

Ann. Mus. civ. Rovereto	Sez.: Arch., St., Sc. nat.	Vol. 12 (1996)	209-218	1998
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OBSERVATIONS ON THE *PSILOCYBE CYANESCENS* COMPLEX OF EUROPE AND NORTH AMERICA

Abstract - JOCHEN GARTZ - Observations on the *Psilocybe cyanescens* complex of Europe and North America.

Several specimens of the *Psilocybe cyanescens* complex from Europe and North America were recently collected for scientific study and herbarium deposit. This paper presents an investigation of the indole derivatives of mushrooms from the Czech Republic (*Psilocybe bohemica*) and some collections from Germany and Austria as well as *Psilocybe cyanescens* from the Pacific Northwest, U.S.A. The content of psilocybin was highest in cultivated and naturally grown mushrooms from the U.S.A. Psilocybin was also found to be contained in the cultured mycelia of this species.

The analysis of all collections revealed only very small amounts of baeocystin. It seems that the collections from Austria and Germany, *Psilocybe bohemica* and *Psilocybe cyanescens* from the U.S.A. are 3 different species with very similar microscopic features. *Psilocybe bohemica* was also cultivated on wet rice and on mulch in a shady outdoor bed. The alkaloid content in cultured basidiocarps was in the same order of magnitude as that found in naturally grown mushrooms.

Key words: *Psilocybe* - Analysis - Alkaloids - Taxonomy.

Riassunto - JOCHEN GARTZ - Osservazioni sul complesso di *Psilocybe cyanescens* in Europa e Nord America.

Numerosi campioni di specie appartenenti al complesso di *Psilocybe cyanescens* sono stati recentemente raccolti in Europa e Nord America, nell'ambito di studi scientifici o per essere inseriti tra gli esemplari d'erbario. Vengono qui presentate indagini sui derivati dell'indolo, condotte su funghi provenienti dalla Repubblica Ceca (*Psilocybe bohemica*), dalla Germania e dall'Austria, oltre che su *Psilocybe cyanescens* proveniente dal Nord-ovest degli U.S.A. Il contenuto in psilocibina è risultato più elevato nei funghi provenienti dagli U.S.A., sia coltivati, sia cresciuti spontaneamente. La psilocibina è pure contenuta nel micelio ottenuto dalla coltivazione di queste specie.

L'analisi di tutti i campioni ha rivelato solo piccole quantità di baeocistina. Le raccolte dall'Austria e dalla Germania, oltre che *Psilocybe bohemica* e *Psilocybe cyanescens* dagli Stati Uniti, sembrano essere tre differenti specie con caratteri microscopici molto simili. *Psilocybe bohemica* è stata pure coltivata all'esterno, su riso umido e su terriccio, in luogo ombroso. Il contenuto alcaloidico dei basidiocarpi coltivati era dello stesso ordine di grandezza di quelli naturalmente tallificanti.

Parole chiave: *Psilocybe* - analisi - alcaloidi - tassonomia.

In the last 20 years ethnomycological and botanical research established that *Psilocybe semilanceata* (FR.) KUMM. is the most important psilocybian species of Europe (GARTZ, 1991, 1993a, 1996; GUZMAN, 1983).

At least one other psychoactive *Psilocybe* species in addition to *Psilocybe semilanceata* is known to exist in various European countries. These strongly blueing mushrooms can be described as belonging to the «*Psilocybe cyanescens* complex» (KRIEGLSTEINER, 1984, 1986).

They grow on raw compost and plant debris in Europe and therefore act as a primary composer (GARTZ, 1993 a, 1996). According to KRIEGLSTEINER (1984, 1986) six taxa including *Psilocybe bohémica* SEBEK and *Psilocybe serbica* MOSER & HORAK are merely synonyms for *Psilocybe cyanescens* WAKEFIELD emend. KRIEGLSTEINER (see also GARTZ, 1996), but the differentiation of single species within the *Psilocybe* genus is subject to considerable controversy among taxonomists (GUZMAN, 1983; GUZMAN & BAS, 1977; KRIEGLSTEINER, 1984, 1986; MOSER & HORAK, 1968; STAMETS, 1993).

