Use of Pale-Yellow Tobacco to Reduce Smoke Polynuclear Aromatic Hydrocarbons

Ray F. Severson,* Richard F. Arrendale, James F. Chaplin, and Ralph E. Williamson

Smoke data (cigarette density, puff number, total particulate matter, nicotine, and polynuclear aromatic hydrocarbons) and lipid data (hydrocarbons, terpenes, fatty acids, docosanol, and sterols) were correlated for five normal green tobacco cultivars (Va 115, Coker 139, NC 2326, NC 95, Coker 187-Hicks) and for two of their pale-yellow selections. Smoke nicotine levels for all pale-yellow selections were higher (2–19%) compared to the normal green parents. Pale-yellow tobaccos had the same or higher levels of docosanol, slightly higher levels of neophytadiene and phytol, lower levels of terpenes, and the same or lower levels of sterols than the normal tobaccos. All but one of the pale-yellow selections produced lower levels (7–25%) of smoke polynuclear aromatic hydrocarbons. The reduction of smoke polynuclear aromatic hydrocarbons in the pale-yellow tobaccos was attributed to their lower leaf solanesol levels.

Bioassays of tobacco smoke condensate fractions have shown that the fractions containing the polynuclear aromatic hydrocarbons (PAH) possess significant biological activity (Akin et al., 1975, 1976; Bock et al., 1970; Hoffmann and Wynder, 1971) and that the PAH are the primary tumor initiators of tobacco smoke (Severson et al., 1978). Pyrolysis studies have shown that the hexane-soluble components of tobacco, the tobacco lipids, are major precursors of PAH in cigarette smoke (Schlotzhauer and Schmeltz, 1968; Schlotzhauer et al., 1976). Therefore, the tobacco lipids are significantly related to tobacco safety. It would be most desirable to determine variations in lipid contents in genetically different flue-cured tobaccos to find a good, low-lipid tobacco for the production of safer cigarettes.

During a screening of various experimental cigarettes for smoke PAH content, cigarettes made from a pale-yellow (PY) selection of NC 95 were found to produce lower levels of smoke PAH than those made from the normal green parent (Severson et al., 1975). This decrease could not be attributed to lower tar levels. Therefore, we decided to look more closely at the relationship between the PY character and smoke PAH constituents.

The PY character in tobacco was first described by Chaplin (1969). The leaves of these plants mature about 10 days earlier and ripen more uniformily than those of normal green tobaccos (Chaplin, 1975). More recently, Chaplin (1977) described the interrelationships between the PY character and certain agronomic and chemical traits of flue-cured tobaccos. Compared to normal green tobacco, the PY tobacco gave lower reducing sugar and starch levels, higher levels of α-amino nitrogen, and slightly reduced yields. However, these differences are probably not of sufficient magnitude to prevent the use of PY tobaccos for cigarette manufacture.

The experiment reported in this paper was conducted to determine whether PY tobacco and normal flue-cured cultivars differ in leaf lipid contents and derived smoke characteristics.

EXPERIMENTAL SECTION

Tobacco Samples. The cultivars chosen for the study were Va 115, Coker 139, NC 2326, NC 95, and Coker 187-Hicks. The genetic factor for PY were transferred to the five cultivars by the back-cross method as described by Chaplin (1977). Plants of the homozygous PY selections and recurrent parents were grown in 1974 at the Oxford Tobacco Research Station, Oxford, N.C.

A randomized three replicate, split-plot design was used. Families comprising the whole plots and subplots consisted of the two PY selections and recurrent parent. Each subplot consisted of 20 plants. Conventional fertilization, culture, and curing practices for flue-cured tobacco was used. The tobacco was primed when judged to be ripe. Harvesting of the PY plants were completed about 10 days earlier than that of the recurrent parent. Leaves from the PY plants were cured separately from those of the green tobacco because they required a different curing schedule. The cured leaves from the same subplot in each replicate were combined into a single sample for cigarette manufacture. The lamina was stemmed and made into 85-mm nonfilter cigarettes for smoke evaluation. The cigarettes were manufactured to a constant diameter and selected for constant draw pressure.

Smoke Analysis. The cigarettes were smoked under standard conditions (Pillsbury et al., 1969) on a Phipps and Bird smoking machine to a 23-mm butt length. The smoke was analyzed for tar and nicotine using the method of Pillsbury et al. (1969). Data on weight, total particulate matter (TPM), tar, nicotine, H_2O, and puff count per cigarette were obtained.

