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Effect of Temperature, Elevated Carbon Dioxide, and Drought during Seed Development on the Isoflavone Content of Dwarf Soybean [*Glycine max* (L.) Merrill] Grown in Controlled Environments

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The effects of elevated temperature, carbon dioxide, and water stress on the isoflavone content of seed from a dwarf soybean line [*Glycine max* (L.) Merrill] were determined, using controlled environment chambers. Increasing the temperature from 18 °C during seed development to 23 °C decreased total isoflavone content by about 65%. A further 5 °C increase to 28 °C decreased the total isoflavone content by about 90%. Combining treatments at elevated temperature with elevated CO_2 (700 ppm) and water stress to determine the possible consequences of global climate change on soybean seed isoflavone content indicated that elevated CO_2 at elevated temperatures could partially reverse the effects of temperature on soybean seed isoflavone content. The addition of drought stress to plants grown at 23 °C and elevated CO_2 returned the total isoflavone levels to the control values obtained at 18 °C and 400 ppm CO_2. The promotive effects of drought and elevated CO_2 at 23 °C on the 6″-O-malonygenistin and genistin levels were additive. The individual isoflavones often had different responses to the various growth conditions during seed maturation, modifying the proportions of the principal isoflavones. Therefore, subtle changes in certain environmental factors may change the isoflavone content of commercially grown soybean, altering the nutritional values of soy products.

KEYWORDS: Carbon dioxide; drought; global climate change; *Glycine max* (L.) Merrill; isoflavone; soybean

INTRODUCTION

The beneficial effects of isoflavone-rich foods have been the subject of numerous studies (*1*, *2*). Although certain contraindications for diets containing high levels of phytoestrogenic isoflavones have been identified (*3*), foods derived from soybeans are generally considered to provide both specific and general health benefits. However, the development of dietary standards for isoflavones and soy products has been hampered by variations in isoflavone concentration and composition in soy-based products (*4*, *5*). Some of this variability can been attributed to the inherent ability of different soybean genotypes to accumulate isoflavones at different levels and proportions (*6*−*9*). However, there is increasing evidence that the environmental conditions under which the soybean seeds develop may play a significant role in determining isoflavone content (*6*−*13*).

Seed from the same soybean cultivar grown in different regions, in different years, or sown at different times in the same field in the same year may have very distinct isoflavone compositions, varying up to 3-fold in total isoflavone content (*6*, *9*, *12*). Plant phenylpropanoid metabolism can be modified by a variety of environmental conditions (*14*). The presence of both abiotic and biotic stresses can alter the phenolic composition of plants (*14*−*17*). Therefore, regional and local variations in temperature, water or nutritional status, insect or microbial attack, atmospheric pollutants, and/or light conditions may influence phenolic metabolism during critical stages of soybean development and modify the ultimate isoflavone composition of the seeds. However, the intrinsic difficulties associated with field studies tend to complicate the identification of specific environmental factors that influence the isoflavone composition of soybeans. While one investigator demonstrated significant reductions in isoflavone concentration in seeds maturing at elevated temperatures (*10*), another study indicated that the effects of temperature during seed development on isoflavone content were minimal, suggesting the importance of photoperiod (*6*). In general, many of the environmental factors known to alter plant phenolic metabolism can be measured, but not controlled in field investigations. Although greenhouse investigations offer a greater degree of control over environmental conditions, plant phenolic composition is strongly influenced by variations in solar light intensity and quality which cannot be easily regulated in greenhouses.

The utilization of controlled environment or growth chambers in soybean research considering the potential effects of global climate change on seed quality has been limited. Standard

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soybean cultivars usually require more space and higher irradiances to produce a reasonable yield than can be reasonably accommodated in growth chambers. However, a dwarf variety of soybean has attributes that allows its use in investigations that require the controlled environments provided by growth chambers (*18*). Therefore, a series of studies were initiated to determine the effects of temperature and other environmental factors during seed development on the isoflavone content of dwarf soybean seeds. Since there appears to be little experimental evidence considering the possible effects of global climate change on the phytonutrient content of crop plants (*19*, *20*), the effects of elevated carbon dioxide and drought were also investigated.

