

## Spread of the fungus *Fomitopsis officinalis* inoculated in stems of living larch in Slovenia

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### Introduction

The fungus *agaricum* or *agariokon* was used by ancient Greeks and Romans for tuberculosis treatments. During history known also as “quinone conk”, “ghost bread”, “tree biscuit” and white agaric is the larch polypore *Fomitopsis officinalis* (Vill.) Bondartsev & Singer (sin. *Laricifomes officinalis* (Vill.) Kotl. & Pouzar) noted for its medicinal properties. In folk medicine is used as a laxative, sometimes was used also as soap and as a substitute of hops in beer production.<sup>1,2</sup> *F. officinalis* is usually associated with *Larix* trees, but it is reported to occur also on different coniferous species.<sup>2,3</sup> The fungus infects the trees through broken branches and causes intensive brown rot in wood.<sup>2,3</sup> Growth of *F. officinalis* is slow and the fruiting bodies vary in shape. They can live up to 70 years and weight up to 10 kg.<sup>2</sup> *F. officinalis* is characterized by diminishing distribution

from south to north and tendency to inhabit mainly mountain regions.<sup>2</sup> It is found in West-Europe, North America, Ural-Siberia and some smaller populations had been detected in Morocco, China, Japan and Korea.<sup>4</sup> In Slovenia, two locations of *F. officinalis* are reported by one source<sup>5</sup> and three by the other.<sup>6</sup> *F. officinalis* is a very rare species and has been suggested already by Domanski<sup>3</sup> to enter it on the list of protected species. It is placed on a list of 33 endangered fungi in Europe which was produced by European Council for Conservation of Fungi.<sup>6</sup> The decrease in localities of *F. officinalis* is regularly reported, probably as a result of mature larch forest reduction and of the gathering of basidiocarps for medicinal purposes.<sup>4</sup> The purpose of this study was to determine successfulness of larch tree inoculation with *F. officinalis*, in order to use it for spreading this fungus in nature and thus promoting and securing its survival in Slovenia.

## **Materials and methods**

Two healthy larches, with diameter at breast height 89 cm and 58 cm, were inoculated with larch wood sticks overgrown with *F. officinalis* mycelium at different tree heights. Mycelium culture was obtained from the Institute for higher fungi, Vinje pri Ljubljani, Slovenia (ZIM culture collection No. 1024).<sup>7</sup> The culture was isolated from a conk of *F. officinales* collected at Podolševa, Slovenia (Longitude: 14° 14' 28'', Latitude 46° 26' 12'') in the year 2000 and kept from that time under oil on potato dextrose agar. One larch tree was inoculated in Ljubljana and one in Gozd Martuljek. Selected sites are distinct in climate conditions (Table 1).

Table 1: Geographical data and climate conditions for Ljubljana and Gozd Martuljek<sup>8</sup>

|                | Longitude  | Latitude         | a.s.l. | Mean annual air temperature <sup>a</sup> | Mean annual precipitation <sup>a</sup> | Max / min air temperature <sup>b</sup> |
|----------------|------------|------------------|--------|--|--|--|
| Ljubljana      | 14°29'03'' | 46°03'07''<br>'' | 308 m  | 10,2°C                                   | 1377 mm                                | 35,9°C / -<br>15,7°C                   |
| Gozd Martuljek | 13°51'54'' | 46°28'54''<br>'' | 901 m  | 6,1°C                                    | 1604 mm                                | 32,6°C / -<br>20,4°C                   |

<sup>a</sup> in the 1971-2000 period; <sup>b</sup> in the year 2006

In April 2006 and December 2006, three years after the inoculation, wood core samples were extracted with an increment borer from the standing trees, at different distances from the primary inoculation points. The wood core samples were sectioned at 3 cm's distance, followed by placing obtained wood particles on the malt extract agar (MEA) plates and incubated at 24°C. The wood material was treated with sterile equipment and sterile techniques were used throughout the isolation procedure. After mycelium had outgrown from the incubated wood samples, the fungus was determined by its microscopic characteristics.<sup>9</sup> Spore and mycelium characteristics of the original *F. officinalis* strain and isolates obtained from our inoculated trees were compared. The inoculation was considered successful, if above mentioned parameters were comparable.

## Results

Table 2: Determination of *F. officinalis* mycelium presence in larch trees inoculated with *F. officinalis*

|  |                   |           |                |
|--|-------------------|-----------|----------------|
|  | Ljubljana (April) | Ljubljana | Gozd Martuljek |
|--|-------------------|-----------|----------------|

|  |      | (December) | (December) |
|--|------|------------|------------|
| inoculation points                                 | 1    | 3          | 4          |
| wood core samples                                  | 6    | 12         | 23         |
| wood core samples with positive determination      | 5    | 1          | 3          |
| % of wood core samples with positive determination | 83,3 | 8,3        | 13,0       |

Maximum length of tree trunk overgrown with *F. officinalis* mycelium was 9 cm below, 10 cm above the inoculation point (Ljubljana, April), 20 cm left, 10 cm below, 10 cm above the inoculation point (Ljubljana, December) and 10 cm above the inoculation point in December at Gozd Martuljek as was showed with laboratory tests.

## Discussion

Inoculation of larch trees with *F. officinalis* is successful using the above mentioned method even on trees in rural and urban areas, where this species of fungus can not be found. After three years of larch tree inoculation, re-isolation of *F. officinalis* mycelium was successful from 83.3% wood core samples. This percentage declined to 8.3% re-isolation success, when isolations following the same procedure were done in the winter time (Table 2). We can only speculate that this decline occurs due to influence of weather conditions to mycelium activity inside the tree trunk. Pietka et al.<sup>10</sup> successfully re-isolated *F. officinalis* mycelium from the larch trees three years after artificial infection in Poland and showed that *in situ* inoculation can be considered as an effective strategy for protection of this endangered fungus. They determined that *F. officinalis* can

develop at the rate of 6 cm per year or less in the heartwood of living trees, parallel to the trunk axis. Slow rates of mycelium progress in living trees were revealed also at the experiment in Slovenia – 6 cm per year, perpendicular to the trunk axis and around 3 cm per year in the direction parallel to the tree axis. The technique of artificial inoculation could be used for preventing *F. officinalis* imminent extinction also in Slovenia as was also indicated with obtained results in our experiment.

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