Smoke Polynuclear Aromatic Hydrocarbons (PAH) Analysis. Cigarettes were smoked to a 30-mm butt length on a 30-port Borgwaldt smoking machine under standard smoking conditions (Pillsbury et al., 1969). The condensate from 90 cigarettes was collected in two dry-ice traps, the last containing a glass wool plug. The PAH were isolated from the cigarette smoke condensate (CSC) using the separation and gas chromatography (GC) method of Severson et al. (1976), shown in Figure 1.

Tobacco Lipid Analysis. The major GC-volatile lipids were analyzed as outlined in Figure 2 by methods developed in this laboratory (Severson et al., 1977, 1978). About 1 g of ground cigarette tobacco was hydrolyzed with ethanolic KOH. The hydrolyzate was adjusted to pH 2 and extracted with hexane. A portion of the extract, after the addition of 1,3-dimyristin internal standard, was analyzed by GC for total solanesol, the solanesenes, and bombiprenone (Severson et al., 1977). After the addition of internal standards (docosane, pentacosanol, and nervonic acid) a 30–40-mg portion of the remaining extract was separated on a silicic acid chromatographic column

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Table I. Comparison of Smoke Data of Cigarettes Prepared from Normal-Green, Flue-Cured Cultivars, and Their Pale-Yellow Selections (per cigarette)

cultivar or selection	av wt,[a] g	puff,[b] no.	TPM,[c] mg	nicotine, mg	H_2O, mg	tar,[d] mg
Va 115	1.13	9.8	36.1	2.66	3.0	30.4
PY Sel 1	1.13	10.5	38.0	3.18	3.1	31.7
PY Sel 2	1.13	10.7	38.3	3.16	3.0	32.1
Coker 139	1.10	9.3	32.7	2.16	2.4	28.1
PY Sel 1	1.05	9.4	32.6	2.19	2.2	28.2
PY Sel 2	1.05	9.6	34.3	2.21	2.7	29.4
NC 2326	1.10	10.4	37.2	3.20	2.8	31.4
PY Sel 1	1.18	10.8	39.1	3.55	2.6	32.8
PY Sel 2	1.10	10.2	37.1	3.43	2.6	31.1
NC 95	1.13	10.5	35.8	3.17	2.5	30.1
PY Sel 1	1.00	9.2	34.3	3.23	2.4	28.7
PY Sel 2	1.13	10.1	36.1	3.54	2.6	30.0
Coker 187-Hicks	1.05	8.2	31.5	2.12	2.4	27.0
PY Sel 1	1.00	8.5	32.2	2.34	2.7	27.0
PY Sel 2	1.00	8.6	32.5	2.24	2.8	27.2

[a] Weight of 85-mm cigarettes made to constant diameter and selected for pressure drop after preconditioning under standard conditions (Pillsbury et al., 1969). [b] Number of puffs required to obtain 23-mm butt length under standard smoking conditions. [c] TPM, total particulate matter. [d] TPM minus nicotine and water.

Table II. Selected Parent PAH Levels in CSC from Normal Green, Flue-Cured Tobacco Cultivars and Their Pale-Yellow Selections

cultivars or selection	fluoranthene, μg/100 cig.[a]	pyrene, μg/100 cig.[a]	1,2-benzanthracene/chrysene/triphenylene, μg/100 cig.[a]	benzofluoranthene, μg/100 cig.[a]	benzopyrene, μg/100 cig.[a]	parent PAH, μg/100 cig.[a]
Va 115	11.4	12.0	7.7	3.4	1.9	36.4
PY Sel 1	11.2	12.3	6.4	2.5	1.7	34.0
PY Sel 2	12.1	12.2	7.0	2.8	1.9	36.0
Coker 139	12.1	11.2	8.2	3.2	2.3	37.0
PY Sel 1	9.8	8.2	5.4	2.3	1.6	27.3
PY Sel 2	9.8	9.2	5.8	2.4	1.7	28.9
NC 2326	12.2	12.8	6.9	3.0	2.0	36.9
PY Sel 1	12.0	12.0	6.1	2.9	1.7	35.0
PY Sel 2	13.2	14.0	6.9	3.1	1.9	39.1
NC 95	11.3	11.4	8.2	3.3	2.2	36.4
PY Sel 1	10.5	10.1	6.6	2.7	1.7	31.6
PY Sel 2	11.5	9.7	6.9	2.5	1.7	32.3
Coker 187-Hicks	12.0	10.9	8.5	3.0	2.0	36.4
PY Sel 1	10.1	9.1	6.2	3.1	1.7	30.2
PY Sel 2	10.7	9.1	6.5	2.6	1.7	30.7

[a] Corrected for differences in detector response.

into the fractions and components shown, and these were quantitated by GC (Severson et al., 1978).

