Amatoxins in wood-rotting Galerina marginata

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Abstract: Amatoxins, bicyclic octapeptide derivatives responsible for severe hepatic failure, are present in several Basidiomycota species belonging to four genera, i.e., Amanita, Conocybe, Galerina and Leptota. DNA studies for G. autumnalis, G. marginata, G. orionensis, G. unicolor and G. venenata (section Naucoria sp.) determined that these species are the same, supporting the concept of Galerina marginata complex. These mostly lignicolous species are designated as white-rot fungi having a broad host range and capable of degrading both hardwoods and softwoods. Twenty-seven G. marginata basidiomes taken from different sites and hosts (three sets) as well as 17 A. phalloides specimens (three sets) were collected in French locations. The 44 basidiomes were examined for amatoxins and phalloxins using high-performance liquid chromatography. Toxinological data for the wood-rotting G. marginata and the ectomycorrhizal A. phalloides species were compared and statistically analyzed. The acidic and neutral phalloxins were not detected in any G. marginata specimen, whereas the acidic (β-Ama) and neutral (α-Ama and γ-Ama) amanitins were found in all basidiomes from either Angiosperms or Gymnosperms hosts. The G. marginata amatoxin content varied from 78.17 to 243.61 μg·mg⁻¹ of fresh weight and was elevated significantly in one set out of three. The amanitin amounts from certain Galerina specimens were higher than those from some A. phalloides basidiomes. Relationship between the amanitin distribution and the chemical composition of substrate was underlined and statistically validated for the white-rot G. marginata. Changes in nutritional components from decayed host due to enzymatic systems and genetic factors as well as environmental conditions seem to play a determinant role in the amanitin profile. Variability noticed in the amanitin distribution for the white-rot G. marginata basidiomes was not observed for the ectomycorrhizal A. phalloides specimens.

Keywords: amanitins, Basidiomycota, Galerina, white-rot fungi

INTRODUCTION

Amatoxins belong to a family of bicyclic octapeptide derivatives composed of an amino acid ring bridged by a sulphur atom and characterized by differences in their side groups (Wieland and Faulstich 1991, Zanotti et al 1992). The main amatoxins, α-, β-, and γ-amanitins inhibit eukaryotic DNA-dependent RNA polymerase II and are hepatotoxic (Faulstich and Wieland 1996, Faulstich and Zilker 1994, Wieland and Faulstich 1983). These toxins first are extracted from the poisonous mushroom Amanita phalloides Fr. and then from other Amanita species. Smaller amounts also are found in species of other genera, such as Conocybe, Galerina and Lepiota (Ammirati et al 1985, Block et al 1955, Brady et al 1975, Bresinsky and Besl 1990, Gérault and Girre 1977, Lincoff 1998, Wieland 1986). Phalloxins are bicyclic heptapeptide derivatives present in A. phalloides basidiomes and related species (Wieland 1983, Wieland and Faulstich 1983). Minor quantities of phalloxins recently were detected in Conocybe lactea (J.E. Lange) Métrod (Hallen et al 2003).

The toxicity of certain Galerina species is well known. Early in the 20th century, Peck (1912) reported a human poisoning case due to G. autumnalis
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(Reck) A.H. Sm. & Singer. Later, Grossman and Malbin (1954) reported a poisoning produced by G. venenata A.H. Sm. (Smith 1953). Over two decades, 10 cases caused by amatoxin-containing Galerinas were mentioned: (i) three European cases, two from Finland (Elonen and Härkönen 1978) and one from France (Bauchet 1983) due to G. marginata (Batsch) Kühner and G. unicolor (Vahl) Singer, respectively; (ii) seven North American exposures, two fatalities from Washington due to G. venenata (McKenney and Stuntz 1987) and five cases reacting positively to treatment, four caused by G. autumnalis from Michigan (two reports) and Kansas (two reports), respectively (Trestall 1991, 1994), and one by Galerina sp. from Ohio (Trestall 1992). A clinical analysis of 12 Chinese patients poisoned with G. autumnalis also was published (Yin and Yang 1999). Over three decades, 53 Japanese patients poisoned by G. fasciculata Hongo, including five fatal cases, were reported (Ishihara and Yamaura 1992). A 6-year-old boy also developed severe hepatic failure after eating a mushroom morphologically identified as G. fasciculata (Kaneko et al. 2001).

