Signaling by Glutamate Dehydrogenase in Response to Pesticide Treatment and Nitrogen Fertilization of Peanut (*Arachis hypogaea* L.)

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The responses of glutamate dehydrogenase (GDH) to NH_4^+ and herbicides offer a new approach for probing the effects of NH_4^+−pesticide interactions at the whole-plant level. Although pesticides and fertilizers have greatly enhanced food production, their combined biochemical effects are not known in detail. Peanut plants were treated with different rates of Basagran (3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide), Bravo 720 (tetrachloroiso-phthalonitrile), and Sevin XLR Plus (1-naphthyl *N*-methylcarbamate), with and without 25 mM NH_4Cl fertilization. Isoelectric focusing, followed by native 7.5% polyacrylamide gel electrophoresis (PAGE) fractionated the peanut seed GDH fully to its isoenzyme population patterns. The pesticide treatments induced positive skewing of the GDH isoenzymes, but NH_4Cl−pesticide cotreatments induced a negatively skewed distribution. Basagran, Sevin, and Bravo increased the amination activities of GDH from 30.0 ± 2.8 units in the control assay to 479.0 ± 20.7, 63.0 ± 5.8, and 35.2 ± 2.2 units, respectively, therefore indicating a direct GDH−pesticide interaction. Neither the NH_4^+ nor the pesticides increased the peanut seed protein yields above the threshold of 3.8 ± 0.7 g per pot. But in the GDH combination of the signals from a pesticide and NH_4^+, at least 70% of the pesticide signal was overridden by NH_4^+ with concomitant increases in peanut seed protein yields to 7.0 ± 1.8 g per pot. Basagran, Sevin, and Bravo possess different pesticidal properties, but their effects on GDH activity were related in the decreasing order of their nucleophilicity, viz. Basagran > Sevin > Bravo.

Keywords: *Arachis hypogaea; glutamate dehydrogenase; signaling; ammonium; pesticides; protein yield*

INTRODUCTION

Organic pesticides have greatly enhanced the production and quality of food, feed, and fiber, as well as the control of disease vectors and pests adversely affecting the health and welfare of the world (Himel et al., 1990). Also, chemical fertilizers have accounted for up to 50% of the increase in crop yields worldwide during this century (Borlaug and Dowswell, 1994). Although agriculture represents the largest single market for pesticides (USEPA, 1979), the effects of pesticide−fertilizer interactions on whole-plant biochemistry are not fully understood. However, the enzymic mechanisms in carbon assimilation (Devine et al., 1993; Daie, 1991; Boger, 1989) and amino acid metabolism (Shaner, 1989) were understood by treatment of plants with pesticides. Conversely, only a few studies were reported on the herbicidal inhibition of NH_4^+ assimmilation by glutamate dehydrogenase (GDH) (Osuji and Madu, 1997a; Osuji, 1997).

Because plants absorb, translocate, and accumulate organic compounds (Thompson, 1983; Briggs et al., 1982; Sirons et al., 1982), the isomerization of GDH in response to herbicides (Osuji, 1997) and phytohormones (Osuji and Madu, 1997b) suggests that the enzyme could be sensitive to organic pesticides in general. Bioregulator inhibition of the glutamine synthetase−glutamate synthase (GS-GOGAT) cycle, but enhancement of the GDH amination activity equally enhanced the protein yields of crops (Osuji and Cuero, 1991a, 1992a,b; Osuji and Madu, 1996). Therefore, the importance of possible GDH response to pesticides is that it may illuminate the metabolic signaling by pesticides and other plant biochemical regulators, and thus provide (1) a method for diagnosing the effects of pesticides on plant NH_4^+ metabolism, and (2) a technology for manipulating the isomerization of the enzyme to optimize crop and crop protein yields with minimum nitrogen fertilizer input. GDH responds to many stress factors including water, salinity, and day/night cycles (Srivastava and Singh, 1987). So far, the GDH isoenzyme population pattern elicited by each inducer has been shown to be unique to the inducer. Because of the enormous differences in the physical and chemical properties of its inducers, GDH is expected to possess correspondingly unique mechanisms that enable it to respond to the diverse stimuli differentially. Some of its regulatory properties appear to be derived from its localization in the mitochondrion and its Schiff base reaction intermediate (Osuji and Cuero, 1991b), which are not shared by many other multisubunit enzymes. Therefore, an analysis of the effects of pesticides on the enzyme is of considerable interest for understanding the mechanisms of signal discrimination and integration by crop plants.

The GDH amination kinetics and isoenzyme population distribution patterns induced by chemically related herbicides displayed relationships that mimicked the chemical similarities among the herbicides (Osuji, 1997; Osuji and Madu, 1997a). GDH isoenzyme patterns were found to be diagnostic of the nutrient status and dry

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10.1021/jf9805303 CCC: $18.00
Published on Web 07/07/1999

matter yield in corn (Osuji et al., 1998a). The above studies, together with the ability of GDH to synthesize glutamate during phytochemical defense response (Osuji and Madu, 1996) established the experimental framework for investigating the effects of pesticide−fertilizer nitrogen interactions on whole plant biochemistry, using the GDH signaling as the probe.

Fertilizer nitrogen generally reduces nodulation and biological nitrogen fixation (Matsunami and Arima, 1997; Fernandes et al., 1982). Consequently, farmers apply nitrogenous fertilizers with caution to their peanuts (Matlick, 1994; Brenneman et al., 1990; Cox et al., 1982). The effects of pesticides on the peanut nitrogen metabolism have not been studied, although the glutamine synthetase, nitrate reductase, and nitrogenase activities (Pacovsky and Fuller, 1991; Abdel-Ghaffar et al., 1982; Juang et al., 1982; Blevins, 1989) and the mobilization of biologically fixed nitrogen (Kvien et al., 1986) were studied in detail. The signaling property of peanut GDH may illuminate the biochemical mechanism of fertilizer nitrogen-induced stress on legume nitrogen metabolism and legume yield. Fertilizer nitrogen application has produced only insignificant increases in peanut yield (Walker et al., 1984). This may be because peanut fixes more than 200 kg of nitrogen per hectare (Wynne and Elkan, 1984).

Prior to the discovery of the GS-GOGAT cycle, GDH was generally considered as the major route for the assimilation of NH_4^+ by plants (Davis, 1965). The large millimolar NH_4^+ Michaelis constants (K_m) of GDH, compared with the micromolar NH_4^+ K_m values for GS, and the inhibition of GS by methionine sulfoximine (MSX) were the initial pieces of evidence that questioned the role of the enzyme in NH_4^+ assimilation (Ray, 1989; Robinson et al., 1991). Evidence in support of the amination function of GDH has slowly accumulated following the deciphering of the subunit composition of its hexameric isoenzymes (Cammaerts and Jacobs, 1983; Loulakakis and Roubelakis-Angelakis, 1991). The inducibility of the isoenzymes was demonstrated to be dependent on the nitrogen nutrient (Loulakakis and Roubelakis-Angelakis, 1991) and concentration (Osuji and Madu, 1995, 1996, 1997b), rather than on the specificity of the plant organs (Srivastava and Singh, 1987; Cammaerts and Jacobs, 1983). Results of the molecular analysis of the gene function also support the aminating function of the enzyme (Melo-Oliveira et al., 1996). Studies on the signaling of the enzyme in response to pesticides will illuminate its in vivo function.

GDH isomerization is a very complex reaction judging from the diversity of the factors that induce it. But the manipulation of its activity with plant nutrients to enhance crop protein yields could be an important approach for improving food and feed supply. Results presented hereunder show that in the GDH integration of signals, up to 70% of the pesticide signals were overridden by the nitrogen nutrient signal, with resultant doubling of peanut seed protein yield in many cases.

MATERIALS AND METHODS

Treatment of Peanuts with Pesticides. Hockley soil containing 51% silt was mixed with *Sphagnum* peat moss in a 1:1 ratio. The experimental soil (10 kg) was placed in each of 60 plastic pots. Peanut seeds (*Arachis hypogaea* L. Starr variety) were planted, six seeds per pot. The raw peanut seeds were purchased from a local green grocery. After 3 weeks when germination was completed, seedlings were thinned to three dominant individuals per pot. The pots were arranged into two groups of 30 pots each, one group was fertilized with 500 mL of 25 mM NH_4Cl solution (i.e., 17.5 ppm N) per pot, the other group was not fertilized. For each group, there were 10 treatments in three replicates. The first treatment was the control without pesticides. The fungicide Bravo 720 (720 g of tetrachloroiso-phthalonitrile/L) was applied at three rates, viz. lower than the recommended (0.012 mL per pot), the recommended (0.036 mL per pot), and higher than the recommended rate (0.072 mL per pot). The herbicide, Basagran (479.4 g of 3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide/L) was applied at three rates, viz. lower than the recommended (0.012 mL per pot), the recommended (0.036 mL per pot), and higher than the recommended rate (0.072 mL per pot). The insecticide Sevin XLR Plus (479.4 g of 1-naphthyl *N*-methyl carbamate/L) was also applied at three rates, viz., lower than the recommended (0.036 mL per pot), the recommended (0.108 mL per pot), and higher than the recommended rate (0.217 mL per pot). The recommended rates of pesticides were as specified by CPCR (1994). Bravo 720 is a product of ISK Biotech Corp., Mentor, OH. Basagran is a product of BASF Corp., Research Triangle Park, NC. Sevin XLR Plus is a product of Rhone-Poulenc AG Co., Research Triangle Park, NC.

