam (6kg pressure for 30 minutes at 120ºC) is used in countries such as Germany but this treatment has to be applied annually and suffers from the dis-advantage that steam sterilization kills all soil borne organisms as well as *Armillaria* (RAZIQ, 1998; RAZIQ, 2000; WEST, 1994)

An air spade is a relatively new technique that cultivates the soil to a depth of 15–25 cm using compressed air to de-compact compacted soil with minimal disturbance or damage to the root system (SMILEY, 2005). Another common operation using an air-spade involves the permanent removal of soil from the base of the tree trunk to the depth at which main roots originate, a technique known as root collar excavation (DAY and HARRIS, 2008; DAY *et al*. 2009). Root collar infection is a critical step in the infection process of trees by *A.mellea*. This involves the formation of mycelium under the bark at the root collar, decomposition of the underlying cambium tissue and decay of secondary xylem. This process girdles the trunk preventing vascular tissue from functioning (BAUMGARTNER and RIZZO, 2002; BAUMGARTNER, 2004). Previous research has shown that air-spading around the root collar potentially offers a cultural means of preventing root collar infection by honey fungus by protecting this important part of the root system from subsequent vascular decay (BAUMGARTNER, 2004; BLISS, 1944; MUNNECKE *et al.,* 1976).

Fungi in the genus *Trichoderma* have been recognised since the 1920s for their ability to act as bio-control agents against both root and foliar plant pathogens (ELAD *et al*. 1982; RAZIQ and FOX, 2003). When added to the soil *Trichoderma* can rapidly colonize a root surface or cortex, depending on the strain. In addition to colonizing roots, *Trichoderma* have developed other mechanisms to attack, parasitize and gain nutrition from other soil borne fungi. These include, mycoparasitism, antibiosis, competition for soil nutrients and/or pore space, solubilization and sequestration of inorganic nutrients (HARMAN, 2006). Previous research under laboratory conditions and using containerized pot trials has consistently shown *Trichoderma* isolates to be highly antagonistic against *A. mellea* with the conclusion that these fungi would make a useful addition to *A. mellea* control strategies (RAZIQ and FOX,

2004b). Trianum is a commercially available T-22 *Trichoderma harzianum* strain sold by Koppert Biological Systems (Veilingweg 14, Postbus 155, 650 AD Berkel en Rodenrijs, Netherlands). According to the manufacturers this strain of *Trichoderma* protects crops from soil-borne diseases such as *Fusarium*, *Pythium*, *Rhizoctonia* and *Sclerotinia*. The potential of T-22 *T. harzianum* strain against *A. mellea* remains unknown.

Objectives of this research are to evaluate the use of air-spading singly and in combination with a commercially available T-22 *T. harzianum* strain as a potential joint cultural/bio-control management strategy for *A. mellea* control.

Materials and Methods

Armillaria mellea isolates

A. mellea isolates were cultured at the John Harborne Building, School of Biological Sciences, University of Reading and maintained on a 2% malt extract agar plates at 25°C ± 1°C in the dark. To ensure their virulence each culture was annually passed through strawberry cv Cambridge Favourite, a highly *A. mellea* susceptible cultivar that is rapidly infected when colonized.

Raised bed construction and inoculation

Eighteen raised beds (1.5 × 3m) were constructed at the University of Reading Shinfield Experimental Site, University of Reading, Berkshire (51°43′ N, –1°08′ W) using 0.3 m^2 breeze blocks. Each raised bed was then filled with a medium suitable for *A. mellea* growth and development (40% general potting compost; 40% John Innes No 2; 20% wood chip). Pure cultures of *A. mellea* (6 agar plates) were then added to a liquidizer containing 2 litres sterile distilled water and an *A. mellea* slurry added to each raised bed except three that were left un-infected for control purposes. These beds were left for one year to allow colonization by honey fungus. After one year strawberry plants cv Cambridge Favourite were potted up using media obtained from each *A. mellea* inoculated raised bed. Symptoms of *A. mellea* infection on all strawberry plants at day 90 after potting up and observation of *A. mellea* root infection on each plant demonstrated that all raised beds were infected (data not shown).