Using thin-layer chromatography, α-amanitin (α-Ama) and β-amanitin (β-Ama) were detected in the fruiting bodies of G. autumnalis, G. marginata and G. venenata (Tyler and Smith 1963, Tyler et al. 1963). Both amanitins were quantified in G. autumnalis (1.5 mg·g⁻¹ dry weight; Johnson et al. 1976) and G. marginata (1.1 mg·g⁻¹ dry weight; Andary et al. 1979). α-Ama and γ-amanitin (γ-Ama) were produced by fermentation from American G. marginata (Benedict and Brady 1967). Then, experiments confirmed the occurrence of α-Ama and β-Ama in German basidiomes of G. autumnalis and G. marginata and revealed the presence of the three amanitins (α-Ama, β-Ama and γ-Ama) in the fruiting-bodies of G. beinrothii Bresinsky, G. sulcipectus (Berk.) Singer and G. unicolor. Furthermore, G. marginata mycelium could produce the three amanitins whereas G. beinrothii and G. unicolor mycelia yielded β-Ama and α-Ama, respectively (Besl et al. 1984). Recently, α-Ama, β-Ama, and γ-Ama were found in cultured mycelia of G. fasciculata and G. helvoleptus (Berk. & M.A. Curtis) Singer (Muraoka et al. 1999, Muraoka and Shinozawa 2000).


Analyses of rDNA sequences carried out on the 35 European and North American Galerina collections belonging to five amatoxin-containing taxa revealed no significant distinctiveness between the species referred to two strīps, Marginata and Autumnalis. According to Guilden et al. (2001), these analyses support the concept that: (i) the boreal Northern Hemisphere constitutes one mycogeographical region; (ii) the North American and European species—G. autumnalis, G. marginata, G. unicolor and G. venenata—should be treated as one species named G. marginata, leaving G. badipes as the other distinct species.

The Galerina marginata complex is widespread in the Northern Hemisphere—Europe and North America (Gilden and Vesterholt 1999) and in Asia (Imazeki et al. 1987). Macroscopic and microscopic characters of G. marginata are described, and its lignicolous habit also is reported (Bon 1990, Guilden et al. 2001, Singer 1986).

A review on the distribution of the 1669 wood-rotting fungi from North America reports that: (i) 93% of these species are white-rotters; (ii) the studied brown-rot fungi do not belong to families of Cortinariaceae and Strophariaceae (Gilbertson 1980). According to the current classifications, G. marginata is a species belonging to one of these two families; it is likely that it could be designated as a white-rot Basidiomycota.

To our knowledge, there is no recent study on the toxin content and profile for the wood-rotting G. marginata. For this reason, G. marginata basidiomes were collected from deciduous and coniferous hosts in French forests and investigated for amatoxins and phallotoxins using high-performance liquid chromatography. Furthermore, toxicological data of the wood-rotting G. marginata and the ectomycorrhizal A. phalloides were compared and the results were validated by statistical analysis.
MATERIALS AND METHODS

Collection conditions of G. marginata and A. phalloides.—Twenty-seven G. marginata basidiomes at various stages of development were collected on decayed wood from three locations in two French regions—Alsace-Lorraine and Franche-Comté in Oct 1996 and 1998, respectively. Sixteen specimens were gathered on fallen trunks of beech (Fagus sylvatica L.) found in two sites from the Lorraine forest of LangouERM and Sarrebourg (Gal L1: n = 10; Gal L2: n = 6). This forest of beech and hornbeam is on either neutral or calcareous soil (at 450 m). Eleven mushrooms (Gal FC) were collected on moss-covered rotted spruce (Picea abies [L.] Karsten) in a mixed pine/spruce/beech wood called the Bois des Tilles (Franche-Comté) at an altitude of 650–675 m on rauracian limestone with pH = 6.8. The three Galerina sets are listed in TABLES I and II. The mushrooms were identified on the basis of macro and micro-morphological descriptions (Bon 1992, Courtecueille and Duhem 2000, Gulden and Vesterholt 1999).

