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Response of Glyphosate-resistant and Conventional Soybean Grafted Plants to Glyphosate

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Abstract

Grafting is a common technique used to impart desirable traits to a plant's scion. Herbicide resistant rootstocks have the potential to confer non–genetically modified (non-GM) scions with herbicide tolerance while mitigating some societal concerns regarding GM crops and food. We examined the impacts of soybean cultivar and growth stage and environmental temperature on transference of glyphosate tolerance to conventional (CN) soybean scions when grafted to glyphosate-resistant (RR) rootstocks. Small CN/RR (scion/rootstock) plants (3-leaf stage) and medium-sized plants (6-leaf stage) were injured more than large plants (10-leaf stage) 34 d after treatment (DAT) with 0.84 and 1.68 kg ae ha^{-1} glyphosate. All CN/CN combinations died, and RR/RR were uninjured. The cultivar of the scion had a greater effect on glyphosate tolerance than the cultivar of the rootstock. CN scions 352 and 5418 were more tolerant than CN scion 5388 across all RR rootstocks 35 DAT when treated with 0.84 kg ha^{-1} glyphosate. CN/RR construct 5388/9392 was more sensitive to temperature compared with 352/9392. Less leaf regrowth of 5388/9392 was observed under the warmer temperature. Our experiments demonstrated that grafting imparted robust glyphosate tolerance across different plant sizes, environmental temperatures, and scion/rootstock cultivars.

Introduction

Herbicides are widely used in agricultural production and landscape management to manipulate the growth of, or kill, undesirable plants. In the United States, more glyphosate is applied than any other herbicide due to the adoption of glyphosate-resistant (Roundup Ready®) crops. Approximately 45 million kg of glyphosate were used on soybean fields alone in 2012 (USDA 2013). Herbicides impart many advantages to the farmer, principally the ability to selectively and efficiently manage weeds; however, significant risks are also associated with their use. Herbicides have the potential to induce human and/or other animal health problems arising from illegal residues in food and from air and water contamination (Myers et al. 2016).

A particular risk in some agricultural areas is herbicide spray droplet and/or vapor drift to sensitive non-target crops growing in adjacent fields. In the US, more than 1,700 drift incident reports in 40 states were investigated in 2004. Investigations confirmed that 1,207 incidents were due to drift from herbicide applications in agricultural fields (Lee et al. 2011). Wolf et al. (1993) reported 2% to 16% drift from ground application in wind speeds of 9 and 30 km h^{-1} when using an 8001 flat-fan nozzle tip. Very small drifting fractions of an application may cause enormous damage to sensitive crops. For example, McNaughton et al. (2012) reported that 2.5% of the recommend rate of glyphosate applied as simulated drift caused a 23% decrease in tomato yield. Lassiter et al. (2007) reported a 30% yield loss of peanut yield when exposed to 0.32 kg ae ha^{-1} glyphosate.

Recently developed 2,4-D- and dicamba-resistant soybeans are likely to be widely planted by farmers who require alternative herbicides to control glyphosate-resistant weeds. Adoption will not only increase the acreage treated with 2,4-D and dicamba, but will result in applications in the late spring through early summer when many specialty crops are entering the reproductive stage that has been identified as most sensitive (Boutin et al. 2014). Also of great significance, certain crops and naturally occurring plants are hypersensitive to dicamba and 2,4-D (Mortensen et al. 2012; Peterson and Hulting 2004). In fact, hundreds of complaints about drift damage have been reported from many states following the commercialization of dicamba-tolerant soybean (EPA 2017). Widespread drift-related damage occurred despite required usage of new dicamba formulations considered to be of low drift potential (Association of American Pesticide Control Officials 2017). Thus, there is a great need to develop technologies to reduce the probability of drift occurrence and methods to protect sensitive crops from off-site movement.

