Volatile Compounds Released during Dry Afterripening of Tabasco Pepper Seeds†

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Afterripening of tabasco pepper (*Capsicum* sp.) seeds (air-dried to 8.1% moisture) for 21 days increased germination from 56% to 81%. However, sealing the tabasco seeds in a glass container during the same period decreased germination to 35%. Enclosure of afterripened seeds in a chamber for a second 21-day period did not affect germination, which remained at 81%. The volatile compounds released during the afterripening of tabasco seeds were identified and compared with those from nonafterripened counterparts by dynamic headspace sampling, capillary glass chromatography, and mass spectrometry. Over 80 peaks were detected, and 65 were identified, including alcohols, aldehydes, ketones, esters, hydrocarbons, and furans.

INTRODUCTION

Seeds of some peppers (*Capsicum* sp.) require a postharvest afterripening treatment of dry storage at room temperature to overcome primary dormancy. Tabasco pepper (*C. frutescens*) afterripening requirements are generally satisfied by a period of dry storage in the dark at 25 °C for 21 days (Edwards and Sundstrom, 1987; Randle and Honma, 1981). Dry afterripening treatments have also been shown to alleviate or reduce dormancy in a number of other species [summarized in Bewley and Black (1983)]. While no mechanism for the loss of dormancy during dry afterripening has been discerned, evaporation or breakdown of germination inhibitors may occur (Wareing and Foda, 1957; Berrie et al., 1975; 1979).

Wesson and Wareing (1969) and Holm (1972) showed that seeds of a number of weed species (*Spergula arvensis*, *Plantago lanceolata*, *Stellaria media*, *Ipmoea purpurea*, *Abutilon theophrasti*, and *Brassica kaber*) failed to germinate when buried in moist soil under laboratory conditions. Artificial aeration partially removed the inhibitory effect and indicated the possible presence of a gaseous inhibitor in the soil.

Endogenous short-chain volatile fatty acids (C_6–C_{10}) have been implicated in maintaining the dormancy of oats (Berrie et al., 1975, 1979) and rice (Majumder et al., 1989). These studies suggest that successful afterripening was related to loss of endogenous volatile fatty acids that inhibit germination. However, Metzger and Sebesta (1982) observed membrane dysfunction and viability loss as the reasons for decreased germination when C_9 acid was applied to embryos of *Avena fatus*; high endogenous C_7–C_9 levels were found in both dormant and afterripened seeds. Furthermore, C_1–C_6 acids applied to dormant dehulled red rice grains actually break dormancy (Cohn et al., 1987). In addition, C_7 and C_8 acids also break dormancy in red rice (Cohn, unpublished results).

Volatile compounds inhibit the germination of other seeds including onion (*Allium cepa*), carrot (*Daucus carota*), *Amaranthus* sp., tomato (*Lycopersicon esculatum*) (Bradow and Connick, 1990, and references cited therein), lettuce (Reynolds, 1989), soybean (Gardner et al., 1990), and species of weed seed (Leather and French, 1990; French and Leather, 1979). Compounds identified as inhibiting germination include allelochemicals within the soil as well as compounds produced by the seeds themselves. Therefore, this study was conducted to analyze the volatiles produced during dry afterripening of tabasco seeds to determine whether loss of such volatiles was responsible for breaking dormancy in these seeds.

Recently, dynamic headspace sampling (DHS) has been used to collect volatiles from *Amaranthus* sp. (Connick et al., 1989), floured chickpea seed (*Cicer arietinum* L.) (Rembold et al., 1989), rice foliage (*Oryza sativa*) (Hernandez et al., 1989), and leaves of *Ceratiola ericoides* (Jordan et al., 1992) for analysis by GC–MS. DHS can be used to trap volatiles without extensive sample preparation and does not require sample heating or solvent evaporation, thus minimizing the formation of artifacts associated with conventional methods of steam distillation or organic solvent extraction. Enhanced separation of compounds can be achieved when flash thermal desorption and cryogenic focusing are used to introduce the volatiles to the GC column (Vejaphan et al., 1988).

The objectives of this study were (1) to evaluate the effects of afterripening on germination potential and viability of tabasco pepper seeds and (2) to analyze the volatiles produced by both afterripened and nonafterripened tabasco seeds by DHS–GC–MS for identification of potential germination-inhibiting compounds.