Raised bed treatments

Each *A. mellea* infected raised bed was then treated as below:

1. Control – no *A. mellea*, no treatment.

2. *A. mellea* only.
3. Air-spading (1 minute per m^2)
4. Air-spading (1 minute per m^2) + Trianum (3.0g per m^2 suspended in 10 L water)
5. Trianum (3.0g per m^2 suspended in 10 L water)

Re-isolation of A. mellea

The presence of *A. mellea* was determined at each assessment date (month 6, 12, 18, 24) using the following techniques. 1. Visual examination of strawberry plant roots by splitting the main root and microscopically observing for the presence of *A. mellea* mycelial sheets in the cambium region (RAZIQ and FOX, 2004a). 2. Removal of root tissue (ideally dying) and isolation on 2% malt extract agar amended with 100mg L streptomycin sulphate and 100mg L oxytetracycline; three 3cm root tissue sections per plant (RAZIQ and FOX, 2004a). 3. Removal of 0.05g soil from each pot (3 soil samples per pot) that was dispersed at the centre of a sterile petri-dish in 0.5ml sterile distilled water. Approximately 15ml of cool 2% malt extract agar was then poured into each dish and each dish rotated and gently shaken to disperse the soil throughout the agar media (RAZIQ and FOX, 2004b). Dishes were then incubated at 25ºC ± 1ºC in the dark. A positive confirmation for *A. mellea* was confirmed by the presence of mycelium and importantly rhizomorphs growing on the agar surface. If only one of the three tests proved positive then this was interpreted as evidence of the presence of *A. mellea.*

Plant Vitality Measurements

Leaf chlorophyll measurements

To keep the physiological age of the leaves comparable throughout the experiment, measurements of chlorophyll content (SPAD) were made only on fully expanded mature leaves. In all cases SPAD measurements were taken from six leaves (two from the top of the crown, two in the centre and two at the base) per tree. A Minolta chlorophyll meter SPAD-502 was used. Chlorophyll was measured at the mid-point of the leaf next to the main leaf vein. Calibration was obtained by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam) after extraction with 80% v/v aqueous acetone (regression equation = 5.58 + 0.053x; r^2 adj = 0.94, P = <0.001), (LICHTENHALER, 1987).

Plant Condition

Plant condition was assessed visually commencing at day 90 after potting up using a visual indexing technique and ratings on the scale: 0 = No yellowing observed, all leaves healthy green colour; 1 = less than 5% of leaves affected and no aesthetic impact; 2 = 5–20% of leaves affected with some yellowing but little or no defoliation; 3 = 21–50% of leaves affected, significant defoliation and/or leaf yellowing; 4 = 51–80% of leaves affected, severe foliar discolouration; 5 = 81–100% of leaves affected with 90-100% defoliation. The individual ratings for each plant in each treatment were used as a measure of condition for statistical analysis. Plant condition ratings used in this study was based on those of stipulated by RAZIQ and FOX (2004a,b)

Statistical Analysis

Mean pathogen severity values for all treatments were transformed using the Arcsin transformation. All data were analyzed using ANOVA and the differences between means were determined using the Tukey *w* procedure ($P = 0.05$) using the Genstat for Windows program. Back transformed pathogen severity values are presented here to ease interpretation of these data.

Results

Damaging outbreaks of *A. mellea* were recorded on strawberry plants cv Cambridge Favourite potted up using media from *A. mellea* infested beds as indicated by plant condition ratings ranging from 3.8-4.4 over the two year study (Table 1). In addition, significant reductions ($P<0.05$) in leaf chlorophyll content, leaf chlorophyll fluorescence Fv/Fm values and fruit yield per plant in comparison with non-*Armillaria* infected control plants indicate *A. mellea* induced detrimental effects on leaf chlorophyll structure, photosynthetic system and growth respectively (Tables 2–4). No symptoms of *A. mellea* were recorded in control plants as demonstrated by plant condition ratings of 0.0 throughout the study (Table 1). Likewise, leaf chlorophyll content, leaf chlorophyll fluorescence Fv/Fm values and fruit yield per plant recorded in non- *A. mellea* infected controls at month 6, 12, 18 and 24 were associated with those of healthy, non-stressed strawberry plants (Tables 2–4).