MATERIALS AND METHODS

Chemicals. Acetyldaidzin, acetylgenistin, acetylglycitin, daidzein, daidzin, genistein, genistin, glycitein, malonyldaidzin, malonylgenistin, and malonylglycitin were obtained from LC Laboratories (Woburn, MA). All other chemicals were obtained from Sigma/Aldrich Chemical Co. (St. Louis, MO).

Plant Material and Growth Conditions. A determinant, early flowering, strongly dwarfed soybean [*Glycine max* (L.) Merrill] was used to minimize vegetative growth and mutual shading of adjacent plants and to facilitate reciprocal transfer of plants between the different growth conditions. The unidentified soybean line used in these experiments was obtained in the late 1960s from the Wye Research Institute (Easton, MD) by scientists from E. I. duPont de Nemours. All efforts to obtain specific information about the breeding of this dwarf soybean line have been unsuccessful. Seed samples have been deposited with the USDA-ARS-Plant Physiology and Genetics Unit (Urbana, IL). Most of the current semi-dwarf soybean cultivars have been evaluated for use in controlled environment experiments and found to have poor growth or yield characteristics.

Six plants were grown in each of two matched controlled environment chambers (1.7 m^2 growing area; model M-18SI, Environmental Growth Chambers, Chagrin Falls, OH). Seeds were sown in 10 in. pots with Hoffman's Professional Growing Media (A. H. Hoffman, Inc., Suffolk, VA) and thinned to one plant per pot about 1 week after emergence. Plants were watered as needed with a complete nutrient solution (*21*).The chambers were set initially for constant air temperature of 25 °C and 60% relative humidity. Plants were illuminated by six 400-W high pressure sodium (Sylvania LU400/ECO, Osram Sylvania, Inc., Danvers, MA) and six metal halide lamps (Sylvania MS400/HOR, Osram Sylvania, Inc., Danvers, MA) configured in three separately controlled banks with two lamps of each type. The banks were programmed to turn on and off at intervals producing stepwise increases and decreases in light that simulated a diurnal cycle of irradiance, as well as, seasonal changes in lighting (*22*). Solstice conditions were 15 h daylength, 44.6 mol^{-2} daily integral of photosynthetically active radiation (PAR, quanta between 400 and 700 nm), and 825 $\mu mol^{-2}\ s^{-1}$ average irradiance of PAR. Daylength was reduced 15 min every week by shortening the high irradiance period in the middle of the light cycle such that autumnal equinox conditions were reached after 12 weeks (12 h daylength, 31.7 mol^{-2} daily integral, and 735 $\mu mol^{-2}\ s^{-1}$ average irradiance). PAR was measured with a quantum sensor and data logger (model LI-1000, LI-COR, Inc., Lincoln, NE).

The limited number of growth chambers required the experiments to be performed in three parts. The first study compared the effects of raising plants at two different levels of atmospheric CO_2 (400 or 700 ppm) from sowing to final harvest. CO_2 was measured and controlled with an infrared gas analyzer (model WMA-3, PP Systems, Haverhill, MA), adding either CO_2 gas during the light period to compensate for photosynthesis or CO_2-depleted air during the dark period to balance increases in CO_2 as result of respiration. At the onset of seed fill (Stage R5), approximately 6 weeks after emergence and 2 weeks after first flowering, chamber air temperatures were reduced from 25 °C to a constant 18 °C for the remaining 6 weeks of seed development to simulate average outdoor temperatures. In addition, three plants from each chamber were exchanged at this time to balance any differences in plant growth that might have resulted during the initial period in different chambers.

In the second and third studies, the effect of elevated temperature and/or drought during seed development were compared either at 400 ppm CO_2 (second study) or at 700 ppm CO_2 (third study). CO_2 levels were controlled as in the first study throughout plant development, and plants were randomized between chambers at the onset of seed fill. At this time, the temperature in one chamber was increased from 25 °C to a constant 28 °C for the duration of seed development, while the temperature in the second chamber was decreased to 23 °C. Some plants in each chamber were also subjected to concurrent long-term water stress by withholding nutrient solution. The degree of drought was controlled based on the average reading of two soil moisture blocks (Soil Moisture Equipment Corp., Santa Barbara, CA) inserted about 5 and 15 cm respectively above the bottom of each pot. The upper sensor was about 5 cm below the surface. The sensors were calibrated gravimetrically for the percent of water saturation of the medium. At 1−2 day intervals, sufficient supplemental water was added to the well-watered controls to maintain approximately 50−60% of saturation, while the droughted plants were adjusted to be approximately 20−30% of saturation.