Because there are significant regulatory hurdles to commercialization of new genetically modified (GM) herbicide-resistant crops as well as consumer resistance in the marketplace, especially for fruits and vegetables, alternate approaches to protecting specialty crops are needed. Grafting, a technology that first appeared in East Asia more than 2,000 yr ago, has been proposed as a method to manage diverse biotic and abiotic stresses. The practice of grafting herbaceous plants, especially tomato, is becoming more common in the United States and Europe (Kubota et al. 2008). Typically, a grafted plant is composed of different rootstock and shoot (scion) tissues. The rootstock is the bottom portion, usually consisting of the root system and a portion of stem tissue, and is used to change or control the character of the entire grafted plant. Scions can be grafted onto a wide range of rootstocks that can modify characteristics like fruit flavor and texture, sugar content, and color (Davis et al. 2008) without modifying the scion genotype. The vascular systems of the scion and rootstock connect gradually after the grafting procedure, allowing assimilates, mineral substances, water, and hormones to be transported between the two parts (Mudge et al. 2009).

Grafting has often been used to provide whole-plant tolerance to drought, soil-borne diseases, and nematodes (Gilardi et al. 2014; Giotis et al. 2012; Ling et al. 2015; Schwarz et al. 2010). Effects of the rootstock on the physiology of grafted plants may be due to root structure; enhanced absorption and translocation of water and nutrients; and the ability to produce and translocate phytohormones, including ABA, cytokinin, and ethylene, to the scion (Zhou et al. 2014). Grafting a conventional (non-GM) scion with a GM rootstock is called transgrafting. This technique has the potential to benefit farmers and the food industry by providing benefits of transgenic traits to a non-GM scion, thus addressing many concerns regarding GM food (Haroldsen et al. 2012). Lusser et al. (2011) recommended development of new transgenic rootstock lines that would be used to produce crop plants with resistance to abiotic and biotic stresses. Further, Haroldsen et al. (2012) noted that a nontransgenic crop scion might obtain tolerance to insects and pathogens when grafted onto a transgenic rootstock with resistance traits.

Expression of a herbicide-resistant trait from a transgenic resistant soybean rootstock in a nontransgenic scion was first reported by Jiang et al. (2012). They created a full factorial of grafted conventional and Roundup Ready® soybean genotypes (CN and RR are used hereafter to designate conventional and Roundup Ready® soybean): CN scion grafted to CN rootstock (CN/CN), RR scion grafted to RR rootstock (RR/RR), CN scion grafted to RR rootstock (CN/RR), and RR scion grafted to CN rootstock (RR/CN). The scion in the CN/RR grafts obtained tolerance to glyphosate from the rootstock; injury of CN/RR following a glyphosate application of 0.84 kg ha^{-1} was about 60% compared with RR/RR. Their results indicated grafting as a potential system to harness molecular technology for the protection of sensitive crops from low doses of drifting glyphosate, and possibly other herbicides, while precluding potential gene flow and providing non-GM food products.

The work by Jiang et al. (2012) left a number of unresolved questions. Because they had used only grafted soybean seedlings at the 3-leaf stage, the potential for plant size to mediate glyphosate tolerance level remained unknown. Our recent unpublished results indicate that cultivar and growing environment might also affect tolerance level. The objective of this research was to investigate the effect of growth stage, cultivar, and air temperature on soybean scion tolerance to the herbicide. We continued to use soybean as a model plant for these experiments due to our experience and success with the system, ease and low cost of propagation, rapid growth rate of grafted plants, and a ready supply of RR and CN genotypes.

