

## BACKGROUND

- Psilocybin containing “magic” or psychedelic mushrooms (PM) affect perception, mood, behavior, and consciousness.
- The neuroplastic, immune modulation and anti-inflammatory effects of psilocybin are currently being explored for a range of conditions including depression, PTSD, anxiety, and substance abuse.
- There is anecdotal evidence of distinctive, subjective experiences with different PM and this may be due to variations in psilocybin content and effects of non-psychedelic tryptamines.
- This phenomenon, the entourage effect, may be the result of the pharmacological interaction between the tryptamines.

## OBJECTIVES

This study aimed to establish the concentration of tryptamines in a variety of popular PM that are reportedly associated with distinct subjective experiences in the non-medical setting and to explore whether there is a correlation between the type of subjective experience and the tryptamine composition of the mushrooms.

## METHODS

### PART 1: QUALITATIVE ASSESSMENT

- A cross-sectional online survey was fielded on July 22-July 25, 2022.
- Participants were recruited from online psychedelic user groups (known users survey) and a general national sample of adults in the US reporting PM experience (national survey).
- A fictional PM was included for quality control (QC).
- Participants were asked to rate their general overall experience and intensity of visual and physical effects (body load) by PM on a 0-to-10-point scale.
- Endpoints were assessed descriptively.

### PART 2: MUSHROOM COMPOSITION DETERMINATION

- *Psilocybe subtropicalis*, *Panaeolus bisporus*, *Psilocybe tampanensis*, *Psilocybe cubensis* - “Enigma”, *Psilocybe natalensis*, *Panaeolus cyanescens*, *Psilocybe stanzii*, *Psilocybe ovoideocystidiata*, and *Psilocybe azurescens* were obtained from field collections.
- Samples of dried ground mushrooms were extracted with methanol. Psilocybin, psilocin, norpsilocin, baeocystin, norbaeocystin, and aeruginascin were quantified using a Waters Acquity H-Class UPLC and Xevo TQ-S Micro MS (Waters Corporation, Milford, MA, USA).
- For 8 of the mushrooms, specimens from 2 different harvests were analyzed.

## SURVEY RESULTS

Of the 2,325 participants who entered the survey, 11.8% (n=112) passed the QC review and completed the PM questionnaire (Table 1).

## PARTICIPANT CHARACTERISTICS

- Of the 112 participants, 50.3% were male, 50.3% were between the ages of 33 and 47 years, and 31.5% were from the Midwest (Table 2).
- The User Population had more older participants (48-63+ years) and the National Survey population had more younger participants (18-47 years).

TABLE 1. SURVEY ATTRITION

Participants	N	%
Total entered National Survey	2,325	100.0
QC1. Did not agree to participate	45	1.9
QC2. Age < 18 years old	51	2.2
QC3. No PM experience	1277	54.9
Total excluded (did not pass QC1-QC3)	1373	59.1
Total continuing to PM questions	952	41.9
QC4. Experience with fictional PM	823	86.4
QC5. Nonsense write-in responses	8	0.8
QC6. AI write-in responses	9	0.9
Total excluded (did not pass QC4-QC6)	840	88.2
<b>Total from National Survey (passed QC1-QC6)</b>	<b>112</b>	<b>11.8</b>
Total from User Survey	37	---
<b>Total Study Population</b>	<b>149</b>	<b>---</b>

TABLE 2. PARTICIPANT CHARACTERISTICS

	Total Population (n=149)	Known User Population (n=37)	National Survey Population (n=112)
Gender	Female	42.3%	49.1%
	Male	50.3%	45.5%
	Non-binary	4.7%	4.5%
	Prefer not to say	2.7%	0.9%
Age	18-32 years old	28.2%	31.3%
	33-47 years old	50.3%	51.8%
	48-62 years old	16.8%	12.5%
	63 years old or more	4.7%	4.5%
US Region	Northeast	22.1%	23.2%
	Midwest	31.5%	29.5%
	South	23.5%	26.8%
	West	22.8%	20.5%

