# Final Report

## Submitted to BP Conservation Programme

# Conservation of Tricholoma matsutake in Shangri-la China

**Project Matsutake Final Report**


## Summary

*Tricholoma matsutake* is an economically important ectomycorrhizal mushroom lauded for centuries in Japan. Recent channels of commerce in rural Shangri-la County, China have made *matsutake* the most valuable and collected resource in the region, eclipsing both logging and pastoralism. *Matsutake* populations in this part of China, the most productive in the nation, appear to be declining steadily, however. Flagging productivity over the past decade has generated fear that present collection methods are not sustainable. This project proposed to collect and disseminate data for establishing viable and sustainable harvest protocols for *Matsutake*. Detailed surveys were conducted on the vegetation and mycorrhizal fungi community associated with *T. matsutake* in order to provide baseline data for long-term *Matsutake* conservation. Some study plots were arranged as an education display, and local stakeholders and policy-makers were invited to help interpret and implement resulting data. The results of this study are useful for local, scientific and government communities eager to establish strategies to conserve this important economic resource.

## Background

*Terma,* texts hidden by the lotus born saint Padmasambhava in the 13th century describe *beyul* –hidden valleys- where aging is halted and plants and animals have miraculous powers. It is from a particularly famous *beyul* that Shangri-la receives its namesake. The stunning scenery and remarkable biodiversity of the ethnically Tibetan county has led to its designation as a Global International Biodiversity Hotspot (Mittermeier *et al.* 1998), one of the Nature Conservancy's "Last Great Places", and a place on WWF's Global 200 Ecoregion list (WWF). This part of Yunnan Province contains roughly one-fifth of the China's vascular plant species, one-fourth of the nation's species of mammals and one-third of its bird species (Xiaokun 2003). Despite this, as late as 1993, 80% of the Shangri-la's prefecture's income was from logging (Yeh 2000). In 1998 the Chinese government enacted a commercial logging ban (Zhang *et al.* 2000) and the *Matsutake* trade quickly stepped in to fill the economic gap.

*Tricholoma matsutake*, an edible and medicinal mushroom, has been consumed in Japan for centuries for its distinguished flavor, medicinal properties and iconic significance. The last century saw dramatic declines in *matsutake* fruiting bodies in Japan. According to Kawai (1981) *matsutake* productivity had declined to less than 10% of its pre WWII levels by the early 1980's. Scarcity combined with a superlative demand by the Japanese populace has made *Matsutake* among the most sought after and expensive mushrooms in the world: retail prices in Tokyo reached $600 US/lb. in the 1990's (Yun 1997). As the mushroom approached near-extirpation in Japan, imports of *T. matsutake* soared. By 1986, Shangri-la County began to export fresh *Matsutake* and is now the largest exporter in China (He 2003). Despite *matsutake* collection's relative novelty here, its impacts on local communities and on the environment have been marked. Today an estimated 60% of Shangri-la's populations is involved in the trade (Winkler 2004).

Tax records from Shangri-la indicate a downward trend in *matsutake* productivity over the past decade, mirroring trends in Japan and Northeastern China. Most collectors recognize that the mushroom is becoming increasingly difficult to find, even as climbing prices persuade persistent collection. The forests of Shangri-la are so filled with collectors that mushrooms are often collected before maturing enough to release spores; many are dug from the ground before they emerge. Two recent studies conducted on the related *T. magnivelare* in North America suggest that microhabitat disturbance is negatively correlated to mushroom biomass (Eberhart et al. 1999, Lefevre 2002) although the studies do not explicitly address the effects of collection. Recent molecular data from Mexico (Schweigkofler et al. 2004), Japan (Yamada 2005), and China (Amend 2006 unpublished data) indicate that fungal colonies (*shiro*) are genetically diverse, suggesting that harvesting mushrooms before they sporulate may inhibit sexual recombination and mushroom formation. Another fear is that over-harvesting may adversely affect the mycorrhizal community, endangering the forest as a whole.