RESULTS

Standard Smoke Analysis. The smoke analysis data obtained under standard smoking conditions and smoking to a 23-mm butt length are given in Table I. The cigarettes were manufactured to a constant volume, so their average weights reflect the densities of the tobacco. The PY character did not result in an appreciable change in tobacco density. About a 5% decrease in tobacco density was observed for the PY selections of the Coker 187-Hicks and Coker 139 cultivars.

The number of puffs per cigarette indicates the rate of tobacco burn. Except for the PY selection 2 of the NC 2326 and PY selections of the NC 95 cultivars, a measurable increase in puff number was observed in the PY selection cigarettes. Tar levels increased or decreased with puff number, ranging from an average 4% decrease in the NC 95 PY selection to a 6% increase in the Va 115 PY tobaccos. This observation is consistent with studies which have shown that tar levels increase with puff number

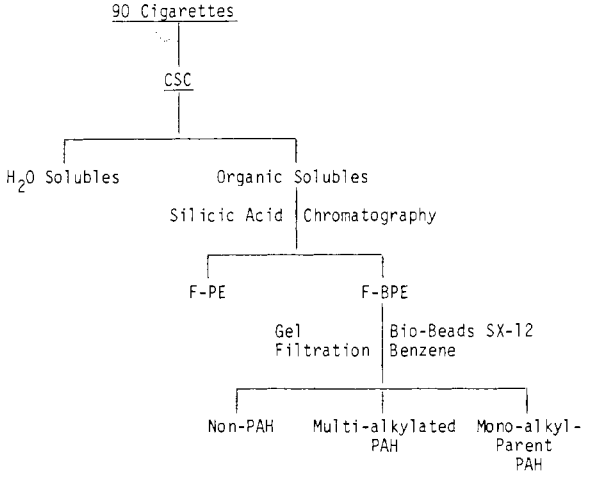

Figure 1. Isolation of PAH from CSC for GC analysis (PE, petroleum ether; BPE, 25% benzene/PE).

(Keith and Tesh, 1965; Brunnemann et al., 1975). There was a trend toward higher smoke nicotine in the PY se-

Table III. Selected Methyl-PAH Levels in CSC from Normal-Green, Flue-Cured Tobacco Cultivars and Their Pale-Yellow Selections

cultivar or selection	methyl-fluoranthenes, μg/100 cig.[a]	methyl-pyrenes, μg/100 cig.[a]	methyl-1,2-benzanthracenes/chrysenes/triphenylenes, μg/100 cig.[a]	methyl PAH, μg/100 cig.[a]
Va 115	23.9	27.6	6.9	58.4
PY Sel 1	26.0	24.0	4.7	54.7
PY Sel 2	24.4	23.9	6.6	54.9
Coker 139	24.5	23.1	7.1	55.3
PY Sel 1	18.0	17.1	4.7	39.8
PY Sel 2	20.5	16.3	4.4	41.2
NC 2326	23.4	21.5	5.9	50.8
PY Sel 1	20.7	20.0	5.1	45.8
PY Sel 2	32.9	34.1	7.3	74.3
NC 95	24.7	25.0	7.6	57.3
PY Sel 1	19.8	19.7	7.2	46.7
PY Sel 2	20.1	19.9	5.6	45.6
Coker 187-Hicks	24.5	21.7	8.1	54.6
PY Sel 1	18.9	16.7	4.2	39.8
PY Sel 2	19.7	18.0	5.5	43.2

[a] Assumed to have detector response of parent.