Materials and Methods

Plant Material and Culture

Soybean seeds were donated by Seed Consultants (648 Miami Trace RD SW, Washington Court House, OH 43160). Seeds were sown in 48-cell flats in a mixture of Wooster silt loam (Fine-loamy, mixed, active, mesic Oxyaquic Fragiudalfs) and Pro-Mix® potting media (Premier Horticulture, 1 avenue Premier, Rivière-du-Loup, QC G5R 6C1, Canada). Seedlings were grown to the unifoliate stage, at which time grafting was done. Following the nomenclature of Jiang et al. (2012), grafted CN/CN, CN/RR, and RR/RR soybean plants were created for all experiments. To determine the effect of growth stage on tolerance to glyphosate, RR/CN plants were also created. We followed the method described by Jiang et al. (2012) with a single modification: both unifoliate leaves were removed from all the seedlings before grafting. A “V”-shaped notch was cut in the rootstock stem above the cotyledons. A wedge-shaped scion was connected to the rootstock, and the junction was supported by a 0.8-cm-long by 2.5-mm-diameter polyethylene tube. Newly grafted plants were cultured in a growth chamber with 280 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), 24/20 C day/night temperatures, a 15/9 h day/night photoperiod, and 98% humidity for a 1-wk recovery period. After the recovery period, seedlings were transplanted into 10-cm-square pots (for 3-leaf-stage plants used in growth stage, cultivar, and environmental effect experiments) or 14-cm-diameter pots (for 6- and 10-leaf-stage plants used in growth stage effect experiments) filled with potting media.

Effect of Soybean Growth Stage on Response of Grafted Plants to Glyphosate

To determine whether plant growth stage affected rootstock-conferred glyphosate tolerance of the CN scion, we used two cultivars, SC5388STS (CN genotype referred to hereafter as 5388) and SCS9351RRTR (RR genotype referred to hereafter as 9351). Grafted soybeans CN/CN (5388/5388), CN/RR (5388/9351), RR/CN (9351/5388), and RR/RR (9351/9351) were grown in a greenhouse with a 16/8 h day/night cycle and a corresponding thermoperiod of 27/22 ± 2 C. Plants were treated with glyphosate (see the Herbicide Treatment section) at the 3-, 6- and 10-leaf stages. The response of grafted soybeans to glyphosate treatments was measured as plant height (defined as the length from the cotyledon node to the tip of the newest leaf) and injury level at 0, 7, 14, 24, and 34 d after treatment (DAT). Injury was assessed visually on a scale of 0% to 100% (with 0% = no injury; 100% = death) and reflected chlorosis, necrosis, overall stunting, and leaf deformation.

Effect of Soybean Scion and Rootstock Cultivar on Response of Grafted Plants to Glyphosate

To determine whether soybean cultivar affected tolerance to glyphosate, three CN cultivars, SC352 (352), 5388, and SC5418STS (5418), and three RR cultivars, SCS9328RR (9328), SCS9392RR (9392), and 9351, were used. Grafted soybeans were cultured in a greenhouse with a 16/8 h day/night cycle and corresponding thermoperiod of 27/22 C. Combinations were CN/CN (352/352, 5388/5388, and 5418/5418), CN/RR (352/9392, 352/9328, 352/9351, 5388/9392, 5388/9328, 5388/9351, 5418/9392, 5418/9328, and 5418/9351) and RR/RR (9392/9392, 9328/9328, and 9351/935). Response of grafted plants to glyphosate was assessed by measuring injury level and leaf number (all the expanded leaves were counted) at 0, 7, 14, 24, and 35 DAT. Shoots were collected at 35 DAT and oven-dried at 60 C to determine final shoot dry weight.

Effect of Day and Night Temperatures on the Response of Grafted Soybean Plants to Glyphosate

Cultivars 352 (CN), 5388 (CN), and 9392 (RR) were used to determine the effect of temperature on tolerance of grafted plants. Grafted seedlings were cultured in two growth chambers (Conviron BDR16), one with a day/night thermoperiod of 28/22 C and the other with a thermoperiod of 24/18 C. Irradiation in both chambers was 480 µmol m^{-2} s^{-1} PPFD, humidity was 78%, and the day/night photoperiod was 15/9 h. The following soybean combinations were used: CN/CN (352/352 and 5388/5388), CN/RR (352/9392 and 5388/9392), and RR/RR (9392/9392). Soybean response to glyphosate was assessed by measuring injury level and leaf number at 0, 7, 14, and 24 DAT.