In 1999, *T. matsutake* was placed on China's National Protected Plant Species List, and an export quota was established for the mushroom by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Authority of China in 2001. Much of the existing literature on the ecology of this taxa, though, is from Japan (Hiromoto 1963, Hamada 1970, Ogawa 1977), and may not apply to idiosyncratic edaphic regimes, ecotypes and endemic flora of the Eastern Himalaya. Efforts to establish sustainable industry in Shangri-la have been based largely on hunches and guesswork. A paucity of information has prohibited a viable conservation plan.

The aim of this project was to collect and disseminate data for establishing viable and sustainable harvest protocols for *T. matsutake*. As such, team Matsutake carried out the following:

## Project Components:

**1) Document the abundance and habitat of *T. matsutake* including detailed vegetation, environmental and mycorrhizal sampling.**

During the 2005 field season, team Matsutake sampled seventy-five Matsutake patches in four communities and three distinct forest ages. Additionally, seventy-five non-Matsutake patches were sampled for comparison. Sites were stratified by forest age as determined by non-structured interviews with local leaders and elders, and verified by samples taken with an increment borer. Multivariate (forest age, understory diversity, mycorrhizal diversity, matsutake abundance, matsutake genotypic diversity) regression analysis was used to determine statistically significant trends potentially important for Matsutake conservation.

**2) Study of the diversity and distribution of ectomycorrhizal fungal community in relation to *T. Matsutake*.**

Little is known about whether there is correlation between the distribution of *T. matsutake* and other EMF species. The answer to this question is interesting on its own because we hardly know anything about positive or negative correlation of distribution among EMF species, which may be a critical factor in structuring EMF communities. Potential anthropogenic changes in abundance and distribution of *T. matsutake* harvest and habitat modification may be manifested through the changes of associated species.

Earlier studies have shown a decrease of soil microbes in the zone of active *T. matsutake* mycorrhizas, and *T. matsutake* is also found to cause soil desiccation. These findings, combined with our field observation of massive distribution of *T. matsutake* mycelia and mycorrhizae within its own genet patch, lead to the prediction that *T. matsutake* may be the dominant species on its host roots and may exclude the coexistence of some other EMF species, or preferentially promote the growth of others.

To answer the above question, we conducted surveys of ectomycorrhizal fungal community. Three forest sites with the age of 20 years, 50 years and 80 years are chosen. At each site, soil cores (2.8 diameter x 10cm) are taken next to 6 matsutake-present trees and 6 matsutake-absent trees. At each tree, soil cores are taken at three directions and three distances for each direction (50cm, 100cm, 150cm). Three cores at the same distance are combined into a composite soil sample. In addition, in order to understand the correlation between vegetation and fungal community, plant survey were also conducted as shown in Fig. 1.

In each plot, mushroom survey is conducted several times. Small fragments of each species are dried with silica gel. Mycorrhizal roots are separated from the soil, cleaned, and morphotyped under dissecting scope. Short roots are grouped by morphological types.

Number of morphotypes in each soil core and abundance of each morphotype are recorded. Root tip samples of each morphotype are dried with silica gel.

DNA extraction, PCR and TFLP are conducted for both mycorrhizal root tips and mushrooms. Some mycorrhizae samples can be identified to species by matching its TFLP data to the mushrooms. Analysis of species richness and composition is

