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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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Table I. Intensive Cropping Systems and Pesticides Applied

system	crop	planting date (approx.)	pesticide name	kg/ha
1	turnip greens[a]	20 Feb	DCPA	8.96
	corn[b]	10 Apr	butylate	3.36
			atrazine	1.68
			ametryne	1.12
			ethoprop	8.96
	snapbeans[a]	25 Aug	trifluralin	0.56
			dinoseb	1.68
2	turnip greens	20 Feb	DCPA	8.96
	peanut[b]	10 Apr	benefin	1.40
			vernolate	2.24
			dinoseb	0.56
			ethoprop	8.96
	snapbean[a]	25 Aug	trifluralin	0.56
			dinoseb	1.68
3	turnip greens	20 Feb	DCPA	8.96
	corn[b]		ethoprop	8.96
			butylate	3.36
			atrazine	1.68
			ametryne	1.12
	turnip greens[a]	1 Sep	DCPA	8.96
4	turnip greens	20 Feb	DCPA	8.96
	peanut[b]	10 Apr	ethoprop	8.96
			benefin	1.40
			vernolate	2.24
			dinoseb	0.56
	turnip greens[a]	1 Sep	DCPA	8.96
5	snapbean[a,b]	20 Mar	ethoprop	8.96
			trifluralin	0.56
			dinoseb	1.68
	soybean	1 Jun	vernolate	2.24
			chloroxuron	1.12
			dinoseb	1.68
			linuron	1.12
	transplanted cabbage	25 Oct	DCPA	8.96
6	turnip greens	20 Feb	DCPA	8.96
	cucumber[a,b]	10 Apr	nitralin	0.84
			ethoprop	8.96
	so. pea[a]	10 Jul	trifluralin	0.56
			dinoseb	1.68
	turnip greens[a]	1 Sep	DCPA	8.96

[a] Crop harvested for analysis. [b] Nematicide applied on this crop at planting time.

Table II. Common and Chemical Names of Pesticides Utilized in the Various Intensive-Cropping Sequences

common	chemical	common	chemical
DCPA	dimethyl tetrachloroterephthalate	ethoprop	*O*-ethyl-*S*,*S*-dipropyl phosphorodithioate
butylate	*S*-ethyl diisobutylthiocarbamate	mezinphos	α isomer 2-carbomethoxy-1-methylvinyl dimethylphosphate
atrazine	2-chloro-4(ethylamino)-6-(isopropylamino)-*s*-triazine	maneb	manganese ethylene-1,2-bis(dithiocarbamate)
ametryne	2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-*s*-triazine	toxaphene	chlorinated camphene
benefin	*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine	benomyl	methyl-*N*-benzimidazole-2-yl-*N*-(butylcarbamoyl)carbamate
vernolate	*S*-propyl dipropylthiocarbamate	chloroxuron	3-[*p*-(*p*-chlorophenoxy)phenyl]-1,1-dimethylurea
dinoseb	2-*sec*-butyl-4,6-dinitrophenol	linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
nitralin	4-(methylsulfonyl)-2,6-dinitro-*N*,*N*-dipropylaniline		
trifluralin	α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine		

Table III. Influence of Previous Cropping History on the Fatty Acid Quantity and Quality, Percent Dry Weight, and Percent Nitrogen of Turnip Greens

C_n	system 1	system 3	system 4	system 6
	Percent Fatty Acid			
I12	0.00 b[a]	0.00 b	0.00 b	0.01 a
A12	0.07 ab	0.02 b	0.02 b	0.15 a
12:1	0.00 b	0.00 b	0.00 b	0.06 a
13	0.09 a	0.00 a	0.02 a	0.08 a
I14	0.00 c	0.01 b	0.00 c	0.04 a
14	0.22 b	0.70 ab	0.24 b	1.20 a
14:1	0.12 a	0.05 a	0.02 a	0.05 a
A15	0.07 a	0.00 a	0.05 a	0.00 a
15	0.16 a	0.33 a	0.27 a	0.54 a
I16	0.08 c	1.18 a	0.48 b	1.56 a
16	33.92 a	27.92 bc	32.26 ab	22.98 a
16:1	0.71 c	2.70 b	4.81 a	3.10 b
A17	1.07 a	2.66 a	1.74 a	4.30 a
I18	0.96 b	0.30 b	0.00 b	4.56 a
18	8.48 b	8.58 b	16.16 a	9.50 a
18:1	14.62 a	14.24 a	18.55 a	13.08 a
19	0.59 a	0.82 a	0.10 a	0.31 a
18:2	16.65 bc	25.55 a	20.48 ab	14.38 c
20	3.00 a	0.30 b	0.73 b	0.14 b
18:3	12.06 b	8.02 b	1.93 c	19.93 a

