



Foliar resistance to ozone injury in the genetic base of U.S. and Canadian soybean and prediction of resistance in descendent cultivars using coefficient of parentage

Kent O. Burkey^{a,c,*}, Thomas E. Carter Jr.^{b,c}

^a USDA-ARS Plant Science Research Unit, 3127 Ligon Street, Raleigh, NC 27607, USA

^b USDA-ARS Soybean and Nitrogen Fixation Unit, 3127 Ligon Street, Raleigh, NC 27607, USA

^c Department of Crop Science, North Carolina State University, Raleigh, NC 27695-7631, USA

ARTICLE INFO

Article history:

Received 31 July 2008

Received in revised form 22 October 2008

Accepted 10 December 2008

Keywords:

Ancestor

Coefficient of parentage

Foliar injury

Ozone tolerance

Soybean

ABSTRACT

Development of ozone (O₃)-resistant cultivars is a potentially important approach for maintaining crop productivity under future climate scenarios in which tropospheric O₃ pollution is projected to rise. A first step in the breeding of resistant cultivars for a crop such as soybean (*Glycine max* (L.) Merr.) is identification of sources of O₃ resistance genes. Thirty ancestral lines of soybean were screened for differences in O₃ foliar injury under greenhouse conditions. The ancestors represented 92% of the genetic base of North American soybean as determined by pedigree analysis. Injury among ancestors ranged from 5 to 50% of leaf area, based on response of the five oldest main stem leaves, indicating both the presence of substantial genetic variation for O₃ injury among the ancestors as well as resistance levels greater than that of the standard control cultivar, resistant Essex (15% injury). Ancestral types Fiskeby 840-7-3 and Fiskeby III exhibited the greatest foliar resistance and PI 88788 the least. A subsequent field study confirmed the foliar resistance of the Fiskeby types. Resistant ancestors identified here are proposed for inheritance and DNA mapping studies to determine the genetic basis of foliar resistance. Because the presence of O₃-resistant ancestors suggested that resistant descendants may exist in addition to the resistant control Essex, a method was developed to facilitate their identification. A predicted O₃-resistance score was calculated for 247 publicly-released cultivars, based on pedigree analysis and ancestral response to ozone. Using this approach, the 32 public cultivars most closely related to resistant ancestors and, thus, most likely to be resistant were identified as priority candidates for future screening efforts. Predicted scores from the analysis suggested that cultivars from the Midwest may be more sensitive to foliar injury, on average, than Southern cultivars.

Published by Elsevier B.V.

1. Introduction

Ozone (O₃) is considered a secondary air pollutant because it is formed in the atmosphere by photochemical reactions between oxygen and primary pollutants such as nitrogen oxides, carbon monoxide, and volatile organic compounds (Prather et al., 2001). Although a greenhouse gas, O₃ is typically not included in discussions of global warming because it is present in the atmosphere at much lower concentrations than carbon dioxide. However, carbon dioxide and O₃ issues are linked in that both are derived either directly or indirectly from the combustion of carbon based fuels. Ozone is widely recognized as a toxic air pollutant affecting both animals (e.g. humans) and plants. Ozone is known to inhibit the growth, biomass production, and yield of both crops and natural vegetation (Heagle, 1989; US EPA, 1996; Fiscus et al., 2005).

Estimates suggest that current ambient O₃ levels are sufficient to reduce yield of sensitive crops (Mauzerall and Wang, 2001; Wang and Mauzerall, 2004; US EPA, 2006). The most pessimistic future climate scenarios project ambient O₃ concentration will rise from the current annual mean of approximately 50 nmol mol⁻¹ to as high as 80 nmol mol⁻¹ in the year 2100 (Prather et al., 2001). If this increase in ambient O₃ were to occur, further declines in crop yields would be expected.

In the absence of successful efforts to reduce O₃ pollution, future crop productivity may depend on the development of O₃-tolerant varieties capable of maintaining yields under elevated ozone conditions. Genetic variation in O₃ sensitivity is commonly observed in most plant species studied to date (Wellburn and Wellburn, 1996) with O₃-sensitive and -resistant cultivars or clones reported for snap bean (*Phaseolus vulgaris* L.) (Guzy and Heath, 1993; Reinert and Eason, 2000), tomato (*Lycopersicon esculentum* Miller) (Temple, 1990), clover (*Trifolium repens* L.) (Heagle et al., 1993), wheat (Heagle et al., 2000), and potato (*Solanum tuberosum* L.) (Heagle et al., 2003) as well as natural vegetation such as black cherry (*Prunus serotina* Ehrh.) (Lee et al.,

* Corresponding author at: USDA-ARS Plant Science Research Unit, 3127 Ligon Street, Raleigh, NC 27607, USA. Tel.: +1 919 515 1620; fax: +1 919 856 4598.

E-mail address: Kent.Burkey@ars.usda.gov (K.O. Burkey).

2002) and *Plantago* spp. (Wolff et al., 2000). The existence of genetic variation in O₃ response within multiple species is encouraging and suggests that genetic manipulation is possible.

Soybean (*Glycine max* (L.) Merr.) is an O₃-sensitive crop. Open-top field chambers have been used to estimate soybean seed yield losses by comparing low O₃ controls, produced via activated charcoal filters, against ambient or higher O₃ treatments. Yield losses of 10–20% have been observed under present day O₃ concentrations of approximately 50 nmol mol⁻¹ (12-h daily average, 0800–2000 h) (Heagle et al., 1998). Greater losses have been reported when O₃ concentrations were elevated to 60–70 nmol mol⁻¹ (8-h daily average, 0900–1700 h) (Mulchi et al., 1988), 70–80 nmol mol⁻¹ (12-h daily average, 0800–2000 h) (Heagle et al., 1998; Booker et al., 2005), or 60–70 nmol mol⁻¹ using an AOT40 approach (Bou Jaoude et al., 2008). Free-air gas concentration enrichment technology, commonly known as FACE, has been used to demonstrate a 20% yield loss for soybean when O₃ levels were increased from an ambient level of 56 to an elevated level of 69 nmol mol⁻¹ (8-h daily average, 0900–1700 h) (Morgan et al., 2006).

Genetic variation in O₃ response has been observed for soybean (Tingey et al., 1972; Heagle, 1979; Heagle and Letchworth, 1982; Mulchi et al., 1988; Foy et al., 1995a, 1995b; Chernikova et al., 2000; Robinson and Britz, 2000). However, most screening studies to date have utilized a narrow range of germplasm such as common cultivars within a maturity group. Perhaps the best known genotypic contrasts in O₃ response are the maturity group V cultivars 'Essex' and 'Forrest' (Smith and Camper, 1973; Hartwig and Epps, 1973). Across multiple environments, direct comparisons have consistently shown Essex to be more O₃-resistant than Forrest in terms of foliar injury (Heagle and Letchworth, 1982; Cheng et al., 2007) and yield loss (Mulchi et al., 1988; Chernikova et al., 2000; Robinson and Britz, 2000). As a result, Essex and Forrest were selected as checks for the present study.

Because so little is known about the range of O₃ resistance in soybean germplasm, a logical next step to expand the knowledge base in North America would be to embark upon a comprehensive assessment of resistance in U.S. and Canadian soybean cultivars. The rationale for prioritizing North American cultivars over exotic germplasm is that, should resistant cultivars be found, they would naturally be more adapted to production and more easily used by breeders who are interested in developing new O₃-resistant cultivars. To date, such a comprehensive assessment has not taken place, because good screening protocols are laborious and expensive to implement. Although more than 500 public cultivars and 2000 private cultivars have been released in North America, few have been rated for O₃ resistance.

In situations where the screening of cultivars for a trait is difficult (the case for O₃ resistance), Carter et al. (2004) have suggested a "short cut" alternative to random or "brute force" screening of cultivars in order to identify resistance. Their approach is to screen a much smaller set of diverse founding ancestors that gave rise to applied breeding programs and then use those results to predict which modern descendant cultivars are most likely to possess similar resistance. This smaller set of likely cultivars is then prioritized for further screening efforts. The approach of screening ancestors not only serves as a guide for identifying adapted resistant cultivars, it also provides other direct benefits such as assessing the range in resistance that may exist in the applied breeding pool and in immediately identifying resistant, albeit unadapted, ancestors for genetic and physiological study.

Gizlice et al. (1994) identified the genetic base of U.S. and Canadian cultivars as 80 extant and lost ancestors that contribute 99% of the pedigree to North American soybean. From pedigree analysis they defined a smaller set of 35 extant ancestors plus immediate descendents of lost ancestors (surrogates) as a 'working

genetic base' for empirical screening. This working base contributes 95% of the ancestry, in terms of pedigree, present in modern U.S. and Canadian cultivars. This set of 35 genotypes is ideal for the short cut screening method proposed by Carter et al. (2004).

To date, the approach of predicting descendent cultivar performance based on ancestor performance (Carter et al., 2004) has usually been limited to visual inspection of pedigrees. Only Villagarcia et al. (2006) have attempted to quantify predictions in a more systematic way using coefficient of parentage (CP) analysis to predict salt tolerance in descendent North American soybean cultivars.