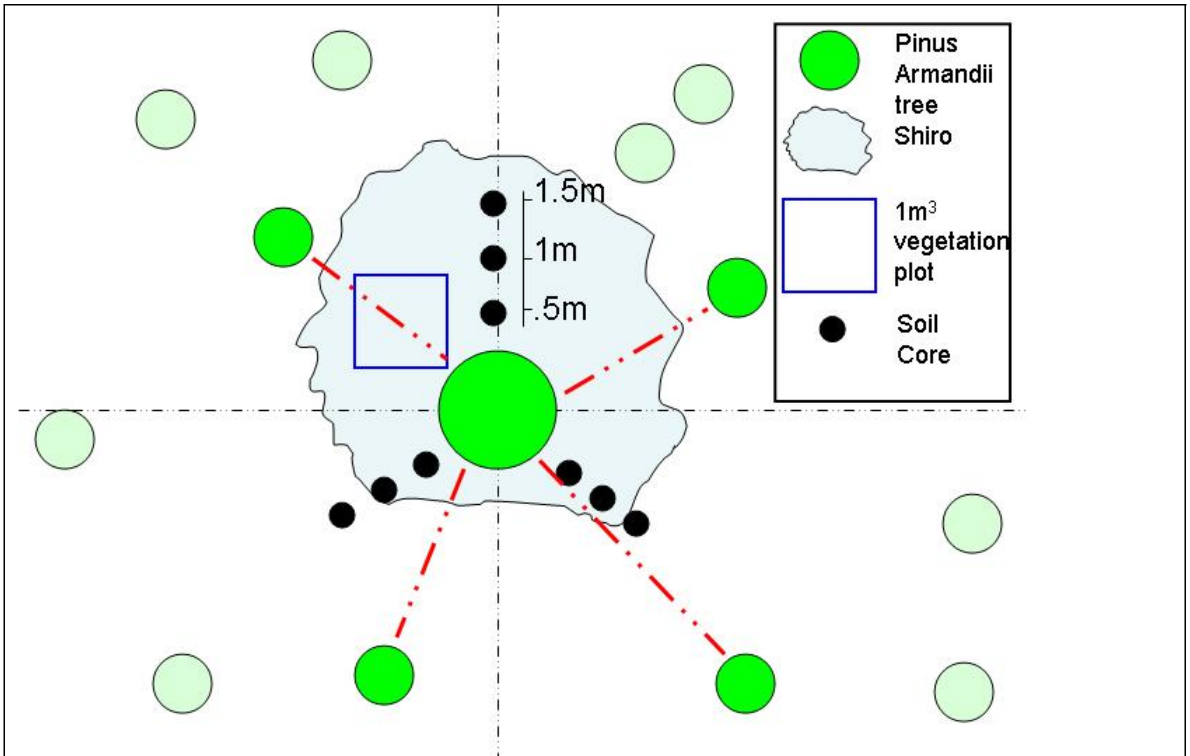


**Figure 1. Diagram of methods used.** Vegetation surveys followed a modified point centered quarter method. Perpendicular lines intersecting at an infected tree were imagined, with one line following the predominant aspect of slope, to divide the site into quarters. Within each quarter, the distance from the center point to the nearest tree with DBH >5cm was recorded as was tree species. A $1m^2$ plot was randomly established as close to the center point as possible within one of the quarters and included herbaceous species and estimated ground cover were recorded. Slope, aspect, GPS location, soil strata depth and ph, and infected tree age (measured by counting annual rings extracted with an increment borer) were also recorded for each Shiro. Three soil cores were extracted at each of three distances: 0.5, 1 and 1.5 m, in each of three directions

**2) Collect baseline data and create permanent monitoring plots to gauge variation with a temporal longitude.**

A subset of sites were analyzed concurrent with a long-term non-timber forest product monitoring initiative conducted by the Kunming Institute of Botany. This project will monitor floristic changes as well as non-timber forest product abundances and extractions. Much of the monitoring from these sites is conducted by community members, and permanent plot demarcations will allow for relatively easy and unambiguous relocation for future monitoring.

**3) Collect historical data on mushroom abundance and markets**.

Recognizing that export and tax records related to matsutake are error prone and unreliable, team matsutake used a multidisciplinary approach to quantify and understand dynamic economic processes underpinning harvest. Allowing for extreme among-year variance, the majority of matsutake exporters cite no significant positive or negative trends in the past eight years. Despite relatively stable returns, collection efforts have increased tremendously. Substantial road construction, increasingly complex trade networks, expansion of commercial harvest into Sichuan and Tibet, and conglomeration of export companies based in Zhongdian have all been well documented. Near-consensus among various strata of the Matsutake trade, is that extracted biomass has not been commensurate with effort.

**4) Assess indigenous knowledge and cultural appropriateness of Matsutake management strategies**

NW Yunnan is as spectacularly diverse in distinct cultures and languages as it is in plants and animals. Diqing prefecture alone counts nine indigenous cultural linguistic groups. Many factors including steep topography, poor roads and cultural linguistic isolation have led to relative autonomy from Chinese authorities (Salick, Yongping, and Amend, 2005), obviating the need for environmental protection policies endorsed by local stakeholders as much as by government organizations.