Furthermore, 17 A. phalloides basidiomes collected from three French locations during the same years were: (i) six specimens from the Alsatan Haguenau forest (Aph A); (ii) three samples gathered from the LangouERM and Sarrebourg forest (Aph L); (iii) eight basidiomes (Aph FC) from the Bois Rodolphe situated in Franche-Comté (TABLES I and II).

Analytical procedure.—Each basidiome was weighed, frozen in liquid nitrogen and ground. Amatoxins and phalloxins were extracted by sonication and separated by reversed-phase liquid chromatography. Identification of both classes of toxins was based on retention times and UV spectral data; the absorbance of the eluate was monitored at 285 nm for the phalloxins and 305 nm for the amatoxins (Wieland 1986, Wieland and Faustich 1983). The toxin detection limit for both amatoxins and phalloxins was 0.5 ng g⁻¹ of fresh fungal material. The concentration of amatoxins (α-Ama, β-Ama and γ-Ama) and phalloxins (phallolidin, phallisin, phalloin, phallacidin and phallisinic) from the 44 basidiomes (27 Galerinas and 17 Amanitas) was determined by means of analytical procedure as previously reported (Enjalbert et al 1992). The amatoxin content corresponding to the sum of the three amanitin amounts assayed in each basidiome (n = 44) taken from the different sites (n = 6) was expressed in μg of toxin per gram of fresh weight of tissue (FW). The amanitin concentrations were expressed as a percentage to determine the toxin profile in the basidiomes of both species G. marginata and A. phalloides. The ratio β-Ama/(α-Ama + γ-Ama) between the acidic and neutral amanitin amounts was calculated for each specimen. The mean data of the five variables—amatoxin content, β-Ama, α-Ama and γ-Ama concentrations as well as β-Ama/(α-Ama + γ-Ama) ratio—for the 27 Galerinas (three sets) and the 17 Amanitas (three sets) were analyzed statistically. This data allowed the comparison of amatoxin content and amanitin profile between the woodrotting and ectomycorrhizal species.

RESULTS

Toxin content and amanitin distribution for G. marginata.—The mean weight (g of fresh basidiome ± sem) for each set of G. marginata was of 0.92 ± 0.11 g (from 0.5 to 1.7 g), 2.90 ± 0.66 g (from 1.2 to 5.5 g) and 1.01 ± 0.24 g (from 0.35 to 2.7 g) for Gal L1, Gal L2 and Gal FC, respectively (TABLE I). The acidic (phallacidin and phallisinic) and neutral (phallolidin, phallisin and phalloin) phalloxins were not present at the toxin HPLC detection limit of 0.5 ng g⁻¹ F.W. in any G. marginata basidiome collected on rotten wood from deciduous and coniferous trees during different years. The amatoxin content corresponding to the sum of three amanitins (mean amounts ± sem) for Gal L1, Gal L2 and Gal FC sets was of 243.61 ± 16.54 μg g⁻¹, 78.17 ± 10.08 μg g⁻¹ and 96.88 ± 12.82 μg g⁻¹, respectively (TABLE I). It was equivalent to 0.024%, 0.008% and 0.009% for Gal L1, Gal L2 and Gal FC, respectively. The statistical analysis by one-way ANOVA showed a significant effect of "Origin" (P_A < 10⁻⁴) for the three sets of G. marginata. The mean amanitin concentrations for the Gal L1 set presented significant difference (PRK < 10⁻⁴) when compared to nearly equivalent amounts for both sets Gal L2 and Gal FC (TABLE I, FIG. 1).

