Chapter 5 Advances in the Cultivation of *Lactarius deliciosus* (Saffron Milk Cap) in New Zealand



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5.1 Introduction

The native flora in New Zealand is composed predominantly of endomycorrhizal plants (Orlovich and Cairney 2004). Thus, compared with the Northern Hemisphere, there are many soils in New Zealand devoid of or with less ectomycorrhizal fungi capable of outcompeting truffles and other edible mycorrhizal fungi (Hall et al. 2007). Land is available to establish edible mycorrhizal fungi orchards in regions with different soil types in temperate to subtropical climates. New Zealand has a worldwide reputation for leading and pioneering the cultivation of edible mycorrhizal fungi. In 1993, the country was second only to the United States for successfully growing the Périgord black truffle (Tuber melanosporum) out of its region of origin in Europe (Wang and Hall 2004). Since then, saffron milk cap (Lactarius deliciosus) was cultivated in New Zealand under field conditions in 2002 (Wang et al. 2002, 2013), bianchetto truffle (T. borchii) was cultivated in 2006 and Burgundy truffle (T. aestivum var. uncinatum) in 2007 (Guerin-Laguette et al. 2009). Brumale, the edible winter truffle (T. brumale) is thought to have been accidentally introduced to T. melanosporum truffières since at least 1995. The first brumale truffles were reported in 2010 (Guerin-Laguette et al. 2013). Two varieties of shoro, Rhizopogon rubescens in 2002 (Wang et al. 2002) and R. roseolus in 2010, were also cultivated (Visnovsky et al. 2010).

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The cultivation of *Lactarius deliciosus* in New Zealand began in the late 1990s (Wang et al. 2013). The fruiting season usually spans from late summer (early February) to late autumn (early June). There are now several plantations producing saffron milk caps from the South to the North, including Neudorf Mushrooms in Nelson, which is commercially managed (https://www.neudorfmushrooms.co.nz/). This chapter focuses on two experimental plantations established at the Plant & Food Research (PFR) Lincoln Farm in Canterbury in December 2007. In a recent study, Guerin-Laguette et al. (2014) detailed the first 3 years (2010 to 2012) of saffron milk cap production in one of these plantations, which contains 41 *Pinus radiata* trees. This was the first intensively monitored saffron milk cap orchard in the world. The purpose of this chapter is to provide an update on the advances made in the ongoing monitoring of this trial since 2013. In addition, we present original results obtained in a similar but smaller trial (ten trees), set up on the same research campus but established with *Pinus sylvestris* (Scots pine). We compare the onset of fruiting and the mushroom yields in both sites.

Over the years, we have accumulated a large amount of saffron milk cap fruiting data over four distinct trial plantations at PFR-Lincoln, trials which were all established with pine seedlings mycorrhized by saffron milk cap under controlled conditions (see also Guerin-Laguette et al. 2000, 2014). The detailed analysis of this data is beyond the scope of this chapter, but we present several general and original observations regarding the fruiting of saffron milk cap at PFR-Lincoln. Finally, we highlight orchard design and management issues potentially affecting the cultivation of *L. deliciosus* in New Zealand and suggest the next research steps required to further improve existing cultivation practices.

5.2 Trial Plantations at PFR-Lincoln, Canterbury

5.2.1 Establishment and Description

Four saffron milk cap plantations, or plots, were established on the PFR-Lincoln Farm between 2005 and 2007. All of them are producing significant quantities of mushrooms (since 2014, the production potential of the four sites combined is \approx 80 kg) and are monitored for yield data. However, for the purpose of this chapter, we will focus on two plantations only. They were established 400 m apart on lawns at the PFR-Lincoln campus in December 2007.

The first, called the "Tank" site $(43^{\circ}38'19.3''S \ 172^{\circ}28'39.2''E)$, was initially established with 42 *P. radiata* seedlings (Figs. 5.1 and 5.2) on a silt loam soil with a clay content of $\approx 18\%$ and a pH of 6.7 (Guerin-Laguette et al. 2014).

Of the 41 trees that survived the transplantation phase, two trees were lost to wind storms in January 2013 and March 2014, leaving the current balance of 39 trees. The site is surrounded by lawns or annual crops and is therefore exposed to the dry summer winds from the northeast and northwest. *Pinus radiata* seedlings

Fig. 5.1 Layout of the Tank trial site and history of mushroom production for each tree. Each square, triangle, and circle symbol indicate a P. radiata tree. The numbers to the left of each tree indicate, from top to bottom starting with the column to the right, the numbers of fruiting bodies harvested from that tree from 2010 to 2016. Tree F52 died during the first year after planting, while trees F22 and F16 were lost due to wind storms in 2013 and 2014, respectively. See also Guerin-Laguette et al. (2014) for the meaning of the tree symbols' shape and the light shadowed disks, which are not directly relevant to the data presented in this chapter. We kept this information for the sake of consistency between both publications





Fig. 5.2 (a) View of the Tank site in March 2017; (b) needle litter became apparent in 2015; (c) one-day harvest under tree F41 in March 2014; (d, e) characteristic saffron milk caps; (f, g) unprecedented winter fruiting on 4 July 2016 on tree F31

were inoculated with two *L. deliciosus* isolates selected from isolates derived from three to five mushrooms collected in a *P. sylvestris* forest at Anglesey, North Wales, in 1997 (I. Hall, pers. comm., Table 5.1). Two other PFR-Lincoln sites, "Demo" and "Barley," were established with trees inoculated with the same batch of isolates.