Our objectives were to (1) assess O₃ resistance in 30 major ancestors and surrogates of U.S. and Canadian soybean based on foliar injury, and (2) use the results in conjunction with pedigree analysis to prioritize for further screening those public cultivars most likely to exhibit reduced O₃ injury. A final objective was to use the predicted injury scores of descendent cultivars to compare Midwest vs. South for trends in predicted O₃ injury.

2. Materials and methods

2.1. Plant culture conditions and ozone treatments

For greenhouse studies, soybean seed were placed in moist paper towels and germinated at 29 °C for 3 days. Seedlings were transplanted into 6 L pots containing Metro-Mix 200 supplemented with 20 g of slow release fertilizer (Osmocote Plus, Scotts-Sierra Horticultural Products, Marysville, OH, USA) and placed in a greenhouse supplied with charcoal-filtered (CF) air (<10 nmol mol⁻¹ O₃). Four seedlings were initially placed in each of five pots and later thinned to one per pot after plants were established. To compare plants of similar developmental stage, supplemental lighting was used to create a day length of 20 h so that plants of all maturity groups were in the vegetative stage throughout the 4-week experiment. All plants were grown for 3 weeks in the CF greenhouse and then two pots of each ancestor were randomly selected for treatment with CF air and two additional pots selected for the charcoal-filtered air plus added O₃ treatment (CF + O₃, 80 nmol mol⁻¹ O₃ target). Pots were assigned to specific chambers according to the experimental design (randomization described in a later section). Plants were exposed for 6 days from 0900 to 1600 h in continuous stirred tank reactors (CSTRs) located in the greenhouse (Table 1). A CSTR is a cylindrical exposure chamber covered with Teflon film, designed for rapid mixing of gases (Rogers et al., 1977; Heck et al., 1978). Supplementary O₃ was generated by electrostatic discharge in dry O₂ (O₃ generator model GTC-1A, Ozonia North America, Elmwood Park, NJ, USA) and dispensed 7 h daily (0900–1600). Relative humidity in the CSTRs was maintained above 50% by adding steam generated by an electric steam boiler to each chamber. After the 6-day exposure, plants were then returned to the CF greenhouse for 2 days to allow complete development of foliar injury symptoms. Independent screenings of all genotypes were conducted in three greenhouse runs: October through November 2003, October through November 2004, and April through May 2005. Thirty ancestors of North American soybean or their immediate descendents and two reference cultivars (Essex and Forrest) were evaluated (Table 2).

A second follow-up experiment was conducted using open-top field chambers in 2005 at a field site located 5 km south of Raleigh, NC, USA. Seven ancestors were compared which contrasted for O₃ response in the greenhouse study, plus control cultivar Essex (Table 3). Seeds were germinated on 06 June and transplanted into 15 L pots of moistened Metro-Mix 200 on 09 June (four seedlings/pot). Nutrition was provided by 60 g of slow release fertilizer

Table 1

Ozone and environmental data. For greenhouse experiments, values are the mean (standard error) of daily averages measured from 0900 to 1600 h in the CSTRs during the 6 days of O₃ exposure. Values marked with different letters are significantly different among runs of the greenhouse experiment ($P \leq 0.05$). For the field experiment, values are the mean (standard error) of daily averages measured from 0800 to 2000 h during the 40 days of exposure with O₃ data measured inside the open-top chambers and the environmental data collected by a micrometeorological station located at the field site.

Environmental factor	Fall 2003 greenhouse experiment	Fall 2004 greenhouse experiment	Spring 2005 greenhouse experiment	Summer 2005 field experiment
CF treatment				
Daily average (nmol mol ⁻¹)	1.0 (0.1) ^b	0.8 (0.1) ^b	2.4 (0.3) ^a	25 (1)
AOT40 [†] (nmol mol ⁻¹ h)	0	0	0	331 (75)
CF + O ₃ treatment				
Daily average (nmol mol ⁻¹)	77 (3)	76 (1)	78 (1)	77 (1)
AOT40 (nmol mol ⁻¹ h)	1513 (44) ^b	1523 (10) ^b	1615 (21) ^a	16106 (206)
Daily temperature (°C)	28.5 (0.4) ^b	27.9 (0.3) ^b	32.5 (2.1) ^a	28.7 (0.4)
Relative humidity (%)	53.0 (0.4)	52.2 (0.7)	59.3 (3.9)	60.0 (1.9)
Photosynthetically active radiation (μmol m ⁻² s ⁻¹)	396 (9)	398 (8)	437 (59)	802 (31)

[†] Accumulated O₃ dose over 40 nmol mol⁻¹ calculated as the sum of differences between hourly mean O₃ concentration and 40 nmol mol⁻¹ during the exposure period.

Table 2

Foliar injury ratings for soybean ancestors, their immediate descendents and Essex and Forrest checks grown in the greenhouse. Values are means combined over three separate greenhouse runs (2003, 2004, and 2005) in which all genotypes were compared with two replications per run.

Plant introduction	Common name	Maturity group	Ancestral Contribution to U.S. and Canadian Soybean Breeding ^{†,‡}			Set in which ancestor was tested [§]	Foliar injury from O ₃ (%)
			Overall (%)	Northern contribution (%)	Southern contribution (%)		
PI 438477	Fiskeby 840-7-3	00	0.78	1.10	0.00	3	5
PI 438471	Fiskeby III	00	0.51	0.72	0.00	3	9
PI 180501		0	0.53	0.75	0.00	3	9
PI 548477	Ogden	V	4.94	4.31	6.44	2	12
PI 548352	Jogun	III	0.53	0.76	0.00	5	13
PI 548461	Improved Pelican	VIII	0.51	0.00	1.78	4	14
PI 548438	Arksoy [†]	VI	0.52	0.04	1.67	2	16
PI 548406	Richland	II	8.21	11.31	0.85	1	16
PI 548657	Jackson	VII	3.25	0.18	10.61	2	18
PI 548485	Roanoke	VII	2.10	0.24	6.54	2	19
FC 33243	Anderson	IV	1.04	1.19	0.70	4	20
PI 548356	Kanro	II	0.78	1.03	0.00	5	20
PI 548603	Perry	IV	2.07	2.08	2.06	3	20
PI 240664	Bilomi #3	X	0.49	0.00	1.64	5	21
PI 548488	S-100	V	7.52	1.75	21.3	2	21
PI 548325	Flambeau	00	0.68	0.97	0.00	5	23
PI 548382	Manitoba Brown	00	1.06	1.50	0.00	1	23
PI 80837	Mejiro	IV	0.68	0.00	2.30	4	23
PI 548311	Capital	0	1.67	2.37	0.00	3	26
FC 31745		VI	0.38	0.04	1.19	4	26
PI 548391	Mukden	II	3.46	4.91	0.00	1	26
PI 548445	CNS	VII	9.38	2.98	24.71	2	27
PI 548318	Dunfield	III	3.62	3.52	3.88	3	28
PI 548298	A.K. (Harrow) [†]	III	4.85	6.88	0.00	1	31
PI 548456	Haberlandt	VI	0.83	0.13	2.50	4	31
PI 548302	Bansei	II	0.78	1.10	0.00	5	33
PI 548362	Lincoln	IV	17.90	24.17	2.90	1	33
PI 548360	Korean	II	0.53	0.76	0.00	5	39
PI 548379	Mandarin (Ottawa)	00	12.15	17.23	0.00	1	40
PI 88788		III	0.49	0.38	0.72	4	49
PI 548667	Essex (check) [‡]	V					15
PI 548655	Forrest (check) [‡]	V					28
LSD (0.05) for ancestors within a set							9
LSD (0.05) for ancestors in different sets							13
Total % of genetic base [†]			92.19	92.39	91.77		

[†] Arksoy is indistinguishable from ancestor Ral soy. Ral soy contributes 0.62, 0.08, and 1.93% of the genetic base for all U.S. and Canadian cultivars, Northern cultivars and Southern cultivars, respectively. A.K. (Harrow) is indistinguishable from ancestor Illini. Illini contributes 2.20, 3.10, and 0.04% of the genetic base for all U.S. and Canadian cultivars, Northern cultivars and Southern cultivars, respectively. By virtue of these surrogates, this O₃ injury study encompasses 95% of the pedigree contribution to U.S. and Canadian soybean.

[‡] From Gizlice et al. (1994). North American soybean breeding has two major breeding pools, Northern and Southern, each of which has a distinct genetic base. The Northern pool is generally regarded as consisting of maturity group IV and less while the Southern pool is regarded as maturity IV and higher. Cultivars in maturity group IV may belong to either group.

[§] Ancestors and immediate descendents were tested in sets of six ancestors plus control genotypes Forrest and Essex to accommodate chamber size for treatment imposition.