Herbicide Treatment

Herbicide application methods followed those of Jiang et al. (2012). Soybean plants were sprayed with glyphosate potassium salt diluted with tap water (Roundup WeatherMax®, Monsanto Company, 800 North Lindbergh Boulevard, St Louis, MO 63167). Plants were in the exponential phase of growth at the time of herbicide application (Board and Kahlon 2011). Three rates (0.28, 0.84, and 1.68 kg ae ha^{-1}, equivalent to ⅓×, 1×, and 2× the label recommended rate) were used in both plant stage and cultivar effect experiments. Two application rates (0.84 and 1.68 kg ae ha^{-1}) were tested in the temperature effect experiments. Nonionic surfactant (Spreader 90, Loveland Products, 3005 Rocky Mountain Avenue, Loveland, CO 80538) was added to herbicide solutions at 0.25% v/v. An indoor track-mounted sprayer equipped with a 3-nozzle boom was used to apply glyphosate treatments, with a pressure of 276 kPa and volume of 234 L ha^{-1}. TeeJet® TTJ60-1102 (Spraying Systems Co., North Avenue and Schmale Road, Wheaton, Ilinois, 60187) nozzles were used in the experiments.

Experimental Design and Statistical Analysis

A completely randomized design was used for all experiments, and ANOVAs were conducted using SAS PROC GLM (SAS v. 9.3, SAS Institute, 100 SAS Campus Drive, Cary, NC 27513). Treatment means were compared using Fisher's protected LSD ($\alpha = 0.05$). Each of the three experiments was conducted two times (referred to as Run 1 and Run 2, where necessary). The number of replications (replication = single potted plant) varied: there were five replications for each run of the growth-stage experiment, four and five replications for Run 1 and Run 2, respectively, of the cultivar experiment, and four replications for each run of the temperature experiment. Experimental runs and replications were considered random effects, and all other variable were considered fixed effects. Data from experimental runs were combined, because the run by treatment interactions were not significant.

Results and Discussion

Effect of Growth Stage on Glyphosate Tolerance

Sensitivity of grafted soybean to glyphosate was affected only by the main factors of plant size and herbicide rate. At 34 DAT with 0.84 kg ha^{-1} glyphosate, small (3-leaf-stage) and medium (6-leaf-stage) plants were injured more than large plants (10-leaf stage), with 65%, 55%, and 45% injury, respectively. Injury of small plants was greater than that of medium plants at 1.68 kg ha^{-1} (Figure 1A). Small and large CN/RR plants initially (14 DAT) expressed similar injury at 0.84 and 1.68 kg ha^{-1} glyphosate (unpublished data). However, injury symptoms of small plants increased over time, while injury of medium and large plants decreased (Figure 1B). All CN/CN and RR/CN plants treated with glyphosate at 0.84 and 1.68 kg ha^{-1} were dead at 7 DAT, but survived treatment at 0.28 kg ha^{-1}, with small plants generally showing more injury than medium and large plants (unpublished data), consistent with the effect of growth stage on the response of CN/RR. Injury symptoms appeared a few days later in the RR/CN constructs than observed in CN/CN. RR/RR grafted plants were not affected by glyphosate (unpublished data).

After treatment with 0.28 kg ha^{-1} glyphosate, small plants of CN/CN, RR/CN, and CN/RR grew at a slower rate than the respective glyphosate treatment–free plants, while large plants were not affected (Figure 1B). The height increase of small plants was one-third that of plants that were not sprayed. CN/RR plants survived glyphosate at the 0.84 kg ha^{-1} rate. Small and large plants increased in height slightly (<10%) between 0 and 34 DAT, whereas medium plants increased in size by about 30% (Figure 1B). The smaller increase in height of small plants was likely related to the fact that they were more severely injured, while for the largest plants, a naturally slower growth rate likely contributed. Symptoms noted on CN/RR plants treated with glyphosate at the rates of 0.84 and 1.68 kg ha^{-1} included mild wilting and chlorosis (7 DAT). The apical meristem was dead (10–14 DAT). At 1.68 kg ha^{-1}, increased height was not observed regardless of plant size (Figure 1B), due to the death of the apical meristem and very limited axillary bud growth (Figure 1C). Following death of the apical meristem, growth of axillary buds was apparent at 14 DAT, followed by appearance of new leaves and side branches over time (Figure 1C). However, leaves that developed after glyphosate treatment were chlorotic and lanceolate shaped, and the adaxial surface was rough. Compared with CN/RR, CN/CN plants showed more severe chlorosis and necrosis on all leaves and were dead at 7 DAT.

