

Review

The cultivation of *Lactarius* with edible mushrooms

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Abstract

From the early 1800s science virtually ignored the cultivation of edible mycorrhizal mushrooms other than the true truffles. The drought was finally broken by Nicole Poitou when she cultivated *Lactarius deliciosus* and *Suillus granulatus* in the Institut National de la Recherche Agronomique's laboratories at Pont-de-la-Maye, France, in the mid-1970s. However, another 20 years were to pass before Yun Wang working at Invermay Agricultural Centre near Dunedin, New Zealand, was able to begin the routine cultivation of *L. deliciosus*. The purpose of this paper is to document the history of the cultivation of *L. deliciosus* in New Zealand since its introduction in the late 1990s, its spread back to Europe and its subsequent introduction into Yunnan, Tibet and Sichuan starting about 2014.

Keywords

saffron milk cap, cultivation, New Zealand, China, productivity

Introduction

The past 50 years has seen several revolutions in the way that plants have been produced for the cultivation of edible mycorrhizal mushrooms. The first method for truffles commonly referred to as "Talon's Technique" (Hall and Zambonelli, 2012; Hall et al., 2007), dates back to the early 1800s and involves growing clean seedlings in the rooting zone of plants already mycorrhized with truffle or in soil known to have produced truffle in the past (Rocchia, 1995). However, the collapse in truffle production particularly in France between 1900 and 1960 (Hall and Zambonelli, 2012) led to calls for alternative methods for producing truffle mycorrhized plants. Three methods were forthcoming. That described by Palenzona (1969) for *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., and *Tuber brumale* Vittad. involved inoculating each plant with about 10⁷ ascospores from blended truffles. Another method replaced spores with sections of root already mycorrhized with truffle (Chevalier and Grente, 1973) a method independently developed for inoculating plants with arbuscular mycorrhizal fungi (Hall, 1973, 1976). A third method employed pure cultures to inoculate clean seedlings



(Chevalier 1972, 1973; Chevalier and Grente, 1973; Fontana and Palenzona, 1969). But by the end of the 1970s it was the first of these techniques that was to be generally adopted for the cultivation of truffles in Europe and by Hall in New Zealand in the mid-1980s (Hall et al., 2007; Murat, 2015).

Sporal inocula have also been used in plantation forestry when it was found essential when plants were raised in soilless media in greenhouses, sufficient inocula could not be expected to blow in from surrounding vegetation, and when outplant sites were devoid of suitable ectomycorrhizal fungi. For example, in New Zealand a suspension of blended *Rhizopogon roseolus* (Corda) Th. Fr. in water is often sprayed onto plants in containerised *Pinus radiata* nurseries (Hall et al., 2003a; Visnovsky et al., 2010) and *Rhizopogon parksii* A.H. Sm. onto Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Hall et al., 2019).

Because ascospores and basidiospores are produced sexually there is the expectation that spores from, for example, *Tuber borchii* Vittad. truffles from the northern reaches of this species' distribution, say near Edinburgh, are likely to be better adapted to the climatic conditions there than *T. borchii* from Sicily. So, when Christina Wedén successfully grew *T. melanosporum* on Gotland off the east coast of Sweden, way north of its natural distribution, it was with plants inoculated with truffles harvested from the northernmost distribution of *T. melanosporum* in France (Wedén et al., 2013).