Some papers have also been published about the occurrence of psilocybin, psilocin and baeocystin in *Psilocybe cyanescens* from the Pacific Northwest, U.S.A. (BEUG & BIGWOOD, 1982; REPKE *et al.*, 1977; UNGER & COOKS, 1979).

According to KRIEGLSTEINER (1986) and STAMETS (1993) these collections possess abundant, capitate pleurocystidia in contrast to mushrooms from Europe (KRIEGLSTEINER, 1984; DENNIS & WAKEFIELD, 1946).

But some doubts still exist about the significance of this single microscopic difference regarding a differentiation of species (KRIEGLSTEINER, 1986).

Additionally, only a few collections of *Psilocybe cyanescens* from Europe and North America were studied at all (GUZMAN, 1983; KRIEGLSTEINER, 1986).

In continuation of earlier studies of *Psilocybe bohémica* (GARTZ, 1993 a, 1996; GARTZ & MUELLER, 1989; SEBELK, 1980; SEMERDZIEVA & NERUD, 1973; SEMERDZIEVA & WURST, 1986) in this work analysis of various collections from the «*Psilocybe cyanescens* complex» including some additional experiments on cultivation is described.

EXPERIMENTAL

Mycelium from *Psilocybe bohémica* (GARTZ & MUELLER, 1989) on 4% malt agar was used to inoculate a rye grain/water mixture identically to the cultivation of other *Psilocybe* species (GARTZ, 1995).

The cultivation on sawdust soaked with water in plastic bags and later on commercial garden mulch was already described in the case of *Psilocybe azurescens* STAMETS & GARTZ (GARTZ, 1995). The duration of the rye based spawn was 6 weeks and of the sawdust mixture 8 weeks.

Then the mulch was spawned in March 1988. In October/November 1989 22 mushrooms appeared on the garden mulch without casing. For additional 5 years 10 up to 33 mushrooms were harvested each year from this German location always in November.

The mushrooms were dried or analysed and analysed as described earlier (GARTZ & MUELLER, 1989).

Laboratory cultivation for the production of fruit bodies of «typical» *Psilocybe cyanescens* from the Pacific Northwest U.S.A. was also attempted. Mycelial cultures were isolated from spores of a dried mushroom (Mason County, 1984) as described earlier for *Psilocybe bohemica* (GARTZ & MUELLER, 1989).

After a 4 weeks cultivation of the mycelia on a 4% malt agar a sterile mixture of 100 g soft rice and 180 ml water was inoculated. The cultivation temperature of 23° C was decreased to 10°C after 10 weeks and this temperature was maintained until the beginning of the fruiting process (2 weeks).

The culture continued to produce mushrooms in additional 3 flushes at temperature from 8 to 14 ° C.

A similar fruiting of *Psilocybe bohemica* (GARTZ & MUELLER, 1989) produced 8 mushrooms in 3 flushes which were analysed like other psilocybian species (GARTZ, 1991, 1995).

Analysed naturally grown fruit bodies:

- a. *Psilocybe cyanescens* U.S.A.:
collection from 1984, 1992 and 1993.
- b. *Psilocybe cyanescens* Germany:
area of the Rheinland: 1993, 1994 and 1995
town Schwerin: 1995.
- c. *Psilocybe cyanescens* Austria: 1992
location near border to Czech Republic.

RESULTS

The investigation showed the occurrence of psilocybin, psilocin and baeocystin in *Psilocybe bohemica* from the garden culture (Table 1). The concentrations of these indole derivatives were similar to the levels found in naturally grown fruit bodies (GARTZ & MUELLER, 1989) or in mushrooms from the cultivation on wet rice grain (Table 1)

In contrast to *Psilocybe semilanceata* (GARTZ, 1991) or *Psilocybe azurescens* (GARTZ, 1995), *Psilocybe bohemica* contains very low amounts of baeocystin.

According to KRIEGLSTEINER (1984, 1986) all studied collections of the *Psilocybe cyanescens* complex had identical spores. In my experience it was also impossible to differentiate the collections from Germany, Austria, U.S.A. as well

TAB. 1 - CONCENTRATION OF ALKALOIDS IN DRIED FRUIT BODIES OF *PSILOCYBE BOHEMICA* FROM THE GARDEN CULTURE (SAMPLES 1-6) AND FROM THE CULTIVATION ON RICE GRAIN (SAMPLES 7-12).

