69, and 55; cf. Medsker et al., 1969). The GLC retention data (Kovat's index 1523) were also consistent with those of an authentic sample of methylisoborneol. The concentration of methylisoborneol in the volatile oil was of the order of 0.4% and therefore of the order of 4 parts per 10^9 parts of the soil.

Other components with earthy odors were detected but could not be characterized in the present work. A number of components with nonearthy odors were also characterized (mass spectrum and GLC retention data consistent with that of an authentic sample). These included benzaldehyde, camphor, hexanol, heptanol, and nonanol.

How much of these compounds is normally transferred to vegetables has yet to be studied although geosmin has recently been characterized in beets (Acree et al., 1976; Murray et al., 1975) and off-flavored dried beans (Buttery et al., 1976).

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Aroma Production by Cultures of *Ceratocystis moniliformis*

Aroma production by the fungus, *Ceratocystis moniliformis*, is described. Sensory evaluation showed the quality and intensity of the aroma to vary with the composition of the culture medium and with the age of the cultures. Combined gas chromatography–mass spectrometry was utilized to identify volatile constituents in cultures characterized as having "fruity-banana", "peach-pear", and "citrus" aromas. The time course of accumulation of volatile constituents in cultures of *C. moniliformis* was followed by headspace gas chromatography. *C. moniliformis* is a potential source of fruit-like essences and/or specific flavor compounds.

There are numerous references in the literature to "fruity" aromas produced by microorganisms. These organisms are potential sources of "natural" fruit essences.

A literature search, plus preliminary studies with several organisms, led to the tentative choice of *Ceratocystis moniliformis*. The present communication describes results which indicate that this organism has potential as a source of flavor essences. *C. moniliformis* could also be useful for biosynthetic studies, since microorganisms generally offer advantages over fruit tissues in terms of introducing potential tracer-labeled precursors and measuring the labeled products (usually excreted into the media).

EXPERIMENTAL SECTION

Cultural Techniques. *Ceratocystis moniliformis* ATCC 12861 was obtained from Dr. Ralph Collins, Department of Botany, University of Connecticut, Storrs. The organism, maintained on 5% potato–dextrose agar slants, was first grown on 5% potato–dextrose agar plates for 4 days at 30 °C. The cells were harvested by flooding each of the plates with 10 ml of sterile, deionized water. The resultant cell suspensions were pooled and a 2-ml aliquot of the cell suspension was used to inoculate 100 ml of liquid media, contained in 250-ml Erlenmyer flasks. The inoculated flasks were plugged with nonabsorbent cotton wool wrapped in cheesecloth and incubated on a rotary shaker at 30 °C. Each 100 ml of the basal liquid medium contained 0.1 g of KH_2PO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.2 mg of $FeSO_4 \cdot 7H_2O$, 0.2 mg of $ZnSO_4 \cdot 7H_2O$, 0.1 mg of $MnCl_2 \cdot 4H_2O$, 0.1 mg of anhydrous $CaCl_2$, 10 mg of thiamin, and 0.5 μg of biotin (Wilson and Lilly, 1958). Various carbohydrate and nitrogen sources were added to the basal medium in quantities which supplied carbon equivalent to 2.5 or 3% dextrose and nitrogen equivalent to 0.1% urea.

Growth. Growth on various sources was assessed by visual comparison, relative to growth on the standard dextrose (2.5 or 3.0%)–urea (0.1%) medium.

Organoleptic Evaluation. A panel consisting of eight members of our Flavor Chemistry Laboratory characterized the aroma of the fungal cultures. The panel members were not restricted to any list of descriptive terms, but they were generally aware that the cultures were being tested for their ability to produce fruitlike aromas. Samples reported to have distinctive or unique flavor notes were reexamined by a highly trained consulting flavorist. He generally confirmed the results of our panel, but pointed out several additional and more subtle notes in the samples.