Seed from three other studies were also utilized to increase the number individual plant samples to four. These studies were performed essentially as described above, and only samples grown under identical growth conditions were included the subsequent isoflavone analyses.

Sample Preparation Procedures. Seeds were harvested progressively as pods matured on each of 4 representative plants for each set of conditions in the various studies. Harvested seeds were stored at −20 °C prior to freeze-drying, weighing and subsequent grinding to a fine flour (Cyclone Sample Mill, UD Corp., Boulder, CO). Ground seed was stored in tightly capped vials at −20 °C until analysis.

Sample extraction was an adaptation of the methods of Wiesman et al. (*5*). Randomly selecting samples from four plant replicates for each treatment condition, approximately 100 mg of the soy flour samples were weighed, placed in 1.5 mL amber microcentrifuge tubes and sufficient 80% (v/v) aqueous ethanol added to yield 100 mg dry weight/mL. After mixing, the samples were incubated at 60 °C for 60 min, briefly removing and remixing the samples at 10 min intervals. The samples were then centrifuged at 17400*g* for 5 min at room temperature. Two 200 μL aliquots of the supernatant from each sample were dried (Savant Speed-Vac) for 90 min and reconstituted in 100 μL of 95% (v/v) aqueous methanol, containing 1% (v/v) formic acid. The reconstituted samples were stored at −20 °C for a maximum of 48 h before HPLC analysis. All extractions were repeated for each soy flour sample.

HPLC Analytical Procedures. All HPLC procedures were performed with a Waters 600 series pump and controller, and a Waters 990 photodiode array detector. Ten μL of the sample was injected into a 250 mm × 4.6 mm Spherisorb ODS-2 (octadecylsilyl, 5 μm particle size) column (Sigma Chemical Co.). Elution was at 1.0 mL/min with the solvent system A = water containing 1% (v/v) formic acid and B = acetonitrile (*5*): 30 min linear gradient from 15 to 22% B, followed by a 20 min linear gradient from 22 to 35% B and 5 min linear gradient from 35 to 70% B. After isocratic elution at 70% B for 5 min, the column was returned to 15% B over 2.5 min and held at the starting condition for 7.5 min before the next analysis. The total analysis time was 60 min followed by a 10 min column regeneration. The chromatograms were monitored and integrated at 262 nm. All chromatograms represent data obtained at one second intervals.

Isoflavone Quantification. The absorbance units/min at 262 nm from the integrated chromatograms of the duplicate sample extractions were averaged and converted to mole isoflavone/g dry weight, as described by Song et al. (*23*). Standard curves were prepared using isoflavone standards at concentrations between 0.25 and 5 μg per HPLC injection. After regression analysis, the linear portion of the curves > 0.995) was used to convert absorbance units/min to mol/g dry weight for each isoflavone. The total isoflavone content was computed by addition. The data are presented as the means with standard deviations of the four plant replicates. Statistical analyses were performed using SigmaStat (SPSS Inc., Chicago, IL).

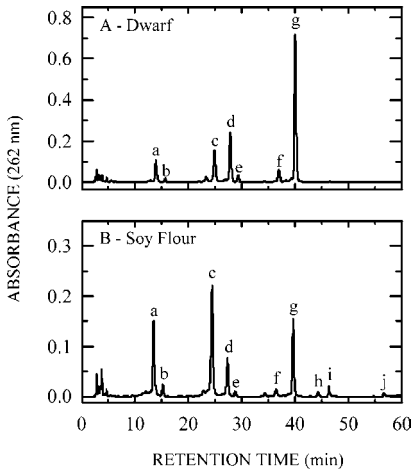

Figure 1. HPLC chromatograms of 80% (v/v) aqueous ethanol extracts of finely ground dwarf *G. max* (L.) Merrill seed (**A**) and a commercial soy flour (**B**). The isoflavone components of the extracts were identified using standards, UV spectra, and comparison to published chromatograms. Peaks: a, daidzin; b, glycitein; c, genistin; d, 6″-O-malonyldaidzin; e, 6″-O-malonylglycitin; f, 6″-O-acetyldaidzin; g, 6″-O-malonylgenistin; h, daidzein; i, glycitein; and j, genistein.