	Date of			
Code	isolation	Host plant	Soil ecology	Geographic origin
NZ	1997	Pinus sylvestris	No data	Anglesey, Wales, UK
D60	1995	P. sylvestris	Calcic brown soil on limestone glacial alluvium	La Joue-du-Loup, Dévoluy, Hautes-Alpes, France
D64	1996	P. nigra	Brown soil on shale	Olette, Pyrénées-Orientales, France
D68	1996	P. pinaster	Brown soil on sandstone	Banières, Gard, France
D74	1996	P. pinaster	Brown soil on sandstone	Banières, Gard, France

Table 5.1 Isolates of *Lactarius deliciosus* used in the PFR-Lincoln trial plantations with ecological information about the source basidiomata

Cultures were isolated from the flesh of fresh fruiting bodies by Ian R. Hall (NZ), Alexis Guerin-Laguette (D60), and Guy Ruiz (D64, D68, and D74)

This initial pool of isolates is termed "NZ" as they were the first *L. deliciosus* isolates to be successfully cultivated in New Zealand (Wang et al. 2002, 2013). Until further genetic analysis is conducted, the number of distinct isolates present in these three sites is unknown. Given the small number of original mushrooms, the genetic variation among the "NZ" isolates is expected to be small.

The second plantation, the "House" site $(43^{\circ}38'19.3"S 172^{\circ}28'19.6"E)$ (Figs. 5.3 and 5.4), was established with ten *P. sylvestris* seedlings mycorrhized by *L. deliciosus* at the same time as the Tank site. The same inoculation method (Guerin-Laguette et al. 2014) and 5×5 m spacing of trees (400 trees/ha) was used. However, in contrast with the Tank site, the *P. sylvestris* seedlings of the House site were each colonized by one of four distinct *L. deliciosus* isolates (Fig. 5.3, Table 5.1) originating from the south of France (Guerin-Laguette 1998). The *Lactarius*-mycorrhized pines in the trial were sheltered from the south and north by a block of another 19 trees of either *P. radiata* or *Q. robur* species which were also planted at 5×5 m spacings. These trees are part of a *Boletus edulis* inoculation trial (Guerin-Laguette and Wang, unpublished). The whole House site (*Lactarius* and *Boletus* trees) is sheltered on all sides but the south by mature conifer and broadleaf trees separated from the trial boundaries by 15–25 m.

5.2.2 Management

5.2.2.1 Planting and Irrigation

Planting holes (approximately $20 \times 20 \times 20$ cm) were moistened with water for 24 h to soften the soil. All seedlings were irrigated after planting, especially during the first summer season. Trees in both sites received consistent irrigation provided by fixed or mobile sprinklers. The trees were irrigated during dry periods, as assessed from the visual examination of soil moisture. Irrigation ran from early summer (December) to early autumn (February), when the mushroom season started. The



Fig. 5.3 Layout of the House trial site and history of mushroom production for each tree. Each square indicates a *P. sylvestris* tree inoculated by a distinct *L. deliciosus* isolate (see also Table 5.1). The numbers to the left of each tree indicate, from top to bottom starting with the column to the right, the numbers of fruiting bodies harvested from that tree from 2010 to 2016. Adjacent *Boletus edulis*-inoculated trees are not shown on this diagram

amount of water applied was not measured. For the first time in 2016, the irrigation was maintained throughout the autumn (March–May).

5.2.2.2 Grass Control

Grass was mown from spring until the first apparition of mushrooms. As trees developed, the ride-on mower could not access an area of 1.5 m radius around the trunk. Grass growth was controlled manually around trees to facilitate subsequent mushroom detection.

5.2.2.3 Pruning

Lower branches (first crown) of the radiata pines at the Tank site were pruned in winter 2014, i.e., 7 years after planting, to facilitate grass control and access to mushrooms below the canopy. The much slower-growing Scots pine at the House site has not been pruned. The lowest branches of *P. sylvestris* have always been in



Fig. 5.4 (a) Growth difference between *P. sylvestris* (small pines at the front) and *P. radiata* (arrows) 4 years after planting; (b) fruiting on the smallest pine (P6) in 2014; (c) the open canopy in March 2017; (d) a harvest in 2015; (e) mushroom cluster in 2016; and (f, g) a one-day harvest around tree ① (see Fig. 5.3) in 2016

contact with the soil surface, providing further shelter for mushrooms growing under the canopy.

