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The role of pH in *Tuber aestivum* syn. *uncinatum* mycorrhiza development within commercial orchards

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The accepted advice when establishing a plantation of *Tuber aestivum* syn. *uncinatum* is that young inoculated trees should be planted on calcareous soils with a naturally high pH level. When a site is employed that has a naturally low pH level, lime is often applied to raise the pH to a considered ideal level of c.7.5. However, this may not be the correct approach. Here we present data from 33 data points taken from commercial truffle orchards in England, UK. Soil pH is correlated to *Tuber aestivum* syn. *uncinatum* mycorrhiza survivorship and development. The optimal observed pH was 7.51 but the actual optimal pH for cultivation may be higher. Sub optimal pH levels lead to a reduction of *Tuber aestivum* syn. *uncinatum* mycorrhiza. This reduction is not permanent and mycorrhization levels may be improved within a 12 month period by amending the soil pH. The importance of understanding the interaction of pH with other variables and the results in relation truffle cultivation are discussed.

Key words: truffle, pH, soil, mycorrhiza, *Tuber aestivum*

INTRODUCTION

Mycorrhizal fungi are those that form a symbiotic association with the roots of a vascular plant host and such species incorporate some of the most widely revered and economically important of all edible fungi species. The consumer demand for many of these edible species leads to high prices paid for fruiting bodies and of all the mycorrhizal species, the genus *Tuber* hold the most prestige and highest value per kg. Consequently, there have been many attempts to cultivate *Tuber* spp. and some of these have been successful. Cultivation involves producing tree saplings with the target *Tuber* spp. growing on the root system and planting these trees into carefully prepared soils. This approach can be very successful and today it is estimated that over 90% of all the fresh *Tuber melanosporum* produced in France originates from inoculated trees that have been cultivated (Gerard Chevalier, personal communication September 27, 2012).

Despite the achievements in truffle cultivation, there remain many obstacles and uncertainties. There are aspects of cultivation that are debated and perceived advances in cultivation methods are often kept within closed groups, due to economic interests and an unwillingness to spread knowledge to rival cultivators. Despite variation in cultivation methods, there are several widely accepted underpinning principals in truffle cultivation based on data taken from wild-production regions. Soil pH is a measure of the activity of hydrogen ions, recorded as an inverse log and is often measured in a soil and water solution. Although pH is treated as of stand-alone importance in truffle cultivation, it is strongly related to the availability of wide range of nutrients such as P, K, Ca, Mg, Zn, Mn, Cu, and Fe.

For truffle cultivation, there are different assumed ideal pH levels, specific to each truffle species. For *Tuber aestivum* syn. *uncinatum*, the pH range within the wild is reported to be primarily 6.8-8.0 (Chevalier, Frochot 1997; Wedén et al. 2004) although within pot/glasshouse cultivation a pH of 7.1 has been reported as ideal (Pruett 2008). Due to a lack of clear evidence indicating the ideal pH level for cultivation, a figure based on wild-site data of c.7.5 is often targeted by truffle cultivators of the species *Tuber aestivum* syn. *uncinatum*.

Due to the perceived ideal pH level, *T. aestivum* syn. *uncinatum* is often grown in calcareous soil. Where the soil pH is below 7.5, the soil is often amended to raise the pH to around this level. Large quantities of material are often used, with applications of over 10 tonnes of limestone per hectare not being uncommon (www.PlantationSystems.com, 10th of January, 2013).

Here we present data collated from a range of commercial truffle orchard sites in order to test the assumption that pH 7.5 is ideal for *T. aestivum* syn. *uncinatum* cultivation.

MATERIALS AND METHODS

33 data points were used in this study from truffle plantations in England (UK) in the years 2010 and 2011. The sites were primarily acidic soils that had been subject to a stage of pH adjustment using crushed and ground calcareous rock. The study also incorporates a number of sites that had a naturally high pH level and were not subject to pH adjustment. An example host tree from one of these plantations is displayed in Figure 1.

Within the 33 data points are 8 sites that were sampled twice. These sites were all sampled once in 2010 and once in 2011. A second level of pH adjustment was used on these 8 sites immediately after the 2010 sampling.

Between the sites there were many variables including soil structure, nutritional profile, tree age, management methods and climate. For example, the annual reported rainfall varied between sites from 650 mm to 2,000 mm per annum.

Soil samples were taken from areas close to the base of trees within the plantations and from a depth of 2-10 cm. Soil samples were then pH tested using a Growth

Fig. 1. An example host tree from one of the data points used in this study. Host tree species is the European hazel *Corylus avellana*.

Technology pH probe and meter calibrated with buffers pH 7.0 and 4.0. Samples were tested as an aqueous solution in a soil to water ratio of 1:1, after agitation to combine the soil with the water.

10 trees from each sample point were randomly selected for root analysis. From each tree a root piece of 4-5 cm length was removed and ectomycorrhiza were identified microscopically.