RESULTS AND DISCUSSION

Isoflavone Composition of Dwarf Soybean Seed. Reverse-phase HPLC analysis of a soybean seed aqueous ethanol extract produced a chromatogram with peaks having retention times, proportions, and spectral characteristics consistent with specific isoflavones (**Figure 1A**). The isoflavone components of the extract were identified using standards, UV spectra, and comparison to published chromatograms (*5*, *12*, *13*, *23*, *24*): a, daidzin; b, glycitein; c, genistin; d, 6″-O-malonyldaidzin; e, 6″-O-malonylglycitin; f, 6″-O-acetyldaidzin; and g, 6″-O-malonylgenistin. These attributions are consistent with the average isoflavone composition of commercial soy flour (*5*) (**Figure 1B**), which also contains the isoflavone aglycones daidzein (h), glycitein (i), and genistein (j). Since long-term storage or processing increased the amounts of isoflavone aglycones (*12*, *24*), the absence of detectable daidzein, glycitein, and genistein in the dwarf soybean sample suggests that the seed sample preparation, storage, and extraction procedures did not significantly alter the original isoflavone composition. As in most commercially grown soybean varieties (*12*, *24*), 6″-O-malonylgenistin is the major isoflavone in dwarf soybean seeds (**Figure 1A**).

Effect of Growth Temperature, Carbon Dioxide Levels, and Drought During Dwarf Soybean Seed Development on Isoflavone Composition. Although the increased yield produced by elevated CO_2 suggests that the projected global climate changes may increase soybean productivity (*25*), the potential effects of the altered environmental conditions, which would also include increased temperatures and altered weather patterns on product quality, have not often been considered experimentally. Using CO_2 levels that approximate the long-term effects of global climate change, the elevation of CO_2 concentrations in field studies increased the levels of total phenolics and phenolic antioxidants in woody species and strawberry (*19*, *26*).

Based on 20 year averages, the mean temperature declines from about 23 to 18 °C during seed development of field-grown soybean at Beltsville, MD. Using growth chambers set at 18 °C during the final 6 weeks of seed development, the growth of dwarf soybean at 400 ppm CO_2 produced seed with a typical isoflavone composition (**Figure 2A**). Although 400 ppm CO_2 is slightly above the average ambient levels at Beltsville, MD, maintenance of constant CO_2 concentrations during the experiments requires supplementing the ambient levels to a concentration that is not routinely exceeded during the normal seasonal fluctuations in atmospheric CO_2. Increasing the CO_2 levels to 700 ppm or about 300 ppm above ambient, slightly modified the seed isoflavone content (**Figure 2B**), increasing the total isoflavone concentration and the 6″-O-malonylgenistin concentration by about 8% (**Table 1**).

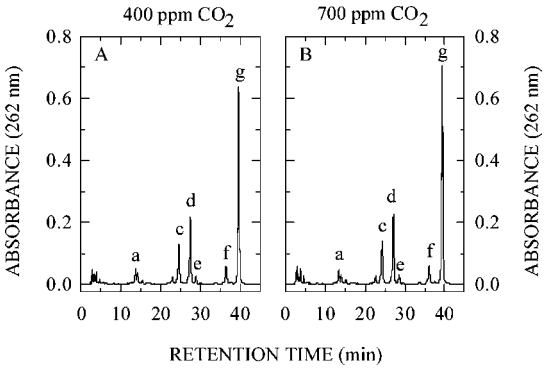

Figure 2. HPLC chromatograms indicating the effect of growth at 18 °C, either 400 (**A**) or 700 (**B**) ppm CO_2 during seed development on the isoflavone contents of *G. max* (L.) Merrill seed. Peak identifications are given in the legend to **Figure 1**.