CN/RR plants treated with 0.84 and 1.68 kg ha^{-1} of glyphosate were more tolerant than CN/CN plants, regardless of their size. Moreover, results indicated a systemic tolerance expressed throughout the entire scion. Larger grafted plants were more tolerant than small plants. The natural tolerance of larger plants to herbicides cannot be discounted as an additional factor that may have contributed to the overall greater tolerance of medium- and large-sized CN/RR plants compared with small plants. It is generally axiomatic that larger annual plants are more difficult to kill than small annual plants (Schweizer and May 1993).

Effect of Cultivar on Glyphosate Tolerance

While inferences to cultivars other than those used in our experiments are not possible, cultivar affected tolerance of grafted CN/RR plants. We found that scion cultivar affected glyphosate tolerance ($P \leq 0.0001$) (Figure 2A); whereas, rootstock cultivar did not

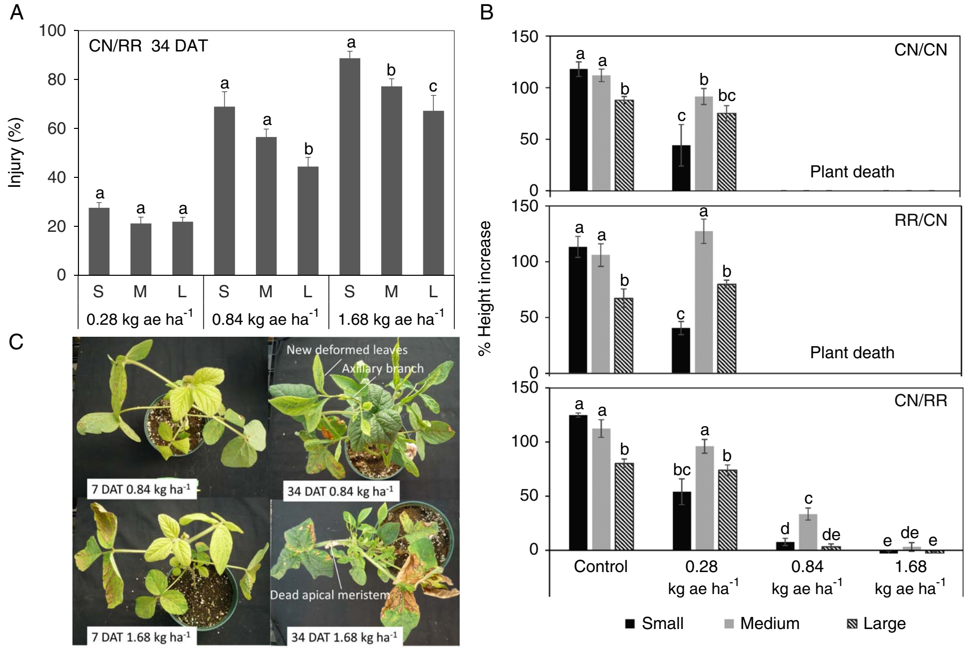

Figure 1. (A) Injury (%) of small (S), medium (M), and large (L) (3-, 6-, and 10-leaf-stage, respectively) grafted CN/RR (conventional scion/glyphosate-resistant rootstock) soybeans to 0.28, 0.84, and 1.68 kg ha^{-1} glyphosate 34 days after treatment (DAT). (B) Height increase (% of plant height at 0 DAT) of S, M, and L grafted CN/RR soybean in response to 0.28, 0.84, and 1.68 kg ha^{-1} glyphosate at 34 DAT. (C) Grafted M-sized CN/RR soybean at 7 and 34 DAT with 0.84 and 1.68 kg ha^{-1} glyphosate. Data from two experimental runs were combined (for each bar, $N = 10$) in A and B. In A, means with the same letter within the same glyphosate rate are not significantly different, and in B, means with the same letter are not significantly different, according to Fisher's protected LSD ($\alpha = 0.05$). Glyphosate did not affect RR/RR constructs.

