Effects of Several Foliar Fungicides on the Fatty Acid Composition and Stability of Peanut Oil

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Foliar fungicides applied to peanuts for the control of Cercospora leafspot during one and/or two growing seasons caused small but statistically significant (p = 0.01) changes in fatty acid composition of peanut oil. Levels of linoleic acid were higher (*ca.* 1 to 2%) in oil obtained from Argentine and Florunner varieties and lower (*ca.* 0.5 to 1%) in Florigiant variety plots treated with fungicide compared with control samples. Treatment effects on oil stability (autoxidation induction period) were small but occasionally statistically significant. Treatment effects on oil stability and fatty acid composition were in all cases, however, no larger than normal year to year fluctuations.

The peanut (*Arachis hypogaea* L.) is highly indeterminate in growth habit and under favorable environmental conditions continues to produce fruit over an extended period of time. As a practical consequence of this characteristic, the harvested crop includes varying proportions of immature seeds that differ from mature seeds in fatty acid composition (Worthington, 1969; Young *et al.*, 1972) and other characteristics (Pang, 1967).

Until recently, fruiting under normal field conditions has been effectively terminated by epidemic levels of *Cercospora arachidicola*, a pathogenic fungus that causes a characteristic leafspot disease and subsequent defoliation. The recent introduction of fungicides highly effective against this organism and rapid adoption of these materials in cultural practices have resulted in a marked change in the physiological condition of plants during the latter period of fruit set and fruit development (Harrison, 1969; Porter, 1970). Under these conditions the leaf canopy is frequently retained intact until harvest, the period of fruit development is lengthened considerably, and yield is greatly increased.

In view of the drastic change in preharvest physiological condition of the plant resulting from the use of fungicides and of possible effects on seed composition, an investigation was made of the effects of these agents on oil stability and fatty acid composition.

MATERIALS AND METHODS

The chemical agents employed in this study were applied according to accepted cultural practices to peanuts planted in randomized blocks with four replications per treatment in 1970 and three replications in 1971. Three application schedules were followed: 14-day, 21-day, and as indicated by meteorological conditions (Jensen and Boyle, 1966). In view of the previously demonstrated relationship between seed maturity and fatty acid composition (Worthington, 1969; Young *et al.*, 1972) and of the possible relationship between fungicide treatments and seed maturity, some of the test plots were harvested at 10- to 20-day intervals to determine possible interactions between treatment and time of harvest. In treatments where time of harvest was an additional variable, harvest dates were treated as subplots. Test varieties included Argentine at Plains, Georgia, during 1970 and 1971 and Florigiant and Florunner at Tifton, Georgia, during 1970 and 1971, respectively.

The fungicides evaluated were: Benlate [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]; Bravo (tetrachloroisophthalonitrile), Fungisperse (45% sulfur + 9.2% zinc ethylenebisdithiocarbamate), Polyram [a mixture of 5.2 parts by weight of ammoniates of ethylenebis(dithiocarbamato)zinc with 1 part by weight of ethylenebis(dithiocarbamic acid) biomolecular and trimolecular cyclic anhydrosulfides and disulfides], and copper–sulfur dust (3.4% copper as cuprous and cupric oxides; 81.5% sulfur) applied in 1970 and 1971; Mertect [2-(4-thiazolyl)-benzimidazole] and DuTer (triphenyltin hydroxide), applied in 1970; and Kocide (cupric hydroxide) and BAS-3021-F [1-([2-(methylthio)ethyl]carbamoyl)-2-(methoxyamino)benzimidazole], applied in 1971. Tests included a total of 120 field plots in 1970 and 138 in 1971.

Peanuts were harvested, dried, and shelled according to accepted practices. Samples were obtained with official Federal-State Inspection Service grading screens. As a check on laboratory procedure, samples from each field plot were analyzed in duplicate for oil stability in 1970 and for fatty acid composition by gas–liquid chromatography in 1970 and 1971. Duplicate values were averaged prior to an analysis of variance. The precision and accuracy of the analytical procedures employed have been described previously (Worthington *et al.*, 1972).

The data were subjected to an analysis of variance to determine significance of treatments and date of harvest on oil stability in 1970 and on fatty acid composition in 1970 and 1971. The means of those treatments found to be significant at the 5% level or higher were submitted to the Duncan's multiple range test. Data from some of the treatments common to both years were combined to test for significance of year effects and treatment × year and other interactions.