By the mid-1990s demand for truffle mycorrhized plants in New Zealand had increased and considerable income was being made from their production and sale by Crop & Food Research (Hall et al., 2007). Despite the commercial revenue from this, research funding was more or less reduced in line. So, Hall's group needed new scientific and hopefully commercial challenges and decided to import Lactarius deliciosus (L.) Gray (saffron milk cap) (Figs 1a, b, c and d). This has an excellent flavour, international market appeal, and importantly, there was some pioneering work that had been published in France by Nicole Poitou and Jacques Delmas that goes back to the mid-1970s showing that it was possible to culture the fungus and get it to fruit in the field (Delmas, 1978; Poitou, 1978, 1982; Poitou et al., 1981, 1984, 1989). Our overlying logic for carrying out this work was because L. deliciosus is a mycorrhizal fungus there was the possibility of growing it as a secondary crop in New Zealand's 1.6 million hectares of Pinus radiata D. Don forest (Berg, 2008). Also, because it forms mycorrhizas exclusively with pines, there was little chance that it would enter New Zealand's native ectomycorrhizal beech forests and compete with the local mycorrhizal fungal flora, we were given permission by New Zealand Biosecurity to import it. In retrospect, perhaps we should have considered trying to import L. sanguifluus and L. vinosus as well which are more popular than L. deliciosus in some countries.

In this paper we outline the initial introduction of *L. deliciosus* into New Zealand, subsequent production of its fruiting bodies, the expansion of research aimed at commercial production, and the establishment of several lines of research that emanated from those early days in Tibet, Sichuan and Yunnan. Whilst *L. deliciosus* is the species that ranks at the top of a list of the most desirable species alongside porcini (*Boletus edulis* Bull.: Fr sensu lato) and chanterelles (*Cantharellus* spp.), elsewhere in the world there are other species of *Lactarius* rated equally highly (Supplementary Table 1). Consequently, a commercial success with *L. deliciosus* on *P. radiata* was likely to create interest with other species of *Lactarius* and on other hosts elsewhere in the world as later outlined by Leonardi et al. (2017). We explore the literature on the edible species of *Lactarius* in the hope that it will shed light on the methods that have been used for their cultivation, what impediments have hobbled their production on commercial scales and, in particular, their cultivation out of season from the Northern Hemisphere like truffles are now being grown in the Southern Hemisphere (Hall et al. 2017a). Hopefully it might also shed light, in particular, on the cultivation of edible *Lactarius* throughout China. The techniques we describe here have worked for us but no doubt methods described by others may have some advantages (e.g. Poitou et al. 1984; Diaz et al. 2009; Wang et al. 2012, 2020).



Fig 1 - Fruiting bodies of *Lactaris deliciosus* (a, b, c and d). Some of the first saffron milk mushrooms to be harvested by Hannes and Theres Krumenacher near Nelson, New Zealand (a) and by John and Portia Barnes from a *Pinus radiata* plantation near Castlemaine, Victoria, Australia (b) Bleeding an orange-coloured sap when the caps or stalks are damaged (c) which become green when exposed to the air (d). Hunan street vendor selling *Lactarius hatsudake*, a popular edible species particularly in China, Japan, Korea and eastern Russia (e). The poisonous brown roll rim (*Paxillus involutus*) (f) has been mistaken for the saffron milk cap but it has a different colour, it does not bleed an orange-coloured sap when damaged, it turns reddish-brown when cut and has a tightly involuted margin to the cap (g).

In New Zealand, a country where mycophagy (mushroom eating) is not strong, the poisonous brown roll rim (*Paxillus involutus* (Batsch) Fr., Figs. 1f and g) has been mistaken for the saffron milk cap. There are also some poisonous *Lactarius* lookalikes such as the woolly milk cap (*Lactarius torminosus* (Schaeff.) Gray) (Hall et al., 2003b) and there are photos on the internet which show there is some justification for making such a mistake (e.g. <u>https://en.wikipedia.org/wiki/Lactarius_torminosus</u>).

In China, plants raised in greenhouses and inoculated with *Lactarius* sect. Deliciosi (a section of the genus *Lactarius*) can be heavily infested by dipterous larvae which render their mushrooms

inedible (Ran et al., 2019). It has been suggested that this is because the antifeedant properties of the mild-tasting latex of the group are relatively ineffective (Ran et al., 2019; Nuytinck, 2004-2005). However, in New Zealand saffron milk cap mushrooms appear to be less affected by fly larvae than in the UK and China perhaps because our native insects have never learned to take a liking to them.