Each rate of pesticide was diluted to 10 mL with distilled water, and delivered to the plant by foliar application with a hand-held sprayer calibrated to deliver 1 mL per spray. The sprayer nozzle was held 3−6 in. from the peanut canopy. The controls were sprayed with distilled water instead. The pesticide dilution to 10 mL ensured uniform treatment of the peanut canopy with each pesticide rate. The pesticides were applied two times at 4 and 6 weeks from seed planting. The peanuts were allowed to grow outside the greenhouse under local June−October (19 weeks) temperature and light conditions, and were watered to 50% saturation 3 days per week. The leaves were allowed to die and dry out before the peanuts were harvested, air-dried, dehusked by hand, and the seeds weighed. The seeds were stored at −20 °C.

Extraction of Storage Proteins. Peanut seeds (2 g) were coarsely ground with mortar and pestle in the presence of hexane, and defatted as described by Basha and Cherry (1976). Storage protein was extracted from the fatfree meal by thoroughly grinding the meal in the presence of 25 mL of 25 mM sodium phosphate buffer, pH 8. The homogenate was centrifuged (20 000*g*, 20 min, 4 °C), and the supernatant was assayed for protein content by the method of Lowry et al. (1951).

GDH Extraction. GDH was extracted from peanut seeds (20 g) or the shoots (4.5 g) and partially purified by 20−65% $(NH_4)_2SO_4$ precipitation as described previously (Osuji and Madu, 1997a). The protein pellet was dissolved in minimum volume of 10 mM Tris-HCl (pH 8.2) and dialyzed against three changes of 3.5 L of the same buffer at 3 °C (Osuji and Madu, 1995).

Isoelectric Focusing of GDH. Partially purified seed GDH containing 0.1−0.2 g or shoot GDH containing about 40 mg total protein was made 4 M with deionized urea and 2% with Bio-Rad Bio-Lyte ampholyte (pH 3−10, 40% w/v). This seed GDH solution (45−50 mL) was applied to the Bio-Rad Rotofor cell and focused (Rotoforated) for 4 h at 4 °C (Osuji and Madu, 1995). The shoot GDH solution after addition of urea and ampholyte was about 17 mL, and was focused in the mini Rotofor cell instead. The 20 Rotofor fractions were harvested, their pH values determined, and their ampholyte contents removed (Osuji and Madu, 1997a) by dialysis at 4 °C.

Gel Electrophoresis. Equal volumes (0.3 mL) of the Rotofor fractions were concentrated 3-fold by freeze-drying and electrophoresed at 4 °C through native 7.5% acrylamide separating gel (Osuji and Madu, 1996). Electrophoresed gels were visualized for GDH activity by staining with phenazine methosulfate-L-glutamate-NAD^+-tetrazolium bromide reagent (Cammaerts and Jacobs, 1983).

GDH Activity. GDH activities were assayed by photometry (Loulakakis and Roubelakis-Angelakis, 1991) at 340 nm. For each experimental treatment, Rotofor fractions 9−17 were pooled together in equal volumes and used for the assays of

GDH activity. Ammonium ion substrate saturation assays were carried out with 7.0 mM α-keto-glutarate (α-KG), 0.2 mM NADH, 1.3 mM $CaCl_2$, 0.2 mL of the GDH solution, and 0.5−125 mM NH_4Cl, in a final volume of 3 mL per assay. For the determination of the NH_4^+ K_m values, 0.3−35.0 mM α-KG, 0.5−12.5 mM NH_4Cl, 0.2 mM NADH, 1.3 mM $CaCl_2$, and 0.2 mL of the GDH solution were used in a final volume of 3 mL per assay. For the determination of the isomerization K_m values, 0.3−35.0 mM α-KG, 9.0−530 mM NH_4Cl, 0.2 mM NADH, 1.3 mM $CaCl_2$, and 0.2 mL of the GDH solution were used in a final volume of 3 mL per assay. All substrates were prepared in 0.1 M Tris-HCl buffer (pH 8.2). Protein concentrations were determined by the method of Lowry et al. (1951). Enzyme and protein assays were done in triplicates, the values reported being the averages ± SD of the triplicate assays. Initial velocities of the GDH reaction at fixed varied NH_4Cl and varying α-KG concentrations were used for the construction of double-reciprocal plots (Osuji and Madu, 1997a; Segel, 1976), the 1/*V*-axis intercepts of which were replotted versus the reciprocals of NH_4Cl concentrations in order to derive the true K_m values. Each unit of GDH activity was micromoles of NADH converted to NAD^+ min^{-1} mg^{-1} protein.

Assay for GDH−Pesticide Interaction. About 60 mg of Rotofor fractionated GDH of the control (no pesticide, no nitrogen fertilization) peanut seed was further purified by electrophoresis through sodium dodecyl sulfate (SDS) 10% polyacrylamide gel (PAG) in the Bio-Rad model 491 Prep Cell (Osuji and Madu, 1997a). Fractions containing polypeptides of molecular weight between 40 and 70 kD were pooled, and the protein content was precipitated by saturation of the solution to 60% with solid $(NH_4)_2SO_4$. The precipitated protein was pelleted by centrifugation (15000*g*, 20 min, 4 °C). It was dissolved in minimum volume of 0.1 M Tris-HCl buffer (pH 8.0) and dialyzed against the same buffer exhaustively to remove NH_4^+ and SDS. The resulting Prep Cell-purified GDH was 9.5 mL, 1.67 mg protein per mL. For the evaluation of GDH−pesticide interaction, 0.01 mmol α-KG and 0.16 mg (0.1 mL) of Prep Cell-purified peanut GDH were added to 1.5 mL of 0.1 M Tris-HCl buffer (pH 8.0) and incubated at 37 °C; after 10 min, 0.15 mmol pesticide was added, and incubation at 37 °C was continued for another 5 min. Pesticide was not added to the control reaction. After equilibration to room temperature, the amination activity of the incubated mixture was assayed photometrically with 0.25−5.0 mM NH_4Cl, 0.2 mM NADH, and 1.3 mM $CaCl_2$ in a final reaction volume of 3 mL per assay, as described above. Assays were repeated three times, and the average activities were applied for the calculation of initial velocities and for the construction of double reciprocal plots (Segel, 1976).

Ammonium Ion Contents of Peanut. NH_4^+ contents of peanut seeds were extracted (Osuji and Cuero, 1991) and assayed by the phenol−hypochlorite method (Weatherburn, 1967).

RESULTS

GDH Response to Pesticide Treatment and NH_4Cl Fertilization of Peanut. Free solution (Rotoforative) isoelectric focusing (IEF) fractionated peanut GDH to its charge isomers, which were in turn fractionated to their isoenzymes by native polyacrylamide gel electrophoresis (PAGE). Because the two steps of electrophoresis produce excellent resolution of GDH to its isoenzyme population, they have made it possible to study the physiological role of the enzyme even in crude extracts (Osuji et al., 1998a; Osuji, 1997; Osuji and Madu, 1995). The results obtained for all of the treatments (Figures 1−4) show that the isoenzymes focused in Rotofor chambers 8−17 correspond to the p*I* values of 4.8, 5.4, 5.8, 6.1, 6.4, 6.6, 7.1, 7.3, 7.5, and 8.1, respectively. The focusing of the isoenzymes in Rotofor chambers 8−17 was generally in agreement with the 7 charge-isomer system of peanut GDH (Osuji, 1997; Osuji and Madu, 1997a) and of plants in general (Chou and Splittstoesser, 1972; Kanamori et al., 1972; Lauriere and Daussant, 1983; Nauen and Hartmann, 1980). The peanut GDH isoenzyme population patterns (Figures 1−4) were also generally in agreement with the theoretically expected binomial distribution of the three subunits in the hexameric isoenzymes, on the basis of the twin nonallelic GDH_1 and GDH_2 gene structure, with the gene (GDH_1) encoding the more acidic subunits (a and α) being heterozygous and codominant, whereas the other gene (GDH_2) encoding the less acidic subunit (β) is homozygous (Cammaerts and Jacobs, 1983).

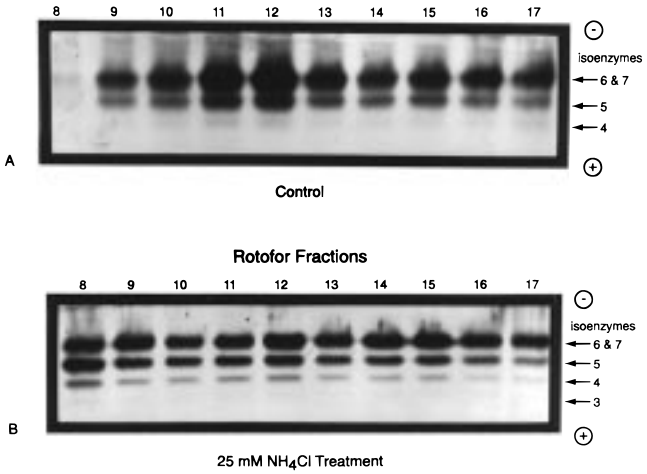

Figure 1. Isomerization of GDH in response (A) to peanut basal growth condition (biological nitrogen fixation) and (B) to 25 mM nitrogen as NH_4Cl fertilization of the peanut. The peanut seed GDH was Rotoforated (free solution IEF) to charge isomers, which were then fractionated to their component isoenzymes by native PAGE. The GDH isoenzyme population distribution pattern was visualized by activity staining of the electrophoresed PAG with tetrazolium blue reagent.