MATERIALS AND METHODS

Plant Material. McIlhenny Select tabasco peppers (*C. frutescens* L.) were grown at Burden Experimental Gardens of Louisiana State University Agricultural Center in 1988. Red-

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ripe fruits were harvested 150 days postanthesis, and the seeds were extracted as described by Edwards and Sundstrom (1987). Seeds were then air-dried to a moisture content of 8.1% and divided into two groups. One group was separated into 55 ± 2 g lots, sealed in glass jars, and stored at −5 °C until analysis of volatile components. The second group was spread out onto paper towels and allowed to afterripen in a dark incubator at 25 °C for 21 days. Following afterripening treatment, seeds were divided into lots, sealed in jars, and stored at 2 °C.

Germination tests were performed on eight replicates of 50 seeds over 14 days at 25 °C as described (Edwards and Sundstrom, 1987). At the conclusion of each germination test, ungerminated seeds were bisected and tested for viability using 1% tetrazolium solution (Grabe, 1970).

Collection of Volatiles. Nonafterripened tabasco seeds were placed in a glass chamber (23 cm height × 5.5 cm i.d.) and ultra-high-purity (UHP) He gas (99.999%, Linde Division, Union Carbide Corp., Danbury, CT) was purged through the chamber at 40 mL/min for 5 min to remove most of the O_2 in the headspace above the seeds. The chamber was sealed and placed in the dark at 25 °C for 21 days to allow for the accumulation of volatiles in the chamber headspace. After 21 days, the volatiles were purged onto a trap (30.48 cm length × 2.5 mm i.d. stainless steel column) packed with Tenax TA (2,6-diphenyl-*p*-phenylene oxide polymer, 0.24 g, 60–80 mesh, Chrompack, Raritan, NJ) as described (Hernandez et al., 1989) except that a UHP He flow rate of 40 mL/min for 18 h was employed. The Tenax TA trap was initially conditioned at 340 °C for 2 h with a He flow of 30 mL/min. Before sample purging and trapping, the Tenax TA trap was baked at 225 °C for 10 min to ensure no carry-over of compounds from previous analyses. The same procedure was followed for collection of volatiles from afterripened tabasco seeds. All volatile trapping experiments were duplicated.

Gas Chromatography–Mass Spectrometry. GC–MS was performed as described (Hernandez et al., 1989) with the following modifications: cryofocusing was achieved in liquid N_2 rather than in an EtOH–dry ice bath; the oven temperature was programmed from 40 to 175 °C at a rate of 2 °C/min and then to 190 °C at a rate of 5 °C/min with the hold times for the three temperatures set at 5, 20, and 15 min, respectively; the MS electron multiplier voltage was set at 2000 V, and there was no solvent delay for the mass selective detector except for alkane-fortified samples, when the solvent delay was 5 min.

Identification of Volatile Compounds. Five microliters of *n*-alkanes (C_8–C_{21}) was dissolved in 5 mL of hexane. Three microliters of this standard mixture was applied to the inside wall of the chamber (no seeds enclosed), and the chamber was purged for 2 h with UHP He at 40 mL/h for the hydrocarbons to accumulate on the Tenax trap. For the final 2 h of headspace purging with UHP He, a 3-μL spike of standard alkane mixture was also applied to the chamber containing tabasco seeds after the 21-day period of volatile accumulation. Subsequent GC–MS analysis was as described above. Chromatographic retention indices were calculated according to the method of Van den Dool and Kratz (1963). Identification of volatile compounds was based on comparison with standards analyzed under identical GC–MS conditions. The unknown mass spectra were computer-matched with the reference mass spectra of the *Wiley/NBS Data Base* (1988) installed on a Hewlett-Packard MSD Chem Station.

RESULTS AND DISCUSSION

Freshly harvested tabasco seeds air-dried at 25 °C to 8.1% moisture (fwb) germinated to 56 ± 7%. Germination potential dropped to 34% when these seeds were enclosed in the volatile collection chamber for a 21-day period. Viability of the seed lot was 81 ± 9% both before and after enclosure. A subsequent attempt to reafterripen the enclosed seeds did not increase germination percentage or affect viability. Conversely, when freshly harvested seeds were allowed to afterripen for 21 days at 25 °C in an open container before enclosure, germinability increased from 56% to 81%. Enclosure of these afterripened seeds in the volatile chamber for a subsequent 21-day period did not affect germination, which remained at 81%. Viability remained at 95 ± 5%.