As important as establishing culturally appropriate harvesting and forestry protocols for sustainable industry is identifying accessible and effective media outlets. Several groups from governmental, commercial and, most recently, non-governmental organizations have begun to broadcast information on environmental protection for Matsutake and other resources. These media range from village meetings, to elementary school lesson plans, to advocacy within religious institutions to radio and television broadcasts.

A principle goal of this study is to assess the efficacy of these broadcast efforts by measuring perceptions of *T. matsutake* ecology and sustainability, trusted sources for information about environmental protection, and the frequency and intensity by which these types of outputs are reaching target audiences. Additionally this study will assess the perceptions and trusted sources of others involved in the trade: local dealers, exporters, NGO staff and government employees.

**5) Develop a highly visible education display.**

With matching funds from the Diqing Export Company, team matsutake successfully completed a professional-quality education display. The display exhibits the wide diversity of ecotypes, vascular plants, animals and fungi of Shangri-la, and the role of the mycorrhizal community within it. Three-dimensional displays depict the Matsutake life cycle from root infection to mushroom spore dispersal, including human harvest. A modular design ensures that the display may be updated as new information and recommendations come in.

**6) Synthesize data into appropriate media and reports for the local, government, conservation and scientific communities.**

Team Matsutake has used several media to disseminate its findings to various stakeholders. Using the education display as a springboard, we held highly successful day-long programs for local school children in matsutake collecting communities. The program includes a guided tour of the display, an opportunity to visit experimental plots in the Shangri-la Alpine Botanical Garden, an interactive matsutake life-cycle "play", mushroom anatomy microscope demonstrations, and matsutake jeopardy. Local school teachers were hired to facilitate and lead some of the excercises, and to ensure that the curriculum could be repeated in subsequent years. Written copies of the lesson plan are deposited at SABG and with the participant teachers.

This summer we also plan to revisit the communities in which we have worked to distribute our findings, make recommendations and answer questions. Meetings with several NGOs active in the region have been scheduled, and a formal presentation at this year's matsutake festival will make our findings available to government officials, matsutake importers and exporters.

## Results

Based on the data collected in 2005, we have the following major findings and outcomes:

**1) Results on the abundance and habitat of *T. matsutake* based on detailed vegetation, environmental and mycorrhizal sampling.**

Analyses indicate that matsutake mycorrhizal abundance and genetic diversity, and mycorrhiza species diversity positively correlate with forest age. ~50 year old and old growth forests appear to produce more matsutake biomass than ~20 year old forests. Surprisingly, matsutake patches contain greater mycorrhiza species diversity than do non-matsutake patches.

Preliminary analysis shows a trend toward greater overall genotypic diversity in old-growth vs. twenty YSL forests, although not yet statistically significant at $\alpha = 0.5$ (Wilcoxon

SRT, P=0.08). Only old growth and 50-60 YSL forests show multiple genotypes within rhizospheres (Figure 2). Twenty YSL forests show lower heterozygosity.

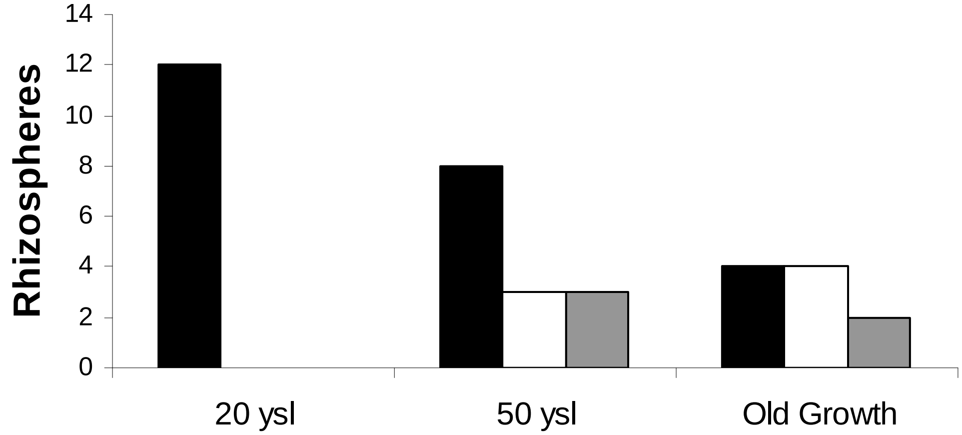


**Figure 2.** Distribution of multiple genotypes per rhizosphere frequency in forest age classes. Black=single genotype, white=two genotypes, grey=three genotypes. A non-parametric Wilcoxon SRT of genotypic diversity between Old Growth and 20yo forests is not significant (*P*=0.08).