The mean concentrations of β-Ama, α-Ama and γ-Ama (expressed as percentage) as well as the mean values of β-Ama/(α-Ama + γ-Ama) ratio in the specimens belonging to the three sets, i.e. Gal L1, Gal L2 and Gal FC are listed in TABLE II. The acidic and neutral amanitin distribution in the G. marginata species obviously was affected by both site and host (TABLE II, FIG. 2a, b) because significant variation (P_A < 10⁻⁴) relative to the Origin factor was found. The Newman-Keuls multiple range tests showed a significant difference (PRK < 10⁻⁴) between the mean concentrations taken two by two for either the three amanitins or the ratios displaying the predominance of acidic and neutral toxins. As regards the Gal L1 and Gal L2 sets, β-Ama and α-Ama were the major toxins but not similarly distributed in both sets. The former,
distinguishable by a high toxin content (243.61 ± 16.54 μg g⁻¹), displayed: (i) an elevated mean concentration of β-Ama (58.69 ± 1.09%); (ii) a small mean concentration of γ-Ama (2.34 ± 0.15%); (iii) a β/(α + γ) ratio of 1.44 ± 0.07 (from 1.21 to 1.38). The latter was characterized by: (i) β-Ama and α-Ama mean concentrations almost identical; (ii) a high mean γ-Ama concentration (9.47 ± 0.33%); (iii) a reversed β/(α + γ) ratio 0.76 ± 0.05 (from 0.61 to 0.87) (TABLE II, Fig. 2a, b). An elevated mean percentage of neutral toxins (α-Ama = 63.43 ± 2.39%), γ-Ama = 13.44 ± 1.08%) and a small value of β/(α + γ) ratio equivalent to 0.31 ± 0.03 (from 0.20 to 0.52) differentiated the Gal FC set (TABLE II, Fig. 2a, b).

**Toxin content and amanitin distribution for A. phalloides.**—The mean weight (g of fresh weight ± sem) for each set of A. phalloides was of 20.81 ± 5.91 g F.W., 32.60 ± 12.74 g F.W. and 18.42 ± 4.78 g F.W. for Aph A, Aph L3 and Aph FC, respectively (TABLE I). Phallotoxins and amanitins were detected by HPLC in all 17 specimens collected from the three sites; amanitin content and amanitin distribution were listed solely (TABLES I and II, respectively).

The mean amanitin content of the Aph A, Aph L3 and Aph FC sets was of 367.09 ± 62.38 μg g⁻¹, 172.07 ± 56.32 μg g⁻¹ and 247.87 ± 14.67 μg g⁻¹ F.W., respectively (TABLE I, Fig. 1). One-way ANOVA showed significant difference between the three sets of A. phalloides (Aph A: n = 6; Aph L3: n = 3; Aph FC: n = 8; P < 0.04). Significant variation in the amanitin contents was observed only between the Aph A and Aph L3 sets (P < 0.04). Origin factor (site) did not affect the amanitin profile of this ectomycorrhizal species because the B-Ama, α-Ama and γ-Ama concentrations as well as β/(α + γ) ratios from the three sets were not statistically different (P > 0.05; TABLE II, Figs. 2a, b).

**Statistical comparison of the toxicological data of G. marginata and A. phalloides.**—The analysis of variance combining Species and Origin as factors was performed on the six sets constituted of 44 specimens (27 Galerinas, three sets; 17 Amanitas, three sets). Significant difference (P < 10⁻⁴) between the six amanitin contents was observed. The Newman-
**Table II. Amanitin distribution in G. marginata (Gal) and A. phalloides (Aph) sets**