GC profiles of headspace volatiles collected over a 21-day period from freshly harvested and from previously afterripened tabasco seeds are shown in Figures 1 and 2, respectively. Approximately 80 peaks were detected in the two profiles, and structures have been assigned to 65 of these compounds on the basis of retention indices and

Table I. Volatiles Released by Tabasco Seeds during or after a Period of Dry Afterripening (AR)

compound	after AR[a]	during AR[b]	peak no.[c]	ref[d]
pentane	+		1	3
hexane	+	+	2	3
heptane	+	+	3	5, 6
octane	+	+	4	3, 5
nonane	+		7	3, 5
tetradecane	+	+		
pentadecane		+		
hexane		+		
butanal	+			11
pentanal	+	+	9	5, 3, 11
hexanal	+	+	11	6, 7, 8, 11
octanal	+		16	7, 11
nonanal	+		18	3, 7
decanal	+			
2-furfural	+	+	21	
benzaldehyde	+	+	23	7, 11
2-heptenal	+			
2-octenal	+			
2-nonenal		+		8
acetone	+		5	9, 11
2-butanone	+			5, 6, 11
3-methyl-2-butanone		+		2, 5, 6, 7
2-methyl-3-pentanone		+		
3-methyl-2-pentanone	+			
2-heptanone	+		12	2, 5, 6, 7, 10
3,4-dimethyl-3-penten-2-one		+		
5-methyl-4-hepten-3-one	+			
6-methyl-5-hepten-2-one	+	+		7, 10
2,2,6-trimethylcyclohexanone		+		
3,5,5-trimethyl-2-cyclohexen-1-one		+		
3-octen-2-one	+			
4-methyl-3-penten-2-one	+			11
2-butoxyethanol	+			
4-methyl-1-pentanol		+		
1-pentanol	+	+	15	1, 3, 4, 12
2-pentanol		+		5, 11
1-hexanol	+	+	17	1, 4, 11
1-octen-3-ol	+		19	7
2-ethyl-1-hexanol	+	+	22	
acetic acid	+	+	20	4
methyl acetate		+	6	3, 5, 6
ethyl acetate	+			3, 4, 5
propyl acetate		+		12
hexyl acetate	+	+		
methyl butanoate		+		12
methyl 2-methylpropanoate		+		
butyl 2-methylpropanoate		+		
butyl 2-methylbutanoate		+		
hexyl 2-methylbutanoate	+	+		
2-ethylfuran	+			3, 5
2-butylfuran	+			
2-pentylfuran	+	+	14	
1-heptene	+			
toluene	+	+	10	
dichloromethane	+	+	8	
chloroform		+		
limonene	+	+	13	3, 10

[a] 81% germination. [b] 34% germination. [c] Reference number of peak pictured in Figures 1 and 2. [d] 1, Bradow and Connick (1988a); 2, Bradow and Connick (1988b); 3, Bradow and Connick (1990); 4, Cohn et al. (1989); 5, Connick et al. (1989); 6, Connick et al. (1987); 7, French and Leather (1979); 8, Gardner et al. (1990); 9, Holm (1972); 10, Reynolds (1987); 11, Reynolds (1977); 12, Reynolds (1989).