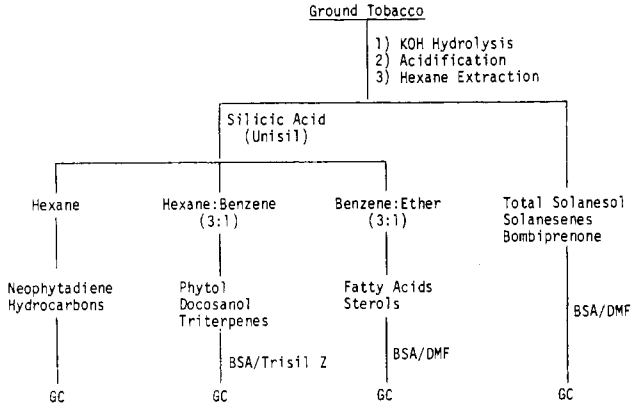

Figure 2. Analytical procedure for tobacco lipids.

lections compared to their recurrent parents.

Smoke PAH Analysis. Data on the PAH analyses of the tobaccos are listed in Tables II and III. As compared to their recurrent parents, all the PH selections, except PY 2 from NC 2326, showed a decrease in overall PAH level: an average 4% parent PAH and 6% methyl PAH for the Va 115 PY selections, an average 25% decrease for both parent and methyl PAH for the Coker 139, a 5% parent and 10% methyl PAH decrease for the NC 95 PY selections, and an average 18% parent and 25% methyl PAH decrease for the Coker 187-Hicks PY selection.

Tobacco Lipid Analysis. Many pyrolysis studies have shown that tobacco lipids are important PAH precursors. In order to determine if the difference in smoke PAH levels could be due to varying lipid levels, the major lipid components in the cigarette tobaccos were determined using the method outlined in Figure 2.

The levels of hydrocarbons, C_{16}–C_{18} fatty acids, the major tobacco fatty alcohol, docosanol (Severson et al., 1978), and sterols in the normal green and PY tobaccos are compared in Table IV. In general, the PY tobaccos yielded slightly lower levels of sterols, the same or slightly higher levels of docosanol, and considerably higher levels of fatty acids. No major differences in hydrocarbon composition could be attributed to the PY character.

The levels of the major terpene components of the tobaccos are compared in Table V. In general, the PY selections had higher levels of neophytadiene and phytol

Table IV. Comparison of Hydrocarbon, Fatty Acids, Fatty Alcohols, and Sterol Levels of Normal-Green, Flue-Cured Tobacco and Their Pale-Yellow Selections

cultivar or selection	hydrocarbons,[a] % dry wt	fatty acids,[b] % dry wt	docosanol,[c] % dry wt	sterols,[d] % dry wt
Va 115	0.099	0.75	0.012	0.164
PY Sel 1	0.092	0.98	0.012	0.128
PY Sel 2	0.081	0.94	0.012	0.143
Coker 139	0.140	1.03	0.011	0.205
PY Sel 1	0.120	1.19	0.011	0.207
PY Sel 2	0.170	1.14	0.012	0.192
NC 2326	0.140	0.65	0.012	0.136
PY Sel 1	0.110	0.81	0.013	0.127
PY Sel 2	0.150	0.99	0.014	0.137
NC 95	0.070	0.68	0.006	0.171
PY Sel 1	0.073	0.72	0.006	0.138
PY Sel 2	0.072	0.85	0.007	0.165
Coker 187-Hicks	0.100	0.74	0.009	0.192
PY Sel 1	0.120	1.12	0.013	0.181
PY Sel 2	0.170	1.13	0.011	0.181

[a] Total C_{25}–C_{34} hydrocarbons; calculated assuming detector response identical with docosane. [b] Total C_{16}, $C_{18}{}^{1,2,3=}$, and C_{18} fatty acids; corrected for difference in detector response. [c] Corrected for difference in detector response. [d] Cholesterol, stigmasterol, campesterol, and sitosterol; corrected for difference in detector response.

and lower levels of the C_{45}-derived terpenes, solanesenes, bombipernones, and solanesol. No trend for the triterpene levels, β-amyrin and cycloartenol, were observed.