5.2.2.4 Fruiting Body Monitoring and Yields

Every year since 2009, sites have been monitored for fruiting-body production. From the start of the mushroom season, sites were visited at least once a week, or several times a week depending on the mushroom production. On the Tank site, between 2010 (the first year of production) and 2012, the position and number of mushrooms surrounding each tree was recorded (Guerin-Laguette et al. 2014). The total mushroom weight (whole site) was also recorded. Subsequently, individual mushroom weights for each tree were recorded. On the House site, the number of mushrooms per tree and the total crop weight for the site were recorded. The mushroom survey allowed us to observe the variation of production per tree and between trees, in functions of time after planting and of changing environmental constraints. The summary of mushroom production in both sites over the first seven fruiting seasons is given in Table 5.2.

5.3 Case Study 1: Evolution of a *Pinus radiata* Plantation

This section covers the mushroom production history of the Tank site.

	Tank					House				
	No. of	No. of	TFW	FW/FB	FW/PT	No. of	No. of	TFW	FW/FB	FW/PT
Year	PT	FB	(kg)	(g)	(g)	PT	FB	(kg)	(g)	(g)
2009	0	0	0	0	0	0	0	0	0	0
2010	8	12	0.47	38.9	59	0	0	0	0	0
2011	28	190	12.05	63.4	430	0	0	0	0	0
2012	32	569	29.4	51.6	918	4	14	0.31	22.1	77
2013	34	536	20.18	37.6	594	5	41	0.96	23.3	191
2014	36	658	39.94	60.7	1109	7	69	4.52	65.5	646
2015	35	462	11.21	24.3	320	10	335	9.16	27.3	916
2016	36	460	14.26	31.0	419	9	626	11.04	17.6	1226

Table 5.2 Saffron milk cap production figures for the first seven fruiting seasons (2010 to 2016)at the Tank and House sites

Trees were planted in December 2007

PT productive trees in the corresponding year, *FB* fruiting body, *T* total harvest, *FW* fresh weight, *FW/PT* average fruiting body yield per tree, i.e., total harvest divided per the number of productive trees in the corresponding year

5.3.1 Tree Growth and Evolution of Mushroom Production for Individual Trees

Trees grew rapidly reaching an average height of about 12 m after 9 years, as measured using an inclinometer (Clino Master, Silva) in November 2016. The history of mushroom production per tree is given in Fig. 5.1. The onset and evolution of mushroom fruiting in the first three productive years (2010-2012) has been described previously (Guerin-Laguette et al. 2014). Since 2013, 10% of trees (trees F28, F33, F38, and F59, Fig. 5.1) began to produce only as late as 5–6 years after planting (Table 5.2). Two further trees began to produce mushrooms 8 years after planting (F23 and F27, Fig. 5.1), while three others (7.3% of the number of trees at the site, F29, F34, and F39) have never produced mushrooms to date (Fig. 5.1). However, because of the indications that the root systems have begun to overlap since the seventh year after planting (please see Sect. 5.5), it is not known which tree supported the production of mushrooms that developed equidistant from trees. Therefore, the actual number of trees that failed to produce to date could be anywhere between 7.3% and 12.2% of the 41 trees successfully planted. All trees except two (F57 and F51, Fig. 5.1) have kept fruiting every year since their first year of mushroom production.

5.3.2 Saffron Milk Cap Production and the Distribution of Rainfall in 2012, 2013, and 2014

The three seasons were marked by very distinct timings of rainfall between February and May (Fig. 5.5). Figure 5.5 illustrates the effect of rainfall on the distribution and intensity of mushroom production in these years. Mushroom production clearly peaked a few weeks after significant rainfalls (Fig. 5.5). The period mid-February to mid-April was exceptionally dry in 2013 compared with 2012 and 2014 (Fig. 5.5). In 2013, the only major rainfall in early autumn (around mid-March) was ≈ 40 mm, or about 37% of the total rainfall developing between early February and mid-March 2012 (≈107 mm, Fig. 5.5). This fall was followed by a moderate peak of mushroom production (≈ 1.0 kg), mid-April 2013 (Fig. 5.5). It is only toward the end of the season (May 2013) that the combined significant rainfall (≈90 mm) triggered the year's most abundant production peak (4.0 kg on May 20, 2013, Fig. 5.5). In 2012 and 2014, abundant rainfalls in February-March were followed by major production peaks in the first half of the fruiting season (Fig. 5.5). In 2013, late rain coincided with dropping temperatures, which in turn could have limited the formation and the development of mushrooms even when soil moisture became favorable to mushroom initiation.