[¶] Essex was compared to Forrest in a preplanned orthogonal comparison as part of the analysis of variance. Injury ratings were significantly different between the two ($P < 0.05$). Sets did not differ significantly for the means of the checks (the F -test ratio was less than 1, data not shown) and, thus, all ancestors are presented with a common mean for Essex and Forrest. The actual means for Essex and Forrest in each testing set were respectively: Set 1: 12, 26; Set 2: 12, 23; Set 3: 14, 26; Set 4: 20, 32; Set 5: 13, 33.

Table 3

Foliar injury ratings for selected soybean ancestors and the Essex check grown in the open-top field chambers during the summer of 2005. Values are mean scores of O₃-induced canopy injury present at 40 days after transplanting. See Table 1 for details on O₃ treatments.

Genotype	Maturity group	Leaves in canopy showing foliar injury [†] (%)
Fiskeby III [‡]	00	17
PI 180501	0	41
Fiskeby 840-7-3	00	44
Essex	V	49
Flambeau	00	50
Capital	0	64
Mandarin (Ottawa)	00	66
Manitoba Brown	00	71
LSD (0.05)		12

[†] Note: a qualitative score of the percentage of leaves showing damage rather than an estimate of the leaf surface showing damage. Actual percentage of the leaf surface showing damage was much less.

[‡] Based on greenhouse results, a preplanned comparison was performed for the field study in the analysis of variance. Fiskeby III, PI 180501 and Fiskeby 840-7-3 (lesser foliar injury in the greenhouse) were compared as a group to Flambeau, Capital, Mandarin (Ottawa), Manitoba Brown (greater foliar injury in the greenhouse). The more resistant group in the greenhouse was also more resistant in the field ($P < 0.01$). The correlation between field and ratings for these genotypes was 0.73 ($P < 0.05$).

(Osmocote Plus, Scotts-Sierra Horticultural Products) incorporated into each pot prior to planting. Pots were placed in open-top field chambers (Heagle et al., 1973) under CF or CF + O₃ conditions (two pots/genotype/chamber). Seedlings were thinned to one plant/pot on 27 June. Plants were irrigated with drip tubes on a daily basis or as needed to prevent water stress. Pot temperatures were moderated with an insulating cylinder composed of 0.6-cm-thick bubble wrap coated on both sides with aluminum (Reflectix™, Markleville, IN, USA) fit tightly around each pot. Ozone dispensing and monitoring were conducted as described by Heagle et al. (1979). Supplementary O₃ was generated by electrostatic discharge in dry O₂ (O₃ generator model GTC-1A, Ozonia North America, Elmwood Park, NJ, USA) and dispensed 12 h daily (0800–2000 EST) in proportion to the average diurnal profile for Raleigh, NC, USA. Ozone concentration in the chambers was monitored at canopy height using UV photometric O₃ analyzers (model 49, Thermo Environmental Instruments Co., Franklin, MA, USA). The O₃ analyzers were calibrated once every 2 weeks (model 49 PS calibrator, Thermo Environmental Instruments Co.).

2.2. Foliar injury assessments

For greenhouse studies, foliar injury was assessed on the five oldest main stem trifoliolate leaves by estimating the percentage of area that exhibited any type of foliar symptom including chlorosis, necrosis, and/or stipple following the approach established by A.S. Heagle (Heagle, 1979; Heagle and Letchworth, 1982). Three independent observers rated each leaf over a 3-day period beginning on day 3 after the end of the exposure period, and the results were averaged. Values for each O₃-treated leaf were corrected for any foliar symptoms observed on the analogous leaf from a paired CF-treated plant. Typically, no correction was required or was less than 5% with the exception being the Forrest check where brown stipple of unknown origin was present on lower leaves prior to O₃ exposure. Corrected ratings for the five leaves of each plant were summed, divided by 500 (the maximum possible injury score), and the percent foliar injury calculated for each plant for use in statistical analyses.

For open-top field chamber studies, foliar injury was estimated visually as the percentage of canopy leaves (including leaves on main stems and branches) exhibiting any O₃-related symptoms of stipple or necrosis without quantitative assessment of the extent of

Table 4

Analysis of variance for foliar injury from O₃ evaluated in the greenhouse and field. For greenhouse experiments, the experimental design was a nested split plot, with whole-plot chambers (i.e. CSTRs) randomly assigned to CF and CF + O₃ treatments. Subplots consisted of pots within a chamber. Because a chamber could hold only eight pots, ancestral genotypes were randomly subdivided into five sets of six genotypes. The cultivars Essex and Forrest were added to each set as tolerant and sensitive controls. All sets were then randomly assigned to chambers. Three independent greenhouse 'runs' (i.e. three separate experiments comparing all genotypes) were conducted. For the field study, a split-plot experimental design was employed. Open-top chambers served as whole plots to which CF and CF + O₃ treatments were assigned. Pots within chambers were the subplots.

Source	d.f.	Mean square
<i>Greenhouse</i>		
Run	2	252.26
Set	4	945.08
Run × set	8	232.50
Rep (run set)	15	329.74
Essex vs. Forrest [†]	1	2728.35*
Essex vs. Forrest × run	2	288.72*
Essex vs. Forrest × set	4	44.60
Essex vs. Forrest × set × run	8	51.95
Error [‡]	15	57.03
Ancestor [§] (set)	25	518.48**
Ancestor × run (set)	49	71.34
Rep × ancestor (run set) [‡]	74	71.36
Ancestor vs. controls (set)	5	195.86
Ancestor vs. controls × run (set)	10	111.75
Rep × ancestor vs. controls (run set) [‡]	15	89.15
<i>Field</i>		
Rep	5	145.21
Entry	7	1830.08**
Essex vs. Ancestor	1	11.56 ns
Ancestors	6	2132.96**
Fiskeby III, Fiskeby 840-73, PI 180501 vs. others	1	7601.74**
Error	34	14.41

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

[†] Sensitive Forrest and resistant Essex checks tested using a preplanned, one-tailed comparison based on performance in previous studies.

[‡] The three partitionings of subplot error were found to be homogeneous using the *F*-max test and, thus, a pooled subplot error was calculated (71.86 with 105 degrees of freedom) and used in testing appropriate higher order interactions.

[§] Ancestor refers to the 30 ancestors or their immediate descendants rated for O₃ response.

injury on individual leaves. Observations were made in the open-top chambers by one observer who rated all plants on the same date. Chamber means for each genotype in a given CF + O₃ open-top chamber were corrected by subtracting the score for the same genotype in a paired CF control open-top chamber. Corrected injury scores were used in statistical analyses.

2.3. Statistical analysis

For greenhouse treatment imposition, the experimental design was a nested split plot, with whole-plot chambers (i.e. CSTRs) randomly assigned to CF and CF + O₃ treatments. Subplots consisted of pots within a chamber. Because a chamber could hold only eight pots, ancestral genotypes were randomly subdivided into five sets of six genotypes. The cultivars Essex and Forrest were added to each set as tolerant and sensitive controls. All sets were then randomly assigned to CSTRs. Three independent greenhouse 'runs' (i.e. three separate experiments comparing all genotypes) were conducted employing two replicates in each case (Table 4). Genotype and O₃ treatments were considered fixed effects in the analysis, and all other effects were treated as random.

For the open-top chamber field study, a split-plot experimental design was employed with six replicates. Open-top chambers

served as whole plots to which CF and CF + O₃ treatments were assigned. Pots within chambers were the subplots. Each of the eight genotypes in the study was randomly assigned to two pots in the chamber for a total of 16 pots per chamber. Pots were arranged as a four by four matrix in the center. Ratings for the two pots of a genotype within a chamber were averaged prior to analysis.

Injury ratings, adjusted for any scores of atypical leaf color in the controls as described above, were subjected to analysis of variance using the general linear models (GLM) procedure (SAS Institute, 2001) with genotype and air quality treatments as fixed variables and other variables as random. Least significant differences (LSDs) were calculated for the nested split plot greenhouse experiment following the approach of Cochran and Cox (1957) where separate LSDs were calculated for within-set vs. across-set comparisons of genotypes. Correlation analysis was completed using the correlation procedure (SAS Institute, 2001). For the field study, Fiskeby III, Fiskeby 840-7-3, and PI 180501 were contrasted as a group to the other ancestors of similar maturity (Mandarin (Ottawa), Mukden, Capital and Flambeau) in a preplanned test, based on greenhouse results. The GLM procedure was used to conduct analysis of variance.

2.4. Coefficient of parentage analysis

2.4.1. Genetic base

For breeding purposes, coefficient of parentage (CP) may be defined as an estimate of the proportion of the genome for which two cultivars share alleles that are identical by descent (Carter et al., 2004). A CP value was calculated for all pairs of U.S. and Canadian cultivars released during 1947–1988. A CP value was also calculated for all ancestors and descendent cultivars. The contribution of an ancestor to the genetic base of North American breeding was defined as the mean CP of that ancestor with all released cultivars. The CP analysis was based on the methods of Malécot (1948). Pedigree information was based on documentations by Carter et al. (1993) and the CP value was calculated using a FORTRAN program by Cui et al. (2000).