Habitat of Lactarius deliciosus

The saffron milk cap is invariably found growing only under Pinaceae. Known hosts include *Pinus halepensis* Mill. (Aleppo Pine, Jerusalem Pine), *Pinus pinea* L. (stone pine), *Pinus pinaster* Aiton (maritime pine), *Pinus radiata* D. Don (radiata pine), *Pinus sylvestris* L. (Scots pine) and *Picea glauca* x *engelmanni*. *Pinus nigra* J.F. Arnold) (black pine) is also a possible host (Díaz et al., 2009; Guerin-Laguette et al. 2000; Kranabetter et al., 2005; Wang et al., 2002; Wang and Hall 2004). The characteristic orange or orange-brown mycorrhizas and rhizomorphs are easily seen in the litter layer and soil under mycorrhized trees with the aid of a hand lens and microscope (Figs. 2a, b, c and e) and the lactifers in the fungal mantle (Fig 2d).

The saffron milk cap is found throughout Continental Europe and as far east as Israel, Macedonia, Turkey, Ukraine and Western Russia, and from the north of Sweden to the south of Spain. It is found throughout the UK (National Biodiversity Network, 2008/2020) but is said to be more common in Scotland although occasionally it can be found in abundance elsewhere such as coastal Anglesey, Wales, during early October. The saffron milk cap has also made the accidental journey to Australia, Chile and South Africa probably on the roots of imported trees. It is found throughout Victoria and New South Wales, Australia, in *P. radiata* forests where it is collected for the restaurant and gournet trade. It has also been found throughout China (Zang et al., 2007) but there it may be confused with *Lactarius vividus* X.H. Wang, Nuytinck & Verbeken (Wang et al., 2015). An unidentified species very similar to *L. deliciosus* is found in the USA but this is molecularly distinct (Kuo, 2011).

It is impossible to estimate total world production of *L. deliciosus* and other edible milk caps but the size of the market can be gauged from the limited literature available. For example, in a paper by Oliach and colleagues (2000), it was reported that in the small village of Laspaúles, Huesca, Spain, one tonne of saffron milk cap were traded each day during the season. In eastern Asia thousands of tonnes of hatsudake (*Lactarius hatsudake* Nobuj. Tanaka) (Fig. 1e), a similar species to the saffron milk cap, are consumed each year. Clearly, the potential for producing these mushrooms in the Southern Hemisphere both as new foods for the home market and, more importantly, for off-season Northern Hemisphere markets is considerable (Voces et al., 2009).

Climatic conditions in productive areas

The wide distribution of the saffron milk cap in Europe from the Spanish productive regions of Catalonia, Castille and Leon, and Aragon (de Román and Boa, 2006) to the top of Scotland and Alpine areas to 2000 m asl (Zambonelli personal observation) shows that this species fruits in a very wide range of climatic regions ranging from cool temperate to Mediterranean.

Although little is known about what constitutes an ideal location some suggestions might be proposed. For example, relatively low temperatures at the start of autumn (September in the Northern Hemisphere and March in the Southern Hemisphere) are likely to be related to early fruiting (Alday et al., 2007). Conversely, a modest autumn rainfall coupled to relatively high temperatures could produce mushrooms with a high moisture content and short shelf life which would be a significant problem if the mushrooms were to be exported. In our experience mushrooms produced in June under relatively dry conditions at Castlemaine, Victoria, Australia, have good keeping qualities. Similarly, mushrooms produced by Hannes and Theres Krummenacher near Nelson, New Zealand, between March and June, have a good shelf life providing rainfall is not excessive.