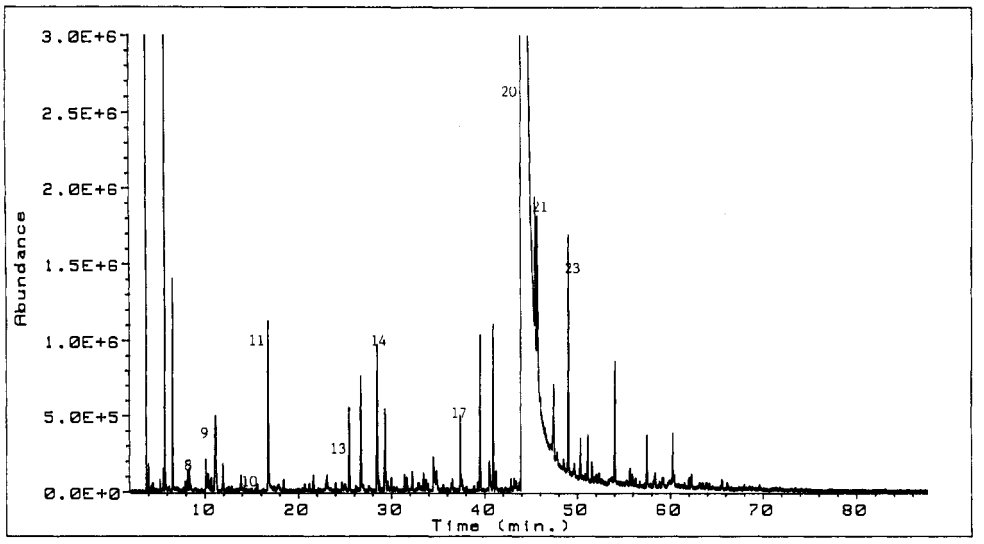

Figure 1. GC/MS total ion chromatogram of volatiles released from freshly harvested tabasco seeds during a 21-day period of dry afterripening.

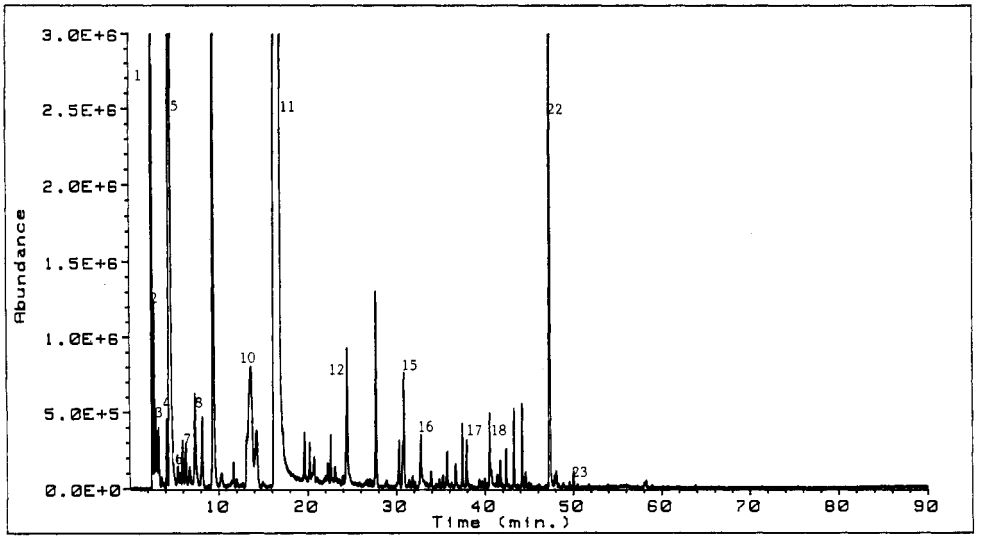

Figure 2. GC/MS total ion chromatogram of volatiles released during a 21-day storage period of previously afterripened tabasco seeds.

mass spectral data (Table I). The compounds identified include alcohols, aldehydes, ketones, esters, hydrocarbons, and furans. Many of these volatile compounds have been shown to be products of lipid oxidation. Some of the compounds identified have been found to affect germination in other seed species (Table I).

It was expected that previously afterripened seeds would release few, if any, volatiles or at the very least that the compounds that were released would be completely different from those released by nonafterripened seeds. This, however, was not the case (Figures 1 and 2; Table I). Although quantitative data were not available from this study, hexanal was noticeably much higher in quantity in previously afterripened seeds. It is not known at this time whether the difference in profiles of the volatile compounds suggests (1) a threshold effect with breakdown or release of those compounds inhibiting germination or a buildup of compounds stimulating germination, (2) a change in seed metabolism during dry afterripening to accommodate those compounds present, or (3) a combination of changes in metabolism triggered by the changes in volatile constituents. The inability to promulgate germination in those seeds that were initially enclosed in the chamber for 21 days and then subsequently subjected to afterripening suggests that the conditions for successful afterripening may change during enclosure, requiring extended time and/or elevated/reduced temperature to break dormancy or that the changes are irreversible and the ability to overcome those hurdles to germination is lost.

The present study was directed toward qualitative determination of volatile compounds produced during dry afterripening of tabasco seeds for identification of potential germination inhibiting compounds. Over 65 compounds were identified in this study; some appeared to be associated with stimulation and others with inhibition of seed germination. Further research will attempt to quantify and screen for key compounds in the germination response as we move toward a better understanding of the mechanism of loss of dormancy during dry afterripening of tabasco seeds.

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