Control peanuts cultivated without pesticide treatment and without nitrogen fertilization gave an almost perfect symmetrical distribution of their GDH isoenzyme populations (Figure 1A). Controls fertilized with NH_4Cl, but without pesticides, displayed a different GDH isoenzyme pattern because it approached the positively skewed distribution (Figure 1B). The positive skewing meant that the nitrogen fertilizer enhanced the GDH acidic isoenzymes relative to the basic isoenzymes (Osuji and Madu, 1997b).

Peanut GDH isomerized in response to the fungicide (Bravo) by displaying isoenzyme population patterns (Figures 2A, C, and E) that were more positively skewed than those induced by nitrogen fertilization (Figure 1B). In response to the Bravo rates in combination with nitrogen fertilization, the GDH isoenzymes departed from the strong positive skewing and approached the symmetrical distribution patterns (Figures 2B, D, and F). Therefore, peanut GDH isomerized in response to each fungicide concentration by making a specific change in its isoenzyme population distribution pattern.

Peanut GDH isomerized in response to the low and high rate of insecticide (Sevin) treatment by displaying positively skewed isoenzyme population patterns (Figures 3A and D); that of the recommended rate was more positively skewed (Figure 3C). In response to the treatments in combination with nitrogen fertilization, the GDH isoenzyme populations (Figures 3B and E) deviated from the positive skewing. The acidic GDH isoenzymes resulting from the nitrogen fertilization/low rate of Sevin treatment were virtually absent so that

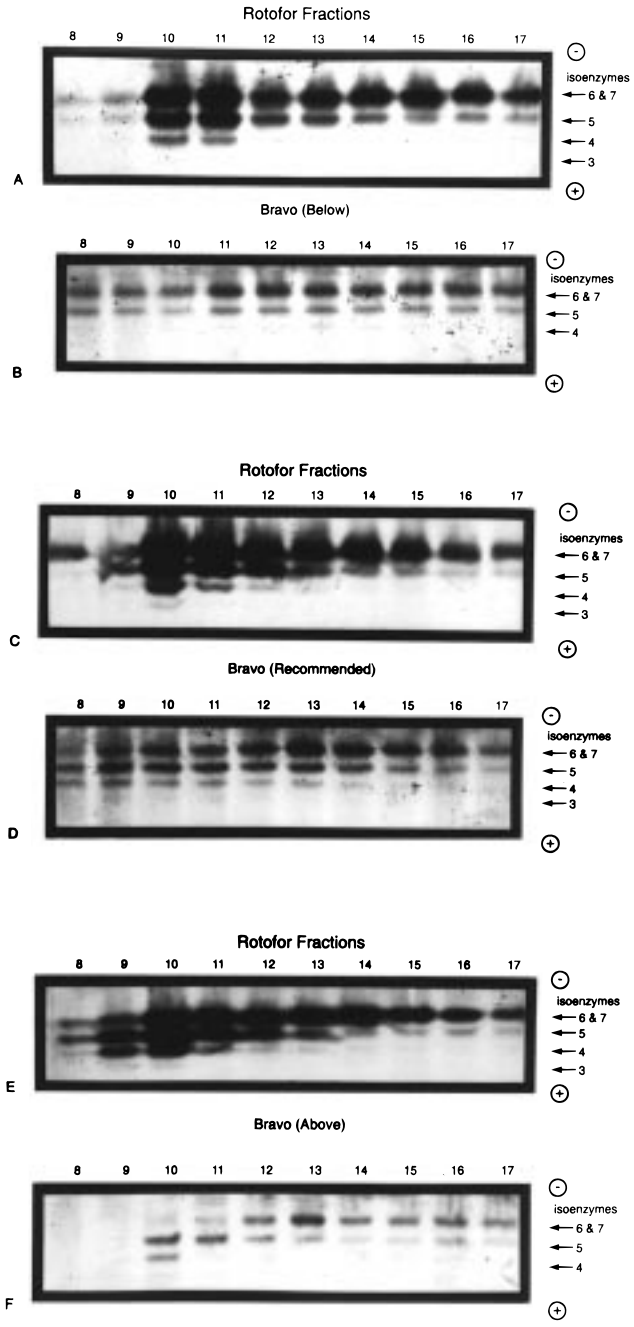

Figure 2. Isomerization of GDH in response to the fungicide (Bravo) protection of peanut: (A), (C), and (E) refer to below, recommended, and above the recommended Bravo rates, respectively. Figures (B), (D), and (F) were the GDH responses to nitrogen fertilization in combination with the low, recommended, and above the recommended Bravo rates, respectively. The peanut seed GDH was Rotoforated to charge isomers, which were then fractionated to their component isoenzymes by native PAGE. The GDH isoenzyme population distribution pattern was visualized by activity staining of the electrophoresed PAG with tetrazolium blue reagent.

the population pattern of the remaining isoenzymes approached negative skewing (Figure 3B). Peanut plants treated with nitrogen fertilizer in combination with the recommended rate of Sevin suffered severe growth retardation and subsequent premature death.

Peanut GDH responded to the herbicide (Basagran) by displaying progressively repressed isoenzyme population patterns (Figures 4A, C, and D). In particular, the recommended and high rates repressed the GDH so much that only the most acidic and the most basic

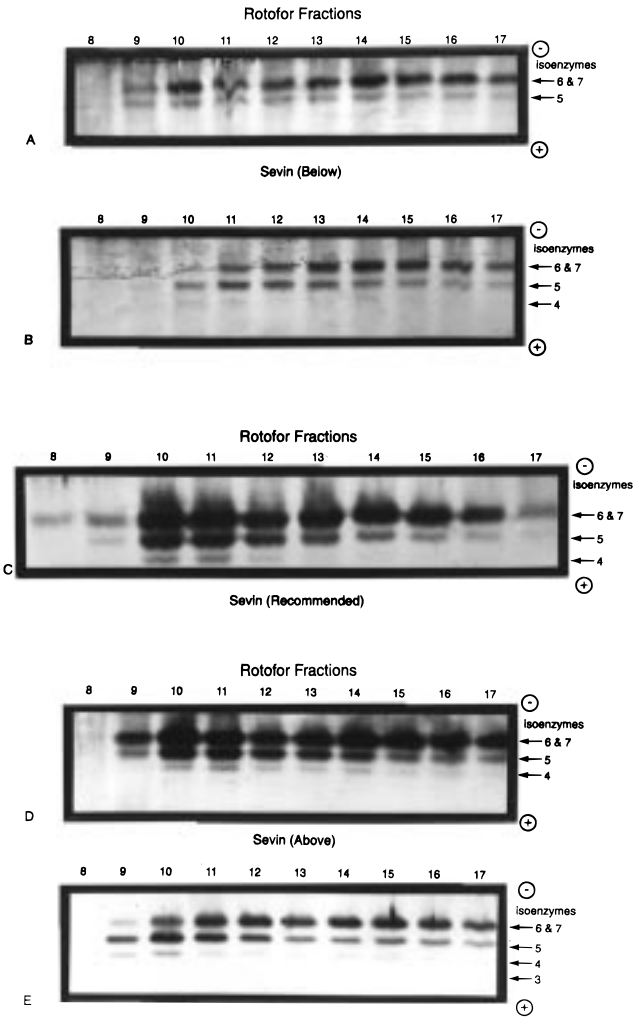

Figure 3. Isomerization of GDH in response to the insecticide (Sevin) protection of peanut: (A), (C), and (D) refer to below, recommended, and above the recommended Sevin rates, respectively. Figures (B) and (E) were the GDH responses to nitrogen fertilization in combination with the low and above the recommended Sevin rates, respectively. The peanut seed GDH was Rotoforated to charge isomers, which were then fractionated to their component isoenzymes by native PAGE. The GDH isoenzyme population distribution pattern was visualized by activity staining of the electrophoresed PAG with tetrazolium blue reagent.

isoenzymes were detectable. In response to the Basagran treatments in combination with nitrogen fertilization, the GDH isoenzyme populations became derepressed, especially in peanuts with high Basagran treatment (Figure 4E), whose GDH isoenzyme population became negatively skewed as a result. As in the insecticide (Sevin) treatments, the peanuts treated with the recommended Basagran rate in combination with fertilizer nitrogen became severely retarded in growth; they did not develop ovaries, and their shoots were harvested and used for GDH analysis. The shoot extracts showed no detectable GDH isoenzymes.