<table>
<thead>
<tr>
<th>Set name</th>
<th>Sample size</th>
<th>$\beta$-Amanitin (%)</th>
<th>$\alpha$-Amanitin (%)</th>
<th>$\gamma$-Amanitin (%)</th>
<th>$\beta/(\alpha + \gamma)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal L1</td>
<td>10</td>
<td>58.60 ± 1.09</td>
<td>38.97 ± 1.03</td>
<td>2.94 ± 0.15</td>
<td>1.44 ± 0.07</td>
</tr>
<tr>
<td>Gal L2</td>
<td>6</td>
<td>43.18 ± 1.49</td>
<td>47.35 ± 1.55</td>
<td>9.47 ± 0.33</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>Gal FC</td>
<td>11</td>
<td>23.13 ± 1.80</td>
<td>63.43 ± 2.39</td>
<td>13.44 ± 1.08</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Aph A</td>
<td>6</td>
<td>52.88 ± 4.08</td>
<td>34.77 ± 2.93</td>
<td>12.48 ± 1.82</td>
<td>1.20 ± 0.20</td>
</tr>
<tr>
<td>Aph L3</td>
<td>3</td>
<td>52.15 ± 1.46</td>
<td>40.77 ± 2.26</td>
<td>7.10 ± 1.11</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>Aph FC</td>
<td>8</td>
<td>46.27 ± 2.98</td>
<td>42.43 ± 2.95</td>
<td>11.30 ± 0.77</td>
<td>0.93 ± 0.12</td>
</tr>
<tr>
<td>G. marginata</td>
<td>27</td>
<td>40.76 ± 3.20</td>
<td>50.8 ± 2.39</td>
<td>8.44 ± 1.05</td>
<td>0.83 ± 0.10</td>
</tr>
<tr>
<td>A. phalloides</td>
<td>17</td>
<td>49.64 ± 2.08</td>
<td>39.43 ± 1.90</td>
<td>10.98 ± 0.86</td>
<td>1.05 ± 0.09</td>
</tr>
</tbody>
</table>

Keuls tests divided the experimented sets into three groups ($P_{\text{FK}} < 10^{-3}$, Fig. 1). The Aph A set representing the first group (a) contained the highest mean amanitin amount (367.09 ± 62.38 $\mu$g g$^{-1}$ F.W.) whereas the third (c) was formed by the Gal L2 and Gal FC sets exhibiting amanitin amounts approximately four times less (78.17 ± 10.08 $\mu$g g$^{-1}$ and 96.88 ± 12.82 $\mu$g g$^{-1}$ F.W., respectively). The second group (b) consisted of the Gal L1 and Gal FC sets showing nearly the same amatoxin content (243.61 ± 16.54 $\mu$g g$^{-1}$ and 247.87 ± 14.67 $\mu$g g$^{-1}$ F.W., respectively). Concerning the Aph L3 set, the basidiomes were classified either in the third (c) or second group (b) (172.07 ± 56.32 $\mu$g g$^{-1}$ F.W.).

The comparison between the amanitin profiles for the six sets of G. marginata and A. phalloides revealed significant difference ($P_{\text{FK}} < 10^{-3}$) in the mean amanitin concentrations and mean values of ratio (Table II). $\beta$-Ama concentration from Gal FC set statistically was lower than that from each of the three Amanitas sets ($P_{\text{FK}} < 0.01$). $\beta$-Amanita concentrations from Gal L1 and Gal L2 sets were significantly different from only those for Aph FC ($P_{\text{FK}} < 0.01$) and Aph A ($P_{\text{FK}} < 0.05$), respectively. Significant difference was recorded between the $\alpha$-Ama concentrations: (i) higher concentration from Gal FC than those from all Amanitas analyzed ($P_{\text{FK}} < 0.01$); (ii) higher concentration from Gal L2 than from Aph A ($P_{\text{FK}} < 0.05$). Significant difference also was revealed between the $\gamma$-Ama concentrations: (i) lower concentration from Gal L1 than those from all Amanitas ($P_{\text{FK}} < 0.01$); (ii) higher concentration from Gal FC than from that from Aph L3 ($P_{\text{FK}} < 0.01$) (Table II, Fig. 2a). Last, the $\beta/(\alpha + \gamma)$ ratio calculated for Gal FC was lower than each one established for the Amanitas series ($P_{\text{FK}} < 0.01$) whereas the low ratio distinguished Gal L1 only from Aph FC ($P_{\text{FK}} < 0.05$) (Table II, Fig. 2b).

Furthermore, the comparison between the amanitin mean concentrations from the three sets of G. marginata showed a significant difference ($P_{\text{FK}} < 10^{-4}$) revealing that the Origin (site/host) clearly affected the ligninolytic G. marginata species. The same analysis carried out on the three sets of A. phalloides showed no statistical difference reporting that the amanitin distribution in the ectomycorrhizal species was not influenced by the Origin (site), as seen in Fig. 3.