2.4.2. Predicted O₃ response of descendent cultivars based on response of ancestors

A predicted O₃ response was obtained for 247 of the 258 cultivars released from 1947 through 1988. Eleven cultivars were excluded in this calculation because less than 75% of their ancestry could be explained by the ancestors or their surrogates in Table 2. The predicted value for a cultivar was calculated by multiplying the O₃ ratings of its ancestors with the fractional contribution of each ancestor to the cultivar. The sum across all ancestors was the predicted O₃ rating. If the total ancestral contribution of the ancestors was less than one for a given cultivar (the case when part of the pedigree was unclear), then each of its known ancestor's contribution was normalized by dividing its predicted rating by the total known ancestor contribution. Previous work has shown that ancestors Illini and A.K. (Harrow) are very similar to each other, if not indistinguishable, as is the ancestral pair Ral soy and Arksoy (Carter et al., 1993; Lorenzen et al., 1995). In these cases, the ratings of A.K. (Harrow) and Arksoy were used as surrogates for Illini and Ral soy, respectively, because Illini and Ral soy were not part of the screening effort.

3. Results

3.1. Ozone treatments and environmental conditions during exposure

The CSTR O₃ exposure system provided consistent treatments across the three independent runs with a 7-h mean O₃ concentration of 76–78 nmol mol⁻¹ in the CF + O₃ treatment

equivalent to accumulated dose over 40 nmol mol⁻¹ (AOT40) values of 1500–1600 nmol mol⁻¹ h (Table 1). The O₃ concentration in the CF treatment chambers was well below the O₃ level required to induce a measurable response, although O₃ concentration was significantly higher ($P < 0.0001$) during the spring 2005 run relative to the 2003 and 2004 fall runs (Table 1). Environmental conditions in the CSTRs during exposure were consistent across experiments with temperature being the exception (Table 1). Temperature was significantly different across runs ($P = 0.038$) with spring 2005 temperatures higher relative to the two fall runs. Relative humidity was maintained above 50% in all greenhouse studies and light levels were similar.

The open-top chamber experiment provided a daily average O₃ concentration of 77 nmol mol⁻¹ in the CF + O₃ treatment similar to the greenhouse study, but the AOT40 values were 10-fold higher as the result of a 40-day exposure period in the field compared to the 6-day exposure in the greenhouse (Table 1).

3.2. Visual symptoms of foliar injury and rating approach

In soybean, as in the case of most plants studied to date, foliar injury on sensitive genotypes was greatest on the older leaves at the bottom of the canopy, with less injury observed on upper canopy leaves (data not shown, see Cheng et al., 2007). The range of injury observed across genotypes in this study was apparent when the second oldest main stem leaves were compared (Fig. 1). No injury was observed on Fiskeby III when CF controls were compared with the CF + O₃ elevated O₃ treatment. Injury under elevated O₃ became apparent on Essex as small necrotic spots, commonly referred to as 'stipple'. Stippling was much more pronounced on the more sensitive Forrest genotype. In the most sensitive ancestor, PI 88788, severe injury was present as large areas of leaf necrosis.

Foliar injury was assessed differently for the greenhouse and open-top chamber studies. Greenhouse plants were younger with a simple canopy structure that allowed quantitative assessment of the percentage of leaf area injured on five main stem leaves following a 6-day exposure. In contrast, plants in the open-top chamber field study were much older with a complex canopy at the time of assessment that made it difficult to identify and assess specific individual leaves. Instead, a qualitative assessment was conducted of the percentage of leaves exhibiting any foliar symptoms after 40 days of exposure without considering the percentage of leaf area affected. Both methods provided a measure of foliar injury, but are based on different approaches.

3.3. Genotypic comparisons of ancestral types and controls

3.3.1. Greenhouse

In the greenhouse study, Essex had significantly lower ($P < 0.05$) foliar injury ratings than did Forrest (Table 2). The magnitude of the difference varied somewhat from run to run, as indicated by the significant ($P < 0.05$) (Essex vs. Forrest) × run interaction in the analysis of variance (Table 4). However, Essex was rated lower than Forrest for injury in 29 of 30 individual within-chambers comparisons in which O₃ was a treatment. Ancestors or their immediate descendents also differed significantly in foliar injury in the greenhouse, with ratings consistent across greenhouse runs (Tables 2 and 4). Because no ancestor × run interaction was evident, only mean data over the three runs are presented.

Foliar injury for the 30 soybean ancestors ranged from approximately 5–50% following the 6-day O₃ treatment employed here (Table 2). The O₃-resistant check Essex developed less injury than the sensitive check Forrest, as would be expected. Ancestral types Fiskeby 840-7-3 and Fiskeby III exhibited the greatest foliar

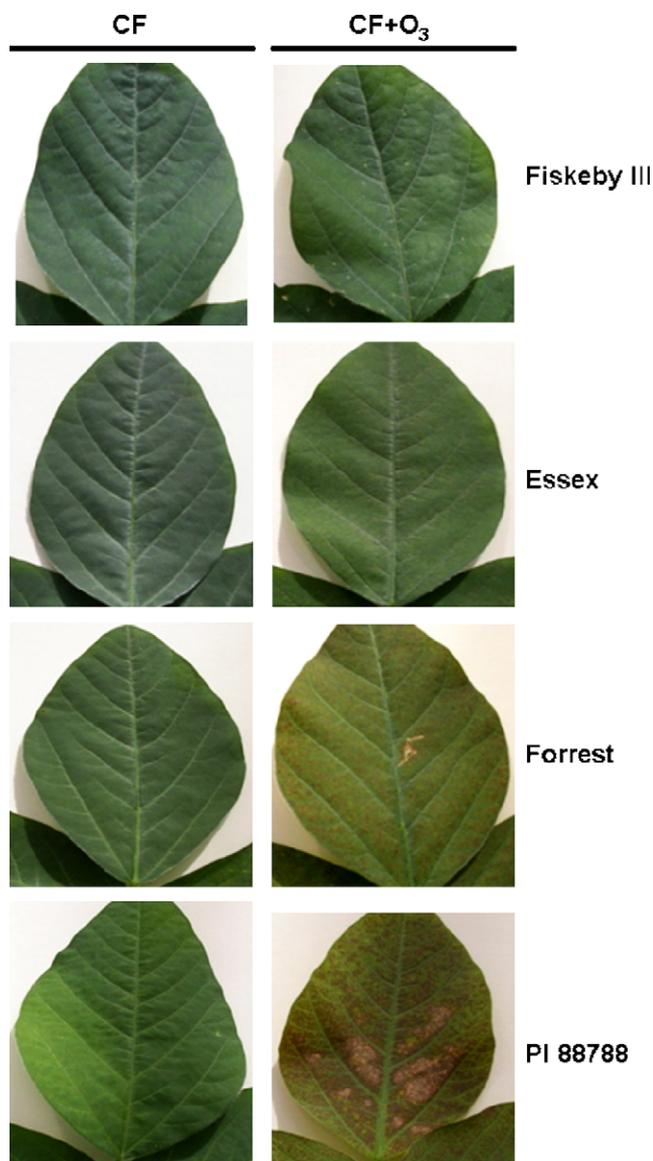


Fig. 1. Foliar injury on the second oldest main stem leaf (counting acropetally) of Fiskeby III, Essex, Forrest, and PI 88788 following a 6-day exposure to $77 \text{ nmol mol}^{-1} \text{ O}_3$ in greenhouse exposure chambers.

resistance with PI 88788 being the most sensitive. Ozone-sensitive and -resistant genotypes were often observed within the same maturity group, a good example being the two Fiskeby ancestral types and Mandarin (Ottawa) from maturity group 00, suggesting that O_3 -induced injury was not related to maturity effects. Conducting these studies in the greenhouse under an extended photoperiod allowed for O_3 injury to be assessed across a wide range of maturity prior to flowering that minimized effects of plant developmental stage on the results.

3.3.2. Field

Selected ancestors that contrasted for O_3 response in the greenhouse were also examined for differences in O_3 response in the field. Using open-top chambers, plants were exposed to elevated O_3 treatment until 40 days after planting and then rated (Table 3). To avoid any potential impact of phenology on injury ratings, ancestors were selected which were similar in maturity (i.e. maturity groups 0 and 00) and rated for injury at the point in development when flowering first initiated in the ancestors. The control Essex was rated on the same day. Although leaf injury

ratings taken in the field were less quantitative than in the greenhouse (percentage of leaves in the field showing damage vs. percentage of leaf area in the greenhouse), genotypic differences in O_3 -induced foliar injury were significant in the field ($P < 0.01$). In a preplanned comparison, the three genotypes with the greatest foliar resistance from the greenhouse study (Fiskeby III, Fiskeby 840-7-3, and PI 180501), taken as a group, exhibited significantly less ($P < 0.01$) injury than did the other ancestors of similar maturity (Mandarin (Ottawa), Mukden, Capital and Flambeau) which also had more severe injury in the greenhouse. Fiskeby III exhibited the least injury of any genotype in the study. Although the Essex control is from a later maturity group and flowered much later than did the groups 0 and 00 ancestors, its injury ranking in relation to these ancestors was very similar to greenhouse results, falling between the tolerant and sensitive ancestors (Table 3).