Fig. 2 - Mycorrhizas of *Lactarius deliciosus* (a, b, c, d, e). A small part of an old mycorrhized section of root showing white fungal hyphae attached to the orange mantle *of L. deliciosus* (a). Extensively mycorrhized plants obtained in Truffles and Mushrooms (Consulting) Limited's facilities (b, e). A single mycorrhized root tip (c). A single root tip showing lactifers growing towards the tip of the root from the darker mantle (d).

Plant production

Because the saffron milk cap grows in a mycorrhizal association with pines it can only be cultivated in plantations established with specially inoculated trees. In the pioneering work by Nicole Poitou starting in the late 1970s, pure cultures of *L. deliciosus* were made from mushrooms (Poitou et al., 1984). From these liquid or solid spawn was made and used to inoculate seedlings of *P. pinaster*. These were then raised in a greenhouse, and in 1980 planted out into a sandy clay soil. Fruiting bodies were formed $3\frac{1}{2}$ years later and then annually.

In the 1990s *L. deliciosus* was being harvested by people like Portia and John Barnes within 100 km of Melbourne (Hall et al., 2003b) where it had probably been accidentally introduced by early European settlers. However, this was an area warmer than much of New Zealand so it was decided to make cultures in the UK instead. Via Dr Tony Lyon's (ex University of Sheffield) vast network of mushroom collector contacts, *L. deliciosus* was located fruiting in northwest Wales (53.2°N) not far from where Ian Hall's aunt had retired to (Hall, 2021). There, the climatic conditions in autumn are similar to those in coastal Canterbury and Otago, New Zealand, (41°S - 46°S) (Hall et al., 2017b) where it was anticipated plantations would be established because the shelf life of mushrooms from those locations would be longer than in the warmer parts of the North Island.

Hall didn't have access to laboratory facilities in Wales but he successfully isolated some pure cultures of *L. deliciosus* in his bedroom using potato dextrose agar slopes in metal capped 2 oz universal bottles.

Sterile seedlings were then prepared and inoculated by Yun Wang using techniques he had become familiar with during a research visit to Corvallis, Oregon, between 1983 and 1985 (Molina, 1980; Molina and Palmer, 1982). The first saffron milk cap infected trees raised in New Zealand in August 2000 (Wang et al. 2002; Wang and Hall, 2004) were produced in greenhouses. Frustratingly, Thelephora contamination probably from a P. radiata plantation 100 m away (Supplementary Fig.1, Figs 4a and c) was sometimes appalling particularly when the trees were felled while we were trying to produce uncontaminated plants (the trees were owned by another research organisation). Contaminating mycorrhizal fungi have posed problems for truffle growers since the late 1970s when truffles were first cultivated by inoculating seedlings with truffle ascospores. Ensuring that plants do not get contaminated with other mycorrhizal fungi has always proved challenging but with experience success can be very rewarding. Even so, other contaminating mycorrhizal fungi, insect pests, nematodes, etc. can creep into the best of research establishments (e.g., Ran et al., 2019). The origin of these can be, for example, piles of waste potting mix, old plants in corners of greenhouses, windblown dust and dirt inside or surrounding greenhouses, and on clothing and boots. Some mycorrhizal contaminants of Lactarius mycorrhized plants are Thelephora spp. (Hall unpublished information) (Figs 3a and c), Peziza ostracoderma (X. He & D. Wang pers. comm.) (Fig, 3b), Aleuria sp. (Fig. 3d), and Sphaerosporella sp. (Di Wang pers. comm.) (Fig. 3e).

After growing the plants for 1 to 2 years they were outplanted into various sites around New Zealand. Encouragingly, saffron milk cap mushrooms were found only 18 months after planting on 8 March 2002 on a coastal North Otago site. Since then, production has been estimated to be as high as 1 tonne per hectare in coastal Canterbury (Guerin-Laguette pers. comm.) and over 3 tonnes per hectare in Nelson. To date our cultures have been made simply on the basis of which mushrooms tasted best in field material but eventually it might be possible to identify the best cultures by measuring odorants either in cultures or mushrooms collected in the field (Miyazawa et al., 2010).