Fig. 5.5 Total yield in the Tank site at each harvest date and weather variables over 4 years from February to June in each year. Weather data was downloaded from MetWatch (http://www.hortp-lus.metwatch.co.nz/) for each of the four seasons 2012–2015. Data from the Lincoln Broadfield (NIWA) site was selected (43°37'34.4"S 172°28'13.4"E), as this is closest to the Tank site

5.3.3 Production During Two Consecutive Autumn Droughts (2015 and 2016)

In both years, rainfall was in severe deficit between February and late April compared with the previous years (see Fig. 5.5 for 2015, data not shown for 2016). In 2015, the combined rainfall during this period reached \approx 70 mm (Fig. 5.5), while in 2012 and 2014, the rainfall over this time amounted to \approx 130 and up to \approx 310 mm, respectively (Fig. 5.5). 2013 was almost as dry as 2015 during this period (\approx 75 mm), but the significant rainfalls from early May had successfully triggered a peak (\approx 4.2 kg) production higher than the late autumn peak of 2015 (3.5 kg, Fig. 5.5). In 2016, irrigation was maintained throughout the autumn for the first time. The average weight of mushrooms produced in 2015 (24.3 g/m) and 2016 (31.0 g/m) was remarkably low in comparison with the values obtained in 2012 (51.6 g/m) and 2014 (60.7 g/m) (Table 5.2).

5.3.4 Overview of the Production During the First Seven Mushroom Seasons

Table 5.2 summarizes for each year the number of productive trees, mushroom numbers, total yields, and average mushroom weights on both the Tank and House sites. On the Tank site, from the third year of production (2012–2014), the average number of mushrooms was around 588 per year (\approx 17 mushrooms/tree). In subsequent drier autumns (2015 and 2016), the total number of mushrooms dropped by about 22%, with 461 mushrooms produced in average for 2015 and 2016 (\approx 13 mushrooms/tree, Table 5.2, Fig. 5.1). Furthermore, the average mushroom weight dropped by as much as 45% when comparing values between the same periods, i.e., 50 g/mushroom (g/m) in average for 2012–2014 and 27.6 g/m in 2015–2016 (Table 5.2). Thus, between 2012–2014 and 2015–2016, the average yield per tree also dropped from 0.87 to 0.37 kg/tree (Table 5.2). Compared with the 2014 highest yield (1.1 kg/tree, Table 5.2), the decrease observed in average for 2015–2016 represents a 67% fall in yield per tree. The decrease in individual mushroom weight seems to be the major reason for the observed fall in total yield (Table 5.2) during dry autumns.

5.3.5 Outlook for the Future

As trees grew, the canopy closed and, from 2015, a needle litter started accumulating (Fig. 5.2b). We also noticed that the production of some trees declined, with up to a 90% drop in mushroom numbers between 2014 and 2015, followed by a weak recovery in 2016 (e.g., Tree F35, Fig. 5.1). It is unknown whether this decline was due to the canopy closure, the succession of dry autumns, or the combination of both factors. The closure of the plantation canopy could be a turning point of the mushroom production because of the likely declines of light and soil temperature. We will keep monitoring this site to better understand the impact of tree ageing and canopy closure on mushroom production.

5.4 Case Study 2: Evolution of a *Pinus sylvestris* Plantation

Although the House site is smaller than the Tank site, a number of observations on the mushroom production history of the House site are very valuable and appear worthwhile as a comparison to the history on the Tank site.

5.4.1 Tree Growth and Onset of Fruiting

Pinus sylvestris seedlings grew much more slowly than the P. radiata seedlings planted the same year on the Tank site. Scots pines reached an average height of 3.7 m after 9 years in comparison with 12.3 m for radiata pines, i.e., only 30% of the growth of *P. radiata* (Fig. 5.4a, c). As a consequence, 10 years after planting, there is still no canopy closure and no needle litter formation (except in the vicinity of the trunk), and the space between trees is still very sunny and covered with dense grass in 2016–2017 (Fig. 5.4c, f). The first mushrooms under P. sylvestris appeared in 2012 or just over 4 years after planting, 2 years later than P. radiata planted at the same time (Table 5.2, Fig. 5.3). In the Barley site established with both pine species on the Lincoln farm (≈1 km away from the Tank site) with the same NZ inoculum as the one used in the Tank site (i.e., of similar genetic origin), we observed again that P. sylvestris started producing mushrooms of NZ isolates 2 years after P. radiata (data not shown). Therefore, it seems likely that the pine species, and its associated growth rate, is the main factor driving the delay required to start mushroom production: the slower the growth of the tree species, the later the mushroom production. Regarding the percentage of trees that became productive, the small size of the House trial limits the extent of comparison with the Tank site. However, we also noticed the progressive increase in the number of trees switching to mushroom production. Furthermore, all P. sylvestris trees became productive within the first four fruiting seasons (Table 5.2, Fig. 5.3). The small size of trees and the fact that mushrooms have not yet reached positions equidistant from neighboring trunks suggest that the root systems of adjacent Scots pines may not have overlapped yet. Two trees stopped fruiting: P2 in 2014 (only) and P6 in 2016 (Fig. 5.3). However, the latter is the smallest tree in this site (data not shown) and has only produced four mushrooms over two fruiting seasons so far (Fig. 5.3).