4. Discussion

4.1. Identification of ozone-resistant soybean ancestors and surrogates

The approach taken here was to induce foliar injury in vegetative plants through short term exposure to O_3 . Major ancestors of U.S. and Canadian soybean were assessed in this way to identify sources of O_3 resistance. A 10-fold difference in O_3 sensitivity was observed within the 30 ancestors selected for study. This range of response was greater than the two-fold difference between tolerant Essex and sensitive Forrest, two cultivars that served as standard reference genotypes in this study (Table 2). Ancestral types Fiskeby 840-7-3, Fiskeby III, and PI 180501 exhibited the greatest numerical O_3 resistance based on foliar injury under greenhouse conditions (Table 2). In general, the ancestors exhibiting the greatest foliar resistance in the greenhouse study were also the least injured in open-top field chambers (Table 3). Although the overall correlation between field and greenhouse genotypic scores was relatively high (0.73 on an ancestral mean basis, $P < 0.05$), some possible discrepancies were noted. For example, foliar injury was similar for Fiskeby III and Fiskeby 840-7-3 in the greenhouse, but Fiskeby III exhibited numerically greater foliar resistance in the field where the higher level of O_3 stress reflected in greater AOT40 values (Table 1) and may have provided conditions necessary to distinguish these ancestors.

The three ancestors with the least numerical injury in this study (Fiskeby 840-7-3, Fiskeby III, and PI 180501, see Table 2) all emanate from Europe. Only one other of the 80 ancestors of U.S. and Canadian soybean, Haberlandt, is from Europe. The two Fiskeby types, developed in Sweden, are related and have a CP value of at least 0.25, the equivalent of half sibs, based on partial records of pedigree. At least some of their pedigree is derived from Northern Japan. Fiskeby types have been noted previously as sources of cold tolerance in Canadian breeding (Hume and Jackson, 1981; Gass et al., 1996; Voldeng et al., 1997). Because cold tolerance and O_3 resistance are both relatively rare traits and physiological in nature rather than disease related, we postulate that there may be a physiological or morphological connection between cold and O_3 response mechanisms in these types.

PI 180501, the other ancestor in this study emanating from Europe, was developed in Germany and is presumed to be derived from the cross of two Chinese landraces. The PI 180501 is from the same cross as three other German accessions in the USDA soybean germplasm collection (PI 180499, PI 180502, PI 180532) and has some pedigree in common with two other accessions (PI 180524 and PI 180525). The O_3 responses of these accessions have not been reported. Examination of these plant introductions for O_3 response seems warranted, based on these associations.

Although the ancestors with the lowest injury ratings were all of very early maturity (groups 0 and 00), other ancestors from maturity groups II through VIII were identified as numerically equivalent to- or better than- the resistant control cultivar Essex. The later maturity ancestors Jogun, Ogden, Improved Pelican, Arksoy, Richland, and Jackson were not significantly different ($P > 0.05$) in O_3 response from the highest rated ancestor, Fiskeby 840-7-3, in the greenhouse. Because the resistant ancestors differ widely in terms of genetic background, it is likely that some carry unique resistance alleles, offering the opportunity to pyramid these alleles through breeding.

The ancestors in the genetic base with the greatest foliar resistance are not major ancestors when considering Canada and the USA on the whole. Only about 10% of the pedigree in North American soybean traces to the more resistant ancestors (Table 2). Ancestral type Ogden (a surrogate for ancestors Tokyo and PI 54610) was the only one that contributed greater than 1% to the genetic base of breeding and also had an injury rating numerically on a par with Essex. Approximately 40% of the pedigree for North American soybean traces to ancestors with injury ratings that are numerically as poor as, or worse than, Forrest, including the dominant ancestors Lincoln, A.K. (Harrow), Dunfield, and Mandarin (Ottawa). PI 88788, a widely used source of soybean cyst nematode (SCN) resistance in the Midwest, was the most susceptible ancestor studied. Thus, while variation in foliar response to O_3 stress appears to exist in the genetic base, pedigree analysis of the base suggests that there may be relatively few descendent cultivars that have good levels of foliar resistance to O_3 .

4.2. Prediction of ozone foliar resistance in descendent cultivars using coefficient of parentage

4.2.1. Utility and rationale

In breeding, a genetic base can be thought of as the complete set of ancestors that contribute to a set of cultivars. The two basic components used to describe the genetic base are the number of ancestors and the percentage contribution of each ancestor to the reference set of cultivars. Percentage contribution is usually estimated by CP. Algebraically, the mean CP of an ancestor with all the cultivars in a set ($\times 100$) is its percentage contribution to the base (Carter et al., 2004). In soybean, the genetic base has been estimated for the U.S. and Canada, Japan and China (Cui et al., 2000; Zhou et al., 2000; Gizlice et al., 1994; Sneller, 1994, 2003).

The genetic base concept has applications in cultivar screening studies. When cultivar information is limited for a trait, perhaps because the trait is difficult to measure, then it may be cost efficient to screen the genetic base prior to surveying the descendent cultivars. The rationale is that the screening results can be used to predict which descendant cultivars are most likely to carry the desirable trait and, thus, those cultivars can be targeted for follow-up screening (Carter et al., 2004). Based on this reasoning, the genetic base of U.S. and Canadian soybean has been screened to detect genetic variation in single sequence repeat (SSR) DNA markers, electron transport in chloroplasts, seed composition traits, resistance to herbicides, ultraviolet radiation, sudden death syndrome disease, and tolerance to salt (Villagarcia et al., 2006; Gizlice et al., 1993; Burkey et al., 1996; Diwan and Cregan, 1997; Reed et al., 1991; Song et al., 1999; Mueller et al., 2003; Hulting et al., 2001; Yaklich et al., 1999).

For O_3 injury, the predicted response for a cultivar can be calculated as weighted average of the foliar injury scores of its ancestors, with the weight being the percentage contribution of each ancestor to the cultivar. When inheritance is primarily additive, the trait is controlled by many genes, breeder selection for the trait is absent, and ancestors are well characterized for the trait, then prediction of performance for descendent cultivars may be

Table 5

Distribution of predicted O_3 foliar injury rating for 247 North American cultivars released during 1947–1988, based on O_3 resistance ratings of ancestors in the greenhouse and their genetic relation to descendent cultivars as measured by coefficient of parentage.

Maturity group	Cultivars No.	Predicted O_3 injury rating of cultivars based on ancestral performance ^{1,§}	
		Mean	Range
00 and 000 [†]	16	28	17 ~ 38
0	20	26	22 ~ 38
I	24	29	21 ~ 36
II	42	29	21 ~ 38
III	34	28	19 ~ 34
IV	43	26	20 ~ 30
V	20	23	21 ~ 27
VI	22	23	17 ~ 26
VII	13	21	18 ~ 24
VIII	9	20	13 ~ 24
IX [*]	4	23	23 ~ 23
b value, score regressed against maturity		-0.8 [*]	-0.2 ns ~ -1.8 [*]

^{*} Significant at the 0.05 probability level.

[†] Only one cultivar of 000 maturity released from 1947 to 1988. Roman numerals converted to Arabic for correlation analysis. The '00 and 000' category was treated as -1 for analysis.

[‡] The predicted O_3 rating for a cultivar is the sum of each ancestor's O_3 rating multiplied by its fractional contribution to the cultivar.

[§] Ozone injury ratings for respective resistant and susceptible controls in greenhouse screening: Essex, 15; Forrest, 28.

^{*} Group IX cultivars did not vary for predicted O_3 response.

relatively accurate across an entire range of predicted performance values, from high to intermediate to low. When reality deviates from this ideal scenario (often the case), then the overall predictive value of the approach is limited to identifying extreme types where assumptions involved in prediction matter less. Fortunately, breeder interest is usually greatest in extreme types.

The greater reliability for prediction of extremes types becomes clear when one examines the pedigree of cultivars predicted to have the greatest foliar resistance to O_3 . Most of their pedigree traces to ancestors which also have foliar resistance to O_3 . It is natural to assume that cultivars descended primarily from resistant types have a reasonable chance of carrying the resistance as well, regardless of the specific inheritance involved. For example, 'Bienville' (Johnson, 1960) traces 100% of its ancestry to resistant ancestors Ogden and Improved Pelican. Bienville, thus, has a reasonable expectation of exhibiting resistance and is worthy of inclusion in future germplasm screening studies aimed at identifying resistance.

