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BRIEF COMMUNICATION

Utility of trigonelline as a biochemical marker for interspecific competition between soybean and the weed common waterhemp

T.L. PFEIFFER*, Y. CHO*, D.J. GIBSON*, B.G. YOUNG** and A.J. WOOD*

Department of Plant Biology, Department of Plant, Soil and General Agriculture**, Center for Excellence in Soybean Research, Teaching and Outreach, Southern Illinois University, Carbondale, IL 62901, USA*

Abstract

Interspecific competition between four soybean cultivars (PI471938, Stressland, Essex and Forrest) and the weed, common waterhemp was investigated under increasing weed densities (*i.e.* 0, 1, 4 and 16 plants per pot). Soybean height and leaflet number were measured over a 45-d period and used to calculate relative growth rates (RGR). Trigonelline (TRG) concentration was determined within the V1 leaf of 45-d-old soybean plants. Soybean leaflet number ($P < 0.05$), soybean height ($P < 0.05$) and soybean RGR_h (expressed in terms of height) differed significantly ($P < 0.05$) according to waterhemp density. At each waterhemp density Stressland matured at a significantly faster rate whereas the maturation rate of Essex decreased in the presence of waterhemp. Final TRG concentrations were affected by the interaction between soybean cultivar and waterhemp density. Under no competition, TRG concentration was significantly lower in Forrest relative to PI471938, Stressland and Essex. TRG concentrations in Essex declined in higher waterhemp densities.

Additional key words: *Amaranthus rudis*, *Glycine max*, relative growth rate.

The influences of abiotic stresses, such as altered temperature, salinity, and drought, upon plant productivity are well characterized (Boyer 1982, Lerner 1999). However the analysis of biotic stresses, such as the competitive interactions between plant species, is critical to our understanding of plant productivity. In particular, the study of interspecific competition allows the dissection of physiological and biochemical parameters altered by plant-to-plant interactions. We have developed soybean (*Glycine max* L.) and common waterhemp (*Amaranthus rudis* L.), a weed of soybean (Johnson *et al.* 1996), as a model for interspecific plant interactions. We are particularly interested in the biosynthesis and accumulation of the plant alkaloid trigonelline (TRG) in soybean (Wood *et al.* 2000). TRG accumulates in response to NaCl and water deficit stress (Tramontano and Jouve 1997, Cho *et al.* 1999, Wood 1999) and is postulated to function as a compatible solute and/or osmoprotectant (Naidu *et al.* 1992). Exogenously applied TRG acts as a cell cycle regulator by lengthening the S phase (Macuzza *et al.* 2000) and promoting cell arrest in the G2 phase (Tramontano and Jouve 1997). We suggest that TRG can serve as a biochemical marker for competition-induced stress, and that TRG concentrations would increase within soybean in response to high competition stress with a weed. The present study is a greenhouse pot trial investigating the competitive relationships between four soybean cultivars and waterhemp using 4 weed densities (0, 1, 4 and 16 plants).

The soybean cultivars Essex and Forrest were

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Abbreviations: RGR - relative growth rate; TRG - trigonelline.

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Correspondence should be addressed to: Andrew J. Wood; fax: (+1) 618 4533441, e-mail: wood@plant.siu.edu.

obtained from Dr. D.A. Lightfoot (SIU-C); cultivar Stressland and PI471938 were obtained from Dr. T. Carter (North Carolina State University, NC, USA). Soybean seedlings (0 - 14 d after planting) were grown under controlled conditions (29/23 °C day/night temperature), and watered daily. 14-d-old soybean seedlings were transplanted to 7.5-dm^3 pots in a 1:1 sand:soil mixture (3 seedlings per pot) containing oversown waterhemp. Waterhemp was thinned to densities of 0, 1, 4, or 16 plants per pot (a target-neighbor competition design; Gibson *et al.* 1999). Each waterhemp seed density had 4 repetitions. 9 d after transplantation, soybean seedlings were thinned to one seedling per pot. The soybean/waterhemp plants were grown on open greenhouse benches (SIU-C; min. temperature = 29 °C), watered 1 - 2 times per day, and pulse fed to soil saturation once every four weeks with a 20:10:20 (N:P:K) fertilizer mix. 9 d after transplanting seedlings, initial measurements of soybean height and leaflet number were taken. Measurements were taken twice a week for 7 weeks giving 14 sets of data. *A. rudis* densities were maintained throughout the study. 45 d after transplanting, the V1 soybean leaf was harvested and analyzed for TRG as described below.