RESULTS AND DISCUSSION

Treatment effects on fatty acid composition, though frequently statistically significant, were small in magnitude and appeared to be of little practical significance. Treatment effects on oil stability were small but occasionally significant (Table III). Some representative data showing treatment effects on fatty acid composition and oil stability in 1970 and on fatty acid composition in 1971 are presented in Tables I–III. Other data show treatment effects similar to those given in Tables I through IV and will appear in the microfilm edition of this volume of the journal.

Those fungicides most effective in control of leafspot, *i.e.*, Benlate and Bravo, gave the most pronounced effects on oil fatty acid composition. In both 1970 and 1971, Benlate and Bravo decreased levels of stearic (18:0) and increased levels of linoleic (18:2) acid over controls in the Argentine variety. These treatment effects produced a maximum increase in calculated iodine number of approximately 1.7 units over those of controls. In a 1 year study (1971), BAS3201-F, a promising new fungicide of the benomyl type, gave treatment effects similar to those observed with Bravo and Benlate. Fungisperse and Polyram produced a similar effect in 1970 (Table I). Mertect

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Table I. Effect of Six Applications (14-Day Schedule) of Certain Foliar Fungicides on the Stability and Fatty Acid Composition of Oil from Argentine Peanuts Harvested in 1970

Treatment	Oil stability[a]	Fatty acid, %[b]							
		16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Control	11.1	12.69ab	4.15a	42.32	33.79b	1.60	0.72b	3.43b	1.30
Benlate	10.9	12.68ab	3.64b	42.01	34.56a	1.54	0.81a	3.44b	1.94
Bravo	11.4	12.42b	3.55b	42.11	34.79a	1.62	0.80a	3.40b	1.33
Fungisperse	12.0	12.48b	3.53b	42.42	34.63a	1.50	0.80a	3.33b	1.35
Polyram	11.5	12.36b	3.72b	42.53	34.52a	1.50	0.77a	3.32b	1.30
Copper-sulfur	11.4	13.05a	4.07a	41.45	33.49b	1.72	0.71b	4.01a	1.52
Significance[c]	N.S.	*	**	N.S.	**	N.S.	**	**	N.S.

[a] Length of autoxidation induction period in days. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *,** mean square values significant at the 5 and 1% level, respectively; N.S., not significant.

Table II. Effect of Six Applications (14-Day Schedule) of Certain Foliar Fungicides on the Fatty Acid Composition of Oil from Argentine Peanuts Harvested in 1971

Treatment	Fatty acid, %[b]							
	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Control[a]	11.42	4.71	43.28	33.31	1.98	0.84	3.25	1.19
Benlate	11.55	4.15d	42.61b	34.24a	1.92c	0.88a	3.39	1.28a
Bravo	11.55	4.42c	43.14a	33.63b	1.91c	0.83b	3.32	1.20b
Fungisperse	11.49	4.70b	43.36a	33.18c	1.97b	0.81b	3.28	1.21b
Polyram	11.48	4.91a	43.45a	32.95d	2.00a	0.81b	3.26	1.18b
Copper-sulfur	11.46	4.73b	43.32a	33.13c	2.00a	0.82b	3.30	1.22ab
Significance[c]	N.S.	**	**	**	*	*	N.S.	*

[a] Control values are average of two harvest dates and were not included in analysis of variance. Treatment values obtained from four harvest dates. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *,** mean square values significant at the 5 and 1% level, respectively; N.S., not significant.

Table III. Effect of Five Applications (14-Day Schedule) of Certain Foliar Fungicides and Time of Harvest on the Stability and Fatty Acid Composition of Oil from Florigiant Peanuts Harvested in 1970

Treatment	Oil stability[a]	Fatty acid, %[b]							
		16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Control	14.5a	10.37a	3.58	52.82c	26.22	1.45	0.95	3.08	1.55
Benlate	14.1ab	9.74c	3.75	53.62ab	25.91	1.44	0.96	2.98	1.59
Bravo	14.0ab	9.66c	3.63	54.15a	25.62	1.52	0.97	2.90	1.54
Polyram	12.6c	10.09b	3.58	53.37bc	26.19	1.42	0.96	2.81	1.57
Copper-sulfur	13.5b	9.82bc	3.59	53.07bc	26.48	1.53	0.98	2.97	1.57
Significance[c]	**	**	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.
Harvest date									
9/4/70	13.5	9.92	3.57	52.64	26.71	1.50	0.96	3.06	1.63
9/24/70	14.1	9.95	3.68	54.16	25.45	1.44	0.97	2.83	1.50
Significance[c]	*	N.S.	**	**	**	*	N.S.	**	**
Treatment × harvest									
Significance[c]	*	N.S.	*	*	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Length of autoxidation induction period in days. [b] Values followed by the same letter are not significantly different at 5% level. [c] *,** mean square values significant at 5 and 1% level, respectively; N.S., not significant.