(Figure 2B). Among the three different CN cultivars, scions 352 and 5418 resulted in greater tolerance than scion 5388 when treated with 0.84 kg ha^{-1} of the herbicide (35 DAT), as represented by overall plant condition (Figure 2A), shoot dry weight (Figure 2B), and plant injury (unpublished data). When 0.28 and 1.68 kg ha^{-1} of glyphosate were applied, plants with 352 as the scion had greater dry weight than constructs using cultivars 5388 and 5418 as scions (Figure 2B). Further evidence that the scion of cultivar 352

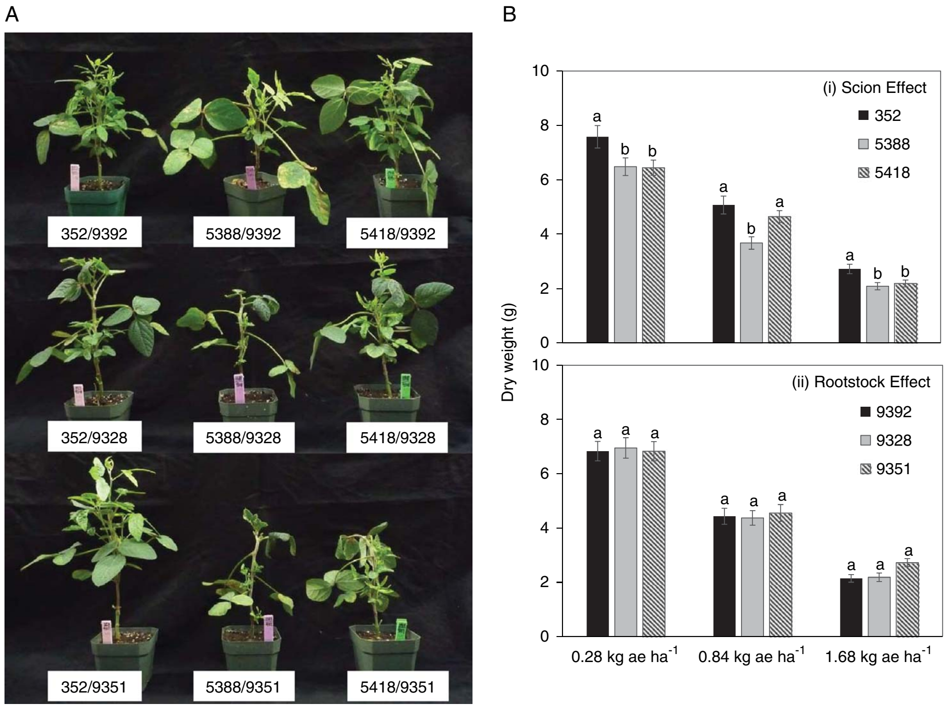

Figure 2. (A) Photo illustrates injury of CN/RR cultivar constructs 35 days after treatment (DAT) with 0.84 kg ha^{-1} glyphosate. Constructs are factorial combinations of three conventional (CN) cultivars (352, 5388, and 5418) and three glyphosate-resistant (RR) cultivars (9392, 9328, and 9351). (B) The effect of CN soybean scions 352, 5388, and 5418 on dry weights of CN/RR constructs at 35 DAT with 0.28, 0.84, and 1.68 kg ha^{-1} glyphosate averaged across rootstock cultivar (i: scion effect).The effect of RR soybean rootstocks 9392, 9328, and 9351 on dry weights of CN/RR constructs at 35 DAT with 0.28, 0.84, and 1.68 kg ha^{-1} glyphosate averaged across scion cultivar (ii: rootstock effect). Vertical bars are an average of 30 replications with standard error. Means with the same letter within a glyphosate rate are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$).