Separation and Identification of Aroma Compounds. Both the headspace of cultures and a Freon-11 extract of the growth broth of *C. moniliformis* were analyzed for aroma compounds. Headspace gas chroma-

tography (GC) was used to separate the lower boiling compounds produced by *C. moniliformis*. A 500 ft × 0.03 in. i.d. SF96(50) open tubular stainless steel column was equipped with a 6 ft × 0.03 in. i.d. stainless steel injection loop. Up to 100 ml of headspace gas was injected into the injection loop while the lower portion of the loop was held in liquid nitrogen. The entire contents of the loop were then flushed onto the GC column by heating the loop to about 200 °C with a heatgun. The oven temperature of the gas chromatograph was then programmed from 60 to 150 °C at the rate of 2 °C/min. The helium carrier gas flow rate was 5 ml/min.

Selected cultures were examined for the presence of higher boiling components utilizing the Freon extraction method. These cultures were filtered to remove the mycelia and the filtrate was extracted with an equal volume of Freon-11 (trichlorofluoromethane, Matheson Gas Products, East Rutherford, N.J.). The usefulness of fluorocarbons for extracting flavor essences has been previously reported (Schultz et al., 1967; Palmer, 1973). The Freon extract was concentrated to about 2 ml in a Flash Evaporator at room temperature. One milliliter of hexane was added to the concentrate and the remaining Freon-11 was removed with a stream of nitrogen gas. (Freon-11 was removed since it tends to corrode the flame ionization detector.) The hexane concentrate was injected onto a 10 ft × 1/8 in. o.d. stainless steel column packed with 5% Carbowax 20M on Chromosorb G. The column was held at 125 °C for 10 min and then programmed at 2 °C/min to 175 °C. The helium carrier gas flow rate was 20 ml/min.

Gas chromatography of both headspace samples and hexane concentrates was carried out on an Aerograph Series 200 gas chromatograph (Varian Associates, Palo Alto, Calif.) coupled to a Hitachi Perkin-Elmer RMU-7 double focusing mass spectrometer (Perkin-Elmer Corp., Norwalk, Conn.). The effluent from the GC column passed through a Watson–Biemann-type separator (Watson and Biemann, 1965) into the ion source. The electron energy was 70 eV and the total ion current chromatograms were recorded from a monitor located between the electrostatic and magnetic analyzers of the mass spectrometer. The GC–MS system used has been described in detail by Issenberg et al. (1969). Identification of all compounds was confirmed by comparison with mass spectra and retention data of reference chemicals run under identical conditions.

RESULTS AND DISCUSSION

Effect of Carbon and Nitrogen Sources on the Aroma Quality and Growth of *C. moniliformis*. Preliminary experiments showed that growth of *C. moniliformis* on dextrose–urea was rapid and that these cultures possessed an intense, predominately banana-like aroma, previously reported as typical of this organism (Hunt, 1950). In all subsequent experiments growth, aroma quality, and aroma intensity of *C. moniliformis* cultures were assessed relative to that found on dextrose–urea.

When the carbon source was altered, a variety of fruity aromas were produced (Table I). The only nonfruity aroma noted was a "mildew" or "leathery" scent produced on oleic acid. Aroma intensity was generally correlated with growth (Table I), the exceptions being ethanol and palmitic acid which yielded good growth, but only faint aromas.

Variation of the nitrogen source also affected the aroma quality and intensity (Table II). Leucine, isoleucine, or norleucine gave rise to an intense overripe banana aroma, similar to that found in dextrose–urea cultures. These amino acids have been reported to be precursors of key aroma volatiles in bananas (Bemelmans, 1970; Myers et al., 1970; Tressel et al., 1970), suggesting that similar biosynthetic pathways may be operating in *C. moniliformis*. Distinctly different aromas were found when glycine and methionine were utilized as nitrogen sources. The organism showed slight growth on inorganic nitrogen, but yielded no aroma.