There was no statistical difference in visual plant condition, leaf chlorophyll content, leaf chlorophyll fluorescence Fv/Fm values and fruit yield per plant between non-*Armillaria* infected control strawberry plants and strawberry plants potted up in media that had been previously air-

TABLE 1. The influence of root collar excavation and *Trichoderma* inoculation on strawberry plant cv 'Cambridge Favourite' condition as a measure of *Armillaria mellea* infection.

	Plant Condition (0–5)			
Treatment	Month 6	Month 12	Month 18	Month 24
No Armillaria, no treatment (control)	0.0a	0.0a	0.0a	0.0a
Armillaria only	4.1c	3.8c	4.4c	4.0b
Air-spading	0.0a	0.0a	0.1a	0.2a
Air-spading + Trianum	0.0a	0.0a	0.0a	0.0a
Trianum	1.3b	2.1b	2.0b	3.5b

All values mean of 9 plants.
Lower case letters indicate significant differences between means for each evaluation date by the Tukey range test ($P = 0.05$).

TABLE 2. The influence of root collar excavation and *Trichoderma* inoculation on strawberry cv 'Cambridge Favourite' leaf chlorophyll content (SPAD values) as a measure of *Armillaria mellea* infection.

	leaf chlorophyll content (SPAD values)			
Treatment	Month 6	Month 12	Month 18	Month 24
No Armillaria, no treatment (control)	0.0a	0.0a	0.0a	0.0a
Armillaria only	11.2b	8.8c	10.4b	14.1b
Air-spading	39.6a	40.5a	38.9a	41.0a
Air-spading + Trianum	42.1a	40.6a	41.5a	43.0a
Trianum	23.0b	18.6b	17.5b	16.2b

All values mean of 9 plants, 5 laves per plant.
Lower case letters indicate significant differences between means for each evaluation date by the Tukey range test ($P = 0.05$).

spaded or media that had been air-spaded and Trianum added (Tables 1–4). Such a response indicated that air-spading *A. mellea* infested soil resulted in at least a two year protective period in which *A. mellea* failed to re-infect a highly susceptible strawberry cultivar. Failure to re-isolate *A. mellea* from media after air-spading indicates the air-spading process may have eradicated *A. mellea* from the infected soil medium (Table 5). Limited efficacy of adding Trianum after air-spading was demonstrated. In all cases plant condition, leaf chlorophyll content, leaf chlorophyll fluorescence Fv/Fm values and fruit yield per plant were comparable with strawberry plants potted up in media that had been air-spaded only (Tables 1–4).

Some degree of efficacy on controlling *A. mellea* was confirmed when applying Trianum only to *A. mellea* infested medium. At months 6, 12, 18 and 24 strawberry plant condition, leaf chlorophyll content, leaf chlorophyll

TABLE 3. The influence of root collar excavation and *Trichoderma* inoculation on strawberry cv 'Cambridge Favourite' leaf chlorophyll fluorescence Fv/Fm values as a measure of *Armillaria mellea* infection.

Treatment	leaf chlorophyll fluorescence Fv/Fm values			
	Month 6	Month 12	Month 18	Month 24
No Armillaria, no treatment (control)	0.820a	0.823a	0.801a	0.812a
Armillaria only	0.444b	0.414c	0.305c	0.392b
Air-spading	0.811a	0.819a	0.822a	0.803a
Air-spading + Trianum	0.820a	0.824a	0.815a	0.815a
Trianum	0.576b	0.516b	0.527b	0.404b

All values mean of 9 plants, 5 laves per plant.
Lower case letters indicate significant differences between means for each evaluation date by the Tukey range test ($P = 0.05$).

TABLE 4. The influence of root collar excavation and *Trichoderma* inoculation on strawberry cv 'Cambridge Favourite' fruit yield (g) as a measure of *Armillaria mellea* infection.

Treatment	Fruit yield (g) per plant			
	Month 6	Month 12	Month 18	Month 24
No Armillaria, no treatment (control)	360.5a	410.3a	338.2a	379.5a
Armillaria only	100.4b	86.2c	115.8b	122.9b
Air-spading	377.2a	446.7a	365.7a	401.4a
Air-spading + Trianum	388.1a	422.5a	365.7a	405.6a
Trianum	166.7b	145.7b	189.4b	133.6b

All values mean of 9 plants
Lower case letters indicate significant differences between means for each evaluation date by the Tukey range test ($P = 0.05$).

fluorescence Fv/Fm values and fruit yield per plant were in some instances significantly improved compared to strawberry plants potted up in medium obtained from *A. mellea* infested beds only (Tables 1–4). For example, based on visual plant condition, application of Trianum alone reduced *A. mellea* severity by 12.5–65.7% over the two year study (Table 1). *A. mellea* was, however, re-isolated from media after addition of Trianum only at months 6–24 (Table 5).