4.2.2. Prediction of ozone response in Midwestern and Southern maturity cultivars

In the present study, a predicted O_3 foliar injury score was developed for 247 cultivars released between 1947 and 1988. Examination of means and ranges of the predicted values for various maturity groups revealed a wide range of predicted injury ratings for cultivars (Table 5). Maturity groups IV and earlier (i.e. Midwestern adapted cultivars) tended to have worse predicted foliar injury scores, on average, than did later maturity groups (i.e. Southern-adapted cultivars) as indicated by the significant regression ($P < 0.05$) of mean predicted score against maturity group (maturity scale converted to equally spaced Arabic numbers for analysis). The Midwest vs. Southern contrast was much larger when comparing the range in predicted injury scores. The Midwestern maturity groups were predicted to have a much wider spread in injury than were Southern maturity groups (Table 5). The reduced range in the Southern groups was attributed primarily to the virtual absence of cultivars predicted to have high

Table 6
North American cultivars predicted to have the lowest O₃ foliar injury ratings among 247 public cultivars released during 1947–1988. Predicted value for O₃ response in a cultivar is a weighted average based on ancestral O₃ injury rating and the percent contribution of those ancestors to pedigree.

Cultivar name	Year of release	Maturity group	Predicted O ₃ injury rating of cultivar based on ancestral performance	Ancestors with lowest O ₃ foliar injury ratings													
				Fiskeby 840-7-3 (5) [†]	Fiskeby III (9)	PI 180501 [‡] (9)	Ogden (12)	Jogun (13) [†]	Improved Pelican (14)	Arksoy (16)	Ralsoy [§] (16)	Richland (16)	Jackson (18)	Roanoke (19)	Anderson (20)	Kanro (20)	Perry (20)
Percentage composition of ancestors to the pedigree of descendant cultivars																	
Bienville	1958	VIII	13	0.0	0.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hardee	1962	VIII	17	0.0	0.0	0.0	12.5	0.0	50.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0
Davis [#]	1965	VI	17	0.0	0.0	0.0	37.5	0.0	0.0	0.0	25.0	0.0	0.0	25.0	0.0	0.0	0.0
Maple Amber	1981	00	17	50.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cobb	1973	VIII	18	0.0	0.0	0.0	12.5	0.0	25.0	0.0	0.0	0.0	46.9	0.0	0.0	0.0	1.6
Kershaw	1982	VI	18	0.0	0.0	0.0	31.3	0.0	0.0	0.0	12.5	0.0	0.0	37.5	0.0	0.0	0.0
Jackson	1953	VII	18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0
Kanrich	1956	III	19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	75.0	0.0
Semmes	1965	VII	19	0.0	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	25.0
Young	1984	VI	19	0.0	0.0	0.0	21.9	0.0	0.0	0.0	12.5	0.0	0.0	18.8	0.0	12.5	0.0
Hood 75	1975	VI	19	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0
Maple Presto	1979	000	19	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maple Ridge	1984	00	20	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0
Perry	1952	IV	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0
Bethel	1967	IV	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	50.0	0.0
Govan	1977	VII	20	0.0	0.0	0.0	12.5	0.0	0.0	0.0	12.5	0.0	25.0	0.0	0.0	0.0	25.0
Gasoy 17	1977	VII	20	0.0	0.0	0.0	12.5	0.0	0.0	0.0	0.0	0.0	25.0	25.0	0.0	0.0	12.5
Sohoma	1978	VI	20	0.0	0.0	0.0	18.8	0.0	0.0	0.8	12.5	0.0	0.0	12.5	0.0	0.0	24.6
York	1967	V	21	0.0	0.0	0.0	12.5	0.0	0.0	25.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
Ransom	1970	VII	21	0.0	0.0	0.0	14.1	0.0	0.0	0.0	3.1	0.0	0.0	3.1	0.0	37.5	0.0
Braxton	1979	VII	21	0.0	0.0	0.0	3.1	0.0	0.0	0.0	0.0	0.0	46.9	0.0	0.0	0.0	23.4
Narrow	1984	V	21	0.0	0.0	0.0	8.0	0.0	0.0	0.0	6.8	0.3	25.0	1.2	0.0	0.0	27.5
Blackhawk	1950	I	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0
Bragg	1963	VII	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0	25.0
Hawkeye 63	1963	III	21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0
Bay	1978	V	21	0.0	0.0	0.0	17.2	0.0	0.0	12.5	3.1	0.0	0.0	15.6	0.0	0.0	9.4
Dare	1965	V	21	0.0	0.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	12.5
Shore	1974	V	21	0.0	0.0	0.0	12.5	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0
Duocrop	1981	VII	21	0.0	0.0	0.0	31.3	0.0	0.0	0.0	12.5	6.3	0.0	12.5	0.0	0.0	0.0
Perrin	1988	VIII	21	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	35.9	0.0	0.0	0.0	24.2
Maple Arrow	1976	00	21	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hutcheson	1987	V	21	0.0	0.0	0.0	6.3	0.0	0.0	6.3	0.0	0.0	0.0	12.5	0.0	12.5	0.0
Mean			20	4.4	1.7	0.0	12.2	0.0	3.7	1.3	3.7	5.5	10.4	10.7	1.5	2.2	6.3
Percentage composition of ancestors to the pedigree of control cultivars																	
Essex	1972	V	22 (15) [¶]	0.0	0.0	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	12.5	0.0	0.0	25.0
Forrest	1972	V	23 (28) [¶]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	0.0	0.0	0.0	28.1

[†] Ozone foliar injury rating of ancestor in parenthesis, based on three greenhouse runs (see Table 2).

[‡] Note: No progeny of PI 180501, Jogun, or Bilomi #3 appeared in the top 15% of cultivars predicted as most likely to have O₃ resistance. However, their progeny may be important for further O₃ resistance research because of their unique pedigree. Of the 258 U.S. and Canadian cultivars released between 1947 and 1988, the following have at least 12.5% of their pedigree tracing to one of these ancestors. From PI 180501, descendent cultivars are Hodgson, Hodgson 78, Simpson, Dawson, Ozzie, Ripley, Sibley and Haroson. From Jogun, descendent cultivars are Disoy, Magna, Prize, Verde and Grande. From Bilomi #3, descendent cultivars are Jupiter, Jupiter-R, and Alamo.

[§] Arksoy is indistinguishable from ancestor Ralsoy. Ralsoy contributes 0.62, 0.08, and 1.93% of the genetic base for all U.S. and Canadian cultivars, Northern cultivars and Southern cultivars, respectively. A.K. (Harrow) is indistinguishable from ancestor Illini. Illini contributes 2.20, 3.10, and 0.04% of the genetic base for all U.S. and Canadian cultivars, Northern cultivars and Southern cultivars, respectively. Thus, Ralsoy and Illini were assigned the same rating scores as Arksoy and A.K. (Harrow), respectively and included in the calculations of predicted values.

[#] Although Davis is predicted to have low injury in this study, a previous rating indicated that Davis' response was intermediate to Essex and Lee (Heagle and Letchworth, 1982).

[¶] Actual rating in greenhouse study in parenthesis.

foliar injury ratings. Based on these injury predictions, one may expect that resistance to foliar injury from O₃ may be more common in the South than in the Midwest, although a range of variation may exist in both.

4.2.3. Descendent cultivars predicted to have highest resistance to ozone

The 32 descendent cultivars with the lowest predicted O₃ foliar injury represent a range of pedigree and maturity group (Table 6). The authors suggest that these may be good candidates for inclusion in future screening efforts aimed at identifying O₃-resistant germplasm. Obviously, however, not all 32 are expected to be validated as resistant to foliar injury from O₃. Davis, for example, which was predicted to have low injury in this study, has been rated in open-top chambers previously as only intermediate to Essex and Forrest for foliar injury (Heagle and Letchworth, 1982). Neither are the 32 expected to capture all resistant cultivars. Essex, for example, was predicted to have an injury score of 22, when its actual rating was somewhat superior (15) and considered as resistant. The goal of the approach is to identify at least some although not all resistant cultivars with a minimum of testing.

The variety Bienville, predicted to be the descendent cultivar with the least O₃ foliar injury, was never used successfully as a parent in the development of a new cultivar in the USA. In Brazil, however, Bienville has been used to develop three soybean progeny that are preserved in the USDA soybean germplasm collection (PI 628804, PI 628806, and PI 628863). The latter two are derived from the cross of Bienville × 'Hood' (Caviness and Walters, 1976). Hood was also predicted to have a low injury rating (a score of 19) in the present study and, thus, progeny of a Bienville × Hood cross would be predicted to have a very desirable foliar injury score of only 16. This score is on a par with Essex's performance in the present study. PI 628855 from Brazil is also predicted to be resistant (a predicted injury rating of 19) because it is from the cross of Jackson × Hood. Jackson had a relatively low foliar injury rating in greenhouse testing as well. Thus, some of the Brazilian material in the USDA germplasm collection is predicted to be more resistant to foliar injury than all U.S. and Canadian varieties except Bienville. The fact that Bienville, Hood, Essex and Jackson are not closely related (i.e. they all have CP values less than 0.20) suggests the Brazilian material could harbor unique recombinant types that might be useful in North American efforts to develop O₃-resistant cultivars.