## PART 1: QUALITATIVE ASSESSMENT RESULTS

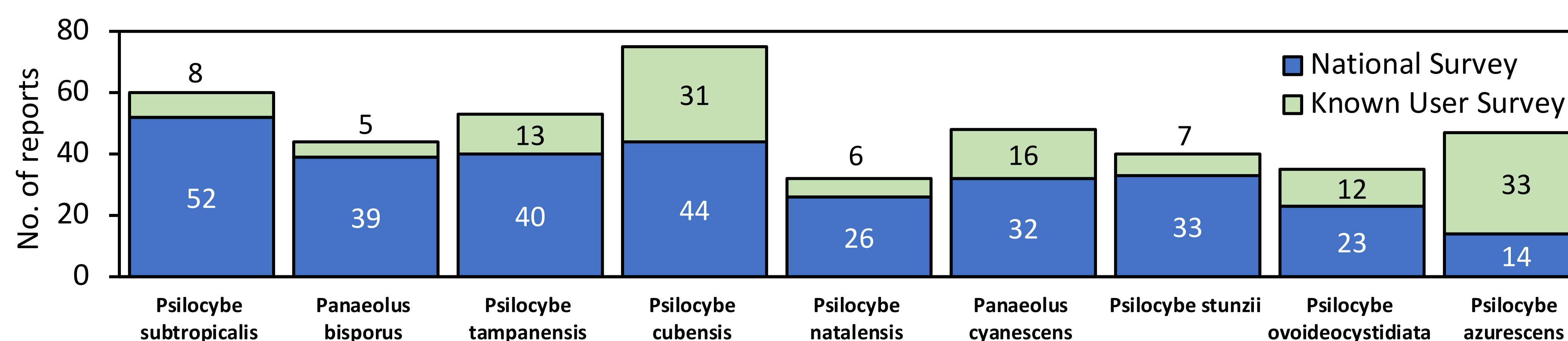


FIGURE 1. MUSHROOMS ASSESSED BY SURVEY POPULATION (n=149)

- *Psilocybe cubensis* was the most reported PM in the known user survey (n=31) and the Total survey population (n=75).
- *Psilocybe subtropicalis* was the most reported PM in the national survey (n=52) and the second highest in the Total survey population (n=60).

	Overall (0 to 10) mean (SD)	Visual (0 to 10) mean (SD)	Body Load (0 to 10) mean (SD)	Higher Visual Experience	Higher Body Load	Visual and Body Equivalent	Visual Experience ≥ 8	Body Load ≥ 8
<i>Psilocybe subtropicalis</i> (n=60)	6.13 (3.24)	6.08 (2.72)	5.93 (2.77)	38.3%	33.3%	28.3%	36.7%	31.7%
<i>Panaeolus bisporus</i> (n=44)	5.98 (2.65)	5.80 (2.97)	5.45 (2.62)	45.5%	31.8%	22.7%	34.1%	22.7%
<i>Psilocybe tampanensis</i> (n=53)	6.53 (2.65)	5.49 (2.83)	5.55 (2.70)	34.0%	37.7%	28.3%	30.2%	24.5%
<i>Psilocybe cubensis</i> (n=75)	7.12 (2.51)	6.35 (2.51)	6.11 (2.54)	40.0%	28.0%	32.0%	34.7%	28.0%
<i>Psilocybe natalensis</i> (n=32)	6.88 (2.54)	<b>6.84 (2.52)</b>	6.03 (2.73)	37.5%	21.9%	40.6%	<b>40.6%</b>	28.1%
<i>Panaeolus cyanescens</i> (n=48)	7.04 (2.99)	6.15 (2.81)	6.00 (2.76)	34.4%	33.3%	31.3%	35.4%	<b>31.1%</b>
<i>Psilocybe stanzii</i> (n=40)	6.35 (2.23)	5.63 (2.73)	5.53 (2.42)	30.0%	32.5%	37.5%	27.5%	25.0%
<i>Psilocybe ovoideocystidiata</i> (n=35)	<b>7.49 (2.50)</b>	<b>6.86 (2.76)</b>	5.97 (2.96)	45.7%	25.7%	28.6%	<b>51.4%</b>	<b>34.3%</b>
<i>Psilocybe azurescens</i> (n=47)	<b>7.51 (2.83)</b>	6.77 (2.97)	<b>6.70 (2.64)</b>	28.9%	31.9%	38.3%	<b>57.4%</b>	<b>42.6%</b>

FIGURE 2. SUBJECTIVE ASSESSMENT OF INDIVIDUAL PSYCHEDELICS MUSHROOMS

- *Psilocybe azurescens* and *Psilocybe ovoideocystidiata* were rated as having the highest overall effect with an average score of 7.5.
- *Psilocybe natalensis* and *Psilocybe ovoideocystidiata* had the highest visual effects with an average score of 6.8 and 6.9, respectively.
- *Psilocybe azurescens* had the highest average physical effect of 6.7.
- Ratings varied greatly as reflected in the standard deviation making it difficult to identify meaningful differences by PM.
- Participants in the known user survey consistently reported lower overall, visual and body scores.