The remaining soybean plant was cut at the soil level, bagged, and dried in a convection oven (80 °C, 72 h) to obtain dry mass data. Harvest for whole plant dry mass of waterhemp was conducted in a similar manner. Leaf tissue (0.1 - 0.5 g) was extracted in methanol at 4 °C in the dark, and phase separated by the addition of distilled water and chloroform (5:3:2 respectively v/v/v) (Wood 1999). TRG was purified by *Dowex-1-OH-* and *Dowex-50-H^+* ion exchange chromatography and quantified spectrophotometrically (UV-VIS spectrophotometer *Lambda 12*, *Perkin-Elmer*, Norwalk, USA) as described (Cho *et al.* 1999).

Repeated measures *ANOVA GLM* procedure with polynomial transformation (*SAS* ver. 6.11; SAS Institute Inc. 1989) was used to analyze the data on soybean height and leaflet number in response to soybean cultivar (4 levels) and waterhemp density (4 levels; 0, 1, 4, and 16 plants). Days since planting were used to represent the level values for data collected on 14 occasions (approximately every 2 - 5 days) in the analysis. The dependent variables were log transformed prior to analysis. Relative growth rate (RGR) was calculated as: $RGR = (\ln M_2 - \ln M_1)/(T_2 - T_1)$, where M_1 and M_2 were the plant height or leaflet number at the beginning (T_1) and the end (T_2) of the period, respectively (Evans 1972). RGR was calculated in terms of height (RGR_h) and leaflet number (RGR_l). The Anderson sphericity test was used to determine if the data met a Type H covariance structure (SAS Institute Inc. 1989). Huynh and Feldt adjusted F-tests were used when the sphericity test was rejected at $P < 0.05$. Final harvest data were analyzed using a two-way *ANOVA* with soybean variety (df = 3) and waterhemp density (df = 3) as main effects. Log and square root transformations were taken on some variables to improve normality and reduce the variance relative to the mean (see Table 1). Total pot biomass was calculated as the sum of soybean and waterhemp dry masses. Soybean relative yield was calculated as soybean d.m. / total pot biomass. Mean waterhemp biomass was calculated as waterhemp d.m. / waterhemp density per pot (data from the zero density treatments were excluded from this analysis).

The effects of the main treatments varied throughout the experiment. Comparing polynomial contrasts for time, soybean height differed significantly ($P < 0.05$) according to waterhemp density on 4 days (13 - 15, 15 - 20, 34 - 37, and 41 - 44). Significant cultivar effects occurred on 3 days (23 - 27, 27 - 29 and 37 - 41). The soybean plants did not possess expanded leaflets on the date of the first data collection, but did from day 13 onwards allowing the analysis of 12 time contrasts. Repeated measures *ANOVA* showed a significant interaction between time (days after planting) and soybean cultivar (Huynh and Feldt adjusted $F_{36, 576} = 2.46$, $P = 0.0002$), and time and waterhemp density (Huynh and Feldt adjusted $F_{36, 576} = 13.56$, $P = 0.0001$), but not the three-way interaction (data not shown). The effects of the main treatments varied throughout the experiment. Comparing polynomial contrasts for time, soybean leaflet number differed significantly ($P < 0.05$) according to the interaction between cultivar and waterhemp density on days 15 - 20. A significant effect of cultivar occurred on 3 days 15 - 27.

When expressed in terms of height, soybean RGR_h differed significantly ($P < 0.05$) according to waterhemp density on two days (9 - 13 and 29 - 34). When expressed in terms of leaflet number, soybean RGR_l was not significantly affected by the interaction of cultivar and waterhemp density. The interaction between soybean cultivar and waterhemp density significantly affected soybean node number. Total pot biomass and soybean reproductive stage were marginally affected by the interaction ($P < 0.1$). Waterhemp density alone as a main effect significantly affected final harvest data for all variables analyzed (Table 1). Waterhemp biomass was marginally affected by soybean cultivar. Trigonelline concentrations were significantly affected by the interaction between soybean cultivar and waterhemp density. In each cultivar (PI471938, Stressland, Essex and Forrest), soybean biomass declined concomitantly as waterhemp density increased (Fig. 1*A*), and there was no further reduction between the 4- and 16-density treatment. The reproductive stage of each cultivar was marginally affected by the interaction with waterhemp density (Fig. 1*B*). At each waterhemp density Stressland matured at a significantly faster rate (*i.e.* maintained a higher reproductive stage value) whereas the maturation rate of Essex decreased in the presence of waterhemp. Similarly, TRG concentrations were affected by the

interaction between soybean cultivar and waterhemp density (Table 1, Fig. 1*C*). As compared to the zero and one waterhemp densities, TRG concentrations in Essex declined in both the 4- and 16-density treatments. Under no competition, final TRG concentrations differed significantly in Forrest relative to PI471938, Stressland and Essex. At the 4-density treatment, each cultivar had a significantly different TRG concentration relative to each other.