The peanut GDH isoenzymes also responded to the ambient environmental conditions which included the level of watering of plants, the summer temperature, and day length. Because these conditions affected all of the experimental plants equally, their total effects were indicated by the GDH isoenzyme pattern of the nil-pesticide/nil-fertilizer control peanut (Figure 1A). Changes in the GDH isoenzyme patterns obtained (Figures 1B, 2, 3, and 4) were therefore due to the effect

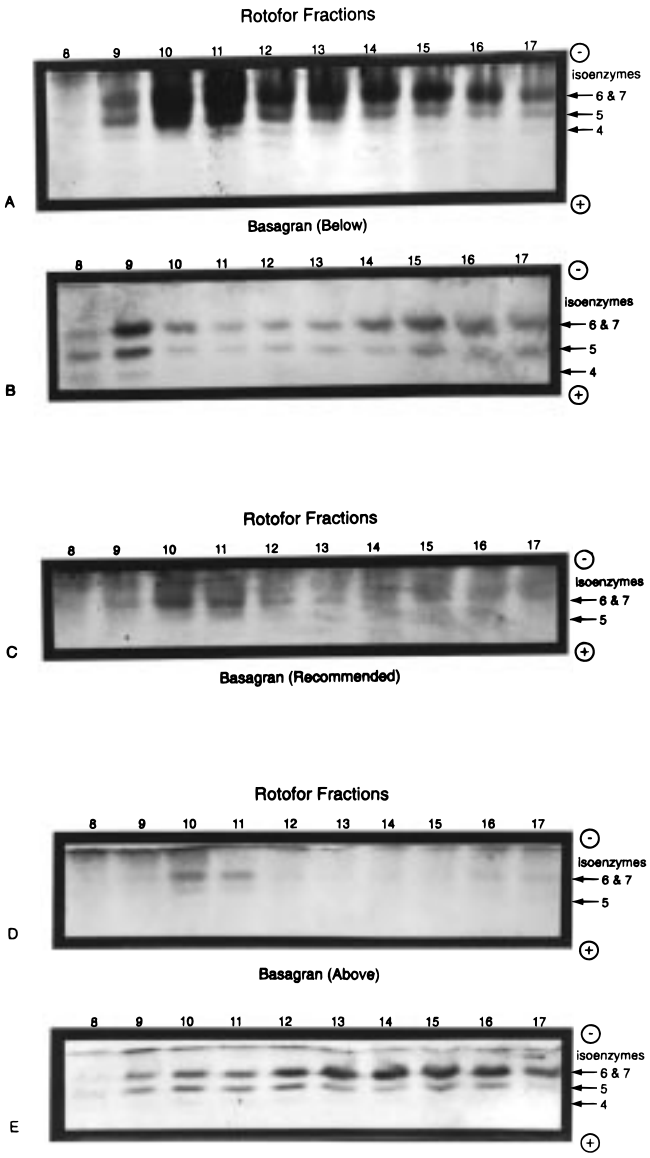

Figure 4. Isomerization of GDH in response to the herbicide (Basagran) protection of peanut: (A), (C), and (D) refer to below, recommended, and above the recommended Basagran rates, respectively. Figures (B) and (E) were the GDH responses to nitrogen fertilization in combination with the low and above the recommended Basagran rates, respectively. The peanut seed GDH was Rotoforated to charge isomers, which were then fractionated to their component isoenzymes by native PAGE. The GDH isoenzyme population distribution pattern was visualized by activity staining of the electrophoresed PAG with tetrazolium blue reagent.

of the specific treatment of the peanut plant. Peanut GDH isomerization in response to treatment of seedlings with herbicides and NH_4Cl solutions were reported (Osuji and Madu, 1997a; Osuji, 1997); the isoenzyme patterns were unique to the inducers applied and different from those in Figures 1−4. Therefore, although there are many inducers of GDH, the response of the enzyme to each appears to be specific.

Isomerization Kinetics. To quantitate the GDH responses to the agrochemical treatment of plants, the amination kinetic properties of the enzyme were determined. Ammonium ion substrate saturation kinetics of the GDH isoenzyme populations displayed a transient saturation plateau in the 3−7 mM NH_4Cl range, beyond which there was no other defined plateau because the amination activity just continued to rise with increases in the NH_4Cl concentration. Similar low NH_4^+ saturation, as well as the inability to saturate the enzyme with high concentrations of NH_4Cl, have been reported for the pea enzyme (Lauriere and Daussant, 1983; Garland and Dennis, 1977). The isomerization of maize GDH also responded to three concentration ranges of NH_4^+, the lowest of which was in the 3−7 mM range (Osuji and Madu, 1995). On the basis of these data, the reductive amination kinetics were studied at low (0.3−6.0 mM) and high (16−250 mM) NH_4Cl concentrations. Intermediate (8−20 mM) NH_4Cl concentrations induced nonlinear kinetics; on the other hand, very low (<0.3 mM) NH_4Cl concentrations gave no measurable reaction.

Figure 5 shows the replots of the amination kinetics at high NH_4Cl reaction conditions. Despite their different isoenzyme population patterns, the GDHs of the two control peanuts and of those treated with low rates of insecticide and fungicide had the same K_m value (30.0 ± 1.7 mM NH_4^+), but different V_{max} values. The replot for the GDH of the herbicide (Basagran) treatment was different from the others, as expected.

The replots at the low NH_4Cl reaction concentrations were similar to Figure 5, and they showed that the GDHs had a low true K_m value of 0.23 ± 0.06 mM NH_4Cl which is far below the NH_4^+ concentration of the mitochondrion (Yamaya et al., 1984). The GDHs of higher plants were previously associated with the possession of large NH_4^+ K_m values (Srivastava and Singh, 1987), which were exploited as strong evidence against an amination function by the enzyme (Ray, 1989; Robinson et al., 1991). The V_{max} values at the low NH_4Cl assay conditions ranged from 0.056 ± 0.007 to 0.105 ± 0.01 units, the GDH V_{max} values of the pesticide treatments being lower than their corresponding combined fertilizer nitrogen/pesticide treatments. This means that the GDHs became noncompetitively inhibited because of the treatment of the peanuts with pesticides, but nitrogen fertilization of the peanuts relieved the inhibition.

All of the replots (Figures 5−7) from assays at the high NH_4Cl concentrations gave large K_m values. Because the pesticide treatments induced different GDH isoenzyme patterns which accordingly displayed K_m values much larger than the amination K_m, the larger K_m values represented the kinetic properties of the isomerization reaction. The large K_m values were previously regarded as the NH_4^+ K_m values of GDH and were used as strong evidence against an amination function by the enzyme (Miflin and Lea, 1980; Suzuki et al., 1981; Rhodes et al., 1988; Robinson et al., 1991). On the basis of the results in Figures 1−5, the isomerization K_m and V_{max} values describe the affinity of the enzyme for its inducers.

The effects of the nitrogen fertilization in combination with pesticide treatments are also presented in Figure 5. Nitrogen fertilization of the control peanut increased the V_{max} value from 1.0 ± 0.06 to 1.7 ± 0.08 units, fertilization of the low rate of fungicide (Bravo) treatment similarly increased the V_{max} value from 1.2 ± 0.06 to 1.5 ± 0.04 units, and fertilization of the low rate of insecticide (Sevin) treatment also increased the V_{max} value from 0.4 ± 0.04 to 1.4 ± 0.03 units. Similar enhancements of the GDH activity following NH_4Cl fertilization of the high rate of pesticide treatments were also observed (Table 1). Therefore, the positive skewing of the GDH isoenzyme populations in the absence of

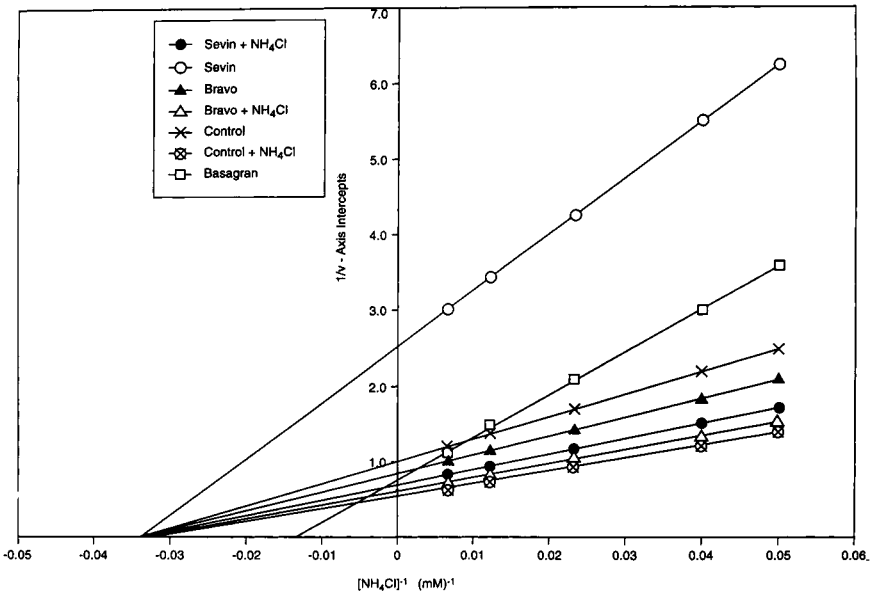

Figure 5. The isomerization kinetics of the GDH of peanuts protected with a third of the recommended rates of the insecticide (Sevin), fungicide (Bravo), and herbicide (Basagran) with and without nitrogen fertilization. Replots of data from double-reciprocal plots in which the activities of the Rotofor-purified charge isomers of peanut seed GDH were assayed at varied α-KG concentrations with NH_4Cl held at varied fixed concentrations. Protein contents of the Rotofor fractions were 2.3 ± 0.6 mg mL^{-1}.