**2) Findings on correlation between T. Matsutake and ectomycorrhizal fungal community.**

The underground Mycorrhizal fungal diversity in studied areas was found to be high. In average, about 15 morphotypes were identified from each composite soil sample. And the species composition varied between different sites.

DNA analysis of this study is still in process. Based on morpotype data, matsutake-present patches do have lower species richness than matsutake-absent patches. Certain morphotypes do have higher chance to coexist with *T. matsutake.*

It is likely that *T. matsutake* changes the soil water and chemistry to such a condition that only certain species can tolerate it, which causes the lower species richness as well as the alteration of species composition in matsutake-present patches.

we chose to combine three soil cores at the same distance under the assumption that *T. matsutake* grows in a complete fairy ring with the host tree as the center. However the field observation showed that this is not true in most cases. *T. matsutake* very often grows in an arch on one side of the tree, and the host tree may or may not be the center of its genet. Therefore this sampling regime may overestimate the spatial distribution of *T. matsutake*. In future study, each soil core should be processed individually.

The vertical stratification of EMF species into different soil layers was not reflected in this sampling regime. But it is found *T. matsutake* mycelia primarily distribute between the organic layer and mineral soil. So it is likely that it has little influence on species above or under this soil horizon.

The sampling regime in this study was designed in such a way that the patch around each individual host tree was treated as a whole unit. However, the proper spatial scale for interspecific interaction of ectomycorrhial fungi as well as positive and negative correlation of EMF species distribution is largely unknown. So these patches (circles with 3 meter diameter) may or may not be the appropriate spatial unit.

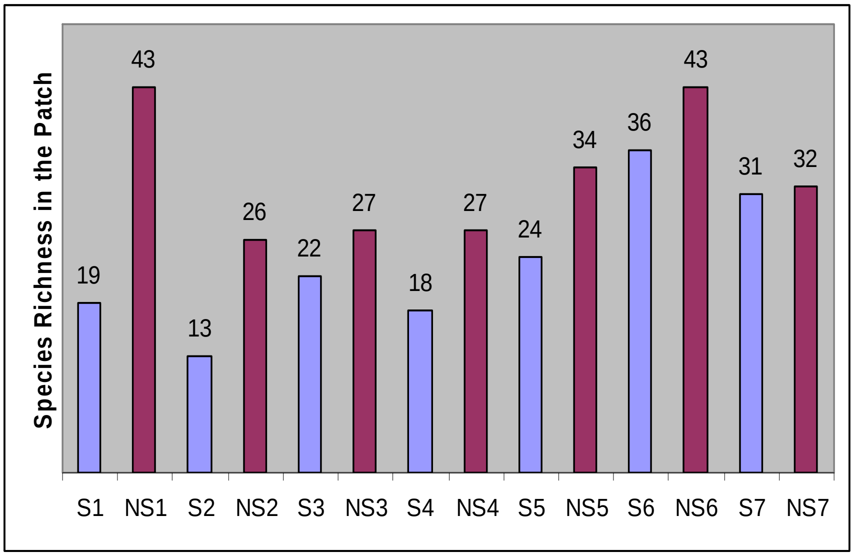


**Figure 3.**
**S**: Shiro patch, the area within the circle of 3.0 m diameter around an individual tree, with *Tricholoma matsutake* present in the soil.
**NS**: Non-shiro patch, the area within the circle of 3.0 m diameter around an individual tree, with no *Tricholoma matsutake present in the soil .*

