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Decreased Lipolysis in Peanuts by a Pyridazinone Bioregulator

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Abstract. Peanut, *Arachis hypogaea,* plants were treated in the field with the bioregulator BAS 105 00W, 4-chloro-5-dimethylamino-2-phenylpyridazin-3-one, a substituted pyridazinone, at different times of development. The seeds were harvested, dried, hand-shelled, and analyzed for lipoxygenase activity and conjugated diene hydroperoxide content. Reduced lipoxygenase activity occurred when the bioregulator was applied to the plants at flowering and pegging. The conjugated diene hydroperoxide content decreased the most in peanuts when the bioregulator was applied at pegging. The apparent K_m for lipoxygenase of treated peanuts with linoleic acid as substrate was the same as that for untreated peanuts.

Genetic breeding is an ideal way to incorporate desirable properties into seeds, but this can take several generations to reach the final goal. Plant growth hormones and/or chemical bioregulators are being investigated as alternative methods for effecting changes within one growing season. Results that demonstrated the possible use of bioregulators for improving the quality of peanuts, cottonseeds, and soybeans have been presented in preliminary reports (Ory et al. 1981, 1982, 1984). In these reports, lipoxygenase activity in treated peanuts and soybeans was decreased. Other reports have shown that substituted pyridazinones play important roles in lipid-metabolizing systems. For example, St. John and Christiansen (1976) showed that the composition of polar lipids of cotton seedlings was changed after treatment with the bioregulator BASF 13 338 by decreasing the relative proportion of linolenic acid in the membranes. This same phenomenon was later shown in bioregulator-treated cereals (St. John et al. 1978) and other food crops (Rittig et al. 1983). Ashworth et al. (1981) reported that bioregulator-treated wheat seedlings had reduced levels of linolenic acid and increased levels of linoleic acid in the phosphatidylcholine,

phosphatidylethanolamine, and total polar lipid fractions. The bioregulator did not affect the levels of palmitate, stearate, and oleate, or the distribution of phospholipid classes. Treatment with pyridazinones has been reported to reduce 18:3 synthesis in wheat (St. John and Hilton 1976) and oats (Jusaitis et al. 1982). More recently, St. John et al. (1984) reported that substituted pyridazinones can inhibit the formation of linolenic acid and also inhibit lipoxygenase in soybeans. Thus, there is growing evidence that pyridazinones, such as BASF 13 338, can play a major role in lipid metabolism. We report data to support our preliminary findings that peanut lipoxygenase activity and the enzyme-substrate reaction products, conjugated diene hydroperoxides, are decreased by treatment of the plants with BAS 105 00W.

Materials and Methods

Materials

The substituted pyridazinone 4-chloro-5-dimethylamino-2-phenylpyridazine-3-one, BAS 105 00W, also known as BAS 13 338 and Sandoz 9785, was the bioregulator used throughout these experiments. Raw peanuts (*Arachis hypogaea*, Florigiant and Spanish varieties) were grown in Greenville, Mississippi. BAS 105 00W was supplied by the BASF Co., Limburgerhof, Federal Republic of Germany. Linoleic acid was purchased from Applied Science Laboratories, Inc.

Methods

Plots were treated at various times of growth as follows: FLO, at flowering; PEG, at pegging. Treatments consisted of applying the bioregulator dissolved in water in concentrations ranging from 0.5 to 2.24 kg/ha. Control plots were untreated. Peanut lipoxygenase was prepared from raw peanuts by the procedure previously described (St. Angelo and Ory 1972). Briefly, quiescent seeds, hulls and testa removed, were homogenized in two volumes of deionized water at 0°C for a total of 45 s at alternating 15-s intervals. The homogenate was squeezed through eight layers of cheesecloth and centrifuged at 30,000*g* for 20 min at 4°C. The supernatant was removed and recentrifuged twice under the same conditions.

Solid ammonium sulfate was added at concentrations from 25% to 40%, and the resulting precipitate (at 40%) was collected, resuspended in deionized water, and dialyzed against deionized water overnight at 4°C. The 40% saturated ammonium sulfate fraction was used as the source of the enzyme. Substrate solutions consisted of 1 ml of 1% linoleic acid solution in ethanol, 99 ml buffer, and 0.1 ml pure Tween 20. The buffer solution contained 0.1 M sodium phosphate, pH 6.2, for measurement of peanut lipoxygenase activity. The substrate solution was sonicated for 30 s, divided into four equal portions, and stored at −20°C until needed. Lipoxygenase activity was measured spectrophotometrically at 234 nm as previously described (St. Angelo and Ory 1972).