Correlation between Smoke PAH Levels and Tobacco Lipid Levels. Simple correlation among several of the tobacco leaf lipid constituents and smoke PAH levels were calculated and are shown in Table VI. Solanesol and total C_{45} isoprenoids (solanesol, bombiprenone, and solanesenes) were the only lipid leaf components to show positive correlation with smoke PAH levels. There were significant positive correlations between solanesol and seven of the PAH entries. The total C_{45} isoprenoids correlated significantly and positively with the fluoranthene, methylfluoranthenes, parent, methyl, and total PAH. The other lipid components generally showed a

Table V. Comparison of the Levels of the Major Terpenes of Normal-Green, Flue-Cured Tobacco Cultivars and Their Pale-Yellow Selections

cultivar or selection	neophytadiene,[a] % dry wt	phytol,[b] % dry wt	β-amyrin,[b] % dry wt	cycloartenol,[c] % dry wt	solanesenes bombi-prenone[d]	solanesol[b]
Va 115	0.11	0.013	0.005	0.006	0.17	1.16
PY Sel 1	0.12	0.011	0.010	0.013	0.18	0.98
PY Sel 2	0.12	0.011	0.005	0.007	0.14	0.99
Coker 139	0.11	0.011	0.009	0.017	0.21	1.11
PY Sel 1	0.12	0.025	0.011	0.018	0.16	0.96
PY Sel 2	0.12	0.018	0.017	0.022	0.23	1.09
NC 2326	0.13	0.010	0.014	0.020	0.28	1.34
PY Sel 1	0.14	0.012	0.011	0.015	0.18	1.14
PY Sel 2	0.16	0.014	0.015	0.018	0.27	1.39
NC 95	0.11	0.011	0.009	0.015	0.19	1.30
PY Sel 1	0.11	0.012	0.005	0.011	0.17	1.15
PY Sel 2	0.13	0.011	0.009	0.016	0.17	1.27
Coker 187-Hicks	0.10	0.016	0.011	0.016	0.24	1.21
PY Sel 1	0.11	0.019	0.010	0.017	0.17	1.01
PY Sel 2	0.13	0.019	0.011	0.015	0.25	1.04

[a] Calculated assuming detector response identical with docosane. [b] Corrected for difference in detector response. [c] Calculated assuming detector response identical with β-amyrin. [d] Calculated assuming detector response identical with solanesol.

Table VI. Simple Correlations between Tobacco Lipids and Smoke PAH

	tobacco lipids							
PAH	solanesol	total C_{45} isoprenoids	$C_{18}{}^{1,2,3}$ fatty acids	total C_{16}–C_{18} fatty acids	neophytadiene	phytol	sterols	triterpenes
fluoranthenes	0.612[a]	0.603[a]	−0.390	−0.461	0.439	−0.641[a]	−0.509	−0.243
methylfluoranthenes	0.513	0.534[a]	−0.277	−0.255	0.374	−0.458	−0.420	−0.249
pyrene	0.521[a]	0.509	−0.504	−0.530[a]	0.404	−0.704[b]	−0.718[b]	−0.306
methylpyrenes	0.536[a]	0.505	−0.332	−0.350	0.368	−0.484	−0.445	−0.490
1,2-benzanthracene/ chrysene/triphenylene	0.467	0.437	−0.519[a]	−0.544[a]	−0.393	−0.531[a]	0.064	−0.337
methyl-1,2-benzanthracenes/ chrysenes/triphenylenes	0.560[a]	0.510	−0.532[a]	−0.583[a]	−0.183	−0.456	−0.126	−0.478
benzofluoranthenes	0.468	0.440	−0.448	−0.493	−0.154	−0.435	−0.052	−0.337
benzopyrenes	0.454	0.453	−0.473	−0.418	−0.215	−0.521[a]	0.087	−0.103
parent PAH	0.616[a]	0.597[a]	−0.553[a]	−0.598[a]	0.192	−0.727[b]	−0.473	−0.337
methyl PAH	0.564[a]	0.550[a]	−0.359	−0.365	0.310	−0.502	−0.411	−0.419
total PAH	0.593[a]	0.579[a]	−0.420	−0.437	0.288	−0.577[a]	−0.436	−0.403

[a] Significant at 0.05 confidence level. [b] Significant at 0.01 confidence level.

negative correlation with the PAH.

There were one or more significant negative correlations between sterols, unsaturated fatty acids, total C_{16}–C_{18} fatty acids, phytol, and some of the PAH. There were highly significant negative correlations between phytol and pyrene and parent PAH. The number of negative correlations shown for phytol suggest that it is probably not a PAH precursor. There were no significant correlations between triterpenes and neophytadiene and PAH.

These data indicate the reduction in smoke PAH in the PY tobacco can be attributed to their lower leaf solanesol levels. This is consistent with pyrolysis data which have shown that solanesol and its esters produce the broad spectrum of PAH found in tobacco smoke and due to their high levels in tobacco are the major tobacco leaf precursors of smoke PAH (Schlotzhauer et al., 1976).