More than a decade later Ian Hall re-isolated *L. deliciosus* from mushrooms collected from a privately owned North Otago, New Zealand, plantation and set up a private plant production facility at Invermay Agricultural Research Centre (Hall, 2021). Because of past experiences with contaminating fungi (Fig. 3) greenhouses were abandoned and internal rooms were used instead in New Zealand (Hall), and subsequently in Sichuan (D. Wang & Hall) and Tibet (Zhang, Sun and Xiong). An autoclaved peat/ perlite/vermiculite potting mix was used and reverse osmosis water used to try and avoid the ingress of contaminating organisms. Daylight LED lighting producing about 10,000 lumens appeared to be sufficient and replaced fluorescent 40 watt tubes which were inadequate (Fig. 4a). Acudam Full Pots (<u>https://www.acudam.com/en/farming/full-pot-truffles</u>) were widely used because these enabled the accurate placement of the inoculum on the roots of the seedlings and the opportunity to open the pots to see what was happening during the 1 to 2 years incubation (Figs 2b and e). Otherwise, methods were probably not too dissimilar to those employed by others, for example, Diaz et al. (2009).



Fig. 3 - Contaminants in pots inoculated with *Lactarius deliciosus*. The characteristic fan-shaped fungal mat of *Thelephora* sp. growing over a polythene sheet below a plastic pot. (a). *Thelephora* growing up and over the lower sections of a pine seedling (c). *Peziza ostracoderma* (Pezizaceae, Pezizales) (b) *Aleuria* sp. (d), and *Sphaerosporella* sp. (e) fruiting below a pine seedling.

Standard forestry planting practices were employed but plant spacing and the subsequent management varied between each of the experimental sites and ranged from carefully tending the trees to almost complete neglect.

Site selection

Ideally, saffron milk cap inoculated plants should be grown in a soil that suits the cultivation of the host pine as well as the fungus – a relatively low pH, low levels of plant available phosphorus, etc (Supplementary Table 2). So comprehensive soil testing during the planning stages has been recommended in New Zealand. Ideally the area should not be too warm and overly wet in autumn because under these conditions the mushrooms may deteriorate faster and have a short shelf life. Pastureland is ideal providing it is not too fertile whilst land where pines have been grown in the past may be unacceptable because of the potential for competition from other ectomycorrhizal fungi. Fruiting appears to have been triggered by a fall in temperature from 20°C to 14°C over a period of

several weeks. The optimum pH seems to be between 5.0 and 5.9 although in Europe the spread in pH is 3.9 to 6.5 (Supplementary Table 2).

Plant husbandry

Ensuring that plants are watered regularly particularly if there is to be a delay of a few days between delivery and planting is particularly important. We don't recommend storing plants for longer than a week before planting and adequate watering is needed in the meantime. We suggest spacing the trees 5 to 6 metres apart and planting during dull, showery weather, when there is not much wind. We do not recommend planting in full sunlight or in windy conditions because the plants do not have access to much water in their containers and can dehydrate in a matter of hours. This might set back their growth by months if not permanently.

Plants that have been raised in Fullpots (Figs 2b, 3b,c, 4b,e) or slotted Plantec-Lannen trays (Figs. 4c, d) (<u>https://www.transplantsystems.co.nz/products/ts-45f-seedling-tray/</u>), can be planted directly into the planting hole without prior treatment but care is needed to ensure that any roots coming out from the sides or bottom of the core are arranged naturally. Stuffing the root ball down into a cramped planting hole is rarely successful particularly if the roots are forced upwards. The root ball should be just covered by 1-2 cm of soil. Irrigating once or twice daily to begin with and making sure that the irrigation water penetrates to a depth below the roots of the plants and not just dampening the top few centimetres of soil is particularly important.