Figure 4. Mycorrhizae of *Tricholoma matsutake*

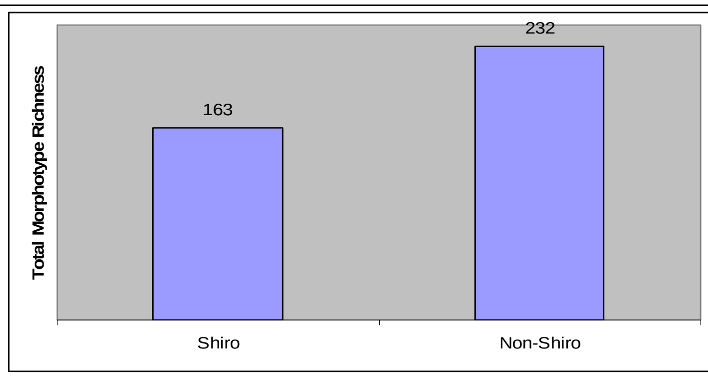


Figure 5. This graph shows the total number of morphotypes identified from all matsutake-present patches verses matsutake-absent patches. Again, it shows matsutake-absent patches have higher morphotype richness than matsutake-present patches.

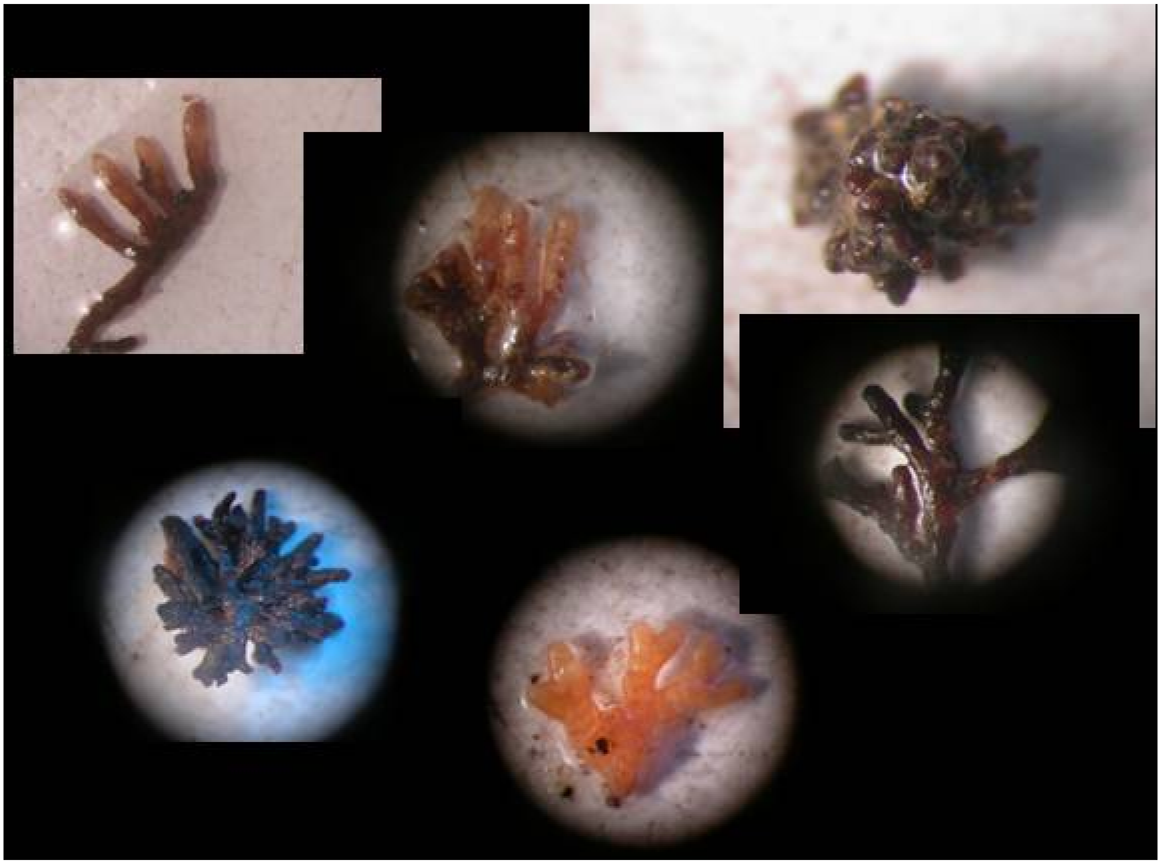

Figure 6. Variety of sampled ectomycorrhizal fungal species

Figure 7. Variety of the sporocarps of sampled ectomycorrhizal fungal species

**3).Indigenous knowledge and cultural appropriateness of Matsutake management strategies**

One hundred twenty two questionnaires were completed by harvesters and matsutake dealers from four ethnic groups in seven communities. Analysis of these data showed several significant trends.