Sample	Psilocybin	Baeocystin (%)	Psilocin
1	0.34	0.03	—
2	0.55	0.01	—
3	0.91	0.02	0.02
4	0.87	0.02	0.02
5	0.62	0.03	0.01
6	0.54	0.05	0.03
7 (1.Flush)	0.44	0.03	0.01
8 (1.Flush)	0.82	0.02	0.03
9 (2.Flush)	0.81	0.03	0.04
10 (2.Flush)	0.62	0.02	—
11 (3.Flush)	0.34	0.02	0.02
12 (3.Flush)	0.53	0.01	—

TAB. 2 - CONTENT OF INDOLE DERIVATIVES IN DRIED MUSHROOMS OF *PSILOCYBE CYANESCENS* FROM THE U.S.A. (%). (SAMPLES 1-5, 1984; 6-10, 1992; 11-15, 1993).

Sample	Psilocybin	Baeocystin	Psilocin
1	0.72	0.03	0.93
2	0.52	0.03	0.23
3	0.41	0.02	0.32
4	0.83	0.04	0.41
5	0.98	0.01	0.28
6	0.88	0.02	0.65
7	0.68	0.04	0.75
8	0.78	0.02	0.62
9	0.55	0.05	0.71
10	0.41	0.04	0.62
11	0.78	0.03	0.58
12	0.59	0.02	0.72
13	0.69	0.01	0.68
14	0.78	0.02	0.71
15	0.48	0.02	0.91

as in mushrooms of *Psilocybe bohemica* with the form and size of the spores. But the macroscopic feature of *Psilocybe bohemica* differs sharply to the fruit bodies from the other locations. I never found any wavy caps in *Psilocybe bohemica* and there were also differences in the colour of the stems as well as in the striate margins which were seen only in this species. In contrast to KRIEGLSTEINER (1984, 1986) I was able to study *Psilocybe bohemica* and the other collections from Europe and the U.S.A. as fresh fruit bodies at the locations and think that there are three different species. Additionally, during crossing experiments, complete reproductive barriers have been found between monokaryons of *Psilocybe bohemica* and *Psilocybe cyanescens*, U.S.A.

Both are autonomous species which do not form hybrid dikaryons. In contrast to these results, monokaryons of the collections from Austria and Germany formed dikaryons together and all features were also identically.

High concentrations of psilocybin and even higher amounts of psilocin were detected in all extracts of *Psilocybe cyanescens* from the U.S.A. (Table 2). The results confirmed the opinion that collections of *Psilocybe cyanescens* from North America are very potent hallucinogenic mushrooms (BEUG & BIGWOOD, 1982). In contrast to the relative *Psilocybe azurescens* (GARTZ, 1995) only minor amounts of baecocystin were found. These concentrations were similar to other published results (REPKE *et al.*, 1977). It seems that the levels of the unstable substance psilocin decreased during storage. The samples from 1984 were analysed 3 years later, the others only a few days after harvesting. I was also able to find the abundant, capitate pleurocystidia only in the collections from the U.S.A.

The content of the alkaloids in cultured basidiocarps of *Psilocybe cyanescens* (U.S.A.) was in the same order of magnitude as that found in naturally grown mushrooms (Table 3).

A photo of such a cultivation was already published (GARTZ, 1993 b)

The amount of psilocybin ranged from 0,22% to 0,34% by dry weight in 5 different mycelia of *Psilocybe cyanescens* (U.S.A.) grown on 4% malt agar. Interestingly, in contrast to the fruit bodies from wet rice grain no other indole derivatives could be detected in extracts.

These differences in the indolic composition of mycelia without fruiting and in cultivated mushrooms were also found earlier in *Psilocybe bohemica* (GARTZ & MUELLER, 1989), *Conocybe cyanopus* (ATK.) KUEHNER (GARTZ, 1991), *Galerina steglichii* BESL and *Psilocybe natalensis* GARTZ, REID, SMITH & EICKER (GARTZ, 1995).