Where there are browsing animals such as rabbits, hares, and possums, and in windy areas, there are considerable advantages in protecting young trees with tree guards or tree shelters. In the truffle industry, tree guards 600 mm (high) x 150 mm x 150 mm have been used extensively. Suppliers include Ascot and Tubex Shrubshelters <u>http://www.ascotindustries.co.nz/stakes--supports/ascot-tree-clips/tree-vine-guards.html</u>, <u>http://www.tubex.com/product-selector.php</u> and <u>https://www.alibaba.com/showroom/polypropylene-tree-shelters.html</u>. Preferably, the shelters should be at least 150 mm wide to allow for air movement within the box. Taller and narrower boxes and plastic sleeves, as used in viticulture, have not been tried but these have caused problems when used for truffle cultivation. We have not observed any problems associated with high temperatures inside shrub shelters providing there has been adequate irrigation although cutting a few 30 mm diameter holes in the lower portion with a hole saw can help (Fig. 4f).

While Fiberguard boxes may also suit some applications, many of our trees tend to be over 40 cm high and might get their tops nibbled off by rabbits. KBC SquareGuards which are 15 cm x 15 cm and 60 cm to 120 cm in height, may be better suited for the tallest of our trees (<u>https://www.advancelandscape.co.nz/shop/Plant+Establishment/KBC+SquareGuard+600mm+to+1.2m.html</u>).

Although saffron milk cap mushrooms have been produced in plantations that were virtually neglected, private research has shown that profitability can be boosted if the trees are managed correctly. Cutting the vegetation close to the soil surface, the judicious use of irrigation, and removing lower branches from the trees are parts of a management strategy that has been shown to pay dividends (Fig. 4g).

Harvesting

It is essential that the mushrooms are harvested carefully to avoid damage and the green discolouration that follows. It is also very important that there are no traces of soil on the stem or in the gills to avoid potential pest and disease organisms contaminating those mushrooms scheduled for export and either being refused a phytosanitary certificate by the exporting country or rejected by biosecurity at their destination (Hall et al., 2007; Hall et al., 2017a).

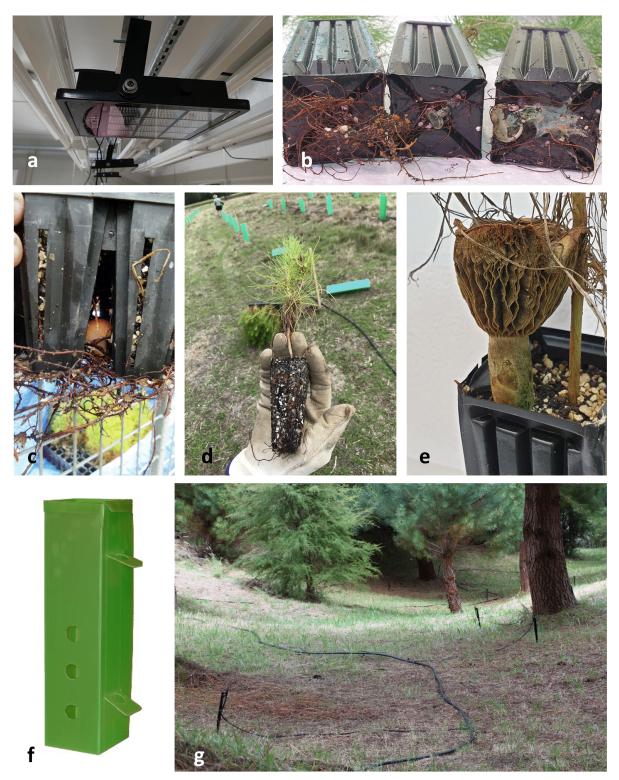


Fig. 4 - Phases of *Lactarius delicious* cultivation. Forty-watt fluorescent tube lights (a) (left and right) produced only 1100 lumens at bench level so these were replaced by Hugo LED white lights (centre) each producing about 10,000 lumens at bench level and adequate plant growth (<u>https://www.hugolighting.co.nz/</u>) (a). Plants produced in Lannen PLANTEK trays (c and d) producing mushrooms before outplanting from the base (c) or from the base and tops of Full Pots (b and e). A 600 x 150 x 150 mm Tree Guard fold-up box with three ventilation holes to provide additional air circulation (f). A crude, but very effective, irrigation system was used in Hannes and Theres Krummenacher's saffron milk cap pine plantation near Nelson, New Zealand. Note the removal of the lower branches from the small tree to the right of centre (g).

