ODOURS AND PIGMENTS IN STINKHORNS

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Summary

A review is given of the literature dealing with attempts to characterise the vile odours released by the big Stinkhorn, Phallus impudicus *Pers. According to recent research, major constituents of the volatiles produced by this fungus are dimethyldisulfide and dimethyltrisulfide, together with linalool, trans-ocimene, phenylacetaldehyde and acetic acid. Several of these compounds were also found in the headspace of a freshly emerged* Clathrus ruber *Mich. ex Pers. These volatiles are undoubtedly emitted to attract flies (which will disperse the spores), a function that may also be served by the bright red colour of the receptaculum of many Phallales.* Phallus impudicus, *which lacks such red pigments, produces a more penetrant odour than the pigmented* Clathrus ruber *and* Anthurus archeri. *Pigments responsible for the orange to red colours in* Mutinus caninus, Phallus rugulosus *and* Clathrus ruber *have been identified as carotenes, principally lycopene and beta carotene.*

Stinkhorns belong to the family Phallaceae (Order Phallales) of which the ripe hymenium mass in fully developed carpophores emits a more or less pronounced carrion-like odour attracting dung flies and scarab beetles. In the world of plants the colourful flowers of the family Araceae have similar characteristics ; insects are lured by the odour from a great distance, and this attraction is enhanced by the bright red or purple colour of the flowers. Among the Phallales the receptacles of many species *e.g. Mutinus ravenellii, Clathrus ruber* and *Aseroe rubra* are also conspicuous because of their red colour. The insects visiting the flowers disperse the pollen, whereas those attracted by Stinkhorns devour the mucous substance on the gleba containing the spores, which are subsequently dispersed via the excrements (Fulton 1889). This means of propagating the species shows that Stinkhorns are specialised organisms, high on the evolutionary ladder. As already pointed out in an earlier paper (Stijve 1994), these curious fungi are anything but endangered species.

Phallales exist in many forms, and it may well be asked if all these varieties are really necessary for efficient spore dispersal. Some forms, such as that of the Big Stinkhorn with its significant Latin name (*Phallus impudicus*) are so intriguing that they were the subject of extensive monographs long before such attention was paid to other and more 'useful' mushroom species. It is beyond the scope of this article to give an historical review of observations and treatises dealing with Stinkhorns. The reader may be interested to know that the oldest treatises (in Latin) were published as early as the 16th century (Hadrianus 1601). Lütjeharms (1931) and Ramsbottom (1953) provided very good reviews on this subject. The latter author devoted chapter 16 of his now classic book to *Stinkhorns and other Phalloids*. The present paper is an attempt to bring together all that is known at present about odours and pigments produced by Stinkhorns.

Odorous compounds

The first scientist who subjected *Phallus impudicus* to what may be called a chemical investigation was Jacobus Christian Schaeffer (1760). His most readable and abundantly illustrated monograph can still be found in specialised antiquarian bookshops, but its price fluctuates between 300 and 600 US dollars. Schaeffer was not only a well-known natural scientist, but also a Protestant clergyman. Because of this last quality, he avoided using the word 'phallus' in the description of his subject and declared: 'I won't mention the comparison made by some authors with a certain part of the human body'. This prudishness is easily forgivable in Schaeffer, because he was not only a keen observer, but also a very good scientific writer. Indeed, anybody studying Stinkhorns should read very carefully the 36 pages of Schaeffer's treatise; the detail and quality of his observations is remarkable! To give just one example: my own comparative study of the essential chemical elements in the different parts of the related *Clathrus ruber* suggested that the gelatinous layer of the egg could serve as a mineral reservoir (placenta) for the budding receptacle (Stijve 1994). This idea was already anticipated in 1760, as demonstrated by paragraph 83 of the said monograph: 'This fungal egg closely resembles that of an animal. It has between its two skins a special tissue which represents the placenta. The outer skin which has not seldom some folds resembles the chorion, whereas the inner smooth skin may be taken for the amnion. Between the two is the moist slimy substance just like the liquor amni of the human embryon'.