expressed greater tolerance to glyphosate than other scion cultivars can be inferred from leaf number of grafted plants. At 35 DAT with glyphosate at 0.84 kg ha^{-1}, the leaf number of CN/RR constructs with the 352 ($P = 0.8434$) and 5418 ($P = 0.7567$) scions were similar to those of plants that were not treated. In comparison, constructs with the 5388 cultivar scion had fewer leaves ($P = 0.0006$) following herbicide treatment. At 1.68 kg ha^{-1}, leaf number increase of all CN/RR constructs was inhibited (unpublished data). The greater glyphosate tolerance of all grafted combinations with the 352 scion may be due to a higher natural tolerance of the cultivar to glyphosate. This phenomenon was also consistent throughout the temperature effect experiment, as discussed in the section Effect of Temperature on Glyphosate Tolerance. Interestingly, compared with the control, the 0.28 kg ha^{-1} glyphosate treatment appeared to induce a leaf number increase for all CN/RR constructs (unpublished data). This phenomenon may be due to the hormesis effect (Cedergreen et al. 2007).

Marinov-Serafimov (2009) demonstrated that conventional soybean genotypes varied in glyphosate tolerance and attributed the phenomenon to genetic differences. Cruz-Hipolito et al. (2009) revealed that the mechanism of glyphosate tolerance in plants includes both target sites and non-target sites. A non–target site mechanism may be responsible for differences in tolerance to sublethal rates of the herbicide among conventional soybean cultivars. Less glyphosate absorption and translocation (Norsworthy et al. 2001) are possible mechanisms.

Effect of Temperature on Glyphosate Tolerance

Glyphosate rate ($P = 0.0002$), cultivar combination ($P < 0.0001$), and temperature (0.0023) affected plant injury. The interaction between glyphosate rate and cultivar combination was significant ($P = 0.0075$), indicating that the effect is likely to be robust across differing environments. Temperature of the growth chamber did not affect tolerance of CN/RR 352/9392 but affected tolerance of CN/RR 5388/9392 at both the 0.84 and 1.68 kg ha^{-1} glyphosate rates (Figure 3). In previous greenhouse and field experiments conducted in different seasons (i.e., winter, spring, or summer), we observed more glyphosate injury of CN/RR plants as day length, light intensity, and diurnal temperatures increased (unpublished data). These data indicate that while temperature may play a role, it is not likely to be the only major factor affecting tolerance of CN/RR grafts to glyphosate. Effects noted in the greenhouse and field experiments were likely a confluence of day length and light intensity, relative humidity, plant morphology, and quite possibly the scion genotype used.

It is important to note that CN/CN 352/352 survived treatment with 0.84 kg ha^{-1} glyphosate under the low-temperature regime (24/18 C), while CN/CN 5388/5388 died (unpublished data). This provided further evidence of greater tolerance of 352 compared with 5388, as discussed above (Figure 3). CN/RR construct 352/9392 had a similar leaf number under low- and high-temperature environments, regardless of glyphosate application rate. However, the difference in leaf number between control and glyphosate-treated plants was less in low-temperature compared with high-temperature conditions. A similar pattern was observed for construct CN/RR 5388/9392. After treatment with 0.84 kg ha^{-1} glyphosate, plants in the low-temperature cabinet had a leaf number similar to that of control plants but had fewer leaves than the control when cultured in the high-temperature cabinet. At 1.68 kg ha^{-1}glyphosate, CN/RR 5388/9392 had 12 leaves on average 24 DAT in the low-temperature cabinet, while plants in the high-temperature cabinet only had 6 leaves on average (Figure 3).