**Discussion**

*Occurrence of G. marginata white-rot fungi.*—G. marginata was reported as wood-rotting fungi occurring predominantly on conifers (Bon 1990, 1992; Courtecuisse and Duhem 2000; Lincoff 1998; Singer 1986). In general, white-rot fungi predominantly de-
grade hardwood. However some of them having a broad host range attack both softwood and hardwood (Blanchette 1991, Rypáček 1977). It is well known that certain ligninolytic fungi, either white-rot or brown-rot as Serpula lacrymans (Wulfen) J. Schröt., Coniophora puteana (Schumach.) P. Karst. and Gloeophyllum trabeum (Pers.) Murrill, grow on coniferous as well as on deciduous trees (Zaremski 1996).

Our investigated specimens were collected on hosts belonging to either hardwoods (Fagus) or softwoods (Picea); so G. marginata can be designated as both a hard- and softwood degrader. European and North American Galerina material known as amatoxin-containing species analyzed for DNA studies also was taken from hosts belonging to Gymnosperms and Angiosperms (Gulden et al 2001).

Different characteristics distinguish softwoods and hardwoods: (i) microstructure of wood (Montgomery 1982); (ii) structural elements building lignin (Blanchette 1991, 2000; Tuor et al 1995); (iii) sugars constituting hemicelluloses (Levy 1982, Montgomery 1982): it should be noted that the major carbohydrates—D-mannose (hardwoods) and D-xylose (softwoods)—provide the energy source for the decay process (Blanchette 1991, Tuor et al 1995). Moreover, the lowest pH values of natural substrates are reported for softwoods (Scheikl 1994). Last, it is well known that the chemical composition of coniferous wood differs also from that of deciduous wood in its content of volatile monoterpenes (Hintikka 1982). Given the variations in microstructure and chemical components between softwoods and hardwoods, differences in physiology and biochemistry could be expected for wild mushrooms growing on either one or the other substrate.

**Amatoxin content of G. marginata and A. phalloides.**—The three amanitins (α-Ama, β-Ama, γ-Ama) were identified in each analyzed G. marginata basidiome from hardwoods (Gal L1: n = 10; Gal L2: n = 6) and softwoods (Gal FC: n = 11). Differences in chemical composition of two substrates could explain the significant variation (FNS < 10⁻⁴) in the mean amatoxin contents between Gal L1 (243.61 ± 16.54 μg.g⁻¹ F.W.), collected on a decayed beech and Gal FC (96.88 ± 12.82 μg.g⁻¹ F.W.) taken from rotten spruce. On the other hand, variation in amatoxin contents between Gal L1 and Gal L2 could be due, at least in part, to the mean weight of fresh basidiomes forming the both sets, 0.92 ± 0.11 g and 2.90 ± 0.66 g, respectively. Approximately 56% of the total
nitrogen in wood is bound to lignin: lignin degradation with simultaneous nitrogen utilization would contribute to mycelial growth (Dill et al 1984). As reported by Muraoka et al (2000), changes in timing between biomass and intracellular α-Ama production are observed for fermentation of G. fasciculata strain GF-060. Furthermore, other parameters such as extrinsic factors (environmental conditions) and intrinsic factors (genetic properties) could contribute to the significant difference between amatoxin contents for Gal L1 and either Gal FC or Gal L2.

Amatoxin content was different between the three A. phalloides sets (Aph A, Aph L3, Aph FC; TABLE I) and as high as those reported in literature (Enjalbert et al 1993, Stijve and Seeger 1979, Tyler et al 1966). Variations in the amatinin mean amounts also were due to different factors. The mean weight of fresh basidiomes of 20.81 ± 5.91 g, 32.60 ± 12.74 g and 18.42 ± 4.78 g, respectively, probably should be involved as suggested above for G. marginata. Moreover, the environment (seasonal conditions of growth, moisture content) and diversity of genetic inheritance play a determinant role on amatoxin content for A. phalloides from different collections (Enjalbert et al 1993, Stijve and Seeger 1979, Tyler et al 1966).