At the state of analytical chemistry in Schaeffer's time, we cannot expect much of his investigations regarding the composition of egg and gleba. Still, his experiments with the egg's slime *e.g.* solubility in water, reconstitution and suitability to paste paper, makes him compare it with vegetable gums such as gum arabic and tragacanth. Two centuries later, it is demonstrated that the egg's slime belongs—just as the said gums—to the class of polysaccharides (Bindler 1967). The clergyman also notices the various odours of the Stinkhorn: the intact egg smells of radish ('Meerrettich') just as an aqueous extract of the eggs remainder after complete eclosion. However, Schaeffer observes that this smell has nothing in common with the stench of the adult Stinkhorn, and he concludes that 'our knowledge of (such) fungi is very incomplete indeed'. His theory about the generation of the foul smell (upon liquefaction of the gleba) is on the scientific level of his time: 'upon exposure to the air, the greenish substance on the cap undergoes a fermentation, which causes the penetrant odour and turns it soon into a dark liquid...'.

Only half a century later the French chemist Braconnot (cited by Freund 1967) analysed the Stinkhorn again. The substances found as 'mucus, matière animale' and 'fongin très animalisé' are not readily identifiable today, but Braconnot also reported the presence of potassium—and ammonium acetate in the egg. In addition, he found 'le sucre des champignons', *i.e.* the sugar alcohol mannitol which occurs in many higher fungi.

We have to wait until the second half of the 20th century before significant progress is achieved in elucidating the chemistry of the Stinkhorn's smell. Indeed, by the 1960s, the techniques for isolating volatile compounds, such as low temperature vacuum distillation, had been much improved. It is thus possible to trap the odorous constituents directly in the headspace over the Stinkhorn in a chemical reagent, and to analyse the derivatives formed. The German, Bernard Freund (1967), applied these techniques when he made the odorous compounds of *Phallus impudicus* the subject of his doctoral thesis at the Marburg Institute for Pharmacy and Food Chemistry. The work involved should not be underestimated. He had to start by gathering more or less ripe eggs in the woods in order to let them eclore in the laboratory until liquefaction of the gleba and formation of the volatiles. The institute must have been a smelly place then! Subsequently, the glebas were deep-frozen and kept at –20°C. It is worth noting that even at this low temperature the material still had an obnoxious smell, indicating that at least part of the odorous compounds were still volatile. After having worked up several kg of Stinkhorns and analysing various fractions, Freund reported the following results:

ISOLATED VOLATILE COMPOUND	TYPE OF ODOUR
hydrogen sulphide	of foul eggs
methylmercaptan	of rotten cabbage
phenylacetaldehyde	of fresh grass
phenylacetic acid	repugnantly sweetish
alpha-phenylcrotonaldehyde	of freshly worked soil
acetaldehyde	pungent
formaldehyde	pungent
propionic acid	pungent
acetic acid	sourish
dihydrochalcone	fresh « greenish » note

The odour of the gleba is not the same in all stages of development. The two compounds on top of the list are most volatile and probably responsible for the carrion-like note. When the ripe egg opens and the Stinkhorn comes out, the gleba is dull green and solid. The smell is still weak then, somewhat radish-like, but the real stench is only produced upon liquefaction and darkening of the green mass, and the sulphur compounds thus formed attract the flies from near and far. The more sweetish and lingering odour of the gleba in a later stage is typical for the presence of the less volatile compounds phenylacetaldehyde, phenylacetic acid and dihydrochalcone. When the spore mass turns liquid, which is undoubtedly an enzymatic process, many chemical reactions occur; some of the odorous compounds are formed from others, *e.g.* the acids by oxidation of the corresponding aldehydes. The gleba is indeed a chemical factory. Surprisingly, the German scientist, in spite of his intensive investigations, disregards the formation mechanism of the volatiles. Even the observation that the smell of *P. impudicus* differs with the state of the gleba does not produce any comment on the various odour components. In Freund's thesis (1967) there is no mention of the fact that during and after eclosion of the egg, several grams of chemical compounds are produced. He finds more or less by accident much free glucose in the liquefied spore mass, but fails to grasp the significance of this important observation.

Upon analysis of comparable material, Stijve (1965, 1966) found about as much bound glucose in the embryonal gleba isolated from the egg as free glucose in the liquid spore mass of the adult Stinkhorn. He supposed therefore that the odour was generated by a gradual enzymatic decomposition of glycosidic compounds, consisting of glucose and, at that time, still unidentified volatile chemicals. This theory is supported by the observation that Stinkhorns found in cold (0–5°C) weather do not smell. Clearly, enzymatic reactions require an adequate temperature range that usually lies well beyond 15°C. Klaassen (1964) observed that, after drying cap and gleba, the odour could be regenerated by simply moistening with water, which can be explained by reactivation of the enzymes. Regrettably, up to now nobody has tried to isolate these hypothetical enzymes.