Discussion

Based on analyses of visual plant condition, leaf chlorophyll content and chlorophyll fluorescence Fv/Fm values as a measure of leaf photochemical efficiency, results of this study indicate air-spading alone or in combination with Trianum provided protection against re-infection by *A. mellea* over

TABLE 5. Re-isolation of *Armillaria mellea* from root systems of strawberry cv 'Cambridge Favourite'.

Treatment	Month 6	Month 12	Month 18	Month 24
No Armillaria, no treatment (control)	–	–	–	–
Armillaria only	+	+	+	+
Air-spading	–	–	–	–
Air-spading + Trianum	–	–	–	–
Trianum	+	+	+	+

All values mean of 9 plants,
+ = Presence of *Armillaria* isolated from either root tissue or soil medium.
– = *Armillaria* not isolated from either root tissue or soil medium.

a period of two years. Likewise analysis of strawberry fruit yield, as a measure of growth, that were statistically comparable with non-*Armillaria* inoculated controls supports this conclusion. Attempts to re-isolate *A. mellea* from any air-spade treated raised bed proved negative indicating that the air-spading process may have eradicated *A. mellea* from the soil medium. Root collar excavation using an air-spade has been shown to affect mycelial fans present at the root collar, but the mechanism by which this occurs is not fully understood (BAUMGARTNER, 2004). Although not explored in this study a number of direct and indirect mechanisms caused by the air-spading process may account for the degree of control recorded in this study. Application of compressed air to the *A. mellea* infested raised bed media may have resulted in the physical destruction of *A. mellea* resting spores and any rhizomorphs present within the soil medium (BLISS, 1944; MUNNECKE *et al.,* 1976; MUNNECKE *et al.,* 1981). *A. mellea* infection is generally higher in soils sub-optimal for root growth i.e. waterlogging or drought (HAGAN, 2004). The air-spading process has been shown to de-compact soils, improve air-porosity and enhance microbial activity, thereby improving conditions for root growth while creating a sub-optimal environmental for *Armillaria* infection. Exposure of the root collar to drying has been shown to kill or inhibit *A. mellea* in infected wood as well as mycelial fan growth (MUNNECKE *et al.,* 1976; MUNNECKE *et al.,* 1981). Under standard working conditions air-spading is generally recommended to be performed at an area 3 × DBH around the tree base. Consequently the area of soil treated will vary depending on tree size. As the rate of rhizomorph growth has been assessed to range from 0.2 to 2.5 m year (PEET *et al*. 1996; VAN DER KAMP, 1993) then air-spading re-treatment may need to occur on an annual basis when undertaken under field or landscape situations (BAUMGARTNER, 2004; BLISS, 1944)

Limited efficacy of adding Trianum after air-spading was demonstrated as plant condition, leaf chlorophyll content, leaf chlorophyll fluorescence Fv/