4.3. Implications to breeding

This study revealed significant O₃-induced foliar injury among soybean ancestors, suggesting that improvement of soybean production is possible in regions where ambient O₃ levels are problematic. A critical question is whether foliar resistance to O₃ injury is related to protection against yield loss. Foliar injury has been associated with yield loss in snap bean (Burkey et al., 2005). However, the literature is very limited for soybean on this point, because genetic variation in O₃-response is typically studied in short term experiments focused on response mechanisms. Yield is not measured, typically, in such studies. As a consequence, foliar injury and yield results are available for only a few cultivars. One case is Essex and Forrest, the resistant and sensitive checks used in this study. The greater foliar injury reported for Forrest (Cheng et al., 2007) is associated with greater yield loss under elevated O₃ (Chernikova et al., 2000; Robinson and Britz, 2000). Essex and Forrest seed yields were similar under low O₃ conditions with elevated O₃ conditions causing a 20–30% yield reduction for Forrest but less than 10% reduction for Essex (Chernikova et al., 2000; Robinson and Britz, 2000).

In an earlier study (Heagle and Letchworth, 1982), the relationship between foliar injury and yield was less clear. In this

case, O₃-induced foliar injury was associated with yield loss in cultivars Davis, Forrest, and Ransom, but a fourth cultivar Bragg exhibited foliar symptoms with no detrimental effect on yield (Heagle and Letchworth, 1982). Based on these results from the cultivar Bragg, Heagle and Letchworth (1982) concluded that foliar resistance is not a good predictor of yield response. However, from the breeder's point of view, use of foliar response to predict yield performance in three out of four cases may be considered promising. The situation that Heagle and Letchworth (1982) detected with Bragg vs. the other cultivars may be analogous to the well known phenomenon of yield tolerance vs. pest resistance encountered with the soybean cyst nematode (Hussey and Boerma, 1989). Several major qualitative genes for SCN resistance are known to exist which protect the soybean root from the cyst nematode (e.g. genes from Peking and PI 88788). Even so, studies have shown that field tolerance to SCN also exists in that the yields of some soybean genotypes do not suffer the same decline in the presence of SCN as do normal susceptible genotypes, even though the root is successfully attacked (Boerma et al., 1993). The fact that 'yield tolerance' to SCN exists is clearly important and has been beneficial to soybean production. Nevertheless, the use of the qualitative resistance genes in SCN breeding programs is currently the dominate approach used in breeding. The approach of yield tolerance is used less, simply because resistance is easier to measure than tolerance.

By analogy, foliar resistance is likely to be an important part of the overall breeding effort to improve soybean yield in the presence of O₃ simply because, as with SCN, yield tolerance will be more difficult to measure. Direct quantitative trait loci (QTL) mapping of yield response may be impractical for O₃ tolerance, because it is beyond the ability of most scientists to apply O₃ stress to sufficient numbers of yield plots in the field. Typically, more than 100 random inbred lines (RILs) are used in a QTL mapping population and precision on yield data requires replication so that, in total, hundreds of yield plots would be needed. A more realistic approach is to first identify RIL extremes which contrast for foliar injury and for QTLs related to foliar injury. Subsequently, one could compare sets of the extremes for yield under O₃ pressure in smaller-sized studies. This approach should begin to clarify the relation of foliar injury and yield.

5. Conclusions

In the absence of successful efforts to reduce O₃ pollution, future soybean productivity may depend on the development of O₃-tolerant varieties capable of maintaining yields under elevated O₃ conditions. Using a foliar injury based screening technique, this study revealed a 10-fold difference in O₃ sensitivity within a group of 30 soybean ancestors following a 6-day exposure to O₃ concentrations equivalent to a 50% increase above current ambient O₃ conditions. These results and those of others (Damicone and Manning, 1987) suggest there is potential for development of cultivars with improved O₃ tolerance. Tolerant ancestors identified here should be further validated and then subjected to inheritance and DNA mapping studies to elucidate the number and nature of genes controlling resistance. Yield should be a part of these genetic studies to clarify the relationship between resistance to foliar injury and yield benefit.

Modern cultivars descended primarily from resistant ancestors were identified using pedigree analysis and are proposed for study to determine their true O₃ resistance. Many of the cultivars predicted to be most resistant are derived from diverse genetic backgrounds (Table 6). If resistance is validated in some of these prioritized cultivars, then there may be an opportunity to pyramid genes from diverse sources without resorting to extensive breeding with the unadapted ancestors which may shatter and

carry disease susceptibility traits. Such efforts may result in greater O₃ tolerance than has been observed to the present. This approach for improving ozone resistance of North American cultivars could be applied to other soybean production regions of the world where the genetic base is sufficiently characterized, such as China. Brazil and European breeding programs may offer important germplasm for O₃ tolerance research.

Acknowledgments

We thank Jeff Barton, Mike Durham, Walt Pursley, Renee Tucker, and Margarita Villagarcia for their technical assistance with this project.