In the 1990s the 'flavour' of the Stinkhorn was re-examined. Three Swedish scientists (Borg-Karlson *et al.* 1994) were intrigued by the fact that the stench of the Voodoo Lily, *Sauromatum guttatum*, closely resembled that emitted by the Stinkhorn. In addition, the carrion flies attracted by both organisms belonged to the same genera. These observations prompted a comparative analysis of the volatile compounds produced by *S. guttatum* and *P. impudicus*. For this purpose, the volatiles were trapped on a small amount of the synthetic polymer Porapak Q, which was subsequently extracted with pentane and diethylether. The extracts thus obtained were analysed by gas chromatography using mass spectrometric detection. A simplified overview of the results is given in Table I. Both *S. guttatum* and *P. impudicus* possess as predominant carrion-like odorous compounds the methylated sulphides among which dimethyltrisulphide is quantitatively the most important. These sulphur compounds are rather volatile and the absence of (even a small amount) dimethylsulphide in *P. impudicus* could probably be explained by losses during extraction and concentration. Both organisms also produce low concentrations of 3-carene and 2-phenylethanol. However, there are important differences: among the volatiles of *S. guttatum* we find indole and skatole which have a pronounced fecal smell. In addition, there is a series of terpenes which are also lacking in the Stinkhorn's 'flavour' profile. On the other hand, trans-ocimene, one of the most important volatiles of *P. impudicus* is not produced by the Voodoo Lily, although it does have a small amount of the cis-isomer.

Their results obtained for the Stinkhorn are quite different from those reported by Freund (1967). As sulphur compounds, this author found only hydrogen sulphide and methylmercaptan, which were not detected by the Swedish team. Freund did not find any linalool or trans-ocimene either, but his classic derivatisation techniques were not suitable for their isolation. On the other hand, he reported acetaldehyde, formaldehyde and phenylacetic acid which were not observed by the Swedes. Although the analyses performed by Borg-Karlson *et al.* (1994) are probably more reliable because of their superior technique, this does not necessarily mean that they have found all compounds present. Freund's method for trapping acetaldehyde and formaldehyde in a derivatising solution is convincing enough, and it is not unthinkable that the Swedes have missed these volatiles. The presence of phenylacetic acid in Freund's extracts is probably due to oxidation of phenylacetaldehyde during the lengthy isolation procedure.

How the odorous compounds are formed has not yet been investigated, but Borg-Karlson *et al.* (1994) draw attention to the fact that both the gleba of the Stinkhorn and the brown-purple top of the flower's spadix become measurably warmer during release of the volatiles, which is probably due to an enzymatic conversion of precursors. The methylated sulphides are probably attractive to flesh flies, because these compounds are also generated during the putrefaction of animal proteins. Tentative experiments demonstrated that flies belonging to the genera *Calliphora*, *Lucilia* and *Sarcophaga* (Sarcophagaceae) are indeed attracted by dimethyl sulphide (Borg-Karlson 1993).

There is no published research yet about the odorous compounds produced by other Phallaceae. During a series of eclosion experiments with *Clathrus ruber* the present author noted that the glass beakers placed over the emerging carpophores retained the stench for a long time after the experiment. This may be explained by tight adsorption of the condensed volatiles to the glass surface. Since this phenomenon offered the possibility of chemical analysis, a 'ripe' egg of *C. ruber* was left under a beaker until fully eclosed with apparent complete liquefaction of the gleba on the inside of the receptacle. Subsequently, the beaker was removed and immediately rinsed with 1 ml of pentane–diethylether 1:1 v/v. Capillary gas chromatography on a DB–Wax column of 30 m × 0.25 mm coupled to a mass spectrometer afforded identification of dimethyl disulphide, dimethyl trisulphide, trans–ocimene, linalool and acetic acid. Although the isolation technique is rather primitive and therefore not quantitative, the results were remarkable since the amounts of sulphur compounds were significantly lower than those of the other odorous components. This could explain why the smell of *C. ruber* is generally more faint than that of *P. impudicus*. The latter species is always found by tracking the odour, whereas this is hardly the case for *C. ruber*. It is indeed possible that a colony of these latticed Stinkhorns remains undetected if one is not specially looking for them. Clathri reported by the author in parks and gardens in France, Spain and Switzerland were rather found because of their conspicuous red colour than for their odour (Stijve 1994).