Table 1. Effect of BAS 105 00W treatment of peanuts on lipoxygenase activity and conjugated diene hydroperoxide.

	Treatment		Lipoxygenase activity	
Sample	Time	Amount applied (kg/ha)	Specific activity (ΔA/min/mg N)	Relative activity (% of total)
Florigiant peanuts				
1	—	None	0.50	100
2	FLO	0.50	0.36	72
3	FLO	2.00	0.21	42
4	PEG	0.50	0.21	42
5	PEG	2.00	0.20	41
	Treatment		CDHP	
Sample	Time	Amount applied (kg/ha)	(μmole/g)	(% of total)
Spanish peanuts				
1	—	None	0.88	100
2	FLO	0.5	0.65	74
3	FLO	2.0	0.82	93
4	PEG	0.5	0.69	78
5	PEG	2.0	0.65	74

N, nitrogen; FLO, at flowering; PEG, at pegging.

Activity was calculated as the rate of change in absorption per minute per milligram of protein nitrogen (Kjeldahl). Conjugated diene hydroperoxide (CDHP) content was measured as previously described (St. Angelo et al. 1975); 24,500 was used as the molar absorptivity (Johnson et al. 1961). CDHP values were reported as micromoles per gram of sample.

Results and Discussion

Total lipoxygenase activity was decreased 28% in peanuts treated with 0.5 kg/ha of BAS 105 00W at flowering (Table 1). When the dosage was increased fourfold, the total activity of the enzyme decreased by 58%. At pegging, the activity was decreased by 58% at both concentrations. In stored raw peanuts, lipoxygenase is the prime catalyst for the production of hydroperoxides (St. Angelo and Ory 1975, St. Angelo et al. 1977a,b).

Hydroperoxides, which result from the oxidation on an unsaturated fatty acid, such as linoleic acid, form a conjugated diene chromophore that has a maximum absorption at 234 nm. By spectrophotometrically measuring the amount of CDHP formed, one can determine the degree of oxidation, or rancidity, in an oilseed, as reported in storage studies with peanuts (St. Angelo and Ory 1975, St. Angelo et al. 1977a,b). Table 1 also lists the CDHP values from peanuts that have been treated with BAS 105 00W in four different applications. In all of the treatments, results showed that CDHP values were reduced in seeds from bioregulator-treated plants, regardless of the method of

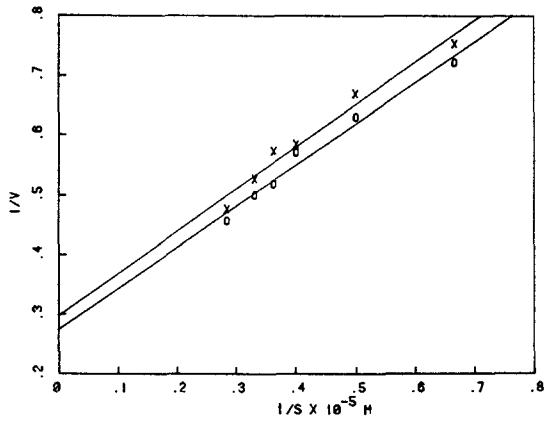

Fig. 1. Lineweaver-Burk plots for partially purified lipoxygenase from raw peanuts. Untreated, O–O; bioregulator-treated, X–X. Substrate solution contained linoleic acid, Tween 20, sodium phosphate buffer, pH 6.2. Enzyme concentration was 6 mg of protein per assay.

application. CDHP values showed decreases ranging from 7% to 26%. Since the reduced CDHP values indicate less oxidation occurring, treated seeds should be expected to have a longer shelf life than the controls.

The apparent Michaelis-Menten constants were calculated from Lineweaver-Burk plots (Fig. 1) for peanut lipoxygenase with linoleic acid as substrate for the treated and untreated peanut enzyme systems. Results indicated that there were no apparent differences between the two constants. The control peanut lipoxygenase had an apparent K_m of 2.53×10^{-5} M, whereas that for treated peanuts was 2.63×10^{-5} M. These data suggest that, although total enzyme activity is reduced, affinity for the substrate remains the same.

In summary, the pyridazinone bioregulator BAS 105 00W, when applied postemergence, is capable of reducing peanut lipoxygenase activity by as much as 59% when 2 kg/ha was added at pegging. CDHP values from treated peanuts also showed decreases. Owing to the decrease in lipoxygenase activity and the reduced CDHP values in peanuts treated with the bioregulator BAS 105 00W, these results suggest a potential for increased shelf life of treated peanuts. Storage studies and effects on calcium content, carbohydrates, polyphenolics, and the biosynthesis of lipoxygenase are currently being investigated in our laboratory.

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