Table III. (*Continued*)

C_n	system 1	3	4	6
	Percent Fatty Acid			
21	0.87 a	0.42 ab	0.08 b	0.23 b
I22	0.02 b	0.05 ab	0.01 b	0.08 a
22	0.81 a	1.29 a	0.59 a	0.46 a
22:1	0.31 a	0.26 a	0.00 b	0.02 b
23	0.54 a	0.25 a	0.00 b	0.04 b
I24	0.18 a	0.03 b	0.00 b	0.26 a
24	1.33 a	1.18 a	0.67 a	1.73 a
24:1	0.02 a	0.08 a	0.02 a	0.06 a
25	0.19 a	0.34 a	0.14 a	0.19 a
I26	0.00 b	0.30 a	0.00 b	0.02 b
26	0.49 a	0.70 a	0.54 a	0.49 a
26:1	0.01 a	0.00 a	0.00 a	0.00 a
27	0.09 ab	0.21 a	0.01 b	0.04 b
28	0.38 b	0.82 b	0.04 b	0.78 a
30	0.00 b	0.38 a	0.00 b	0.16 b
total sat.	6666.8 b	6715.9 b	9625.6 b	19551.4 a[b]
even	49.82 a	41.88 a	51.25 a	37.60 b
odd	2.53 a	2.37 a	0.63 b	1.44 ab
unsat.	44.54 a	50.91 a	45.83 a	50.68 a
iso[c]	1.23 bc	1.85 b	0.51 c	6.54 a
% dry wt	13.82 a	10.45 b	9.16 c	9.06 c
% N (Kjel)	5.61 a	5.62 a	5.56 a	5.37 a

[a] Values in a line in a group of columns followed by the same letter or letters are not significantly different at the 5% level. [b] Total is listed as micrograms of fatty acid/gram of dry weight. [c] Iso- and anteiso-fatty acid identification by relative retention technique. Possible that anteiso is the diunsaturated even-numbered compound immediately preceding (i.e., A17 or 16:2) and iso compound may be the triunsaturated even-numbered compound immediately preceding (i.e., I18 or 16:3).

Table IV. Cucumber Percentage Fatty Acid Composition from Crops Grown in an Intensive-Cropping Sequence

C_n	treatment CK[a]	nem.	herb.	nem. + herb	year 1	2
	Percent Fatty Acid					
15	1.1 a[b]	1.8 a	2.5 a	1.2 a	0.2 a	3.1 a
I16	1.0 a	1.0 a	1.5 a	1.9 a		2.7 a
16	45.8 a	44.9 a	41.7 a	39.8 a	53.6 a	33.2 a
16:1	a	a	a	1.3 a	0.1 a	0.6 a
A17	1.0 a	1.3 a	2.2 a	2.2 a	a	3.4 a
18	17.7 a	14.5 a	20.7 a	17.2 a	24.7 a	10.3 a
18:1	14.6 a	11.8 a	12.9 a	13.8 a	3.8 a	22.7 a
19	a	0.7 a	0.1 a	0.5 a	0.2 a	0.5 a
18:2	8.3 a	6.6 a	6.2 a	5.3 a	0.4 a	12.9 a
20	1.3 a	1.5 a	1.8 a	3.0 a	2.2 a	1.6 a
21	0.2 a	0.4 a	0.3 a	1.7 a	0.3 a	0.9 a
22	2.1 a	2.9 a	2.8 a	3.7 a	3.9 a	1.8 a
22:1	a	0.1 a	0.5 a	0.3 a	0.2 a	0.2 a
23	0.4 b	1.0 a	0.4 b	0.5 b	0.7 a	0.4 a
24	2.8 a	4.6 a	3.6 a	3.3 a	5.4 a	1.8 a
24:1	a	1.6 a	a	0.3 a	a	1.0 a
25	0.5 a	0.7 a	0.4 a	1.4 a	0.7 a	0.7 a
26	1.6 a	3.5 a	2.1 a	2.0 a	2.8 a	1.8 a
27	0.1 a	0.3 a	0.2 a	0.3 a	0.2 a	0.2 a
28	0.5 a	0.8 a	0.1 a	0.3 a	0.5 a	0.3 a
sat. even	73.0 a	72.8 a	72.9 a	69.4 a	93.1 a	50.9 a
sat. odd	2.1 a	5.0 a	3.7 a	5.6 a	2.4 a	5.8 a
unsat.	22.9 a	20.2 a	19.6 a	21.0 a	4.5 a	37.3 a
iso[c]	1.0 a	1.0 a	1.5 a	1.9 a	a	2.7 a
anteiso	1.0 a	1.3 a	2.2 a	2.2 a	a	3.4 a
% dry wt	3.43 a	3.18 a	3.30 a	3.32 a	3.88 a	2.74 a
% N (Kjel)	2.82 a	2.77 a	2.80 a	2.84 a	2.40 a	3.22 a