Like *Psilocybe cyanescens* from the U.S.A. all collections from Europe displayed a strong blueing reaction, despite the fact that levels of psilocin in these mushrooms are very low (Table 4). From 1960 on, it was found that only psilocin can be oxidized into products of bluish-green colour (GARTZ, 1996). The phosphate group in psilocybin and baecocystin prevents direct oxidation of the



Fig. 1 - *Psilocybe bohémica* from the garden culture. (photo: J. Gartz).



Fig. 2 - Mushrooms of the *Psilocybe cyanescens* complex from Austria. (photo: J. Gartz).



Fig. 3 - *Psilocybe cyanescens* in the Pacific Northwest, U.S.A. (photo: J.W. Allen).

alkaloids. However, the typical blueing phenomenon does occur when this protective group is removed by enzymes, such as various phosphates, which are very common also in mushroom tissue. Apparently, the enzymatic removal of the phosphate group from the psilocybin occurs quite quickly. This is how psilocin is formed after injuries of the fruit bodies. Immediately afterwards psilocin continues to break down into blue-coloured compounds.

In comparison to *Psilocybe cyanescens* from the U.S.A. it seems generally that the naturally grown mushrooms of this species complex in Europe contain smaller amounts of alkaloids especially in the case of psilocin (Table 1-4).

However it should be pointed out that this clear difference will be invalid in a few years.

Beside this new location in the town Schwerin I know from other collections in recent years:

Hamburg 1993, Berlin 1994 (3 locations) and some locations in Frankfurt for about 5 years.

It is now well known that some new locations in Germany or the Netherlands are really artificial cultivations on wood material or mulch mainly in parks and gardens of large towns (STAMETS & CHILTON, 1983; GARTZ, 1993).

TAB. 3 - THE DRY WEIGHT VARIATION OF PSILOCYBIN, BAEOCYSTIN AND PSILOCIN LEVELS IN CULTIVATED *PSILOCYBE CYANESCENS* (U.S.A.) AS A FUNCTION OF FLUSH NUMBER.

Flush N.	Psilocybin	Baeocystin (%)	Psilocin
1	0.68	0.03	0.72
2	0.51	0.02	0.63
3	0.81	0.02	0.41
4	0.71	0.03	0.92

TAB. 4 - INDOLE ALKALOIDS IN MUSHROOMS OF THE *PSILOCYBE CYANESCENS* COMPLEX FROM EUROPE (% DRY WEIGHT).

	Sample	Psilocybin	Baeocystin	Psilocin	
Rheinland	1993	1	0.61	0.01	0.05
	»	2	0.48	0.02	0.03
	1994	3	0.51	0.01	0.02
	»	4	0.71	0.02	0.03
	1995	5	0.43	0.03	0.02
	»	6	0.38	0.01	0.05
Schwerin	1995	7	0.45	0.02	0.03
	»	8	0.33	0.03	0.04
	»	9	0.62	0.01	0.05
	»	10	0.52	0.02	0.04
Austria	1992	11	0.51	0.02	0.02
	»	12	0.44	0.03	0.05
	»	13	0.43	0.01	0.02
	»	14	0.38	0.01	0.03
	»	15	0.45	0.03	0.04

Even in Europe the spawn for these cultivations derived from spores of *Psilocybe cyanescens* from the Pacific Northwest, U.S.A.

In the past mycologists have experienced that North American species can spread very quickly across Europe, for example *Stropharia rugoso-annulata* FARLOW (the Giant Stropharia).

Generally, we can expect that the genuine *Psilocybe cyanescens* complex from Europe will achieve a remarkably wide area of distribution in the future because of the modern use of mulch in parks and gardens. But because of the «under-

ground» cultivation which produce huge amounts of spores we can expect similar or even larger distribution waves of *Psilocybe cyanescens* from the U.S.A. in the wake of environmental changes including the use of fertilizers and wood substrates in Europe.

ACKNOWLEDGEMENTS

The author thanks M. Semerdzieva (†), S. Trebes, B. Schurig and P. Rausch, who generously supplied valuable information and mushrooms for analysis.

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