Table 1 : Volatile constituents isolated from Sauromatum guttatum and Phallus impudicus. + = < 2% of the total volatiles, ++ = 2 – 20 %, +++ = > 20 %

	S. guttatum	*P. impudicus*
Sulphur compounds		
Dimethyl sulphide	+	
Dimethyl disulphide	++	++
Dimethyl trisulphide+++	+++	+++
Dimethyl tetrasulphide	+	
Nitrogen compounds		
Indole	+	
Skatole	+	
Terpenoids		
6-methyl-5-hepten-2-one		+
3-carene	+	+
myrcene		+
cis-ocimene	+	
trans-ocimene		+++
alpha-pinene		+
alpha-terpinene	+	
geraniol	+	
linalool		++
alpha-farnesene		+
alpha-caryophyllene		+
beta-caryophyllene	+	
Aromatic compounds		
anisole	+	
benzyl alcohol		+
acetophenone	+	
phenylacetaldehyde		+++
2-phenylethanol	+	++
Aliphatic compounds		
acetic acid		+

Pigments

As mentioned earlier, colours, especially reddish ones, also appear to play a role in attracting carrion flies. It was therefore considered interesting to investigate the pigments responsible for the red colour of representatives of *Mutinus* and *Clathrus*. About 30 years ago (Stijve unpublished) the author immersed the reddish coloured top of a *Mutinus caninus* specimen in alcohol, which readily dissolved the pigment in a few days. The resulting yellow extract was found to have absorption maxima at 445, 470 and 500 nm, which is characteristic for carotenoids, the group of pigments giving the beautiful orange-red colour to the common carrot. Such pigments are also encountered in other mushrooms such as chanterelles and cup fungi, *e.g. Aleuria aurantia* and *Caloscypha fulgens*. Their presence can be used as a taxonomic characteristic. There is a good review article on this subject by Valadon (1976). However, the presence of carotenoids in macromycetes should not be assumed to be generalised. The Fly Agaric (*Amanita muscaria*) and the colourful representatives of the genera *Dermocybe* and *Hygrocybe* contain quite different pigments. About 14 years after my analysis of the *Mutinus caninus*' pigments, Harashima (1978) analysed *Phallus regulosus* (Fisch.) O. Kuntze, a species resembling a big *M. caninus*, but having a removable and conical pileus. The long stipe is yellow to orange red (Imazeki & Hondo 1981). This Stinkhorn is probably identical with *P. rubicundus* (Bosc.) Fr., a rather common species in the South of the USA. The Japanese investigator managed to isolate two crystalline pigments from 13 specimens—totalling 67 g—which he identified as beta-carotene and lycopene, *i.e.* the substances that give their colour to respectively carrots and tomatoes! Five years earlier Fiasson & Petersen (1973) had already detected the same pigments in the red receptacle of *Clathrus ruber*. Only in the 1990s, when it had become possible to determine those pigments easily and rapidly by means of liquid chromatography, the analysis of the said Stinkhorn was repeated at the Nestlé Research Centre (Stijve & Tagliaferri 1994). Fragments of 50–100 mg weight were cut from the emerging receptacle and analysed individually. The pigments present were indeed mainly lycopene and carotene, although some neurosporene was also observed. The quantities measured varied of course with the intensity of the receptacle's colour, which can be pale red, orange or even deep red.

Typical concentrations for an 'average' red colour were 1% lycopene and 0.07% beta carotene. It is clear that carrion flies are mainly attracted by the cadaverous odour of the *Clathrus*. The red colour may help at a short distance, but is alone not attractive as demonstrated by the fact that tomatoes and carrots are not visited by these flies!

It would undoubtedly be interesting to further investigate the odorous compounds and their generation during the liquefaction of the gleba. The differences between the reported results are great, but this can be largely attributed to the analytical methods used. With state-of-the-art instrumentation it should now be possible to follow the formation of the odorous compounds by periodically sampling the headspace over the pileus and to analyse it by gas chromatography and mass spectrometry. In addition, investigation of the enzymatic mobilisation of the chemical compounds present in the budding receptacle is also highly desirable. The chemistry of the Stinkhorns is still far from being completely understood.

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