It is worthy of note that certain G. marginata specimens were more toxic than some A. phalloides basidiomes. European species considered as the richest in amatinins (TABLE I, FIG. 1). The amatoxin content in Gal L1 (243.61 ± 16.54 μg.g⁻¹ F.W.) was as high as that in Aph FC (247.87 ± 14.67 μg.g⁻¹ F.W.) and more elevated than that in Aph L3 (172.07 ± 56.32 μg.g⁻¹ F.W.). Given the lethal dose of amatoxins estimated to be about 0.1 mg.kg⁻¹ human body weight, or even lower (Wieland 1986), the ingestion of 10 G. marginata basidiomes containing about 250 μg of amatinins per g of fresh tissue should poison a child weighing approximately 20 kg. G. marginata is a toadstool easily confused with such edible mushrooms as Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sm. and Armillaria mellea (Vahl) P. Kumm. In a 20-year retrospective recording clinical data from 2108 amatoxin exposures in North America and Europe, few cases due to ingestion of Galerinas were listed. Scarcity of these wood-rotting fungi often unobserved by collectors explains the infrequency of poisoning. Moreover, it has been shown that 21% of amatoxin poisonings are caused by unidentified species (Enjalbert et al 2002).

As regards comparison of the amatoxin contents between G. marginata and other species of Galerina genus, our findings are in agreement with the Czech survey of amatinin-containing mushrooms, recording that G. sulcipes is more toxic than A. phalloides (Klan 1993). Furthermore, according to Muraoka et al (2000), the amatinin mean amount in the strains of both Japanese G. helvoticeps (n = 3; 207.7 ± 130.11 μg.g⁻¹ F.W., from 47.81 to 556.22 μg.g⁻¹) and G. fasciculata (n = 18; 245.96 ± 20.58 μg.g⁻¹ F.W., from 222.33 to 259.96 μg.g⁻¹) could be as high as that from A. phalloides. Numerous fatalities consequently are caused by G. fasciculata in Japan (Ishihara and Yamaura 1992).

**Toxin distribution of G. marginata and A. phalloides.**—Regarding the toxin profile, any acidic and neutral phallootoxins were not detected in the 27 French G. marginata specimens using HPLC analyses. Our results confirmed that these bicyclic heptapeptide derivatives related to amatoxins are not present in the Galerinas (Wieland 1983, Wieland 1986, Wieland and Falustich 1983). On the other hand, the γ-Ama previously HPLC detected in only one specimen (Enjalbert et al 1992) was identified, associated with β-Ama and α-Ama, in each G. marginata basidiomes (n = 27) from either hardwoods or softwoods. Significant variations (P< 10⁻⁴) between the β-Ama, α-Ama and γ-Ama concentrations as well as the values of the β/(α + γ) ratio observed for the three sets underlined the effect of Origin factor (site/host) on the amatinin distribution in G. marginata. Predominance of β-Ama (acid amatinin) distinguished Gal L1 growing on hardwoods whereas that of α-Ama + γ-Ama (neutral amatinins) individualized Gal FC collected from the most acidic substrate (softwoods; FIG. 2b). Relationship between the distribution of either acidic or neutral toxins and pH of the collection site is observed for A. phalloides. Basidiomes of the ectomycorrhizal species collected from acidic soil (siliceous soil and clay and chert; pH = 4.5-5) are distinguishable by the predominance of phalloidin, a neutral phallootoxin (Enjalbert et al 1996, 1999). Difference between amatinin profiles for the two sets (Gal L1 and Gal L2) growing on Fagus hosts could not be due to the chemical composition of natural substrate but to the different components from decayed wood. Indeed, the decomposition process leads to various chemical patterns of substrate (Barras et al 1992). White-rot Basidiomycota have the capacity to degrade the three wood polymers (lignin, cellulose, hemicellulose) at different rates and extent. Within that group, fungi are either simultaneous degraders of lignin along with wood poly saccharides or selective lignin degraders or may show both types of decay (Blanchette 1991, Eriksson et al 1996, Solov’ev et al 1985). Many enzyme systems leading to oxidative and reductive conversions are involved in lignin biodegradation. Enzyme multiplicity can explain the heterogeneity of the substrate at suc-
cessive stages of wood decay (Tuor et al 1995, Watanabe and Kuwahara 2000). Advances on the molecular genetics of ligninolytic fungi have shown that different genes encoded enzymatic systems responsible for lignin degradation (Blanchette 1991, Cullen 1997, Varela et al 2000). Furthermore, environmental conditions, such as temperature, humidity, microclimate, low nitrogen content, elevated carbon dioxide and pH values, must be crucial in governing the selectivity of fungal biodegradation of wood components. Such factors giving rise to series of ecological niches filled by a range of micro-organisms improve carbohydrate degradation (Blanchette 2000, Levy 1982). Fungal lignin degradation results in the formation of low molecular weight compounds, mostly aromatic carboxylic acids (oxalic, citric, formic and butyric acids) metabolized by bacteria (Tuor et al 1995). Changes in glucide and organic acid contents of substrate affect the production and regulation of secondary metabolites (Frank 1998) and therefore could play a role in amanitin profile of G. marginata. Experiments on the three strains of G. fasciculata and 18 strains of G. helvus have shown that fermentation conditions are involved in the distribution of acidic and neutral amanitins. In liquid-cultured mycelia, α-Ama and small γ-Ama amounts are accumulated whereas β-Ama is only detectable; in solid-cultured mycelia, α-Ama and β-Ama are the main toxins and only trace of γ-Ama is detected (Muraoka and Shinozawa 1999, 2000). Significant difference in amanitin distribution between the three sets of G. marginata due to components of substrate is in agreement with the findings for the Japanese Galerina species considered generally as wood-rotting fungi. Unlike the DNA studies performed on the G. marginata complex showing the absence of correlation between the substrate and the distribution of genetic types (Gulden et al 2001), our findings pointed out the relationship between the substrate and distribution of amanitins being nitrogen-containing toxins. In the same way, changes in the chemical components of edible Pleurotus sp. mainly the protein content are correlated with the substrate composition (Sturion and Oetterer 1995).