The level of PAH in cigarette smoke thus appears to be genetically controllable, that is, different flue-cured tobacco cultivars yield different quantities of smoke PAH. Therefore, appropriate selections of a tobacco may be a means of reducing the resulting levels of PAH in cigarette smoke and thereby produce a safer cigarette. However, since the carcinogenic PAH threshold of cigarette smoke had not been determined, animal bioassay studies are required to determine if the smoke PAH reduction observed in the PY tobacco is biologically significant. The higher levels of α-amino nitrogen and nicotine in the PY tobaccos may have undesirable biological effects.

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Turnip Green, Cucumber, Snapbean, and Southern Pea Response to Pesticides in Intensive-Cropping Sequences

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Fatty acid contents and percent total nitrogen determinations were made for turnip greens, cucumber, snapbeans, and southern peas grown in intensive-cropping sequences utilizing multiple pesticide applications. Relatively minor changes in fatty acid quantity and quality or percent nitrogen were found. The major observation is the stability of crop quality in plants exposed to multiple pesticide applications or residues.

Muliple- or intensive-cropping depends upon the use of various pesticides in the individual crops. This results in the possibility of crop damage from pesticides applied to previous crops grown on the same land in the same year. Previous research has shown a lack of herbicide influence on the oil quality and quantity in corn (Wilkinson and Hardcastle, 1974), soybean (Wilkinson and Hardcastle, 1972d; Hardcastle et al., 1974), and cottonseed (Wilkinson and Hardcastle, 1971, 1972a,b,c), which further demonstrated the isolation of the maturing seed from deleterious influence by pesticides. However, the lipid composition of vegetative tissues has been shown to be altered by environment and herbicides (Wilkinson and Kasperbauer, 1972; Wilkinson, 1974; Wilkinson and Smith, 1975a,b) and the possibility of vegetative crop quality change remains. Turnip greens (*Brassica rapa* L.), cucumber (*Cucumis sativus* L.), and snapbeans (*Phaseoleus vulgaris* L.) are leafy and pericarp crop tissues which should be highly responsive to pesticides. Therefore these crop tissues plus southern peas (*Vigna unquiculata* (L.) Walp.) were harvested from intensive-cropping sequences for quality analysis.

METHODS AND MATERIALS

Land preparation procedures followed those common to the area. Fertility was maintained at a high level with soil pH maintained at 6.0-6.5. Land was turned and bedded to eliminate crop residues from the soil surface immediately before each crop was planted. Crops planted and pesticides utilized in each of the six intensive-cropping sequences are shown in Table I. Chemical and common names of pesticides are listed in Table II.

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Fungicides were applied as needed. Benomyl was applied to peanuts and maneb was applied to turnip greens, cabbage, and cucumbers. Insecticides (i.e., mazinphos or toxaphene) were applied as needed.

Lipid analyses were by procedures presented earlier (Wilkinson, 1974). Protein analyses were by macro-Kjeldahl analyses (AOCS, 1957). Samples were collected from two crops replicated five times in each of 1971, 1972, or 1973. Analyses of variance were conducted on micrograms of lipid/gram of dry weight for lipid quantity analyses and on percent composition for qualitative analyses, or percent nitrogen content.

RESULTS AND DISCUSSION

Turnip Greens. Fatty acid contents of turnip greens treated with seasonal combinations of pesticides were not significantly different from the untreated crops in either quantity or quality. Percent protein contents and percent dry weight were not influenced by pesticide applications from the untreated check.

Significant differences in qualities of fatty acids were found in samples taken from different cropping systems (Table III). This was an effect of previous cropping rather than previous pesticide influence since the pesticide-treated plot areas were the same in each successive crop and significant differences were not obtained between the untreated plots and pesticide treated areas. Fall grown turnip greens (system 6) preceded by turnip greens, cucumbers, and southern peas had a significantly greater total fatty acid content than turnip greens grown in the other three systems of multiple cropping (Table III). This increased percentage of fatty acids was due to significantly increased quantities of total saturated even numbered, unsaturated, iso-, and anteiso-fatty acids (Table III). With few exceptions, these increases in total fatty acids of the various structural subclasses were reflected in the various individual constituents (Table III).

The increased quantities found in system 6 were not reflected in similar changes in fatty acid quality (Table III).

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