## PART 2: MUSHROOM COMPOSITION DETERMINATION RESULTS

	JULY	NOV	JULY	NOV	JULY	NOV	JULY	NOV	JULY	NOV	JULY	NOV	JULY	NOV	
	µg/mL	Psilocybin	Psilocybin	Psilocin	Psilocin	Norpsilocin	Norpsilocin	Baeocystin	Baeocystin	Norbaeocystin	Norbaeocystin	Aeruginascin	Aeruginascin	Min/Maj	Min/Maj
<i>Psilocybe cubensis</i> (PE)	0.08767	0.04220	0.05580	0.00800	0.02607	0.00600	0.01560	0.00070	0.00107	0.00085	0.00173	0.00360	0.15724	0.22211	
<i>Psilocybe subtropicalis</i>	0.25040	0.42260	0.08680	0.02450	0.00853	0.00625	0.01133	0.00605	0.00333	0.00435	0.00627	0.00470	0.07259	0.04775	
<i>Panaeolus bisporus</i>	0.64887	0.00720	0.18587	0.06230	0.02680	0.00730	0.02140	0.00000	0.00567	0.00000	0.01260	0.00000	0.08739	0.10504	
<i>Psilocybe natalensis</i>	0.49760	0.00260	0.10873	0.02540	0.01587	0.00660	0.01727	0.00000	0.00587	0.00200	0.00947	0.00000	0.07993	0.30714	
<i>Panaeolus cyanescens</i>	0.47867	0.35730	0.01653	0.10120	0.02580	0.00880	0.01373	0.00850	0.00260	0.00490	0.01127	0.00000	0.12034	0.04842	
<i>Psilocybe tampanensis</i> fruit	0.01900	0.00530	0.04033	0.08190	0.00993	0.00000	0.00180	0.00090	0.00000	0.00400	0.00280	0.00000	0.10784	0.05619	
<i>Psilocybe ovoideocystidiata</i>	0.14533		0.02880		0.00820		0.01020		0.00120		0.00920		0.24494		
<i>Psilocybe azurescens</i>	0.08333	0.05990	0.17947	0.08950	0.01053	0.00960	0.00673	0.00000	0.00227	0.00260	0.00313	0.00320	0.08625	0.10308	
<i>Psilocybe enigma</i>	0.35133	0.73590	0.14473	0.01490	0.04787	0.00000	0.01973	0.00000	0.00627	0.00270	0.00413	0.00670	0.08845	0.01252	
<i>Psilocybe stanzii</i>	0.02960		0.06480		0.01440		0.01300		0.00000		0.00000		0.31854		