[a] CK = untreated, hand-hoed check; nem. = nematicide treated; herb. = herbicide treated; nem. + herb. = nematicide + herbicide treated. [b] Values in a line within treatments or years followed by the same letter are not significantly different at the 5% level. [c] Iso- and anteiso-fatty acids identified by relative retention technique. Possible that anteiso is the diunsaturated even-numbered compound immediately preceding (i.e., A17 or 16:2) and iso may be triunsaturated even-numbered compound immediately preceding (I18 or 16:3).

Table V. Cucumber Fatty Alcohol Percentage Composition from Crop Grown in Intensive-Cropping Sequences

	treatment				year	
FAlc	CK[a]	nem.	herb.	nem. + herb	1	2
			Percent Fatty Alcohol			
14	a[b]	a	0.5 a	a	0.0 a	0.2 a
A15	a	a	0.1 a	0.2 a	0.1 a	0.0 a
15	b	b	4.4 a	0.1 b	0.3 a	2.0 a
I16	a	a	0.3 a	0.2 a	0.0 a	0.2 a
16	10.6 a	10.0 a	11.4 a	9.4 a	9.3 a	11.4 a
A17	1.6 a	0.6 a	0.8 a	2.2 a	0.8 a	1.8 a
I18	1.2 a	2.3 a	5.3 a	1.0 a	4.5 a	0.4 a
18	26.6 a	25.9 a	24.6 a	14.6 a	13.9 a	31.9 a
18:1	42.4 a	39.2 a	33.3 a	43.4 a	39.5 a	39.6 a
18:2	7.2 a	4.6 a	7.6 a	11.5 a	13.3 a	2.1 a
18:3	0.9 a	1.2 a	2.7 a	2.0 a	1.9 a	1.4 a
19	a	a	a	1.0 a	a	0.5 a
20	0.9 a	3.0 a	1.6 a	1.4 a	2.8 a	0.7 a
22	0.4 b	0.6 b	3.7 a	0.8 b	2.0 a	0.8 a
23	a	a	0.3 a	a	a	0.1 a
24	2.3 a	3.5 a	4.0 a	3.4 a	3.2 a	3.4 a
25	a	a	0.1 a	a	a	a
26	5.0 a	5.2 a	3.4 a	5.9 a	4.0 a	5.7 a
27	a	0.1 a	0.1 a	0.1 a	0.2 a	0.0 a
28	0.8 a	0.9 a	0.8 a	0.4 a	1.2 a	0.3 a
30	a	2.9 a	0.7 a	2.6 a	3.1 a	a
sat. even	46.7 a	52.1 a	50.6 a	38.5 a	39.6 a	54.4 a
sat. odd	0.0 b	0.1 b	4.9 a	1.3 b	0.4 a	2.7 a
unsat.	50.5 a	44.9 a	43.6 a	56.8 a	54.7 a	43.2 a
iso[d]	1.2 a	2.3 a	5.6 a	1.2 a	4.5 a	0.6 a
anteiso	1.6 a	0.6 a	0.9 a	2.4 a	1.0 a	1.8 a
total[c]	5200.8 b	3573.2 b	8926.1 a	4045.8 b	5354.6 a	5518.3 a

[a] CK = untreated, hand-hoed check; nem. = nematicide; herb. = herbicide treated; nem. + herb. = nematicide + herbicide treated. [b] Values in a line within treatment or year followed by the same letter are not significantly different at the 5% level. [c] Total fatty alcohol = micrograms/gram of dry weight. [d] Iso- and anteiso-fatty alcohols identified by relative retention technique. Possible that anteiso- is the diunsaturated even-numbered compound immediately preceding (i.e., A17 or 16:2) and iso- may be triunsaturated even-numbered compound immediately preceding (I18 or 16:3).