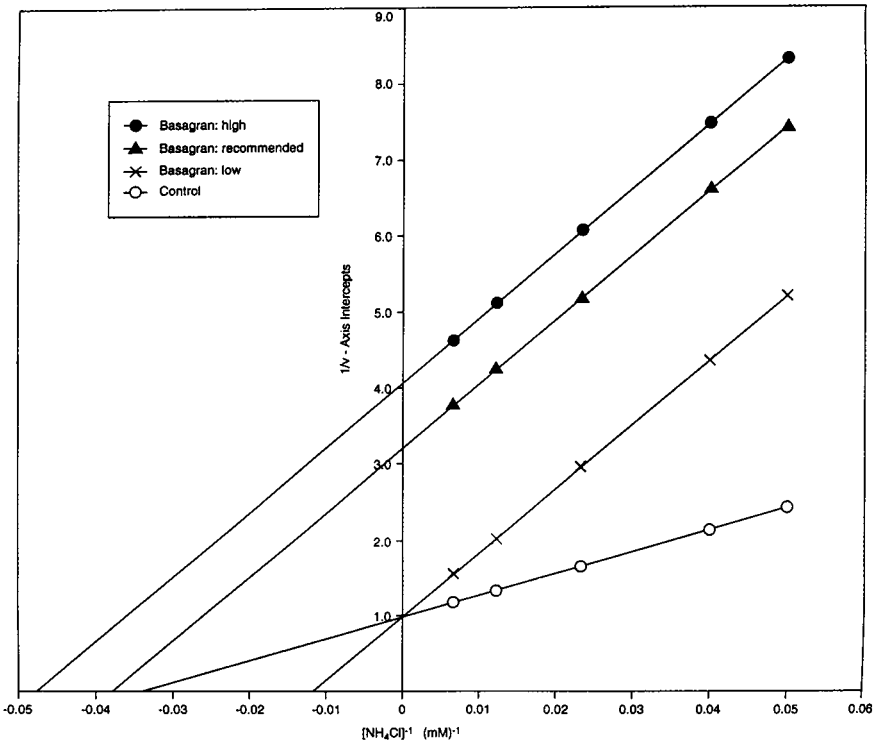

Figure 6. The isomerization kinetics of the GDH of peanuts protected with different rates of the herbicide (Basagran). Replots of data from double-reciprocal plots in which the activities of the Rotofor-purified charge isomers of peanut seed GDH were assayed at varied α-KG concentrations with NH_4Cl held at varied fixed concentrations. Protein contents of the Rotofor fractions were 2.5 ± 0.7 mg mL^{-1}.

nitrogen fertilization indicated enzyme inhibition, but the NH_4Cl fertilization of the pesticide-treated crops relieved the inhibition.

Figure 6 shows the GDH kinetics in response to the different concentrations of the herbicide (Basagran). The GDHs of the low, recommended, and high rates of herbicide treatments gave parallel replots in which the V_{max} values decreased with the increasing herbicide concentration. The parallel plots are similar to the effects of an uncompetitive inhibitor on an enzyme (Segel, 1976; Garland and Dennis, 1977), with the GDH isoenzyme population induced by the high rate of Basagran treatment being the most inhibited. The GDH isomerization K_m values were large: 83.3 ± 7.2, 26.3 ± 1.8, and 20.8 ± 1.4 mM NH_4Cl for the low, recommended, and high rates of herbicide applications, respectively.

The GDHs induced by the recommended and the high rates of the fungicide (Bravo) had a competitive inhibition relationship; also, the GDHs of the low and high rates had an uncompetitive inhibition relationship (Figure 7). Unlike the herbicide application in which the

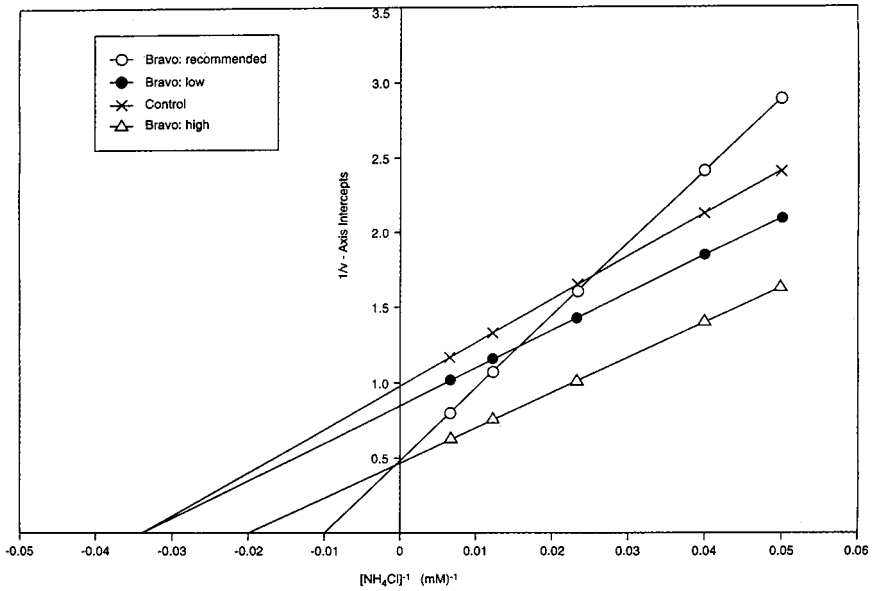

Figure 7. The isomerization kinetics of the GDH of peanuts protected with different rates of the fungicide (Bravo). Replots of data from double-reciprocal plots in which the activities of the Rotofor-purified charge isomers of peanut seed GDH were assayed at varied α-KG concentrations with NH_4Cl held at varied fixed concentrations. Protein contents of the Rotofor fractions were 2.6 ± 0.5 mg mL^{-1}.

Table 1. Isomerization Kinetic Constants of GDH in Response to Pesticide and Fertilizer Nitrogen Treatment of Peanut

	without fertilization		with nitrogen fertilization	
treatments	V_{max}[a]	K_m[b]	V_{max}[a]	K_m[b]
controls	1.0 ± 0.06	30.0 ± 1.7	1.70 ± 0.08	30.0 ± 1.7
low Basagran rate	1.0 ± 0.06	83.3 ± 7.2	0.91 ± 0.07	30.0 ± 1.7
recommended Basagran rate	0.31 ± 0.01	26.3 ± 1.8	–	–
high Basagran rate	0.24 ± 0.01	20.8 ± 1.4	1.53 ± 0.04	25.0 ± 0.6
low Bravo rate	1.05 ± 0.05	30.0 ± 1.7	1.50 ± 0.04	30.0 ± 1.7
recommended Bravo rate	2.0 ± 0.08	100.0 ± 8.6	3.03 ± 0.06	31.7 ± 2.8
high Bravo rate	2.0 ± 0.08	50.0 ± 3.4	1.53 ± 0.06	62.5 ± 3.8
low Sevin rate	0.40 ± 0.05	30.0 ± 1.7	1.40 ± 0.03	30.0 ± 1.7
recommended Sevin rate	1.0 ± 0.03	30.0 ± 1.7	–	–
high Sevin rate	1.0 ± 0.03	50.0 ± 3.5	1.25 ± 0.04	35.7 ± 1.7

[a] NH_4^+ was the fixed varied substrate, whereas α-KG was the varied substrate. V_{max} = μmol^{-1} mg^{-1} protein = 1 unit. [b] K_m = mM NH_4^+.

high rate induced the most inhibited form of GDH, the low fungicide rate induced the most inhibited form of the enzyme because its V_{max} value (1.05 ± 0.05 units) was lower than that (2.0 ± 0.08 units) for the recommended and high rates. There was a noncompetitive inhibition relationship between the GDHs of the control and of the low rate of Bravo treatment (Figure 7). The K_m values of the Bravo-induced isomerization were again large, being 30.0 ± 1.7, 100.0 ± 8.6, and 50.0 ± 3.4 mM NH_4Cl for the low, high, and recommended rates of Bravo treatment, respectively. The GDH K_m and V_{max} values induced by the cotreatment with fertilizer nitrogen/recommended rate of Bravo were 31.7 ± 2.8 mM NH_4Cl and 3.03 ± 0.06 units, respectively.

The changes induced by the insecticide (Sevin) on the isomerization of peanut GDH shared a similarity with those induced by the fungicide (Bravo) in that the GDH induced by the low rate was the most inhibited (V_{max} value of 0.4 ± 0.04 units) as compared with the GDHs induced by the recommended and high rates (V_{max} value of 1.0 ± 0.3 units). Also, the GDHs induced by the low and recommended rates of Sevin treatment had noncompetitive inhibition relationship (figure not presented). Therefore, the GDHs induced by the low and recommended rates of Sevin treatment had the same large isomerization K_m value (30.0 ± 1.7 mM NH_4Cl), which was lower than that of the GDH induced by the high rate (50.0 ± 3.5 mM NH_4Cl). The GDH K_m values induced by the high rates of pesticide treatments/nitrogen fertilization were large: 62.5 ± 8.8, 35.7 ± 1.7, and 25.0 ± 0.6 mM NH_4Cl for Bravo, Sevin, and Basagran, respectively (Table 1).