Foraging behavior is significantly partitioned by gender, ethnicity, and community. While the males and females surveyed earn similar income from matsutake (and similar income overall), female harvesters will travel farther distances than males to collect them (Wilcoxon SRT, $P$=0.02). Among communities, there were no significant differences between effort (days per month spent collecting and forage bout distance traveled). Overall income from Matsutake was found to positively correlate with average distance traveled to collect.

Consensus analysis of rankings supports the conclusion that there is general agreement among informants. A standard criterion for a significant consensus (the ratio of the first eigenvalue to the second >= 3.0) was met. Analysis showed strong consensus within and among community, gender, education, income and ethnic groupings.

Village leaders and Government officials appear to be the most trusted sources for Matsutake overall (Figure 8). Rankings of other trusted sources of information appear to follow approximately normal (bell curve) distributions with the exception of "Other Pickers" which is skewed towards low ranks.

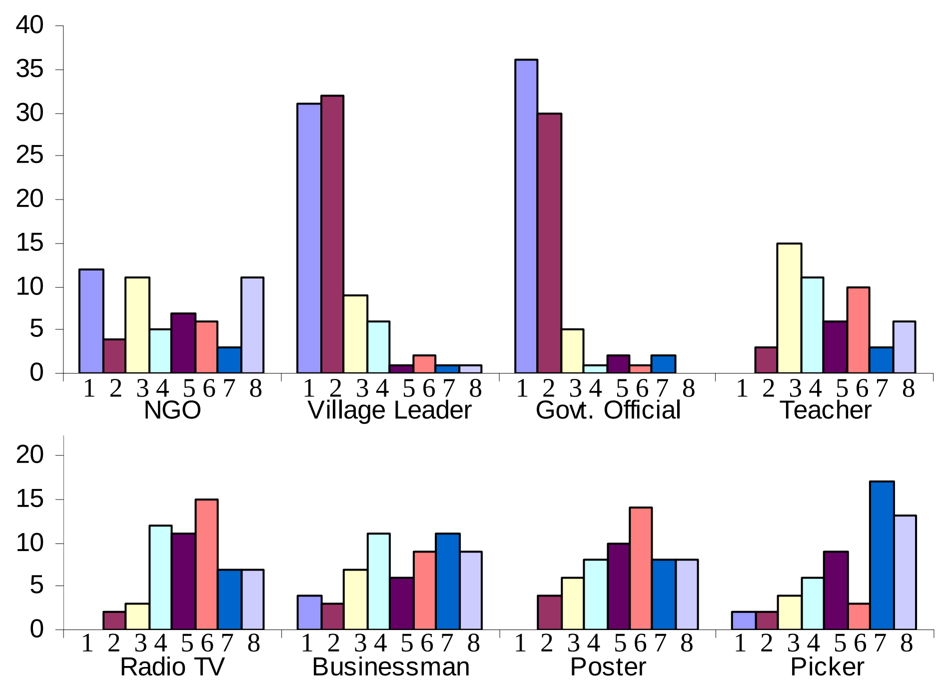


**Figure 8.** Rankings of trusted sources for *T. matsutake* information shows strong consensus among village, ethnic, gender, education and income groupings. For each category, columns indicate the frequency with which an informant ranked an information source (1-8 from left to right). Village leaders and government officials are the highest ranked sources overall.

**4) Community Education and Outreach**

An additional outcome of this project was the inauguration of an ongoing Matsutake instructional series at the Shangri-la Alpine Botanical Garden. Lesson plans for a day-long curriculum in which pickers learn about fungal life-cycles, anatomy and forest symbioses were developed and taught in conjunction with local grade-school teachers (Figure 4). Young pickers from nearby communities were transported to and from the activities by bus.

A permanent display was also established on the grounds of the Shangri-la Export Company's factory in Zhongdian (Figure 9, 10). Within the first week of the display's opening, it received nearly 500 visitors, including mushroom harvesters, school children, village leaders, mushroom exporters and even the vice-governor of Shangri-la.