Although the results presented in **Table 1** suggest slight changes in the relative proportions of the soy isoflavones from seed produced at elevated CO_2 and normal growth temperatures, the effects of elevated CO_2 were minimal and unlikely to adversely effect product quality. However, increased levels of atmospheric CO_2 are likely to be accompanied by elevated average temperatures. Consistent with earlier research (*10*), increasing the temperature to 23 °C during dwarf soybean seed development reduced the total isoflavone content at both CO_2 levels (**Figure 3A**,**B** and **Table 1**). The 5 °C temperature increase decreased total isoflavone levels by about 65 and 30% for the plants grown at 400 and 700 ppm CO_2, respectively, relative to the seed produced at 18 °C and 400 ppm CO_2 (**Table 1**). With the exception of 6″-O-malonylglycitin, all the individual isoflavones contributed to this decline in total isoflavone content. However, the reduction in the levels of the individual isoflavones was not uniform, suggesting that differences in metabolic responses to hyperthermia may produce soybean seed with altered isoflavone proportions. Computing the ratios between the concentrations of the principal isoflavones in dwarf soybean seeds demonstrates the altered proportions of the isoflavones in seeds that developed at 23 °C (**Table 2**). Furthermore, the growth of dwarf soybean at 700 ppm CO_2 reduced the effects of hyperthermia on the seed isoflavone content, suggesting a decreased sensitivity to elevated temperatures at higher CO_2 levels.

Although unlikely to occur under current climatic conditions, the temperature during dwarf soybean seed development was further increased to 28 °C (**Figure 4**). The 10 °C temperature increase reduced total isoflavone levels by 87 and 74% for the plants grown at 400 and 700 ppm CO_2, respectively, relative to the seed produced at 18 °C and 400 ppm CO_2 (**Table 1**). Similar to the seed produced at 23 °C, 6″-O-malonylglycitin levels responded differently to the increased temperature relative to the other isoflavones. Although growth under elevated CO_2 reduced the effects of the 10 °C temperature increase on total isoflavone concentrations, the promotive effects of elevated CO_2

Table 1. Effects of Temperature (T), Elevated CO_2, and Drought (D) during Seed Development on the Isoflavones in *G. max* (L.) Merrill Seed[a]

T (°C)	CO_2 (ppm)	D (±)	total		genistin		6″-O-malonyldaidzin		6″-O-malonylglycitin		6″-O-malonylgenistin	
			μmol/g	% change	μmol/g	% change	μmol/g	% change	μmol/g	% change	μmol/g	% change
18	400	–	8.55 ± 0.44A	0.0 (0.0)	1.18 ± 0.11A	0.0 (0.0)	2.41 ± 0.07A	0.0 (0.0)	0.24 ± 0.02A	0.0 (0.0)	4.72 ± 0.24A	0.0 (0.0)
18	700	–	9.21 ± 0.37AN	7.7 (7.7)	1.36 ± 0.09B	15.3 (15.3)	2.48 ± 0.06AN	2.9 (2.9)	0.26 ± 0.04AN	8.3 (8.3)	5.11 ± 0.18B	8.3 (8.3)
23	400	–	3.02 ± 0.15A	–64.7 (0.0)	0.30 ± 0.03A	–74.6 (0.0)	0.80 ± 0.04A	–66.8 (0.0)	0.37 ± 0.03A	54.2 (0.0)	1.55 ± 0.05A	–67.2 (0.0)
23	700	–	6.16 ± 0.21B	–28.0 (104.0)	0.81 ± 0.04B	–31.4 (170.0)	1.21 ± 0.04B	–49.8 (51.3)	0.50 ± 0.03B	108.3 (35.1)	3.64 ± 0.10B	–22.9 (134.8)
23	400	+	3.98 ± 0.20C	–53.5 (31.8)	0.44 ± 0.04C	–62.7 (47.0)	0.96 ± 0.03C	–60.2 (20.0)	0.36 ± 0.02A	50.0 (–2.7)	2.22 ± 0.11C	–53.0 (43.2)
23	700	+	8.53 ± 0.39DN	–0.2 (182.5)	1.04 ± 0.05D	–11.9 (246.7)	2.19 ± 0.06D	–9.1 (173.8)	0.81 ± 0.02C	237.5 (118.9)	4.49 ± 0.26DN	–4.9 (189.7)
28	400	–	1.12 ± 0.05A	–86.9 (0.0)	0.09 ± 0.01A	–92.4 (0.0)	0.37 ± 0.01A	–84.7 (0.0)	0.24 ± 0.01AN	0.0 (0.0)	0.42 ± 0.02A	–91.1 (0.0)
28	700	–	2.25 ± 0.09B	–73.7 (100.9)	0.23 ± 0.01B	–80.5 (155.6)	0.62 ± 0.02B	–74.3 (67.6)	0.40 ± 0.01B	66.7 (66.7)	1.00 ± 0.05B	–78.8 (138.1)
28	400	+	1.03 ± 0.07A	–88.0 (–8.0)	0.10 ± 0.01A	–91.5 (11.1)	0.34 ± 0.03A	–85.9 (–8.1)	0.23 ± 0.01AN	–4.2 (–4.2)	0.36 ± 0.02C	–92.4 (–14.3)
28	700	+	1.54 ± 0.08C	–82.0 (37.5)	0.15 ± 0.01C	–87.3 (66.7)	0.51 ± 0.01C	–78.8 (37.8)	0.26 ± 0.02AN	8.3 (8.3)	0.62 ± 0.04D	–86.9 (47.6)