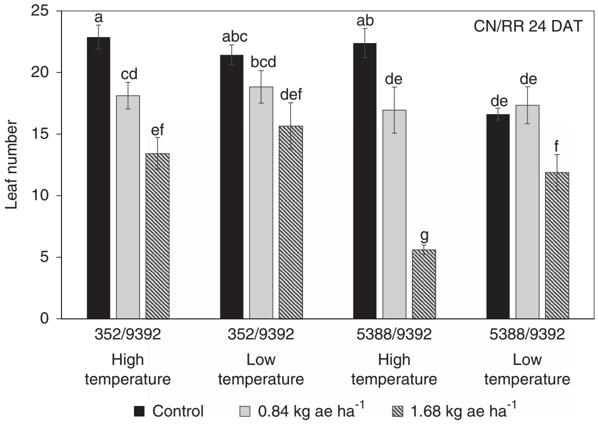

Figure 3. Effect of temperature on CN/RR leaf number at 24 DAT with 0, 0.84, and 1.68 kg ha^{-1} glyphosate. Grafted soybeans were cultured under two different temperature conditions: high temperature, 28/22 C (H); and low temperature, 24/18 C (L). Vertical bars represent a mean of eight replications with standard error. Means with the same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$). RR/RR constructs were not affected by glyphosate. CN/CN constructs were killed by glyphosate, except for construct 352/352 at the low temperature when treated with 0.84 kg ha^{-1}.

Temperature, light intensity, and humidity affect the efficacy and selectivity of herbicides (Cole 1983; Pline et al. 1999; Stewart et al. 2009; Waltz et al. 2004). These factors contribute to the time-of-day application effect of glyphosate. Glyphosate efficacy was reduced when applied at 0600 hours or after 2100 hours compared with 0900 and 1800 hours (Martinson et al. 2005; Mohr et al. 2007). More glyphosate was transferred to the apical meristem in RR soybean under higher temperature and induced greater chlorophyll loss (Pline et al. 1999). Temperature generally interacts with light and humidity when affecting herbicide behavior. Glyphosate efficacy was higher under higher air temperature/high soil moisture (Adkins et al. 1998), which may be due to faster plant growth. These factors likely played a role in the greater injury under the higher-temperature regime of the 5388/9392 plants following glyphosate treatment.

Our research adds further evidence to the value of transgrafting as a potential alternative to conventional breeding (Flores et al. 2010; Schwarz et al. 2010) and gene modification approaches to manage environmental stresses. Grafting using a glyphosate-tolerant rootstock imparted glyphosate-susceptible soybean scions with a partial but stable herbicide tolerance. The phenomenon was robust across different plant sizes, environmental temperatures, and scion/rootstock cultivars, though each factor modulated the level of tolerance somewhat. The basis for tolerance has not been determined but may be induced by the existence of CP4-EPSPS in the scions as result of translocation of *cp4-epsps* mRNA or/and CP4-EPSPS enzyme from the rootstock, translocation of glyphosate from CN scion to RR rootstock, less herbicide absorption in scions of grafted plants, greater glyphosate metabolism, or supply of aromatic amino acids by RR rootstocks (Jiang et al. 2012).

Spray drift from glyphosate, dicamba, and 2, 4-D misapplication plays a major role in damaging sensitive agronomic and specialty crops (Bhatti et al. 1997; Colquhoun et al. 2017; Egan et al. 2014; Everitt and Keeling 2009; Kruger et al. 2012). Expanding use of new GM crops with resistance to 2,4-D and dicamba, along with resistance to glyphosate, is likely to result in even more incidents producing crop injury (Egan et al. 2014; Mortensen et al. 2012). Our model system indicates that grafting is a technique that may be employed to help mitigate injury from low concentrations of drifting herbicides. In particular, such an approach is an attractive option for high-value crops such as tomato, in which grafting is already commercially practiced. Additional research using herbicide-resistant rootstocks of otherwise sensitive specialty crops is justified.

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