The techniques used by Portia and John Barnes to harvest wild mushrooms in Australia involves gently lifting the mushroom with two fingers either side of the stalk and then sliding a knife under the fingers to severe the stalk as close as possible to the soil surface. The stalk is then trimmed to remove any adhering soil and the cap then placed upside down in flat-based baskets (Fig.1b). They are then kept cool and rushed to the market so as to arrive in perfect condition.

Productivity

In 2000 when Portia and John Barnes took Ian Hall to several *P. radiata* plantations that produced saffron milk cap near Castlemaine and Daylesford in Victoria, Australia, he estimated the standing crop to be somewhere between 100 and 200 kg per hectare (Supplementary Fig. 2). This would mean that over the whole of the season the crop must have been several times this. Romá et al. (1997) also noted production of more than 100 kg per hectare on Scot's pine (*Pinus sylvestris*) in forests in the central Pyrenees. However, much higher production figures have been achieved by Theres and Hannes Krummenacher in Nelson, New Zealand, from their saffron milk cap inoculated *P. radiata* trees (Figure 5, Supplementary Fig. 3). Subsequently, their saffron milk cap glade produced far more mushrooms than ever expected (Fig. 5). In year 9 production averaged 6 kg per tree. With the trees planted at about 600 per hectare it is estimated that the potential value of the mushrooms based on farmer's market prices in year 9 alone would exceed the estimated value of the timber at year 30.

Production in a small research plantation in a lawn at Plant & Food Research at Lincoln, New Zealand, was somewhat less productive (Guerin-Laguette et al., 2020). It was planted with 5 m x 5 m spacing, began fruiting 5 years after planting and reached a maximum at year 10 when production averaged 1.25 kg per tree. Production then began to fall which was attributed to canopy closure and/or dry conditions (Guerin-Laguette et al., 2020). However, inadequate irrigation, desiccating winds and a lack of aggressive tree pruning might also have been responsible (Hall unpublished; Krummenacher pers. comm., 2008).

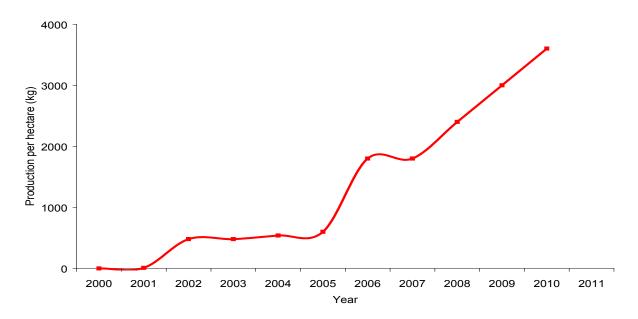


Fig. 5 - Production of saffron milk cap extrapolated to per hectare in Theres and Hannes Krummenacher's saffron milk cap glade from planting in 2000 to the 2009-2010 season.

Prices

In their 2006 paper on marketing the saffron milk cap in Northern Spain, Miriam de Roman and Eric Boa recorded that pickers were paid between 1 to $12 \notin kg$ but on average 478 tonnes were sold in Mercabarna every year worth $\notin 1.5$ million with prices ranging between $3.6 \notin kg$ and $7.2 \notin kg$.