5.4.2 Overview of the Production During the First Five Mushroom Seasons, Comparison with the Tank Site, and Outlook for the Future

The number of mushrooms produced kept increasing over time (Table 5.2, Fig. 5.3). Given that the same spacing has been adopted between both sites, located only 400 m apart on a very similar lawn of the PFR campus, we assume that it is

reasonable to compare the productivity of pines in both sites, i.e., the mushroom yield per productive tree over time (Fig. 5.6). We can observe that the average yield per tree on *P. sylvestris* kept increasing and has overtaken that of *P. radiata* in 2016: the production per *P. sylvestris* tree was 1.23 kg/tree compared with 0.42 kg/tree for *P. radiata* (Table 5.2, Fig. 5.6). We suggest that the following factors may help to explain the differences observed in yields between the two pine species: (1) the slow growth and compact shape of *P. sylvestris* trees; (2) the smaller size of the House site achieved a more consistent, and probably more intense, irrigation; and (3) this site is more sheltered than the Tank site from Northwest and Northeast dry winds. We will keep monitoring the yield in the House site to determine the long-term evolution of yield, again in relation to the environment changes caused by tree ageing and canopy closure.

With a tree spacing of 5×5 m, both plantations represent a density of 400 trees per ha. Production values described here for the two most productive years, assuming that all trees are productive, represent 444 kg/ha for *P. radiata* in 2014 (7 years after planting) and 490 kg/ha for *P. sylvestris* in 2016 (9 years after planting). These values are well above maximal values (≈ 30 kg/ha) reported for naturally established populations of *L. deliciosus* (Martínez-Peña et al. 2012), thereby supporting the hypothesis that mushrooms could be farmed in pine plantations established with mycorrhizal seedlings.



Fig. 5.6 Comparison of the yields (kg per tree) on the Tank (*Pinus radiata*) and on the House (*Pinus sylvestris*) sites since the establishment of the plantations in December 2007

5.5 Mushroom Fruiting Observations

These observations were made over the four saffron milk cap trials at PFR-Lincoln.

5.5.1 Fruiting Distribution Around Trees

Mushrooms can appear anywhere around the trunk. However, for *P. radiata* trees that are partly in full sun (i.e., edge trees not sheltered by other trees) and exposed to the wind, we noticed that the mushrooms tended to develop preferably on the shady sides of the trees (south side). Each year, for both pine species, the distance of mushrooms from the tree trunk increased, and after 7 years of fruiting, it is possible to find mushrooms up to 2.5 m from the tree trunk (over 1 m away from the drip line of trees) although mushrooms still emerged very close to the trunk. Over the years, the root systems of neighboring trees are expected to overlap each other. It is difficult to know precisely when this phenomenon started, but our field notes on the Tank site (when mushrooms started to be found at equidistant locations from trunks) suggested that such an overlap began in 2015 or ≈ 7 years after planting. This means that it gradually became almost impossible to assign with certainty mushrooms to particular trees, either because mushrooms grew at equidistant locations from neighbor trunks or because the underground mycelial network started to act as one individual developing multiple mycorrhizal connections with the root systems of several trees.

Mushrooms tend to grow bigger when developing within dense grass, as opposed to mushrooms forming over bare ground or below the needle litter. A grass cover is therefore desirable, but the height of the grass above the ground should preferably not exceed 15 cm. A taller grass canopy greatly increases the probability of missing mushrooms or of finding them late, when they are overmature.

5.5.2 Sustained Production from Fallen Trees

Fast-growing *Pinus radiata* trees are very sensitive to wind storms. In the Tank site to date, we have lost 6% of productive *P. radiata* trees to this phenomenon. The wind-exposed Barley site incurred similar tree losses (although none of the 11 *P. sylvestris* there were uprooted), and it is likely that more *P. radiata* trees could be lost in the future especially if a wind storm follows a rain episode that would have previously softened the ground. Interestingly, we found mushrooms fruiting all over the area in which these trees were centered up to 3 years following their death. Given the impossibility of determining precisely the date when root systems began to overlap, it is unclear how long the mycelium could fruit after the death of the associated tree. We suggest that the mushrooms could still fruit for at least one

season following the tree death but cannot demonstrate this with the present data. Indeed, we cannot rule out that roots from neighboring trees have reached the area left by fallen trees and have become mycorrhizal with the mycelium that once colonized the dead tree, thus enabling the mycelium to keep fruiting.