Table I. Effect of Carbon Source on Growth and Aroma of *C. moniliformis*

Carbon source[a]	Growth[b]	Aroma[c]
Sugars		
Dextrose	++++	Fruity, banana
Galactose	+++	Citrus, grapefruit, lemon
Fructose	+++	Fruity
Sucrose	+++	Fruity
Xylose	+	Faint
Ribose	++	Faint
Esters		
Ethyl acetate	++	Faint
Propyl acetate	0	None
Isobutyl acetate	0	None
Isoamyl acetate	0	None
Acids		
Acetic	0	None
Oleic	+++	Faint fruity note, mildew, leathery
Palmitic	+++	Faint fruity note
Alcohols		
Ethyl alcohol	+++	Faint
Isoamyl alcohol	0	None
Others		
Glycerol	++	Canned pear, peach
Corn starch	++++	Cantelope, tropical flower, banana
Tripalmitin	0	None

[a] Media contained carbon equivalent to 2.5% dextrose, in a basal medium containing 0.1% urea as the nitrogen source. [b] ++++, excellent; +++, good; ++, fair; +, poor; 0, no growth. [c] Most common descriptive terms given by MIT flavor panel.

Table II. Effect of Nitrogen Source on Growth and Aroma of *C. moniliformis*

Nitrogen source[a]	Growth[b]	Aroma[c]
Amides		
Urea	++++	Fruity, banana
Asparagine	+++	Fruity, sour
Amino acids		
Leucine, isoleucine, or norleucine	+++	Fruity, strong overripe banana
Alanine	+++	Weak fruity
Methionine	++	Weak potato
Glycine	+++	Sweet, pineapple, lemony
Aspartic acid	0	None
Inorganic		
$NaNO_3$	++	None
$(NH_4)_2SO_4$	++	None

[a] Media contained nitrogen equivalent to 0.1% urea, in a basal medium containing 3.0% dextrose as the carbon source. [b,c] See Table I.

Identification of Aroma Compounds from Selected Cultures. Four cultures with different characteristic aromas were chosen to further investigate the compounds responsible for their aroma. These were cultures grown on dextrose–urea (fruity, banana), dextrose–leucine (fruity, strong overripe banana), glycerol–urea (canned pear, peach), and galactose–urea (citrus, grapefruit, lemon).

GC headspace analysis of all four cultures showed the presence of ethanol and short-chained esters (Figure 1A). These esters are commonly found in fruits (Nursten, 1970) and are probably responsible for the frequent charac-

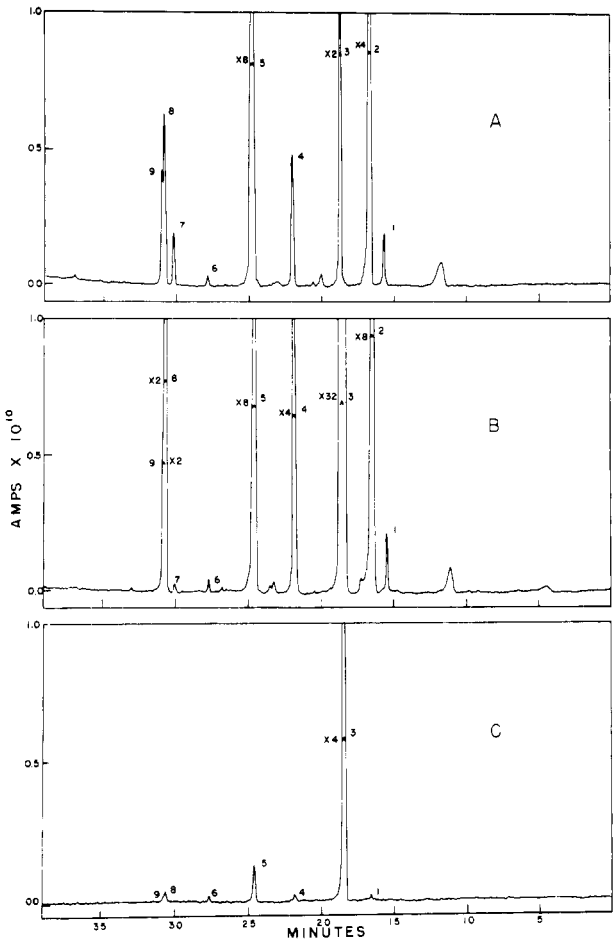

Figure 1. Chromatograms of headspace vapor from cultures of *C. moniliformis* grown on dextrose-urea for (A) 4 days, (B) 6 days, and (C) 10 days. Volatile components identified by gas chromatography–mass spectrometry: peak (2) ethanol, (3) ethyl acetate, (4) *n*-propyl acetate, (5) isobutyl acetate, (8) isoamyl acetate. Compound responsible for peak 9 tentatively identified as active amyl acetate (2-methyl butyl acetate). Attenuation: ×1 except where indicated.

terization of *C. moniliformis* cultures as having a fruity aroma. All of these compounds except *n*-propyl acetate and active amyl acetate (2-methyl butyl acetate) have been previously reported to be present in cultures of *C. moniliformis* (Collins and Morgan, 1962).