**Figure 9.** Students examine a spore print at a Matsutake instructional series at SABG (left). A permanent Matsutake information display reaches out to various levels of the Matsutake business.

Figure 10. Team members at the education program with local students.

**5) Publications and presentations:**

Select photos and text from the education display have been published as a book: Plant Diversity  of Shangri-La Comprehensive Volume. Our initial findings were presented as a keynote speech at the 2nd annual matsutake festival in Zhongdian.  Subsequently, team members have presented at the Society for Economic Botany meeting in Thailand, and two paper abstracts have been accepted for the International Conference on Mycorrhizae in July 2006 in Spain.  At least three peer-review papers will be submitted following final analyses.

## Reccomendations

Based on our data from the 2005 field season team Matsutake has made the following observations and recommendations:

Increasing effort has not lead to commensurate levels of matsutake harvest. Although exact productivity levels are difficult to measure, there is reason to believe that harvesting activity, which may include leaf-litter disturbance, digging, a shift towards harvesting smaller size classes and diminished spore rain may have adverse affects on matsutake biomass production. Additionally, forest management practices, both extant and historical, have been shown to affect matsutake reproduction, matsutake mycorrhizal abundance, mycorrhizae species diversity and potentially, biomass produced.

While many forests are variously protected via cultural, religious and political mechanisms, others are logged for timber or fuelwood, or are burned to clear pasture. As older pine forests seem more favorable to Matsutake abundance and diversity, we recommend that villages refrain from logging in matsutake producing areas. Because the annual value of matsutake extracted from a given forest far exceeds the value of that forest's trees, selectively avoiding disturbance in productive areas is economically sound.

Additionally, older forests have been shown to harbor more genetic diversity, and that diversity is manifest in smaller spatial scales than previously assumed. That multiple genotypes are found within a single soil core suggests that shiro are actually a mosaic of repeated inoculation by ambient spores. Of all known studies on ectomycorrhizal genera, only one other, *Suillus*, shares this pattern of genetic mosaicism. This trait is potentially significant for Matsutake conservation, because it seems to indicate that infection by airborne spores is a frequent and important aspect of Matsutake life history. Thus, a system of harvesting matsutake which allows for spore rain should be implemented. In several of the villages surveyed in this project, there was already some sort of harvest rotation in place: several days of collection followed by several days without collection. Aside from increasing the average biomass of mushrooms collected, this system could also allow a percentage of mushrooms to mature to spore-releasing stage. Some villages mentioned a system of "privatization", whereby mushroom patches are tenured to specific custodians for a given amount of time. Inherent problems of resource inequality and the system's conflict with a federal policy banning private land ownership are two factors which have prevented implementation of this system to date.

## Future studies and projects

This project has indicated the need for future work, both in terms of scientific study, as well as community outreach. Because everyone, from harvesters, to middlemen, to exporters and eventually consumers has a vested interest in Matsutake conservation, there are open networks of communication which can be utilized to inform the community of viable conservation strategies. Previous efforts have used posters, however these seldom have the desired effect as few people bother to read them and they remain inaccessible to illiterate community members. A more culturally appropriate medium should be applied: VCDs are popular and players are abundant, and village meetings are suitable fora for disseminating results and strategies.

There is still much to learn about Matsutake ecology, much of it pertinent to conservation. A regional study of population genetics would be useful for determining spatial scales of genetic differentiation. This would help managers establish boundaries for genetic reservoirs, determine whether or not populations harbor unique genetic resources, and determine how much geneflow there is among populations.

A second line of inquiry would be to empirically determine spore rain within populations and whether or not this correlates with forest management or harvest protocol. Matsutake spores are nearly identical to many other species of mushroom, so a molecular technique using taxon-specific genetic primers would have to be employed. Such primers both from the multicopy ITS region, as well as from microsattelite loci have been developed by researchers, making such a study feasible and relatively economical.

A third study would be to examine how matsutake, and its harvest, effect the mycorrhizal community. Over 95% of vascular plants rely on fungal symbionts for nutrient and water uptake. Changes in the mycorrhizal community via anthropogenic forces would likely not be detectable in the plant community until many years later, making a thorough study of mycorrhizal population dynamics and species interactions a timely and essential predictor of future forest health.

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