[a] The principal isoflavones found in the soybean seeds were quantified as μmol/g seed flour dry weight and presented as the means ± SD ($n = 4$ plants, eight analyses). The percent change in concentration produced by a given treatment was computed both in terms of the control value obtained at 18 °C and 400 ppm CO_2 and in parentheses a control value obtained at 400 ppm CO_2 and the same growth temperature. For samples obtained at the same growth temperature, means followed by the same capital letters are not statistically different ($P > 0.05$, $n = 4$). For a given isoflavone, means followed by a capital N are not statistically different ($P > 0.05$, $n = 4$) from the control value obtained with plants grown at 18 °C and 400 ppm CO_2.

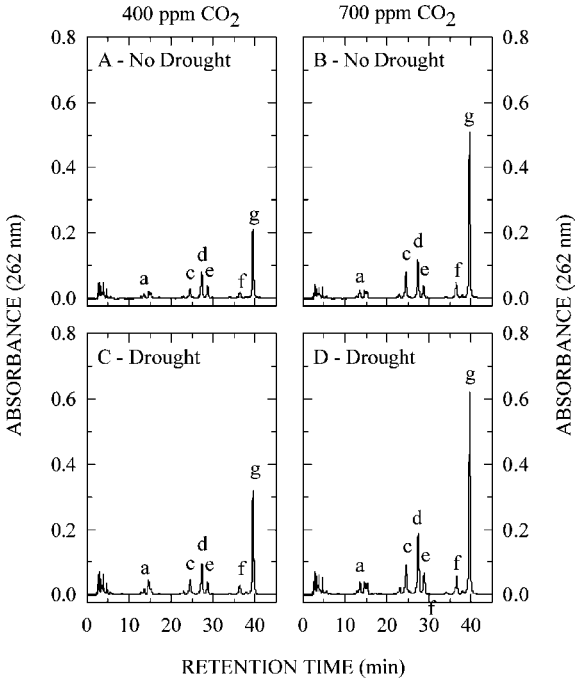

Figure 3. HPLC chromatograms indicating the effect of growth at 23 °C, either 400 (**A**, **C**) or 700 (**B**, **D**) ppm CO_2 and with (**C**, **D**) or without (**A**, **B**) moderate drought stress during seed development on the isoflavone contents of *G. max* (L.) Merrill seed. Peak identifications are given in the legend to **Figure 1**.

on the levels of certain individual isoflavones, such as 6″-O-malonylgenistin, were reduced (**Table 1**).

The results presented above clearly indicate significant changes in the isoflavone content of dwarf soybean seed that developed at different temperatures and CO_2 levels. However, variations in weather patterns that result in regional increases in drought are also a likely consequence of global climate change. Therefore, a moderate drought stress was imposed on the plants during seed development at 23 and 28 °C and the two CO_2 levels. As shown in **Figure 2**, drought increased the isoflavone levels at both 400 and 700 ppm CO_2 when compared to seed from plants grown at 23 °C at the same CO_2 levels. Combining drought and 700 ppm CO_2 during seed development at 23 °C produced total isoflavone concentrations equivalent to the 18 °C control (**Table 1**), reversing the temperature-dependent decrease in isoflavone levels. Furthermore, the effects of drought and elevated CO_2 on the levels of 6″-O-malonylgenistin and genistin in seed produced at 23 °C appear to be additive.