There was considerable variation in the total amounts and relative proportions of these esters in the four cultures. For example, considerably more isoamyl acetate (3-methyl butyl acetate) was present in the headspace of the dextrose–leucine culture. This is consistent with the strong, overripe banana aroma and with leucine being a direct precursor of isoamyl acetate, as shown earlier in bananas (Myers et al., 1970) and in yeasts (Yoshizawa, 1966).

Despite the similarity in the headspace chromatograms of the four cultures, the galactose–urea and glycerol–urea cultures each had a unique aroma (Table I). This led us to investigate the less volatile components (via Freon-11 extraction) as a possible source of the distinctive aromas.

The Freon-11 extract of the canned pear, peach, glycerol–urea cultures contained a number of compounds. Two were identified as γ- and δ-decalactone, compounds known for their characteristic peach aroma (Furia and Bellanca, 1971).

The Freon-11 extract of the citrus, grapefruit, lemon galactose–urea cultures also contained a number of compounds. The monoterpenes geranial and citronellol were identified. Geranial (as the predominant constituent of citral) is a character impact compound in lemons and undoubtedly contributes to the characteristic aroma of these cultures. Citronellol has a roselike aroma and could also contribute. Collins and Halim (1970) reported the presence of monoterpenes in cultures of *Ceratocystis variospora*.

Although the components identified above represent only a few of the volatile components in cultures of *C. moniliformis*, the results already indicate the presence of character impact compounds.

Time-Course Study of the Production of Aroma in Dextrose–Urea Cultures. The development of aroma compounds in cultures of *C. moniliformis* was studied by organoleptic evaluation and by analyzing the headspace after 4, 6, and 10 days of growth on dextrose–urea. By day 4, cultures already possessed a recognizable fruity aroma, and headspace chromatograms showed the presence of esters and ethanol (Figure 1A). Preliminary experiments had indicated that cultures were entering the stationary phase of growth at day 4. By day 6, the aroma of cultures was more intense and distinctly banana-like, correlating with the increased concentration of acetate esters in the headspace (Figure 1B). The fruitlike aroma of the cultures disappeared by day 10 and the only component present in significant quantity in the headspace was ethyl acetate (Figure 1C).

The chromatograms suggested that the cultures began to produce volatiles by day 3 or 4, reached a peak of production within the next few days, and then stopped production. The disappearance of volatiles between days 6 and 10 presumably resulted from either reutilization of the volatiles by the organism or by evaporation from the culture flasks.

In order to decide which of the above hypotheses was correct, cultures were: (1) grown on the various esters and ethanol and (2) the evaporative loss of volatiles from the shake cultures was measured.

C. moniliformis grew weakly on ethyl acetate and not at all on other acetate esters when these compounds were the sole carbon source (Table I). In contrast, cultures grew well on ethanol (Table I).

Aqueous solutions of esters and ethanol were prepared so that headspace chromatograms approximated those from day 6 cultures (Figure 1B). When these solutions were incubated in shake flasks the esters disappeared by evaporation in about 5 days, while little or no ethanol was lost.

These results tend to support the hypothesis that the esters are lost by evaporation, while ethanol is reutilized by the organism. Additional studies will be necessary to establish the actual rate and total production of esters and alcohols during growth.

DISCUSSION AND CONCLUSIONS

The results described here indicate that a variety of distinctive fruitlike aromas are produced by *C. moniliformis*, depending on the carbon and nitrogen source in the growth medium. Selected cultures with aromas characteristic of a particular fruit contained character impact compounds which occur in these same fruits.