GDH in Vitro Response to Pesticides. The changes induced on the GDH kinetics of pesticide-treated peanut relative to the untreated control strongly suggested a pesticide−GDH interaction. Figure 8 shows the double-reciprocal plot of the GDH amination activity assayed in the presence of equal concentration of pesticide. The apparent V_{max} values were 30.0 ± 2.8, 35.2 ± 2.2, 63.0 ± 5.8, and 479.0 ± 20.7 units for the control assay and assays in the presence of Bravo, Sevin, and Basagran, respectively. The results in the figure suggest that GDH has binding sites for the pesticides. The binding of herbicides to GDH was first suggested following the observed inhibition kinetics of the enzyme in peanuts germinated in the presence of herbicides (Osuji, 1997). The water solubilities of Bravo, Sevin, and Basagran are 0.6, 113, and 500 mg/L, respectively (Weber, 1994). Their order of increasing hydrophilicity is also their order of increasing nucleophilicity. This means that the effect of pesticides on GDH isomerization depends on the nucleophilicity of the pesticide; the more nucleophilic, the more severe is the nucleophilic interference by the pesticide on the GDH amination. Bravo and Sevin are nonionic pesticides (Weber, 1994); therefore, the pH values of the three pesticides could not be the factor determining their interaction with GDH. Peanut GDH used for the GDH−pesticide interaction assay was purified by removal of low molecular weight polypeptides in order to minimize the interference by the degraded polypeptides of the enzyme, most of which possess molecular weights less than 40 kD. The GDH−pesticide interaction was assayed only at low NH_4^+

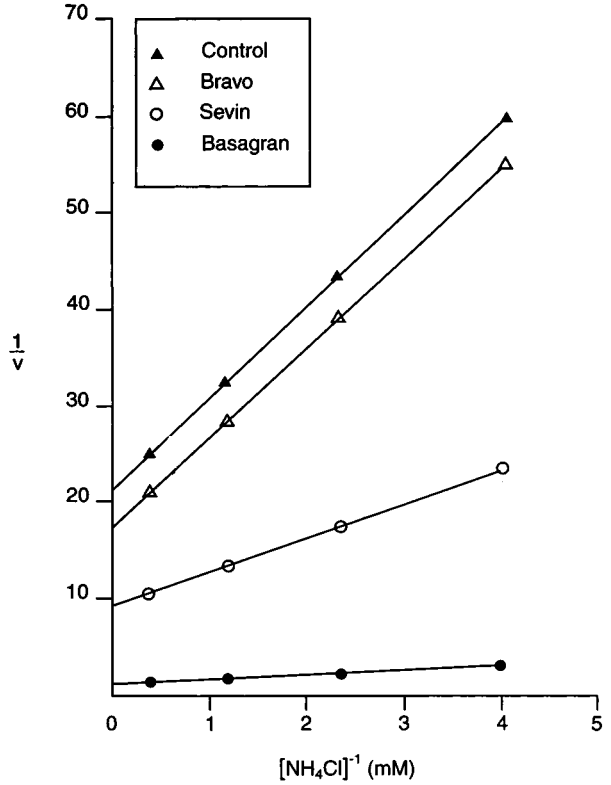

Figure 8. Double-reciprocal plots of the velocity of peanut seed GDH against varied NH_4Cl concentrations in the presence of constant pesticide (50 mM), and NADH (0.1 mM) concentrations with α-KG held at varied fixed levels, in a volume of 3.0 mL per assay. Velocities are mmol min^{-1} mL^{-1}, the enzyme being 1.67 mg protein mL^{-1}.

concentrations because higher concentrations induced nonlinear kinetics of the enzyme. Also, GDH interaction with pesticide was assayed at 50 mM pesticide concentration because that was the range of pesticide concentration sprayed on the experimental plants.

Signaling by GDH. The in vivo and in vitro GDH−pesticide interactions suggested a signaling function by the enzyme. Figure 1A represents the signaling by GDH in response to the basal nutrient condition, which was mainly due to the biologically fixed nitrogen by the peanut and the soil water content. Figures 2A, C, and E were the resultant signaling in response to the treatments with low, recommended, and high rates of Bravo, respectively. A comparison of the isoenzyme pattern in Figure 1A with those in Figures 2A, C, and E shows that the Bravo-induced signals superseded that due to the basal nutrient conditions. This illustrates the signal discrimination function of GDH. Similar comparison of the isoenzyme pattern in Figure 1A with those induced by Sevin in Figures 3A, C, and D, and those induced by Basagran in Figures 4A, C, and D show that the pesticide-induced signals superseded that induced by the basal nutrient condition.

Figures 1A and B show the existence of signal discrimination between the nitrogen nutrient supplied via biological fixation and via NH_4Cl application. The reduced number of GDH isoenzymes during the supply of biologically fixed nitrogen compared with that during the supply of fertilizer nitrogen suggests that the biologically fixed nitrogen was more concentrated than the fertilizer nitrogen. Peanut fixes more than 200 kg nitrogen per hectare (Wynne and Elkan, 1984). This explanation is supported by the inhibition of the GDH of the unfertilized peanut, whereas the GDH of the fertilized peanut was not inhibited (Figure 1). The fertilizer nitrogen inhibition of nodulation and nitrogen fixation (Matsunami and Arima, 1997; Fernandes et al., 1982), with concomitant relief of NH_4^+-dependent inhibition of GDH isomerization, was visually demonstrated (Figures 1A and B) by the GDH signal discrimination function. Because concentrations of NH_4^+ induced specific patterns of GDH isomerization (Osuji and Madu, 1995, 1997b), the foregoing results show that signal discrimination by GDH is based on the concentration, as well as on the nucleophilicity of the inducer.

Signal integration for the combined pesticide treatment/nitrogen fertilization is best explained by comparing the GDH kinetic constants (V_{max} and K_m) of the fertilizer nitrogen-treated control, the pesticide treatment, and the corresponding pesticide/fertilizer nitrogen treatment because the enzyme combined the signals from the biologically fixed nitrogen, the fertilizer nitrogen, and the pesticide. On this basis, the 18 treatments analyzed in this study fell into four modes of signal integrations.

Pesticide treatments which induced increases in the V_{max} values of the GDH as a result of cotreatment with NH_4Cl included the low Sevin, low Bravo, and the high Basagran rates. The GDH V_{max} value increased from 0.4 units in the low rate of Sevin treatment to 1.4 units in the corresponding combined Sevin and NH_4Cl treatment (Table 1). Because the GDH V_{max} value of the NH_4Cl-fertilized control peanut was 1.7 units, the increase of V_{max} from 0.4 to 1.4 units represented about 76% override of the pesticide signal by the nitrogen nutrient signal. The GDH V_{max} value increased from 1.0 in the low rate of Bravo treatment to 1.5 units in the corresponding combined Bravo/NH_4Cl treatment. This represented an override of about 71% of the pesticide signal by the nitrogen nutrient signal. The GDH V_{max} value increased from 0.24 in the high rate of Basagran treatment to 1.53 units in the corresponding combined Basagran/NH_4Cl treatment. Therefore, in the integration of the signals by GDH, about 88% of the Basagran signal was overridden by the nitrogen nutrient signal. In the three cases, the increase in V_{max} values at constant K_m suggested that the nitrogen nutrient noncompetitively displaced the pesticide molecules from their interaction with the enzyme.

Pesticide treatments which induced increases in the GDH V_{max} values and decreases in the K_m values as a result of the combined NH_4Cl/pesticide treatment included the recommended rate of Bravo and the high rate of Sevin treatments. This signal integration mode is better explained on the basis of the K_m values because the V_{max} values remained as high as in the NH_4Cl-fertilized control peanut. The GDH K_m value decreased from 100 mM NH_4^+ in the recommended rate of Bravo treatment to 31.7 in the corresponding combined Bravo/NH_4Cl treatment. Because the K_m value of the NH_4Cl-fertilized control peanut was 30 mM, the decrease represented about 97% override of the pesticide signal by the nitrogen nutrient signal. For the high rate of Sevin treatment, the GDH K_m values decreased from 50 mM to 35.7 mM in the corresponding combined Sevin/NH_4Cl treatment. The decrease represented an override of 71% of the pesticide signal by the nitrogen nutrient signal.

Pesticide treatments which induced decreases in the GDH K_m values while the V_{max} values remained un-

changed as a result of the combined NH_4Cl/pesticide treatment of the peanuts were illustrated by the low rate of Basagran treatments. The GDH K_m value decreased from 83 mM NH_4^+ in the low rate of Basagran treatment to 30 mM in the corresponding combined Basagran/NH_4Cl treatment. Because the GDH K_m value of the fertilized control peanut was 30 mM, the decrease from 83 to 30 mM represented a 100% override of the pesticide signal by the nitrogen nutrient signal. The decrease in K_m value while the V_{max} value remained unchanged suggested that in the signal integration by GDH, the nitrogen nutrient competitively displaced the pesticide molecules from their interaction with the enzyme.

Pesticide treatments which induced increases in the GDH K_m values and decreases in the V_{max} values as a result of the combined NH_4Cl treatment of the peanuts were exemplified by the high rate of Bravo treatments. Because the GDH V_{max} value of the fertilized control peanut was 1.7 units, the decrease of the V_{max} value from 2.0 units in the high rate of Bravo-treated peanut to 1.53 units in the corresponding combined Bravo/NH_4Cl treatment suggested that the nitrogen nutrient concentration was insufficient to displace the high concentration of Bravo molecules from their interaction with the enzyme.