Interspecific competition of soybean has been studied in a relation to a number of plants (Bussan *et al.* 1997, Vitta *et al.* 1994). However to our knowledge, this is the first analysis of soybean-waterhemp interspecific competition using a biochemical marker (*i.e.* TRG accumulation). TRG, the *N*-methyl conjugate of nicotinic acid, is part of the nicotinamide biochemical responsible for the production of NAD^+, $NADP^+$, and ribonucleotides. We postulate that altered TRG

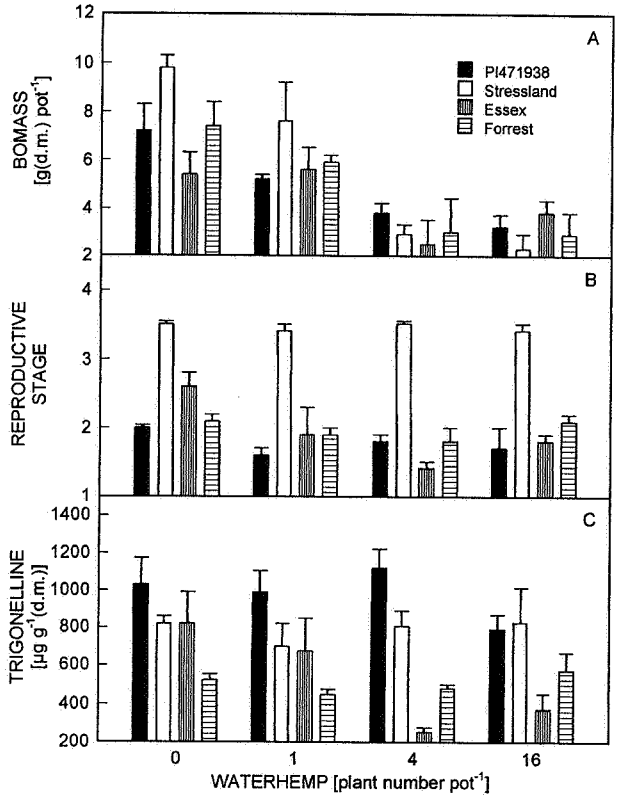

Fig. 1. Competition effects of increasing waterhemp (*A. rudis*) density upon soybean (*G. max*) biomass (*A*), reproductive stage (*B*) and trigonelline concentration (*C*) at final harvest (55 d). Reproductive stage refers to the standardized soybean developmental scheme from flowering to seed maturity (*i.e.* R1 - R8). Each data point represents the mean measurement on 3 individual plants ± SE.

Table 1. *F* values from *ANOVA* of final harvest data. $^+ P < 0.1$, * $P < 0.05$, ** $P < 0.01$. Error df = 48 except for trigonelline content (error df = 47), soybean nodes & reproductive stage (error df = 46) due to missing values.

Variable	Effect		
	soybean cultivar (df = 3)	waterhemp density (df = 3)	interaction (df = 9)
Soybean biomass	1.58	25.72**	1.69
Soybean nodes[1]	5.01**	7.65**	2.17*
Reproductive stage[1]	72.52**	5.85**	1.92$^+$
Soybean relative yield[2]	1.68	44.25**	1.33
Waterhemp biomass[2]	2.74$^+$	69.20**	1.50
Waterhemp mean biomass[2, 3]	1.99	21.56**	1.29
Total biomass	6.14**	4.03*	2.03$^+$
Trigonelline[1]	21.79**	2.94*	3.48**

[1] Square-root transformed variable; [2] Log transformed variable; [3] df = 2 for waterhemp density effect, df = 6 for interaction, and error df = 36 because zero waterhemp density treatment excluded from calculation.

concentration, particularly between cultivars, is either an adaptive response to water and/or nutrient stress, or a reflection of altered demand for the metabolites of the nicotinamide pathway. Alternatively, TRG accumulation may be a sensitive indicator of oxidative stress (Berglund 1994). The cultivars showed a response to interspecific competition. A number of whole plant characters such as plant height, leaflet and node number and relative growth rate all differed by cultivar. These differences among cultivars in their response to a problematic weed have important implications for the development of sustainable agroecosystems (Mohler 2001). Variation in a biochemical marker such as TRG accumulation allows us to address the mechanistic details responsible for interspecific competition.

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