Table VI. Snapbean Fatty Acid Percentage Composition from Crops Grown in Intensive-Cropping Sequences

	treatment				system		
C_n	CK[a]	nem.	herb.	nem. + herb.	1	2	5
			Percent Fatty Acid				
12	0.38 a[b]	0.14 a	0.10 a	0.23 a	0.10 b	0.48 a	0.06 a
I14	0.06 a	0.07 a	0.08 a	0.12 a	0.04 a	0.10 a	0.11 a
14	0.40 a	0.30 a	0.43 a	0.43 a	0.28 b	0.63 a	0.26 a
15	0.59 a	0.44 a	0.42 a	0.40 a	0.27 b	0.84 a	0.27 b
I16[e]	0.51 a	0.62 a	0.51 a	0.33 a	0.70 a	0.68 a	0.11 a
16	25.85 a	24.85 a	25.89 a	30.25 a	27.91 a	24.75 a	27.46 a
16:1	1.29 a	0.90 a	0.57 a	0.97 a	2.06 a	0.70 ab	0.03 b
A17	0.34 a	0.35 a	0.14 ab	b	0.24 a	0.38 a	a
I18	0.73 a	0.38 a	0.52 a	0.73 a	0.84 a	0.74 a	0.19 b
18	8.49 a	7.62 a	9.70 a	9.59 a	10.87 a	6.79 a	8.88 a
18:1	12.43 a	12.18 a	14.52 a	14.13 a	22.16 a	10.91 b	6.87 b
18:2	22.47 a	22.79 a	24.07 a	21.74 a	18.40 b	25.35 a	24.56 a
18:3	12.27 b	14.63 a	13.30 ab	12.06 b	3.03 c	15.78 b	20.38 a
21	1.04 a	1.30 a	0.84 ab	0.38 b	0.86 a	1.27 a	0.54 a
22	2.69 a	2.15 b	2.05 b	1.75 b	1.97 a	2.31 a	2.20 a
23	0.95 a	1.42 a	1.00 a	0.80 a	1.09 a	1.32 a	0.72 a
24	2.67 a	2.78 a	2.22 a	2.02 a	2.46 a	2.60 a	2.20 a
25	0.88 a	1.22 a	0.90 a	0.51 a	1.07 a	0.98 a	0.58 a
26	1.39 a	1.94 a	0.90 a	0.83 a	1.69 a	1.34 a	0.76 a
27	0.80 a	0.81 a	0.18 b	0.08 b	1.08 a	0.27 ab	0.05 b
28	0.61 a	1.05 a	0.25 a	0.37 a	0.94 a	0.15 a	0.62 a
total[c]	6151.0 a	6949.6 a	5383.0 a	6118.1 a	7489.8 a	5837.1 a	5121.3 a

Table VI. (*Continued*)

C_n	treatment				system		
	CK[a]	nem.	herb.	nem. + herb.	1	2	5
			Percent Fatty Acid				
% dry wt	6.27 b	5.85 c	6.74 a	6.60 ab	5.12 b	6.95 a	7.02 a
% H_2O	11.41 a	11.82 a	11.42 a	11.31 a	10.67 a	11.89 a	11.90 a
% N[d]	3.04 a	3.10 a	2.91 b	2.89 b	2.79 b	3.06 a	3.10 a

[a] CK = untreated, hand-hoed check; nem. = nematicide treated; herb. = herbicide treated; nem. + herb. = nematicide + herbicide treated. [b] Values in a line within treatment or system followed by the same letter are not significantly different at the 5% level. [c] Total = micrograms/gram of dry weight. [d] N = % Kjeldahl nitrogen. [e] Iso- and anteiso-fatty alcohols identified by relative retention technique. Possible that anteiso- is the diunsaturated even-numbered compound immediately preceding (i.e., A17 or 16:2) and iso- may be triunsaturated even-numbered compound immediately preceding (i.e., I18 or 16:3).