TABLE 3. TRYPTAMINE CONTENT BY GROWING PERIODS

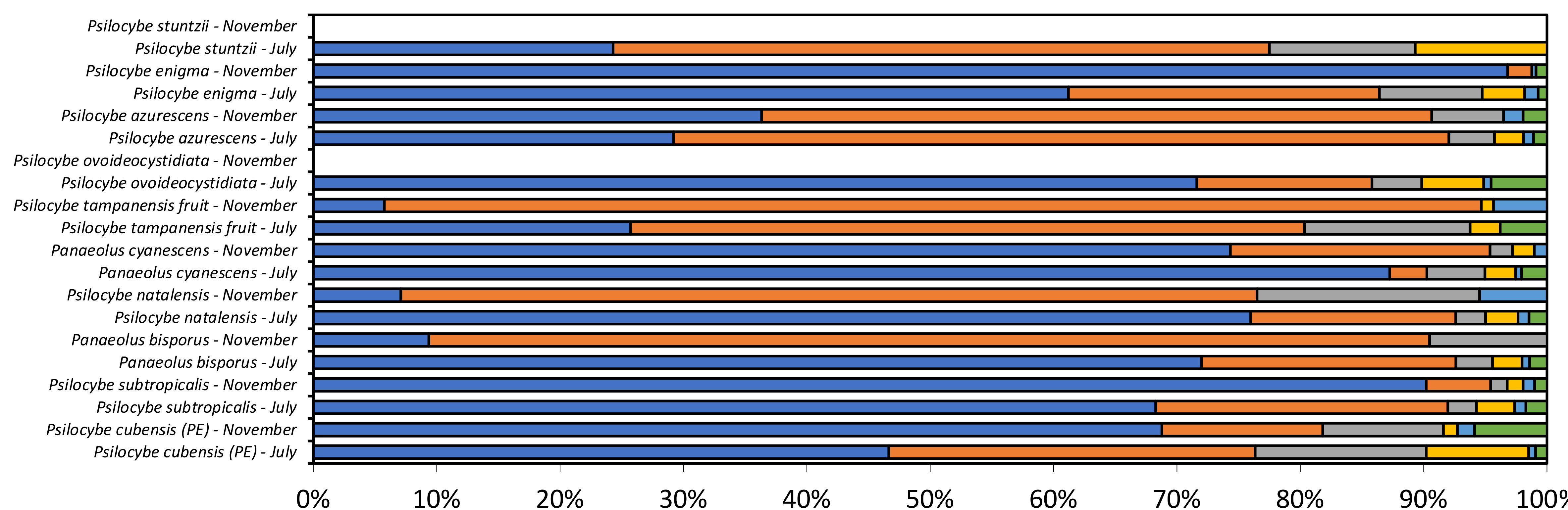


FIGURE 3. PERCENTAGE TRYPTAMINE BETWEEN 2 GROWING PERIODS

## RESULTS AND DISCUSSION

The national survey screened out 840 (88.4%) potential participants for selecting a fictional PM (86.5%) and other quality control screens (1.8%). A total of 149 individuals completed the survey with 37 (22%) individuals from known psychedelic user groups and 112 (78%) individuals from the general national sample. Participants in the PM user groups, relative to those in the national sample, were more likely to be male (77.4% vs. 45.5%) and older with a greater percentage between 43-52 years old (32.3% vs. 16.0%). Overall, *Psilocybe cubensis* (n=75), *Psilocybe subtropicalis* (n=60) and *Panaeolus cyanescens* (n=48) were the 3 most commonly used PM. Participants rated *Psilocybe azurescens* and *Psilocybe ovoideocystidiata* with the highest overall effect with an average score of 7.5; *Psilocybe natalensis* and *Psilocybe ovoideocystidiata* had the highest reported impact on visual effects with an average score of 6.8 and 6.9, respectively; *Psilocybe azurescens* had the highest average physical effect of 6.7. For the majority of PM there was a wide range of ratings from 0 to 10. Psilocybin, the most abundant component, ranged from 0.02 (*Psilocybe tampanensis* fruit) to 0.65 (*Panaeolus bisporus*) µg/mL. Analyses of different harvests of the same type of mushroom showed no consistency in the composition and proportion of any of the mushrooms with variances up to 122% for the non-psychoactive tryptamines and up to 395% for psilocybin and psilocin (Table 3). The total content of other tryptamines varied proportionally to the combined psilocybin and psilocin content. Of note is that strong body and weaker visual effects seem to be recorded for mushrooms with a relatively lower psilocybin and higher baeocystin content while mushrooms with an equal ratio of psilocybin vs. the other tryptamines seem to produce more visual effects.

## CONCLUSION

The large number of participants screened out by control measures highlights the challenges in conducting surveys on psychedelics. Furthermore, the dose of the psychedelic substances taken and even the type of mushroom cannot be ascertained via survey research. There is substantial interobserver variation in the assessment of experiences with 10 different PM, which is in line with our findings of varying tryptamine content of different harvests of the same mushroom. Furthermore, components other than the ones analyzed, could modify individual experiences. A hypothesis-generating pattern seems to emerge pointing towards distinct visual and physical experiences which may be explained by the ratio of non-psychedelic tryptamines to psilocybin and the amount of baeocystin in different mushrooms.

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