These findings indicate *C. moniliformis* to be a potential source of individual flavor chemicals, total fruit essences, or enzymes capable of catalyzing specific bioconversions of inexpensive substrates to such flavors or essences.

In more recent studies (E. Lanza and J. K. Palmer, unpublished results) *C. moniliformis* has proven to be an excellent experimental subject for studying the biosynthesis of monoterpenes.

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1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone, a New Herbicide

1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone (EL-171), a new chemical compound, is herbicidally active at low dosages and is safe for use on cotton. EL-171 controls a wide variety of annual grass and broadleaf weeds and is more active preemergence than postemergence. Susceptible plants treated preemergence with EL-171 emerge with chlorotic leaves, become necrotic, and subsequently die. EL-171 is a slow-acting, translocated herbicide, and treated plants appear unable to direct the synthesis of chlorophyll.

In the course of a greenhouse screening program evaluating herbicidal activity of various substituted pyridinones and related chemicals, many were found to be very effective in controlling several weed species. 1-Methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone (coded EL-171), a new chemical compound, was synthesized and found to be safe on cotton and particularly effective for the control of a broad spectrum of annual grass and broadleaf weeds. Data which demonstrate the herbicidal activity and crop selectivity of EL-171 are presented in this report.

CHEMICAL METHODS

EL-171 was synthesized from the appropriate ketone [prepared by the method of Coan and Becker (1954)] by the procedures suggested by Benary and Bitter (1928) and El-Kholy et al. (1973). After recrystallization from ethyl acetate–Skellysolve B, the compound was obtained as a white crystalline solid which melts at 153–155 °C. Nuclear magnetic resonance and infrared spectra and microanalysis data are in accord with the proposed structure (Figure 1).

The compound is moderately soluble in chloroform, ethanol, acetone, and ethyl acetate, somewhat less soluble in ether and benzene, and almost insoluble in hexane and Skellysolve B. Solubility in water at pH 7 is approximately 12 ppm.

Preliminary toxicological data indicate that EL-171 has a low order of mammalian toxicity. The acute LD_0 of EL-171 by oral administration is greater than 10 g/kg of body weight for rats, 500 mg/kg for dogs, and 250 mg/kg for cats. The LD_{50} to fasted female mice is greater than 10 g/kg. The LC_{50} at 96 h for bluegill is 7 ppm. In subacute studies, a dosage of 2000 ppm was the no effect level when fed to rats for a period of 91 days. Body weight gain was suppressed in rats fed diets containing both 4000 and 8000 ppm. However, these high dosages did not affect the hematology, clinical chemistry values, or the histological findings.

Table I. Percent Control of Annual Grass and Broadleaf Weeds with Preemergence Application of EL-171 (Greenhouse Test)

Weed species	Dosage, kg of AI/ha 0.3	0.6	1.2	2.4
Barnyardgrass (*Echinochloa crus-galli*)	100	100	100	100
Crabgrass (*Digitaria sanguinalis*)	100	100	100	100
Foxtail (*Setaria italica*)	95	95	100	100
Johnsongrass (*Sorghum halepense*)	100	100	100	100
Ryegrass (*Lolium multiflorum*)	60	100	100	100
Wild Oat (*Avena fatua*)	80	90	95	100
Cocklebur (*Xanthium pensylvanicum*)	55	70	85	100
Jimsonweed (*Datura stramonium*)	80	100	100	100
Lambsquarters (*Chenopodium album*)	80	80	100	100
Morningglory (*Ipomoea purpurea*)	70	95	95	100
Mustard (*Brassica nigra*)	60	80	80	100
Nightshade (*Solanum quineense*)	100	100	100	100
Pigweed (*Amaranthus retroflexus*)	100	100	100	100
Sicklepod (*Cassia obtusifolia*)	100	100	100	100

HERBICIDE PROPERTIES

EL-171 was evaluated in the greenhouse as pre- and postemergence applications on several weed species.

In a preemergence greenhouse test (Table I), seeds of 14 weeds were sown approximately 3 cm deep in rows across galvanized metal flats (seven species per flat) and