Three modes of signal integration were not represented in the 18 treatments studied. These were increases in K_m values at constant V_{max}, decreases in V_{max} values at constant K_m and increases in both of the V_{max} and K_m values. These unrepresented signal integration modes appear to lead to growth retardation because each is an antagonistic kinetic combination. In this regard, two treatments, viz. the recommended Basagran/NH_4Cl fertilization and the recommended Sevin/NH_4Cl fertilization, severely retarded the peanut growth and led to their premature death. This might have been due to possible inactivation of the GDH during its integration of the antagonistic signals from the combined NH_4Cl and the specific concentration of the pesticides. In support of this explanation was the observation that the GDH extracted from the shoot of the retarded peanuts had suffered more than 95% degradation to polypeptides lower in molecular weight than 30 kD (Osuji et al., 1999). Pesticide-mediated inhibition of carbon metabolism (Daie, 1991) might have also contributed to the premature death, although peanut is not a target crop for Basagran and Sevin. Peanut GDH subunit molecular weights are in the range of 45−69 kD (Osuji and Madu, 1997a). The degradation of GDH was not indicative of the senescence of the peanut because GDH activity increases during senescence (Loulakakis et al., 1994). The GDH of peanut seeds germinated in the presence of organo-nitrogen herbicides suffered more degradation than the controls (Osuji, 1997). This, however, suggests that GDH degradation is part of the reactions that control the signaling function.

Peanut Seed and Seed Protein Yield Increases. Table 2 shows that the fertilization of the control peanut did not increase the seed yield. This is one of the reasons why farmers are reluctant to apply nitrogenous fertilizers to their peanut fields (Matlick, 1994; Brenneman, 1990). Peanut fixes great amounts of nitrogen and so may not require fertilizer nitrogen for normal growth (Wynne and Elkan, 1984). Table 2 also shows that the increasing rates of herbicide (Basagran) application progressively decreased peanut yield, but the increasing rates of fungicide (Bravo) progressively increased yield. These are the expected opposite effects of the herbicide (Figure 4) and fungicide (Figure 2) on yield, based on the GDH−pesticide in vitro interactions (Figure 8) and the different amination mechanisms of the isoenzyme patterns they induced (Figures 6 and 7). Therefore, the effects of the pesticides on the GDH influenced peanut yield. However, the pesticide-induced yields (average of 26.5 ± 3.2 g per pot) in the absence of fertilizer nitrogen were statistically insignificant as compared with the controls. This may possibly be because no pests (such as weeds, fungi, or insects) actually attacked the plants. It is known that field protection of peanut plants with Bravo (Besler et al., 1996) and Basagran (Grichar and Colburn, 1995) increased the yields. Insecticide application does not normally affect yield (Smith and Sams, 1977).

Table 2. Effects of Pesticide Protection of Peanut Plants with or without 25 mM NH_4Cl Fertilization on Peanut Seed and Seed Protein Yields*

	seed yield*		seed protein yield*	
pesticide treatments	without NH_4Cl	with NH_4Cl	without NH_4Cl	with NH_4Cl
controls	28.4 ± 4.1[a]	26.0 ± 3.9[a]	3.9 ± 0.8[a]	4.7 ± 1.1[a]
herbicide Basagran				
low rate	31.2 ± 4.6[a]	31.9 ± 15.2[a]	4.9 ± 1.8[a]	5.0 ± 0.9[a]
recommended rate	27.5 ± 4.4	−	4.7 ± 2.0	−
high rate	24.1 ± 3.1[a]	46.6 ± 6.2[b]	3.2 ± 0.5[a]	7.7 ± 1.7[b]
fungicide Bravo				
low rate	19.4 ± 6.4[a]	54.6 ± 7.3[b]	2.8 ± 1.2[a]	8.4 ± 0.9[b]
recommended rate	23.8 ± 3.7[a]	50.0 ± 6.8[b]	3.3 ± 1.1[a]	8.4 ± 0.8[b]
high rate	28.4 ± 3.2[a]	37.2 ± 10.8[a]	3.7 ± 1.4[a]	5.1 ± 2.3[a]
insecticide Sevin				
low rate	27.9 ± 3.5[a]	59.9 ± 4.6[b]	4.2 ± 1.7[a]	9.3 ± 1.5[b]
recommended rate	26.1 ± 2.8	−	4.0 ± 0.7	−
high rate	28.9 ± 3.5[a]	53.2 ± 3.9[b]	4.4 ± 1.0[a]	7.6 ± 1.8[b]

* Means ± SD with the same letter under both yields are not different ($P > 0.05$) with or without NH_4Cl fertilization within a treatment. * Yield is grams per pot of three peanut plants.

Apart from a few exceptions, seed yields were dramatically increased (average of 47.6 ± 9.9 g per pot) by the combined pesticide treatments/NH_4Cl fertilization and were increased up to 280% in the combined NH_4Cl fertilization/low rate of Bravo treatment (Table 2). The peanuts which gave increased yield produced 145 ± 15 seeds per pot, whereas those that had no yield increase possessed 74 ± 12 seeds per pot. Developmental and environmental factors regulate the sink-source capacity (Daie, 1991). Therefore, the combined pesticide treatment/NH_4Cl fertilization was the environmental factor that induced the GDH increase in V_{max} and the attendant doubling of the source-sink capacity.

Seed protein content was 152.2 ± 17.3 mg/g of seed in the nil-fertilized/nil-pesticide-treated control peanut, and it remained statistically unchanged irrespective of whether the peanut was fertilized with nitrogen, protected with pesticide, or treated with pesticide in combination with NH_4Cl fertilization. However, because most of the combined pesticide/fertilization treatments gave increased peanut seed yields and doubled number of seeds per pot, they also induced almost doubled seed protein yield (average of from 3.8 ± 0.7 g to 7.0 ± 1.8 g) per pot. This result is in agreement with the doubling of the storage protein contents of crops following the increases in the amination activity of their GDHs (Osuji and Cuero, 1991a, 1992a,b). Similar doubling of legume seed yield following the treatment of legume plant with gibberellin was reported (Belucci et al., 1982), although

no enzyme activity was investigated. GDH accounted for 50−100% of the NH_4^+ assimilated in corn, especially when externally supplied NH_4^+ increased the GDH activity and inhibited the GS-GOGAT cycle (Osuji and Madu, 1995, 1996). The 25 mM NH_4Cl fertilization was adopted in this study because previous results (Osuji and Madu, 1995, 1996, 1997b) showed that GDH activity was optimum, whereas GOGAT was inhibited, when crops were supplied with that level of nitrogen fertilization. Those signal integration modes which led to increases in the V_{max} values also led to increases in peanut seed and seed protein yields (Table 2). This included the high rate of Basagran treatment from which peanut seed protein yield increased from 3.2 ± 0.5 g without NH_4Cl fertilization to 7.7 ± 1.7 g per pot with NH_4Cl fertilization; low rate of Bravo treatment from which peanut seed protein yield increased from 2.8 ± 1.2 g without NH_4Cl fertilization to 8.4 ± 0.9 g per pot with NH_4Cl fertilization; and the recommended rate of Bravo treatment from which peanut seed protein yield increased from 3.3 ± 1.1 g without NH_4Cl fertilization to 8.4 ± 0.8 g per pot with NH_4Cl fertilization. Other examples where signal integration led to increases in V_{max} values and to increases in peanut seed protein yield are the low rate of Sevin treatment from which yield increased from 4.2 ± 1.7 g without NH_4Cl fertilization to 9.3 ± 1.6 g per pot with NH_4Cl fertilization; and the high rate of Sevin treatment from which yield increased from 4.4 ± 1.0 g without NH_4Cl fertilization to 7.6 ± 1.8 g per pot with NH_4Cl fertilization. Signal integration modes which led to decreases in the K_m values did not increase the peanut seed protein yield. This was exemplified by the low rate of Basagran treatment to peanut from which seed protein yield (4.9 ± 1.8 g) without NH_4Cl fertilization was not significantly different from that (5.0 ± 0.9 g per pot) with NH_4Cl fertilization. Similarly, those signal integration modes which led to increases in K_m values did not induce increased peanut seed protein yields. For example, the yield (3.7 ± 1.4 g) of the high rate of Bravo treatment to peanut without NH_4Cl fertilization was not significantly different from that (5.1 ± 2.3 g per pot) with NH_4Cl fertilization. The signal integration modes which merely led to changes in the GDH K_m values did not induce increased peanut seed protein yields probably because NH_4^+ is not limiting in peanut, and/or the large K_m values do not refer to the in vivo NH_4^+ concentration. The isomerization of GDH in response to environmental factors is very complex. Therefore, the GDH signal integration modes offer a simple framework for explaining the effects of combined pesticide/fertilizer nitrogen applications on the protein yield. The agriculturally beneficial rates of pesticide application to peanuts are those which when combined with fertilizer nitrogen induce increases both in the GDH V_{max} values and in the peanut seed protein yield. Because of their high protein contents, peanut seeds are excellent sources of food ingredients (Ory, 1986).