Fm values and fruit yield per plant were comparable with strawberry plants potted up in media that had been air-spaded only. However, a useful degree of *A. mellea* protectant properties (12.5 to 66.75% based on visual plant condition) was demonstrated by the addition of Trianum alone to *A. mellea* infested soil medium. With respect to *Armillaria*, electron microscopy has shown that certain *Trichoderma* species attacked, penetrated and destroyed the outer tissue of the *Armillaria* rhizomorphs and, once inside, killed *Armillaria* hyphae by coiling and direct penetration. After one week, the rhizomorphs infected with each of these *Trichoderma* species were devoid of hyphae (DUMAS and BOYONOSKI, 1992). Other mechanisms of *Trichoderma* induced root protection against soil borne pathogens was achieved via the production of fungitoxic compounds such as antibodies and/or chitonolytic enzymes and colonisation of the root system (a primary site of tissue injury during disease attack) as well as improving tree health and vigour by enhanced nutrient uptake and "buffering" against sub-optimal soil conditions (ELAD *et al*. 1982; HOYOS-CARVAJAL *et al*. 2009). Soil applications of *Trichoderma* under field conditions induced greater degrees of control than that recorded in this investigation. For example *Armillaria* infested soil supplemented with commercially available strains of *T. koningii* and *T. harzianum* prevented further mortality of grapevines while *Trichoderma* treated vines subsequently yielded a full crop with new callusing of infected bark and new cane growth recorded (COOK, 1993; ELKINS *et al*. 1997; HUNT, 2004). One of the factors that determine the success of *Trichoderma* in controlling *Armillaria* is population density. Studies of the relationship between incidences of *Armillaria* with that of *Trichoderma* indicated higher incidence of *Armillaria* at low incidence of *Trichoderma* (PATALE and MUKADAM, 2007) Therefore at low population densities, antagonistic strains of *Trichoderma* would be limited in their efficacy against *Armillaria.* Consequently, the introduction of *Trichoderma* spp annually into the soil is strongly recommended with *Trichoderma* populations maintained on a regular basis to keep soils inhibitive to *Armillaria* (OTIENI *et al.* 2003a,b). Possibly addition of Trianum at a higher concentration into the *A. mellea* infested soil medium used in this study may have induced a higher degree of control. Likewise annual supplementation as recommended by OTIENI *et al.* (2003a,b) may also have induced a greater degree of protection against *Armillaria* infection. Interestingly Trianum induced protection against *A. mellea* infection ranged from 48.5 to 65.7% from months 6 to 18 based on visual plant condition. At month 24 however, Trianum induced protection against *A. mellea* infection was only 12.5% based on visual plant condition. Such a result indicates a lowering *Trichoderma* population at month 24 after inoculation that would account for the reduced degree of *A. mellea* protection conferred. Indeed manufacturers of Trianum recommend three monthly "top up" inoculations when using their product. Ongoing research

is exploring the influence of Trianum applied at higher population densities and/or at regular intervals to further improve *A. mellea* management strategies.

Symptomatic strawberry plants in *A. mellea* infected beds at each 6 monthly sampling date had significantly less vitality (lower leaf chlorophyll content and chlorophyll fluorescence values) and fruit yield than healthy control plants, as demonstrated by significant differences in vitality and yield parameters. As the presence of *A. mellea* was re-isolated from around the root system results demonstrate that the presence of *A. mellea* are associated with lower vitality and yield parameters in symptomatic strawberry plants. Therefore, it appears that using vitality and yield data to evaluate the efficacy of air-spading for future management strategies of *Armillaria* root disease is justified. This disease assessment approach may also be useful for studies by other workers investigating alternative control systems as a means of quantifying *Armillaria* root disease on other plants.

Application of Findings

Armillaria root rot is notoriously difficult to control (WEST, 1994, WEST and FOX, 2002). Traditional management systems of *Armillaria* through the removal of tree stumps and major roots are an expensive time consuming process. No chemical controls exist in the UK for the management of this pathogen (WEST and FOX, 2002). Where chemical controls have been field tested elsewhere for the eradication of this pathogen, both systemic and non-systemic fungicides failed to fully eradicate the rhizomorphs from which the pathogen can spread (WEST and FOX, 2002). Consequently there is a need for an alternative approach to the problem (RAZIQ, 1998). Air-spading is neither reliant on fungicides, nor does it require expensive equipment (BAUMGARTNER, 2004). In combination with the application of a *Trichoderma* based bio-control agents this may improve long term efficacy against *Armillaria* infection and be adopted both therapeutically provided *Armillaria* infection is still in the early stages or be applied to prevent infection of healthy susceptible species.

Results of this study should, however, be interpreted with some degree of caution. The experiments were conducted under controlled conditions where the soil rhizosphere differs from that in a woodland, forest or urban environment. This may influence results especially with respect to *Trichoderma* inoculation and colonization of plant root systems. However, our results indicate that air-spading followed by inoculation with Trianum appears to offer promise as a joint cultural/bio-control strategy for the management of *A. mellea*. Peer review published research supports these conclusions (BAUMGARTNER, 2004; BLISS, 1944; MUNNECKE *et al.,* 1976).

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