5.5.3 Mushroom Yields per Tree

The number of mushrooms per tree can vary greatly among the trees but also over time for some trees (Figs. 5.1 and 5.3). Some trees produced just one or two mushrooms every year, while others produced tens of mushrooms every year. The production of some trees regularly increases until it reaches a plateau, while that of others oscillates or even declines sharply. In terms of the number of mushrooms produced, P. sylvestris became the most productive tree species after 5 years. In the House site in 2016, three trees produced over 100 mushrooms each (Fig. 5.3) with tree P4 producing up to 163 mushrooms (or 2.9 kg). In the Tank site, the most productive P. radiata tree (F41) achieved 5.9 kg in 2014 with only 71 mushrooms (83 g/m in average), which is less than half the number of mushrooms produced by the most productive P. sylvestris trees in 2016 (Fig. 5.4). The variation of yield between trees is shown for the two most productive years for both sites, 2014 for Tank and 2016 for House (Fig. 5.7). In case of *P. radiata*, two trees (F41 and F36) really stood out in terms of yield, while it appears that the majority of trees produced less than the mean value (Fig. 5.7). In the case of P. sylvestris, there seemed to be less variation in yield between trees. It is largely unknown why trees F41 (Tank) and P4 (House) were so productive (e.g., possible environmental reasons) or if there will be a means (e.g., genetic basis) to select for such trees, or for the right tree/isolate combination, in order to maximize yields. It may be possible to select fungus/tree partners for high mushroom yields based on genotyping; therefore, having all trees at the current spacing producing mushroom yields equivalent to the best performing trees could achieve significant yield increases 5 years after planting, equal to the 2.3 tons per ha from P. radiata tree F41 and 1.1 t/ha from P. sylvestris tree P4. These production values are not realistic targets at this stage but indicate the maximum potentials that could ultimately be achieved with each pine species. Sustained high production from P. radiata would require careful and heavy pruning in order to maintain a favorable mushroom environment and irrigation may also be crucial during drought-filled years as discussed below. Despite yields theoretically lower than P. radiata, at least over the first five fruiting seasons, slow-growing pine species such as P. sylvestris still appear very attractive for saffron milk cap cultivation in New Zealand conditions: they are more resistant to wind storm and will need less pruning than P. radiata to maintain a mushroom-friendly lawn environment. The slow pine growth delays canopy closure and needle accumulation. It also allows for more light to reach the ground in the development of the canopy. The variation of mushroom production per tree may also be related to the soil condition and water availability in addition to the tree's original mycorrhization.



Fig. 5.7 Dot histogram of the yield per tree for the most productive fruiting seasons for the Tank (2014) and House (2016) sites. Data for the Tank site are obtained from actual individual mushroom weights. Data for the House site are inferred from the number of mushrooms per tree and the average weight of mushrooms in 2016. The red bar indicates the mean yield per tree

5.5.4 Mushroom Size

Mushrooms tended to be smaller under *P. sylvestris* than under *P. radiata* (Table 5.2). They also occurred more frequently in clusters under *P. sylvestris* (Fig. 5.4e). The largest mushroom harvested from the Lincoln trials was 250 g from *P. radiata* (Barley site). On the same host tree in Nelson, with a warmer climate than Christchurch, mushrooms have grown as big as 375 g (Theres and Hannes Krummenacher, pers. comm.). However, from a commercial point of view, smaller mushrooms may be more desirable, at least for the retail market.

5.5.5 Fruiting Season Span and Unprecedented Winter Fruiting in 2016

The mushroom season typically runs from early January until early June, with mushroom peaks distributed between February and May depending on the season. In 2016, for the first time since the mushroom surveys at Lincoln commenced in 2008, we harvested large and fresh fruiting bodies as late as July 4 from seven trees in the Tank site (Fig. 5.2) and three trees in the House site (data not shown). We

suggest that the timing of the mushroom season is mainly related to the soil temperature. These observations would suggest that late June to early July 2016 temperatures were particularly mild. Indeed, between June 20 and 24, 2016, the soil temperatures 10 cm below ground were slightly higher than the maximal temperatures recorded for the same days between 2008 and 2015 and three to six degrees higher than the average temperature recorded over this period (unpublished). Soil temperatures were obtained from New Zealand's National Climate Database (https://cliflo.niwa.co.nz/) using data from the Lincoln Broadfield Electronic Weather Station (43°37′34.4″S 172°28′13.4″E), which is only \approx 1.4 km away from both sites.

5.6 Cultivation Research: The Next Steps

As this chapter clearly shows, there are crucial factors in saffron milk cap production that are unknown. This is to be expected with every new crop where a large number of cultivation parameters require testing. We list below a few areas of research that could further improve *L. deliciosus* cultivation in pine orchards in New Zealand.