Concerning the toxin distribution for the three A. phalloides sets (Aph A, Aph L3 and Aph FC), our HPLC results showed that phallootoxins and amatoxins were detected in all specimens (n = 17). The occurrence of both groups of toxins for this species is consistent with literature (Wieland 1983, Wieland 1986, Wieland and Faulstich 1983). Amounts of acidic and neutral phallootoxins assayed in many French A. phalloides specimens are indicated (Enjalbert et al 1989, 1993). Unlike American materials of A. verna Fr. (Benedict et al 1970, Tyler et al 1966), no amanitin-free A. phalloides specimen from either our collections or various French locations was found (Enjalbert et al 1992, 1996, 1999). The major amanitins were always β-Ama and α-Ama; predominance of acidic over the neutral toxins seems to be the rule as previously reported (Enjalbert et al 1993, Stijve and Seeger 1979, Tyler et al 1966, Wieland 1986). Studies concerning this ectomycorrhizal species revealed that associated woody plants do not appear to have an effect on the toxin distribution and that the geological and pedological characteristics of the collection sites have a greater influence on phallootoxin profile than amanitin distribution (Enjalbert et al 1996, 1999). No significant difference was found between the amanitin distributions for the A. phalloides sets taken from the three sites having nearly the same type of soil.

Overall, our findings underlined intraspecific variability in the amanitin profile for the wood-rotting G. marginata resulting from the host/fungus combination. Relationship between the chemical composition of the substrate and the expression of toxic secondary metabolites depends on many intrinsic and extrinsic factors. Structural and chemical differences in wooden substrate (hardwoods and softwoods) should constitute only a part of the parameters determining the nitrogen-containing toxin pattern. Decaying process leading to changes in nutritional substrate certainly linked on host/fungus gene activity also could be considered as crucial factors in the acidic and neutral amanitin production. An extensive research should be done to validate these hypotheses. First, analyses of additional G. marginata specimens from various hosts and sites over the years should be carried out to verify the variability in the amanitin distribution. Second, a thorough understanding of enzyme system from the white-rot fungi should lead to identify the nutritional components from the substrate participating in the regulation of toxic secondary metabolites for G. marginata.

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LITERATURE CITED