Table VII. Southern Pea Fatty Acid Content from Crops Grown in Intensive-Cropping Sequences and Multiple Pesticide Applications

	CK[a]	nem.	herb.	nem. + herb.
		Fatty Acids		
15	2.0 a[b]	0.2 a	0.3 a	0.6 a
16	31.2 a	27.2 a	29.9 a	28.1 a
16:1	1.9 a	3.1 a	3.9 a	3.5 a
18	13.4 a	12.1 a	11.7 a	11.6 a
18:1	10.7 a	13.1 a	9.9 a	10.7 a
18:2	17.6 a	19.9 a	18.6 a	21.9 a
20	10.9 a	12.0 a	11.7 a	11.6 a
21	0.4 a	0.7 a	0.8 a	0.7 a
22	5.9 a	5.5 a	6.4 a	5.7 a
23	1.2 a	0.6 a	0.7 a	0.6 a
24	3.0 a	3.4 a	3.5 a	3.3 a
25	0.5 a	0.7 a	0.3 a	0.3 a
26	0.4 a	0.6 a	0.5 a	0.6 a
27	0.3 a	0.1 a	0.1 a	0.2 a
28	0.5 a	0.1 a	0.1 a	0.1 a
total[c]	5863 a	6175 a	5670 a	6456 a
% N[d]	3.84 a	3.81 a	3.90 a	3.84 a

[a] CK = untreated, hand-hoed check; nem. = nematicide treated; herb. = herbicide treated; nem. + herb. = nematicide + herbicide treated. [b] Values in a line followed by the same letter are not significantly different at the 5% level. [c] Total = micrograms/gram of dry weight. [d] N = % Kjeldahl basis.

For example, palmitic acid (C_{16}) was present in greater quantity in system 6 but this represented a lower percentage composition of palmitic acid in system 6 than in systems 1 and 4. Stearic acid (C_{18}) quantity was greatest in system 6 but the percentage composition of stearic acid was greatest in system 4. Oleic acid ($C_{18:1}$) quantity was greatest in system 6 but the percentage composition of stearic acid was not statistically different between the different multiple cropping systems. Similar observations were made in many of the individual constituents and the percentage totals of the various structural components. Percentage dry weight was significantly greatest in system 1 and least in system 6. Percentage total N did not vary between the various multiple cropping systems. Thus, pesticide applications influenced neither fatty acid quantity and quality, percentage dry matter, nor percentage protein as greatly as previous cropping history.

Cucumber. The quantity and quality of cucumber fatty acids was not influenced by either pesticide treatment or year of growth (Table IV). The quality of fatty alcohol extract was influenced by pesticide treatment. These were C_{15}, C_{16}, C_{I18}, C_{22}, C_{24}, C_{27} (Table V). The only major constituent that was significantly influenced by pesticide applications was C_{16} and the remaining constituents showed minor changes. Total fatty alochol content was increased by herbicide treatment, and this was consistent throughout the subclass totals (Table V). Years of production did not alter the quantity of fatty alcohols found in cucumbers. Percentage composition of fatty alcohols present in cucumbers was not significantly altered by pesticides or years of production with the exception of the minor constituent C_{22} which was increased by herbicide treatment (Table V). Total N and percent dry weight were not influenced by pesticide treatment or years (Table V).

Snapbean. Percent nitrogen was influenced by pesticides and systems. On a percentage composition basis, the major constituent $C_{18:3}$ was influenced by pesticides as were also the minor constituents C_{21}, C_{22}, and C_{27} (Table VI). But on an intensive-cropping system basis, major constituents $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ were significantly different between systems as were, also, the minor constituents C_{15}, $C_{16:1}$, C_{18}, and C_{27}.

Southern Peas. Qualitative changes were not induced by pesticide applications in southern peas (Table VII).

CONCLUSIONS

Four crops were grown in intensive-cropping sequences where each was subjected to multiple pesticide or residue interactions. In general, each of these pesticides is registered for use as a single application. And, in some instances, the registration restricts the use of the land for any other crop until the following growing season. Thus, the overall pattern demonstrated by these data is the remarkable lack of pesticide influence on vegetative crop quality. In conjunction with the seed oil data presented earlier, these data strongly suggest that there is no need for concern about the quality of crops grown using pesticides.

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COMMUNICATIONS

Biosynthesis of [^{14}C]Patulin by *Penicillium urticae*

Carbon-14-labeled patulin was biosynthesized by *Pencillium urticae* using sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, or [U-^{14}C]glucose. Patulin yield ranged from 455 to 897 mg/100 mL of potato dextrose media after incubation at 20 °C for 14 days. The percentage conversion of carbon-14 label into patulin was 9.0–9.3% for sodium [2-^{14}C]acetate, 0.3–0.6% for sodium [1-^{14}C]acetate, and 6.0–7.6% for [U-^{14}C]glucose. Ten microCuries of sodium [2-^{14}C]acetate produced the highest specific activity of 0.189 μCi/mmol of patulin.