5.6.1 Plantation Design: Tree Composition, Plantation Layout, and Grass Cover

We recommend diversifying the pine species in order to assess the potential of each species for mushroom cultivation. New Zealand has 16 Pinus species, of which 13 are naturalized (http://www.nzflora.info/factsheet/Taxon/Pinus.html). It would be useful to set up plantation trials with species offering various advantages such as ornamental (P. pinea or P. pinaster), nut production (P. pinea), suitability to local climates (P. canariensis), and wind resistance (P. sylvestris). Depending on the location in New Zealand, pine species not causing a threat as wilding species would also be preferred. It would also be essential to compare the productivity between different pine species. Mixing pine species or pines and other tree species could also prove beneficial. Besides the tree composition, open canopy conditions should be maintained as long as possible. There are several means to achieve this, separately or in combination: (1) low density planting; (2) different planting layouts, e.g., rows or hedges instead of bidimensional planting; (3) slow-growing species; and (4) pruning. It is possible to prune pines as long as this is restricted to the active candle zones in early summer. However, the optimal pruning regime has yet to be discovered for each pine species. Finally, it is recommended that the plantation site is sheltered from dominant winds. Wind breaks could be used as long as they remain low cost. A grass cover provides an ideal micro-habitat for mushroom formation.

However, as it can generate additional cost and disturbance (soil compaction), considering naturally short grass varieties, such as turf-type tall fescues, could prove beneficial.

5.6.2 Other Management and Mushroom Monitoring Aspects

In areas with regular drought periods, such as the east of New Zealand, it is strongly advised that there is access to irrigation in order to maintain, or limit, the decline in production because of insufficient rainfall in summer and autumn. In dry areas, mulching (e.g., bark) at planting is recommended even though this may delay the onset of fruiting (Guerin-Laguette et al. 2014). The timing and quantity of irrigation and other parameters such as the water temperature or oxygenation are far from understood and would need to be thoroughly investigated. However, based on our experience during very dry spans, we recommend a few abundant water regimes, 30-50 mm every 2-3 weeks, over a regular shallow irrigation. The use of soil temperature and humidity sensors would be essential to better understand the conditions conducive to mushroom flushes. Such sensors, coupled with light intensity detectors, could monitor the changes in micro-climate resulting from plantation ageing, canopy closure, and climatic factors (drought, wind). In an intensive farming model, one would also need to consider optimizing the frequency of the mushroom checks and harvests, as well as limiting the soil compaction through repeated stepping, which seems to affect the subsequent harvest yields more than the direct act of harvesting mushrooms (Egli et al. 2006).

5.6.3 Future Perspectives

Farming edible mycorrhizal mushrooms is a very recent development in human cropping history. Many field trials will be required to improve and optimize sustainable cultural practices for new crops such as *L. deliciosus*. Research will be best achieved by a partnership between industry (farm, forestry, and mushroom industries) and science providers. The high scale supply of high-quality, controlled mycorrhizal seedlings will require technology transfer and quality control in order to avoid the pitfalls seen in the truffle industry (Murat 2015). In this chapter, we focus on the development of small relatively intensive mushroom orchards (i.e., a few well-maintained trees) as a means to diversify on-farm production while growing timber or firewood. Another possible approach would use controlled mycorrhizal seedlings for extensive large-scale production of mushrooms under a forestry model with limited tree management and natural rain. However, one difficulty of this approach is the harvest of mushrooms occurs over a short time and over a very large area.

5.7 Conclusions

The mushroom monitoring data from the Tank and House sites highlight the advances made so far in the cultivation of saffron milk cap, with annual yields of up to ≈ 0.5 t/ha, and strongly suggest that its efficient cultivation is achievable in the near future in the South Island of New Zealand. There are many limitations: under P. radiata, we know that a combination of canopy closure and dry autumns has contributed to declining yields, but we are unable to determine what the most important factor was. Irrigation seems to have tempered the effect of drought only to a very small extent. Under P. sylvestris, irrigation did seem to compensate for low rainfall, but the canopy was still open (favorable grass conditions) and the small number of trees enabled us to achieve a more consistent and intense irrigation. Furthermore, the House site was more sheltered than the Tank site, and the impact of this environmental context could not be measured precisely. In the case of canopy closure, it is unknown whether the main factor is over-shading (i.e., lack of light, soil temperature), increased competition between trees for limited resources (including water), or modification of the soil characteristics (needle litter, acidification, etc.). Despite these limitations, we hope that this work present enough convincing evidence that an abundant mushroom crop is already achievable and is likely to be much higher once we will have acquired the basic cultivation knowledge.

Since 2013, mushrooms from all PFR-Lincoln blocks were sold to the public and to restaurants in order to introduce them to New Zealanders (often for the first time), assess their popularity, and generate income for the trial maintenance. The feedback received from the public and the professionals has been very positive, including a constant demand from some of New Zealand's most prestigious chefs. These observations suggest that saffron milk caps could become a popular autumn crop within the next few years in New Zealand. We hope that this work can contribute to and stimulate the innovative and sustainable farming of other edible mycorrhizal mushrooms. There are over 500 of these species worldwide (Honrubia 2007) not yet studied for cultivation and which could bring multiple benefits: farm diversification and yearly income for local populations, non-meat source of protein, environment protection (preventing soil erosion and protecting the climate through carbon fixation), and health benefits such as antioxidant properties (Palacios et al. 2011). A comprehensive review of health benefits in edible ectomycorrhizal mushrooms can be found in Pérez-Moreno and Martínez-Reyes (2014).