Table 2. Effects of Temperature (T), Elevated CO_2, and Drought (D) during Seed Development on the Relative Proportions of the Malonyl Isoflavones in *G. max* (L.) Merrill Seed[a]

T (°C)	CO_2 (ppm)	D (±)	6″-O-malonylgenistin/ 6″-O-malonyldaidzin	6″-O-malonylgenistin/ 6″-O-malonylglycitin	6″-O-malonyldaidzin/ 6″-O-malonylglycitin
18	400	–	1.96	19.67	10.04
18	700	–	2.06	19.65	9.54
23	400	–	1.94	4.19	2.16
23	700	–	3.01	7.25	2.42
23	400	+	2.31	6.17	2.67
23	700	+	2.05	5.54	2.70
28	400	–	1.13	1.75	1.54
28	700	–	1.61	2.50	1.55
28	400	+	1.06	1.57	1.48
28	700	+	1.22	2.39	1.96

[a] The ratios of malonyl isoflavones found in the soybean seeds were computed from the data presented in **Table 1**.

Increasing the CO_2 level from 400 to 700 ppm, raised 6″-O-malonylgenistin levels by 134.8% in the seed of well-watered plants relative to the control values obtained at 23 °C and 400 ppm CO_2 (**Table 1**). In plants grown at 400 ppm CO_2, drought increased 6″-O-malonylgenistin levels by 43.2%. Adding these percent change values yields 178.0%, which is similar to the experimental value of 189.7% for seed from plants grown under both elevated CO_2 and drought. Although a similar response was observed in genistin levels, none of the other isoflavones produced these additive reactions to elevated CO_2 and drought (**Table 1**).

Unlike the isoflavone content of seed developed at 23 °C, the addition of a drought stress to plants producing seed at 28 °C and 700 ppm CO_2 reduced the total, relative isoflavone content of the seed (**Figure 4B**,**D** and **Table 1**). Although the percent changes in 6″-O-malonylgenistin (138.1%) and genistin (155.6%) levels produced by increasing the CO_2 concentration from 400 to 700 ppm at 28 °C were similar to those obtained at 23 °C, 134.8 and 170.0%, respectively, there were no apparent additive effects of drought and elevated CO_2 on any of the individual isoflavone in seeds produced at 28 °C. Furthermore, considering the ratios for the various isoflavones (**Table 2**), variations in CO_2 levels may have greater influence on the relative proportions of the isoflavones than drought.

In conclusion, the results of this study demonstrate the utility of controlled environment experiments in determining the effects of combinations of environmental factors that may influence the quality of field-grown agricultural products. By precise control of growth temperature, soil moisture content, and CO_2 concentration, it was possible to demonstrate the possible

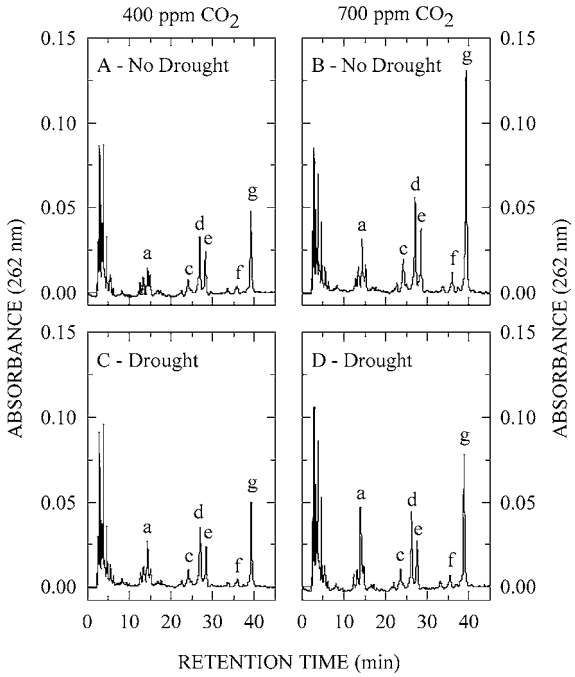

Figure 4. HPLC chromatograms indicating the effect of growth at 28 °C, either 400 (**A**, **C**) or 700 (**B**, **D**) ppm CO_2 and with (**C**, **D**) or without (**A**, **B**) moderate drought stress during seed development on the isoflavone contents of *G. max* (L.) Merrill seed. Peak identifications are given in the legend to **Figure 1**.

consequences of global climate change on soybean seed isoflavone composition. Furthermore, the magnitude of the plant responses to these different growth conditions suggests that subtle changes in certain environmental factors may play a significant role in determining the isoflavone content of commercially grown soybean.

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