RESULTS

Root samples from 33 data points were analysed along with corresponding soil samples. Of the 33 sampling points, the sample with the lowest soil pH level (5.68) also had the lowest mycorrhization index of 9.27. This mycorrhization figure was 66.4% below the average for the dataset. The data point with the highest mycorrhization

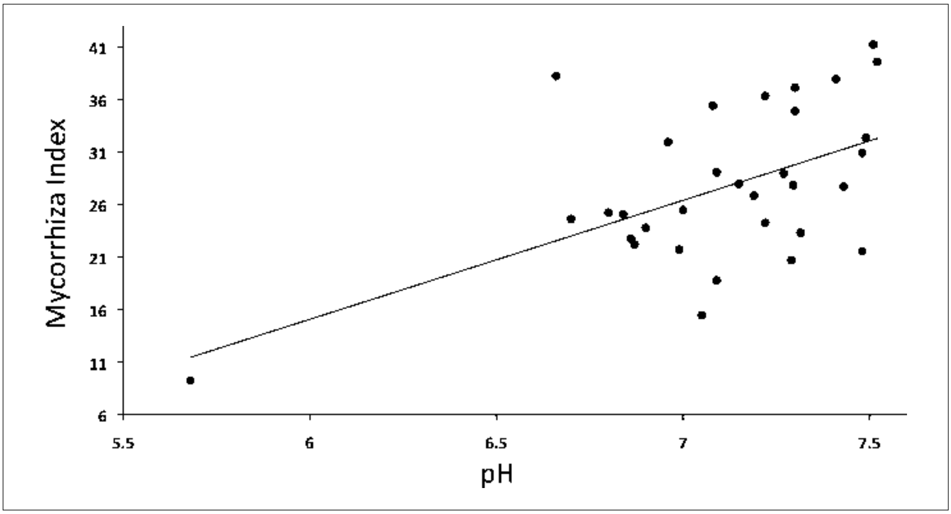

Fig. 2. Mycorrhization index of *T. aestivum* syn. *uncinatum* on host plant tree roots significantly correlated with soil pH level. ($R^2 = 0.2969$, $p<0.005$).

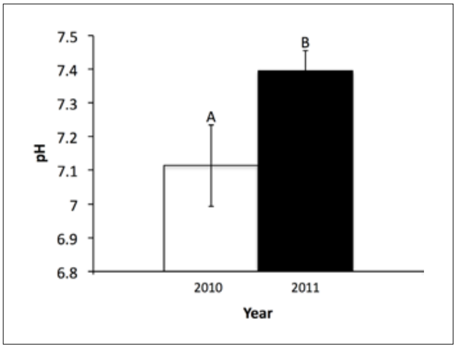

Fig. 3. Mean values ± SE for the pH of soil from 8 sites subject to soil amendment in 2010. 2010 figures are prior to soil amendment and 2011 figures are post soil amendment. Error bars are one standard error. Values followed by different letter codes are significantly different (*T*-test, $P < 0.05$).

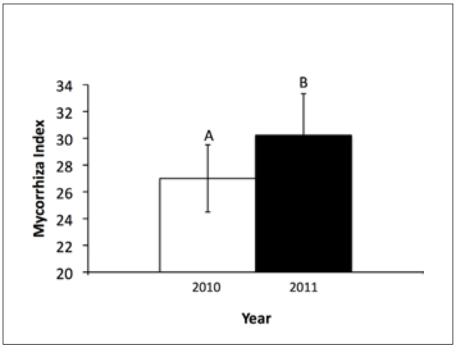

Fig. 4. Mean values ± SE for mycorrhization index of *T. aestivum syn. uncinatum* on host plant tree roots from 8 sites subject to soil amendment in 2010. 2010 figures are prior to soil amendment and 2011 figures are post soil amendment. Error bars are one standard error. Values followed by different letter codes are significantly different (*T*-test, $P < 0.05$).

index of 41.26 had the second highest measured soil pH level of 7.51. The highest mycorrhization level of 41.26 was 49.4% above the average for the dataset.

There was a significant correlation between soil pH and the mycorrhization index of a host plant. The results displayed in Figure 2 clearly show that the mycorrhization level increases with increasing soil pH, up to the second highest tested pH level in this study of 7.51. This relationship was highly significant with 29.7% of mycorrhizal variation between sites being attributed to soil pH levels ($R^2 = 0.2969$, $p<0.005$).

Of the data points displayed in Figure 2, 16 represent 8 sites that underwent additional liming and therefore, pH adjustment over the course of 12 months. The average pH of these 8 sites increased significantly from 7.1 in 2010 to 7.4 in 2011 (paired t-test, $p<0.05$). This significant increase in pH, displayed in Figure 3, was in parallel with a significant increase in mycorrhization levels, displayed in figure 4 (paired t-test, $p<0.05$). The average mycohrrization levels of the 8 sites was 27.0 in 2010 and rose to 30.2 in 2011.