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References

- Egli S, Peter M, Buser C, Stahel W, Ayer F (2006) Mushroom picking does not impair future harvests results of a long-term study in Switzerland. Biol Conserv 129:271–276
- Guerin-Laguette A (1998) Les lactaires à lait rouge: mycorhization contrôlée des pins et caractérisation moléculaire. Application à l'étude de la compétence écologique et de la compétitivité d'isolats de *Lactarius deliciosus*. [The red milk cap mushrooms: controlled mycorrhization of pines and molecular characterization. Application to study the ecological competence and the competitiveness of isolates of *Lactarius deliciosus*.] in French with English summary. Ph.D. thesis, Ecole Nationale Supérieure Agronomique de Montpellier
- Guerin-Laguette A, Plassard C, Mousain D (2000) Effects of experimental conditions on mycorrhizal relationships between *Pinus sylvestris* and *Lactarius deliciosus* and unprecedented fruitbody formation of the saffron milk cap under controlled soilless conditions. Can J Microbiol 46:790–799
- Guerin-Laguette A, Hesom-Williams N, Parmenter G, Strong G, Wang Y (2009) Field research and cultivation of truffles in New Zealand: an update. In: Li DZ et al. (ed) The mycorrhizal edible mushroom resources and their sustainable utilization. The proceeding of the 5th international workshop on edible mycorrhizal mushrooms, IWEMM5, Chuxiong, 26–29 August 2007, Acta Botanica Yunnanica Suppl. XVI, pp 90–93
- Guerin-Laguette A, Cummings N, Butler RC, Willows A, Hesom-Williams N, Li S, Wang Y (2014) Lactarius deliciosus and Pinus radiata in New Zealand: towards the development of innovative gournet mushroom orchards. Mycorrhiza 24:511–523
- Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y (2013) Mycorrhiza analyses in New Zealand truffières reveal frequent but variable persistence of Tuber melanosporum in co-existence with other truffle species. Mycorrhiza 23(2):87–98
- Hall IR, Brown GT, Zambonelli A (2007) Taming the truffle the history, lore and science of the ultimate mushroom. Timber Press, Portland, OR, 304 p
- Honrubia M (2007) Wild edible fungi and rural development: a global and critical point of view. In: Abstract book of the 5th international workshop on edible mycorrhizal mushrooms, IWEMM5, Chuxiong, 26–29 August 2007, p 29
- Martínez-Peña F, Ágreda T, Águeda B, Ortega-Martínez P, Fernández-Toirán LM (2012) Edible sporocarp production by age class in a Scots pine stand in Northern Spain. Mycorrhiza 22:167–174
- Murat C (2015) Forty years of inoculating seedlings with truffle fungi: past and future perspectives. Mycorrhiza 25:77–81
- Orlovich D, Cairney JWG (2004) Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. N Z J Bot 42:721–738
- Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno MA, Martínez JA, García-Lafuente A, Guillamón E, Villares A (2011) Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem 128:674–678
- Pérez-Moreno J, Martínez-Reyes M (2014) Edible ectomycorrhizal mushrooms: biofactories for sustainable development. In: Guevara-Gonzalez R, Torres-Pacheco I (eds) Biosystems engineering: biofactories for food production in the century XXI. Springer, Heidelberg, pp 151–233 https://doi.org/10.1007/978-3-319-03880-3_6
- Visnovsky SB, Guerin-Laguette A, Wang Y, Pitman AR (2010) Traceability of marketable Japanese shoro in New Zealand: using multiplex PCR to exploit phylogeographic variation among taxa in the *Rhizopogon* subgenus *roseoli*. Appl Environ Microbiol 76:294–302

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- Wang Y, Hall IR (2004) Edible ectomycorrhizal mushrooms: challenges and achievements. Can J Bot 82:1063–1073
- Wang Y, Hall IR, Dixon C, Hance-Halloy M, Strong G, Brass P (2002) The cultivation of *Lactarius deliciosus* (saffron milk cap) and *Rhizopogon rubescens* (shoro) in New Zealand. In: Hall IR et al. (eds) Edible mycorrhizal mushrooms and their cultivation. Proceedings of the second international conference on edible mycorrhizal mushrooms, New Zealand Institute for Crop & Food Research Limited, Christchurch, New Zealand, CD-ROM, 3–6 July 2001
- Wang Y, Cummings N, Guerin-Laguette A (2013) Cultivation of basidiomycete edible ectomycorrhizal mushrooms: *Tricholoma, Lactarius*, and *Rhizopogon* – Chapter 16. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms. Soil biology, vol 34. Springer-Verlag, Berlin/Heidelberg, pp 281–304