Table IV. Effect of Delayed Harvest on Fatty Acid Composition of Florunner Peanuts Treated with Fungicides in 1971[a]

Harvest date	Fatty acid, %[b]							
	16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0
Sept 10	10.14	2.20c	48.80b	31.21a	1.34	1.43	3.13	1.76
Sept 20	10.11	2.27b	49.01b	31.06a	1.33	1.41	3.10	1.71
Sept 30	10.13	2.34a	49.57a	30.39b	1.36	1.41	3.08	1.71
Significance[c]	N.S.	**	**	**	N.S.	N.S.	N.S.	N.S.

[a] Average values from plots treated with Benlate, Bravo, Kocide, copper-sulfur, and the appropriate control. [b] Values followed by the same letter are not significantly different at the 5% level. [c] *,** mean square values significant at the 5 and 1% level, respectively; N.S., not significant.

and DuTer produced a small but insignificant increase in linoleic acid and a decrease in stearic acid when applied to the Argentine variety in 1970. Treatment effects of Benlate and Bravo on the Florunner variety were similar to those observed with the Argentine variety but were smaller in magnitude. Significant effects (decrease) were observed for palmitic (16:0) and stearic acid. Kocide produced a small but significant decrease (16:0) when applied to the Florunner variety. In the Florigiant variety (Table III), Benlate and Bravo decreased levels of palmitic and linoleic acid and increased levels of stearic and oleic (18:1) acid over controls.

Effect of time of harvest was most pronounced in the Florigiant and Florunner varieties (Tables III and IV). With the Argentine variety, the effect of delayed harvest was occasionally significant for some fatty acids. This effect was small, however, and apparently random in that observed differences did not follow a consistent pattern with respect to time of harvest. Treatment × time-of-harvest interaction was significant only in the Florigiant variety (Table III).

When data from the treatments shown in Tables I and II were combined for both years, an analysis of variance showed highly significant year effects for all fatty acids, significant treatment effects for all fatty acids except arachidic (20:0), and significant year × treatment interaction for all fatty acids except arachidic and eicosenoic (20:1). Date of harvest and other interaction terms were nonsignificant for the combined data.

The differences in fatty acid composition due to treatment observed in this study were no larger than usual year to year differences observed in this and previous studies (Worthington and Hammons, 1971; Worthington *et al.*, 1972) and are probably of little practical importance. The source of these differences is unknown but may be associated with an extended period of plant vigor and a change in the proportions of mature and immature seeds in samples from treated plots as compared with samples from untreated controls.

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Reaction Gas Chromatographic Analysis of Pesticides. II. On-Column Transesterification of Organophosphates by Methanol

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A method is described for the on-column transesterification of four classes of organophosphate pesticides to the corresponding methyl esters, chromatography on Porapak P or Q, and detection by Rb_2SO_4 pellet alkali flame ionization detector. Reproducible conversions were obtained for transesterification with methanol. Distinguishing gas chromatographic peaks were also obtained when the organophosphates were injected in ethanol, 1-propanol, and 1-butanol. Chromatographic and detector conditions are given for the simultaneous analysis of a carbamate (Mobam) with the four classes of organophosphate pesticides.

Organophosphate pesticides have been analyzed using gas chromatographic techniques that would be applicable for screening purposes utilizing either the phosphorus-sensitive flame photometric detector or the alkali flame ionization detector (Beroza and Bowman, 1968; Bowman and Beroza, 1970; Watts and Storherr, 1969). Thin-layer techniques have also been employed using both one-dimensional and two-dimensional development (Gardner, 1971). With the approximately 60 organophosphate pesticides that are now marketed, it has become increasingly difficult to verify the identity of a particular gas chromatographic peak or thin-layer spot. Usually multiple columns must be employed in gas chromatography or multiple solvent systems and visualization reagents in thin-layer chromatography. A gas chromatographic method which would give responses characteristic of the various classes of organophosphates would seem to be of value.

The term reaction gas chromatography was coined in 1960 (Drawert *et al.*, 1960) and has evolved to include any structural change of a compound occurring within the gas chromatograph. High temperature pyrolysis of a compound to obtain a fingerprint is the most common technique and is used frequently in the petroleum industry.

Esposito and Swann (1969) formed trimethylsilyl derivatives of some polyols by an on-column reaction. Almost concurrently, Jaglan and coworkers (1969) esterified the dealkyl metabolites of methyl parathion and methyl paraoxon on a gas chromatographic column. Spengler and

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