Patulin, 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one, is a toxic secondary metabolite produced by several species of *Aspergillus*, *Penicillium*, and other related fungi isolated from food material (Buchanan et al., 1974; Ciegler et al., 1971; Scott, 1974). Patulin was originally isolated for its antibiotic activity (Katzman et al., 1944), but has since been demonstrated to be a carcinogenic (Dickens and Jones, 1961) and mutagenic mycotoxin (Mayer and Legator, 1969). The occurrence of patulin in foods and feeds (Buchanan et al., 1974; Harwig et al., 1973; Scott et al., 1972; Stott and Bullerman, 1975a) represents considerable environmental hazard to humans and animals.

Metabolism studies of patulin require labeled toxin of high specific activity. Several methods have been reported for possible incorporation of carbon-14 into patulin with *P. patulum* from readily available, inexpensive precursors, acetate and glucose (Bassett and Tannenbaum, 1960; Scott and Beadling, 1974).

MATERIALS AND METHODS

Media and Cultures. *Penicillium urticae* was obtained from the culture collection at Washington State University (Pullman, WA) and transferred twice on potato dextrose agar (Difco, Detroit, MI). A suspension was made from the mycelia. One-liter culture flasks containing 100 mL of potato dextrose media autoclaved at 120 °C for 45 min were inoculated with 10-mL suspensions of *P. urticae* according to the method of Norstadt and McCalla (1969). Ten microCuries of sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, or [U-^{14}C]glucose was added to duplicate culture flasks. Flasks were placed on their sides for maximum surface area and incubated without agitation at 20 °C for 14 days.

Extraction and Semipurification. The media and mycelial mat were extracted three times with 100 mL of ethyl acetate in a large separatory funnel. Extracts were combined and rotary evaporated to 5 mL. Semipurification of the patulin was accomplished on an alumina column as described by Norstadt and McCalla (1969).

Quantitation of ^{14}C. The semipurified patulin samples were quantified using a fluorodensitometric thin-layer chromatography (TLC) method developed by Salem and Swanson (1976). Activated 250 μm TLC silica gel G plates were spotted with semipurified [^{14}C]patulin samples along with known concentration of patulin standard and run in benzene–methanol–acetic acid (90:5:5). Spots with a R_f of 0.20 were developed in concentrated ammonia fumes for 30 min and then quantified with a fluorodensitometer equipped with a TLC scanning unit. The maximum excitation wavelength was 254 nm and the maximum emission wavelength was 415 nm. Patulin spots were scraped, dissolved in Scinti Verse (Fisher Scientific Co., Fair Lawn, NJ), counted in a liquid scintillation counter (Packard Model 3255 Tri-Carb), and specific activity calculated.

Radiopurity. Radioactive patulin samples from TLC were run on a high-pressure liquid chromatograph (LC) to determine radiopurity. LC equipment consisted of a Waters Associates 6000A pump, Model U6K injector, and a Micrometrics Model 785 variable-wavelength detector set at 320 nm. Separation was achieved with a Lichrosorb ODS precolumn (4.6 mm i.d. × 5 cm), followed by a Zorbax ODS column (4.6 mm i.d. × 25 cm) using a solvent system of acidified acetonitrile (9 vol of acetonitrile to 91 vol of 0.5% formic acid) previously filtered through a 0.45-μm Millipore filter. Flow rate was 2.5 mL/min. Retention times were measured with a Spectra Physics Minigrator and compared with known patulin standards.

Antibiotic Activity. In addition to chemical analyses, biological assays were used to determine the antibiotic activity of patulin using streak plate methods (Geiger and Conn, 1945) or disc assay methods (Stott and Bullerman, 1975b). Blank Bacto half-inch sterile sensitivity discs were immersed in TLC purified solutions of [^{14}C]patulin and a known standard (3 mg/mL) until saturated. Discs were immediately placed on a violet red bile agar plate which was inoculated with a lawn of *Escherichia coli*. The plates were incubated at 37 °C for 48 h and zones of inhibition were observed.

RESULTS AND DISCUSSION

The yield of patulin obtained from the potato dextrose cultures ranged from 455–897 mg/100 mL of medium (Table I). This represents an increase of two–three times that reported previously by Norstadt and McCalla (1969) with *P. urticae*. This discrepancy may be due to the method of purification. In this study, TLC rather than crystallization was used to purify patulin. Crystallization techniques often result in the loss of some product. LC demonstrated that the TLC purified [^{14}C]patulin had a

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