From the yield increases (Table 2), it appears that a certain threshold of signals (production threshold) was attained before the enzyme was able to increase its amination velocity for seed protein yield to increase from 3.8 to 7.0 g per pot, on the average. A similar threshold dry matter yield also occurred in the response of maize GDH to phosphate supply status (Osuji et al., 1998a).

DISCUSSION

GDH−Pesticide Interaction. The alteration of the GDH activity in vitro (Figure 8) by the three pesticides, in the sequence Basagran > Sevin > Bravo > control, provided the evidence for a direct GDH−pesticide interaction based on the nucleophilicity of the pesticide molecule. This was also the case with the organonitrogen herbicides (Osuji, 1997). Although the three pesticides, Sevin, Bravo, and Basagran, are not structurally related (Figure 9), the different GDHs they induced still displayed close relationships in their isomerization kinetics. The insecticide Sevin is a naphthylcarbamate, the organochlorine fungicide Bravo is a phthalonitrile, and the herbicide Basagran is a benzothiadiazin (Figure 9). Therefore, the GDH isoenzyme populations they induced are not based on the functional groups of the pesticide molecules because they possess different functional groups. Also, the GDH−pesticide interaction is not based on the pH values of the pesticides because some pesticide molecules are not ionizable. However, pesticide molecules possess comparable nucleophilicities. Xenobiotic nucleophilicity is also important in the detoxification mechanism of the P450 monooxygenases (Cole, 1994; Kreuz et al., 1996; Wagenet and Rao, 1990). These reasons illuminate the differential effects of Sevin, Bravo, and Basagran on the isomerization of peanut GDH.

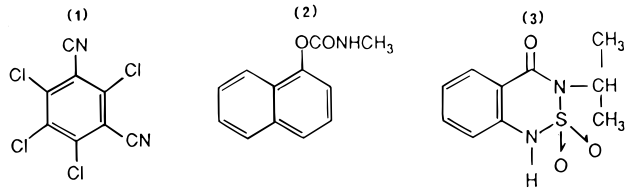

Figure 9. Chemical structures of (1) tetrachloroiso-phthalonitrile (Bravo 720), (2) 1-naphthyl *N*-methylcarbamate (Sevin XLR), and (3) 3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide (Basagran).

The interaction of GDH with pesticides in vitro alters the amination kinetics of the enzyme (Figure 8). A similar interaction could occur in vivo, enabling the pesticide to alter the amination function of the enzyme. Other factors including light/dark cycle, temperature, soil water activity, and insect predation which also induce the GDH isomerization were constant for all the plants; their effects being manifested by the GDH isomerization pattern of the control plants (Figure 1A). Therefore, the changes (Figures 2−4) in the GDH isomerization patterns were specific to the pesticides. A natural substrate of the enzyme is α-KG, a member of the tricarboxylic acid (TCA) cycle. The interaction of the enzyme with pesticides, and the consequent isomerization reaction, constitute a signal to the TCA cycle and the cellular energy metabolism which become altered accordingly. This is the metabolic basis of the signaling response by the enzyme.

The pesticides and xenobiotics that interact with GDH to induce its isomerization are so diverse that the binding sites in the GDH must exhibit an enormously low specificity. Perhaps this explains why the isomerization K_m values are equally large and variable (Table 1). It is also possible that the oxidative stress induced by the pesticides elicited the release of NH_4^+ from the phenylpropanoid pathway (Osuji, 1997; Osuji and Madu, 1996); the NH_4^+ thus produced then induced the isomerization of GDH. The NH_4^+ contents of all the harvested peanut seeds were determined, but the NH_4^+ contents of the pesticide-treated and the control peanuts were not significantly different. Therefore, it was unlikely that the isomerizations of the peanut GDH were due to

the metabolically derived NH_4^+. This shows that the pesticides had induced the GDH isomerization long before any induction of the phenylpropanoid pathway. GDH isomerization is an early reaction in the cascades of responses of plants to environmental stress. This is an important mechanism because in this way the mitochondrion is alerted in advance of imminent changes in the environment. GDH is a mitochondrial enzyme.

Signaling by GDH in Relation to Crop Yield. The isomerizations of the peanut enzyme in response to the different pesticides and their concentrations (Figures 1−4) are visual demonstrations of the signaling reaction of the enzyme. The redox-dependent changes (changes in the isoenzyme populations) were substantial judging from the magnitude of the changes in the kinetic constants (Table 1). Signal transduction by nitrogenase is similarly mediated through redox-dependent changes in the structure of the enzyme (Schindelin et al., 1997), during which process the enzyme also undergoes substantial conformational changes.

The combination of the signals from the nitrogen fertilizer and the pesticide so as to deliver one response in the form of a unique isoenzyme distribution pattern of GDH was by integration but not by summation because the kinetic constants of the GDHs of the pesticide/fertilizer nitrogen treatments were not the cumulative totals of those of the corresponding separate pesticide and fertilizer nitrogen treatments. In the GDH integration of signals, those from the more nucleophilic inducers override those from the less nucleophilic inducers, the degree of override depending on the relative nucleophilicities of the inducers (Osuji et al., 1998a). On this basis, the integration of antagonistic signals by GDH may lead to reduced crop growth and perhaps to premature crop death. It is not, however, clearly known why pesticides kill plants (Devine et al., 1993). Basagran is an inhibitor of photosynthetic electron transport, but peanut is not a target plant species (CPCR, 1994). Phenyl-ring hydroxylation of Basagran (Buschmann and Prehn, 1986) increases its nucleophilicity. Sevin is a carbamate insecticide, but 1-naphthol, one of its hydrolysis products, is a strong nucleophile. Phenolic compounds, including 1-naphthol, are plant growth regulators (Singh et al., 1991). Also, Bravo is an organochlorine fungicide, but its dehalogenated products (Sato and Tanaka, 1987) are stronger nucleophiles. From their regulation of the GDH activity, the three pesticides functioned also like *N*-(carboxymethyl) chitosan and similar plant biochemical regulators (Osuji and Cuero, 1991a, 1992a,b). However, it is possible that GDH was only one of the biochemical target sites of the three pesticides. Many bioregulators, phytohormones, and herbicides control starch synthesis and sink-source interaction (Daie, 1991; Sonnewald and Willmitzer, 1992). Therefore, in the cases of the premature deaths of the peanuts treated with the recommended rates of Basagran, or Sevin in combination with NH_4Cl fertilization, the cumulative effect of the treatments on the GDH, and possibly on sucrose synthesis, would more fully account for the result observed. This discussion shows that the GDH signaling mechanism is unique and especially different from the protein kinase cascades, because whereas GDH is a mitochondrial signaling system, the kinase cascades are cytosolic where they relay information from the cell-surface receptors to the cell nucleus (Davis, 1994).

Although biochemical regulators induced increased protein contents in cotton (Hedin et al., 1988), large increases (≥100%) in peanut seed yields are not easy to achieve in the field. For example, peanut seed yield increase of up to 7% was achieved by foliar application of urea (Walker et al., 1984), when it was understood that fertilizer nitrogen application to the soil was ineffective. Also, a yield increase of up to 35% was achieved by treatment of peanuts with phenolic compounds, although the biochemical basis of the effect was not explained (Singh et al., 1991). Tertiary amines also enhance crop yield by increasing the net-carbon assimilation (Keithly et al., 1991), but no enzyme was associated with the bioregulation process. Therefore, the manipulation of the signaling of GDH in response to nitrogen nutrients, pesticides, and similar nucleophiles (Osuji and Cuero, 1991, 1992a,b) could be a potentially useful technology not only for increasing crop yields, but also crop protein yields.

The use of nucleophiles, at minimal fertilizer nitrogen input, to manipulate crop and crop protein yield via the GDH signaling property is a potentially important technology in view of the increasing world population and the attendant increased demand for food and fiber, especially in the developing countries. The GDH-mediated increase in crop protein yield is even more important because arable land may not increase to meet the increasing world demand for food and fiber.

Evaluation of the Pesticide Burden of Harvested Crops. On the basis of the results in Figures 1−4, evaluation of the pesticide content of crop tissue by the GDH method is potentially feasible because it would only require that control crops be set up without the pesticides, but with the same fertilization, irrigation, and other conditions as the test crop. Signaling by GDH is particularly useful for evaluating the activity of pesticides because the activity of the enzyme affects whole-plant biochemistry via its relationship to plant growth (Osuji et al., 1998a; Osuji and Madu, 1997b). Similar to spectroscopic methods for pesticide analysis (Cairns and Sherma, 1992), the GDH isomerization responded differently to each pesticide concentration. Other advantages are that the response to each pesticide is unique; it indicates the interaction between the metabolism of a pesticide and the other agrochemicals, and it does not require the preparative extraction and cleanup works which are the costly and exacting steps in the chromatographic analysis of pesticide residues (Nemoto and Lehotay, 1998; Richter, 1992; Sawyer et al., 1990). The redistribution kinetics of pesticides in the environment determines pesticide use efficiency. Therefore, the relationship between the GDH isomerization and the crop yield suggests that the isomerization could be a sensitive method for the evaluation of pesticide sink-source redistribution kinetics in plants.

ACKNOWLEDGMENT

We thank Drs. Robert D. Stipanovic and Steven J. Lehotay for their assistance in the revision of the research proposal.

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Received for review May 18, 1998. Revised manuscript received November 12, 1998. Accepted January 7, 1999.

JF9805303