DISCUSSION

Our observations that Mycorrhization levels within commercial orchards are higher at higher pH levels, up to an observed pH level of 7.52 is consistent with the known sensitivity of fungi to pH. With fungi in general, increases in biomass with corresponding reductions in pH levels have been observed in soil-based systems (Rousk et al. 2009) In culture, a range of fungi species are also consistently shown to be sensitive to pH levels with some isolates even displaying distinct infraspecific variation (Hung, Trappe 1983).

The optimal pH level we observed within this study was 7.5, well within the reported wild-site range of 6.8-8.0 (Chevalier, Frochot 1997; Wedén et al. 2004) However, as the data shows a linear relationship, it cannot be claimed that pH 7.51 is the optimal level to maintain the highest level of mycorrhization on the host plants roots. The exact optimal pH level may be higher and more sampling is needed to ascertain the exact figure in soil-based systems. Pruett (2008) observed that the optimal pH level for root colonisation, with pot-grown host plants was 7.1. Our results display that this is not the case within commercial truffle orchards and that a higher pH level is beneficial.

At a pH level of 5.8 *T. aestivum* syn. *uncinatum* has been cultured, although mycelia expansion is reported as quicker on higher pH medium (Michaels 1982). The observed lower level of *T. aestivum* syn. *uncinatum* colonized host roots at pH levels below 6 within this study demonstrates a low tolerance of this species for sub-optimal soil pH levels and conflicts with those reported in-culture. Clearly, cultured isolates have different requirements to those growing in symbiosis. This is mirrored in the published data for *T. magnatum,* where pH levels of 6.0 have been reported as ideal in culture (Mischiati, Fontana 1993) but in fruiting sites, soil pH is consistently reported to be above 7.23 (Bragato et al. 2004).

An exception to the stated importance of pH for species of the *Tuber* genus that are cultivated was reported by Garcia-Montero et al. (2006) where it was observed that pH has a very limited impact on production of *Tuber melanosporum*, although the study was constrained to the pH range 7.00 to 8.17. Our observations for *T. aestivum* syn. *uncinatum* contrast with the conclusions by Garcia-Montero et al. (2006).

Further, we observe that the impact of exposure to a sub-optimal pH level within commercial truffle orchards is not one characterised by an irreversible deleterious outcome but rather the relationship is more plastic. As we have shown here, a lower mycorrhization level can be increased by adjusting the pH level of the truffle orchard soil. Further, an increase in mycorrhization level is observed within a timeframe of 12 months. This observation is of importance to truffle cultivators as it suggests that attempts to improve the performance of a plantation can be successful.

The relationship between soil pH and mycorrhization is very clearly displayed in this study but it is important to note that pH alone only accounts for c.30% of the mycorrhization variation between sites. It is also important to understand that pH does not operate in isolation and recognize that other factors that can impact on mycorrhization can be further modified by soil pH. An example of this is the presence of the bacteria *Pseudomonas fluorescens* that was reported by Dominguez et al. (2012) to have a positive impact on colonization levels of *Tuber melanosporum* at sub-optimal pH levels.

There are many variables within commercial orchards, such as soil tilling and irrigation, climatic variables, flora and fauna, soil nutrition and soil structure which may be important in maintaining good *Tuber* spp. colonization levels. The interplay of these variables warrants in-depth study. For example, soil flora and fauna may be impacted by management methods such as tilling (Dorr de Quadros et al. 2012) as well as alterations in soil pH levels (Auclerc et al. 2012) and the presence/absence of such impacted flora has been directly linked to *Tuber* spp. colonization levels (Dominguez et al. 2012). Therefore, the interplay of soil pH with other variables on the success of a truffle plantation is important and should be addressed by truffle cultivators. However, as a stand-alone measurement, pH is clearly of critical importance for truffle cultivation.

Mycorrhizal Systems Ltd has access to unified truffle research sites in 23 countries. This creates a large databank, a portion of which is displayed in this study. Clearly, more analysis is needed to deduce an optimal pH level for cultivation but the interplay of pH with other factors, such as climate, also warrants deeper investigation.

CONCLUSION 104 (113)

The observed optimal pH for mycorrhization of *T. aestivum* syn. *unicinatum* within commercial orchards is 7.51. Host plant mycorrhization rates, plotted against soil pH, showed a linear relationship with pH 7.52 being the highest level tested in this study. The exact optimal soil pH level for mycorrhization levels may be higher.

Sub-optimal pH levels lead to the loss of *T. aestivum* syn. *unicinatum* mycorrhiza. However, utilizing soil additives to increase the soil pH may reverse this decline. Significant increases in mycorrhization rates were observed 12 months after pH adjustment application, demonstrating that the potential of a truffle plantation can be successfully improved.

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