References

- Boerma, H.R., Hussey, R.S., Reese Jr., P.F., Finnerty, S.L., Wood, E.D., 1993. Registration of soybean cyst nematode tolerant germplasm line G88-20092. *Crop Sci.* 33, 216.
- Booker, F.L., Miller, J.E., Fiscus, E.L., Pursley, W.A., Stefanski, L.A., 2005. Comparative responses of container-versus ground-grown soybean to elevated carbon dioxide and ozone. *Crop Sci.* 45, 883–895.
- Bou Jaoude, M., Katerji, N., Mastrorilli, M., Rana, G., 2008. Analysis of the ozone effect on soybean in the Mediterranean region II. The consequences on growth, yield and water use efficiency. *Eur. J. Agron.* 28, 519–525.
- Burkey, K.O., Miller, J.E., Fiscus, E.L., 2005. Assessment of ambient ozone effects on vegetation using snap bean as a bioindicator species. *J. Environ. Qual.* 34, 1081–1086.
- Burkey, K.O., Gizlice, Z., Carter Jr., T.E., 1996. Genetic variation in photosynthetic electron transport capacity is related to plastocyanin concentration in the chloroplast. *Photosynth. Res.* 49, 141–149.
- Carter Jr., T.E., Nelson, R.L., Sneller, C., Cui, Z., 2004. Genetic diversity in soybean. In: Boerma, H.R., Specht, J.E. (Eds.), *Soybean Monograph*. 3rd edition. Am. Soc. Agron., Madison, WI, pp. 303–416.
- Carter Jr., T.E., Gizlice, Z., Burton, J.W., 1993. Coefficient-of-parentage and genetic similarity estimates for 258 North American soybean cultivars released by public agencies during 1945–1988. U.S. Dep. Agric. Tech. Bull. Num. 1814. U.S. Govt. Print. Office, Washington, DC.
- Caviness, C.E., Walters, H.J., 1976. Registration of Hood 75 soybean. *Crop Sci.* 16, 741.
- Cheng, F.-Y., Burkey, K.O., Robinson, J.M., Booker, F.L., 2007. Leaf extracellular ascorbate in relation to O₃ tolerance of two soybean cultivars. *Environ. Pollut.* 150, 355–362.
- Chernikova, T., Robinson, J.M., Lee, E.H., Mulchi, C.L., 2000. Ozone tolerance and antioxidant enzyme activity in soybean cultivars. *Photosynth. Res.* 64, 15–26.
- Cochran, W.G., Cox, G.M., 1957. *Experimental Designs*. John Wiley and Son, New York.
- Cui, Z., Carter Jr., T.E., Burton, J.W., 2000. Genetic base of 651 Chinese soybean cultivars released during 1923 to 1995. *Crop Sci.* 40, 1470–1481.
- Damicone, J.P., Manning, W.J., 1987. Foliar sensitivity of soybeans from early maturity groups to ozone and inheritance of injury response. *Plant Dis.* 71, 332–336.
- Diwan, N., Cregan, P.B., 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. *Theor. Appl. Genet.* 95, 723–733.
- Fiscus, E.L., Booker, F.L., Burkey, K.O., 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant Cell Environ.* 28, 997–1011.
- Foy, C.D., Lee, E.H., Shalunova, L.P., Divine, T.E., 1995a. Acid soil (aluminum) tolerance in soybean cultivars related to ozone tolerance. *J. Plant Nutr.* 18, 361–371.
- Foy, C.D., Lee, E.H., Rowland, R.A., Devine, T.E., 1995b. Ozone tolerances of soybean cultivars and near isogenic lines in a fumigation chamber. *J. Plant Nutr.* 18, 649–667.
- Gass, T., Schori, A., Fossati, A., Soldati, A., Stamp, P., 1996. Cold tolerance of soybean (*Glycine max* (L.) Merr.) during the reproductive phase. *Eur. J. Agron.* 5, 71–88.
- Gizlice, Z., Carter Jr., T.E., Burton, J.W., 1993. Genetic diversity in North American soybean: I. Multivariate analysis of founding stock and relation to coefficient of parentage. *Crop Sci.* 33, 614–620.
- Gizlice, Z., Carter Jr., T.E., Burton, J.W., 1994. Genetic base for North American public soybean cultivars released between 1947 and 1988. *Crop Sci.* 34, 1143–1151.
- Guzy, M.R., Heath, R.L., 1993. Responses to ozone of varieties of common bean (*Phaseolus vulgaris* L.). *New Phytologist* 124, 617–625.
- Hartwig, E.E., Epps, J.M., 1973. Registration of Forrest soybean. *Crop Sci.* 13, 287.
- Heagle, A.S., Body, D.E., Heck, W.W., 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2, 365–368.
- Heagle, A.S., Philbeck, R.B., Rogers, H.H., Letchworth, M.B., 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. *Phytopathology* 69, 15–20.
- Heagle, A.S., 1979. Ranking of soybean cultivars for resistance to ozone using different ozone doses and response measures. *Environ. Pollut.* 19, 1–10.
- Heagle, A.S., 1989. Ozone and crop yield. *Ann. Rev. Phytopathol.* 27, 397–423.
- Heagle, A.S., Letchworth, M.B., 1982. Relationships among injury, growth, and yield responses of soybean cultivars exposed to ozone at different light intensities. *J. Environ. Qual.* 11, 690–694.
- Heagle, A.S., Miller, J.E., Pursley, W.A., 1998. Influence of ozone stress on soybean response to carbon dioxide enrichment: III. Yield and seed quality. *Crop Sci.* 38, 128–134.
- Heagle, A.S., Miller, J.E., Sherrill, D.E., Rawlings, J.O., 1993. Effects of ozone and carbon dioxide mixtures on two clones of white clover. *New Phytologist* 123, 751–762.
- Heagle, A.S., Miller, J.E., Pursley, W.A., 2000. Growth and yield responses of winter wheat to mixtures of ozone and carbon dioxide. *Crop Sci.* 40, 1656–1664.
- Heagle, A.S., Miller, J.E., Pursley, W.A., 2003. Growth and yield responses of potato to mixtures of carbon dioxide and ozone. *J. Environ. Qual.* 32, 1603–1610.
- Heck, W.W., Philbeck, R.B., Dunning, J.A., 1978. A Continuous Stirred Tank Reactor (CSTR) System for Exposing Plants to Gaseous Air Contaminants: Principles, Specifications, Construction, and Operation. USDA Agricultural Research Service, Washington, DC.
- Hulting, A.G., Wax, L.M., Nelson, R.L., Simmons, F.W., 2001. Soybean (*Glycine max* (L.) Merr.) cultivar tolerance to sulfentrazone. *Crop Prot.* 20, 679–683.
- Hume, D.J., Jackson, A.K.H., 1981. Pod formation in soybeans at low temperatures. *Crop Sci.* 21, 933–937.
- Hussey, R.S., Boerma, H.R., 1989. Tolerance in maturity Groups V–VIII soybean cultivars to *Heterodera glycines*. *Suppl. J. Nematol.* 21 (No. 45), 686–692.
- Johnson, H.D., 1960. Registration of soybean varieties, VII. *Agron. J.* 52, 659.
- Lee, J.C., Steiner, K.C., Zhang, J.W., Skelly, J.M., 2002. Heritability of ozone sensitivity in open-pollinated families of black cherry (*Prunus serotina* Ehrh.). *Forest Sci.* 48, 111–117.
- Lorenzen, L.L., Boutin, S., Young, N., Specht, J.E., Shoemaker, R.C., 1995. Soybean pedigree analysis using map-based molecular markers: I. Tracking RFLP markers in cultivars. *Crop Sci.* 35, 1326–1336.
- Malécot, G., 1948. *Les mathématiques de l'hérédité* (The mathematics of heredity), Masson, Paris. W.H. Freeman and Co., San Francisco, CA.
- Mauzerall, D.L., Wang, X.P., 2001. Protecting agricultural crops from the effects of tropospheric ozone exposure: reconciling science and standard setting in the United States, Europe, and Asia. *Annu. Rev. Energy Environ.* 26, 237–268.
- Morgan, P.B., Mies, T.A., Bollero, G.A., Nelson, R.L., Long, S.P., 2006. Season-long elevation of ozone concentration to project 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. *New Phytologist* 170, 333–343.
- Mueller, D.S., Hartman, G.L., Nelson, R.L., Pedersen, W.L., 2003. Response of US soybean cultivars and ancestral soybean lines to *Fusarium solani* f. sp. *Glycines*. *Plant Dis.* 87, 827–831.
- Mulchi, C.L., Lee, E., Tuthill, K., Olinick, E.V., 1988. Influence of ozone stress on growth processes, yields and grain quality characteristics among soybean cultivars. *Environ. Pollut.* 53, 151–169.
- Prather, M., Ehhalt, D., Dentener, F., Derwent, R., Dlugokencky, E., Holland, E.A., Isaksen, I., Katima, J., Kirchhoff, V., Matson, P., Midgley, P., Wang, M., 2001. Atmospheric chemistry and greenhouse gases. In: Houghton, J.T., et al. (Eds.), *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, pp. 239–287.
- Reed, E.H., Teramura, A.H., Kenworthy, W.J., 1991. Ancestral U.S. soybean cultivars characterized for tolerance to ultraviolet-B radiation. *Crop Sci.* 32, 1214–1219.
- Reinert, R.A., Eason, G., 2000. Genetic control of O₃ sensitivity in a cross between two cultivars of snap bean. *J. Am. Soc. Hort. Sci.* 125, 222–227.
- Robinson, J.M., Britz, S.J., 2000. Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status, and long term photosynthetic productivity. *Photosynth. Res.* 64, 77–87.
- Rogers, H.H., Jeffries, H.E., Stahel, E.P., Heck, W.W., Ripperton, L.A., Witherspoon, A.M., 1977. Measuring air pollutant uptake by plants: a direct kinetic technique. *J. Air Pollut. Control Assoc.* 27, 1192–1197.
- SAS Institute, 2001. SAS system for Windows, Release 8.2. SAS Institute, Cary, NC.
- Smith, T.J., Camper, H.M., 1973. Registration of Essex soybean. *Crop Sci.* 13, 495.
- Sneller, C.H., 1994. Pedigree analysis of elite soybean lines. *Crop Sci.* 34, 1515–1522.
- Sneller, C.H., 2003. Impact of transgenic genotypes and subdivision on diversity within elite North American soybean. *Crop Sci.* 43, 409–414.
- Song, Q.J., Quigley, C.V., Nelson, R.L., Carter, T.E., Boerma, H.R., Strachan, J.L., Cregan, P.B., 1999. A selected set of trinucleotide simple sequence repeat markers for soybean cultivar identification. *Plant Var. Seeds* 12, 207–220.
- Temple, P.J., 1990. Growth and yield of processing tomato (*Lycopersicon esculentum* Mill.) cultivars to ozone. *Environ. Exp. Bot.* 30, 283–291.
- Tingey, D.T., Reinert, R.A., Carter, H.B., 1972. Soybean cultivars: acute foliar response to ozone. *Crop Sci.* 12, 268–270.
- U.S. E.P.A., 2006. Air Quality Criteria for Ozone and Related Photochemical Oxidants EPA/600/R-05/004aF-cF. U.S. Environmental Protection Agency, Washington, DC.
- U.S. E.P.A., 1996. Air Quality Criteria for Ozone and Other Photochemical Oxidants. U.S. EPA report no. EPA/600/P-93/004bF ed. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards, Research Triangle Park, NC.
- Villagarcia, M., Cardinal, A., Carter Jr., T.E., Shannon, J.G., Boerma, H.R., 2006. Salt tolerance in the genetic base of US and Canadian soybean. *Crop Science Society of America, Annual meeting abstracts*.

- Voldeng, H.D., Cober, E.R., Hume, D.J., Gilard, C., Morrison, M.J., 1997. Fifty-eight years of genetic improvement of short-season soybean cultivars in Canada. *Crop Sci.* 37, 428–431.
- Wang, X., Mauzerall, D.L., 2004. Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. *Atmos. Environ.* 38, 4383–4402.
- Wellburn, F.A.M., Wellburn, A.R., 1996. Variable patterns of antioxidant protection but similar ethene emission differences in several ozone-sensitive and ozone-tolerant plant selections. *Plant Cell Environ.* 19, 754–760.
- Wolff, K., Morgan-Richards, M., Davison, A.W., 2000. Patterns of molecular genetic variation in *Plantago major* and *P. intermedia* in relation to ozone resistance. *New Phytologist* 145, 501–509.
- Yaklich, R.W., Helm, R.M., Cockrell, G., Herman, E.M., 1999. Analysis of the distribution of major soybean seed allergens in a core collection of *G. max* accessions. *Crop Sci.* 39, 1444–1447.
- Zhou, X., Carter, T.E., Cui, Z., Miyazaki, S., Burton, J.W., 2000. Genetic base of Japanese soybean cultivars released during 1950 to 1988. *Crop Sci.* 40, 1794–1802.