About the same time that de Roman & Boa were doing their work, the price of Australian grade 1 saffron milk cap was A\$ 40/kg in Melbourne which is currently a little more than saffron milk cap sells for in New Zealand. Clearly, a European price of 40 €/kg Ian Hall was quoted in 2000 was either exceptional or exaggerated, although these are the normal prices in upmarket retail stores such as Harrod's in London. At the time of writing (mid-June 2020) the only price Ian Hall could find was on Woodland Foods web site (https://woodlandfoods.com/products/saffron-milk-cap-mushrooms/c-23/p-13778) where dried saffron milk cap pieces were for sale for US\$65.44/lb (= NZ\$225/kg).

Discussion

In the early 1990s New Zealand's state forestry assets in Northland, the Wairarapa and East Cape covering nearly 600 km² were privatised and a new company called Juken Nissho established. Mr Yusho Nakamoto, a part owner of this company, and the President of WoodOne in Hiroshima Prefecture, heard about our work on the production of *L. deliciosus* on pine plantations and a joint venture First Light Mushrooms, was established between Mr Nakamoto and Crop & Food Research. Mycorrhized trees were initially produced at Invermay but in 2005 Maria-Lidia Hance left Crop & Food Research to join the staff of Juken Nissho in Gisborne. There she produced more than half a million *L. deliciosus* inoculated pines. This soon showed success (Anderton, 2008; Scoop, 2008).

Di Wang, Ian Hall and Wei-Ping Xiong again visited the plantations near Gisborne in 2020 but this time the owners were cautious about the general public entering their forests to pick mushrooms – the accident rates in New Zealand's plantation forests are very high and the owners wished to reduce accidents and their insurance premiums (Forestry New Zealand, 2019; Hutching, 2018; Sunlive, 2020). There was also some indication that the management of the plantation had shifted more towards that of a standard New Zealand *P. radiata* forest. We now wonder if in the first 20 years of the life of a *L. deliciosus* production and then retired as the stand moves into its third decade. Even as little as 1 tonne of *L. deliciosus* per hectare per year for 15 years and selling for as little as 15 would surely be worth exploring. However, if forestry companies did not find this attractive then specialist plantations like Hannes and Theres Krummenacher's might be the only way to proceed.

In upland parts of New Zealand small-seeded exotic conifers are encroaching on an unstable tussock grassland which has led to the introduction of rules and regulations that ban their cultivation in certain areas and the establishment of a National Wilding Conifer Control Programme funded by government agencies (Hall et al., 2019). Some of these small seeded trees like *P. radiata* and *P. sylvestris* are good hosts for the saffron milk cap (Guerin-Laguette et al. 2014; 2020) but there has been a growing reluctance amongst potential saffron milk cap growers to avoid these species. In contrast, *P. pinea*, has become particularly attractive because buyers often reason that if the mushrooms fail to eventuate, they can always switch to producing nuts – a developing industry in New Zealand (Hart and Newcomb, 2007). Perhaps if wilding pines were to produce edible mycorrhizal mushrooms instead of, for example, *Rhizopogon roseolus* (Corda) Th. Fr., this might lead to a deeper understanding of the role mycorrhizas play on the roots of trees, the underground warfare that is occurring on tree roots, and that the revenue from the sale of edible mushrooms from wilding pines might even help pay the cost of the removal of their host tree.

It seems almost certain that the next advance in the cultivation of truffles and in particular, the bianchetto truffle and maybe the Italian white truffle (*Tuber magnatum* Picco (Hall et al. 1998) will come from successfully inoculating trees with cultures of *T. borchii* and *T. magnatum* under controlled

conditions similar to those we have used in this paper and those employed by Iotti et al. (2016). The main problems to overcome will be maintaining a level of cleanliness and rate of growth first in vitro and then during incubation of the fungus on the host root for one or two years. Hopefully there will be no complicating factors such as the need for co-organisms and maintaining three or more organisms in balance while stable mycorrhizas are established perhaps over two years.

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