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Pollen-Mediated Dispersal of Glyphosate-Resistance in Palmer Amaranth under Field Conditions

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In addition to being a strong competitor with cotton and other row crops, Palmer amaranth has developed resistance to numerous important agricultural herbicides, including glyphosate. The objective of this study was to determine if the glyphosate-resistance trait can be transferred via pollen movement from a glyphosate-resistant Palmer amaranth source to a glyphosate-susceptible sink. In 2006 and 2007 glyphosate-resistant Palmer amaranth plants were transplanted in the center of a 30-ha cotton field. Susceptible Palmer amaranth plants were transplanted into plots located at distances up to 300 m from the edge of the resistant pollen source in each of the four cardinal and ordinal directions. Except for the study plots, the interior of the field and surrounding acreage were kept free of Palmer amaranth by chemical and physical means. Seed was harvested from 249 and 292 mature females in October 2006 and 2007, respectively. Offspring, 14,037 in 2006 and 13,685 in 2007, from glyphosate-susceptible mother plants were treated with glyphosate when the plants were 5 to 7 cm tall. The proportion of glyphosate-resistant progeny decreased with increased distance from the pollen source; approximately 50 to 60% of the offspring at the 1- and 5-m distances were resistant to glyphosate, whereas 20 to 40% of the offspring were resistant at the furthest distances. The development of resistance was not affected by direction; winds were variable with respect to both speed and direction during the peak pollination hours throughout the growing season. Results from this study indicate that the glyphosate-resistance trait can be transferred via pollen movement in Palmer amaranth.

Nomenclature: Glyphosate; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; cotton, *Gossypium hirsutum* L.
Key words: Glyphosate resistance, pollen movement, long distance dispersal.

The profitability of U.S. cotton has been greatly improved since the commercial release of genetically-modified (GM) herbicide-tolerant (HT) cultivars. Between 1997 and 2008, the adoption of HT cotton provided a cumulative net farm income benefit of $799 million (Brookes and Barfoot 2010). The majority of these savings were directly related to the replacement of expensive, soil-applied chemicals required for early-season weed control with the low-cost, broad-spectrum herbicide, glyphosate (Brookes and Barfoot 2010). The use of HT cotton cultivars has provided growers with supplementary benefits beyond simply an increase in farm profits. Studies evaluating the implementation of GM crops state that the adoption of conservation tillage (CT) systems is one of the most significant advantages of HT technology (Brookes and Barfoot 2010). The cumulative (1997 to 2008) value of nonpecuniary benefits derived from GM HT cotton was an estimated $461 million for U.S. growers. The extensive use of HT crops, including cotton, has not come without problems; the espousal of glyphosate-resistant (GR) cotton has facilitated the development of GR Palmer amaranth, one of the most serious weed pests in the southeastern and mid-southern United States.

Palmer amaranth, also commonly known as carelessweed, is a tall, upright, and dioecious summer annual that is native to the Mexican states of Sonora and Baja California, as well parts of southern Arizona and California (Ehleringer 1983). Palmer amaranth was first described by Sereno Watson in 1877, working from specimens collected from San Diego County, CA, and from along the banks of the Rio Grande River (Watson 1877). The species has since spread into the Midsouth and southeastern United States, where it is a common and competitive weed in row crop production. Interference from Palmer amaranth has been shown to significantly affect the growth and yield of corn (*Zea mays* L.) (Massinga et al. 2001), soybean [*Glycine max* (L.) Merr.] (Bensch et al. 2003; Klingaman and Oliver 1994), peanut (*Arachis hypogaea* L.) (Burke et al. 2007), and cotton (Morgan et al. 2001; Rowland et al. 1999). Morgan et al. (2001) reported that Palmer amaranth at a density of 10 plants per 9.1 m of row (1-m row spacing at College Station, TX) reduced cotton canopy volume, relative to the weed-free check, by 45% at 10 wk after crop emergence (WAE); lint yield was reduced by 54% for the same weed density. Rowland et al. (1999) determined that lint yield was reduced 5 to 9% per plot (10 m long by 3.6 m wide at Perkins, OK; 10 m long by 4.1 m wide at Chickasha, OK) for each 1 kg increase in weed biomass. Research done in Georgia during 2006 and 2007 demonstrated that two GR Palmer amaranth spaced every 7 m of row (0.9 m row spacing) reduced cotton yield 23% (MacRae et al. 2007).

In 2004, a grower in Macon County, Georgia reported that he was unable to manage Palmer amaranth on a 250-ha site using glyphosate (840 g ae ha^{-1}) (Culpepper et al. 2006). Glyphosate applied at 12 times the recommended dose failed to control this biotype in the field. As of 2011, the presence of GR Palmer amaranth has been confirmed in 65 counties in Georgia (A. S. Culpepper, data not shown). Currently, GR Palmer amaranth infests more than two million ha in 10 states (Alabama, Arkansas, Georgia, Louisiana, Missouri, Mississippi, North Carolina, New Mexico, South Carolina, and Tennessee) (Heap 2011). This is approximately 50% of the total acreage devoted to upland cotton production in the United States (NASS 2011). Within 3 yr of its discovery, GR Palmer amaranth became the single greatest threat to the economic sustainability of cotton production, largely due to the lack of control provided by available POST herbicides.

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Pyrithiobac, an acetolactate synthase (ALS) -inhibiting herbicide historically has been effective at controlling escaped Palmer amaranth in cotton. However, there is now resistance to this herbicide throughout Georgia (Wise et al. 2009) and the southeastern United States (Heap 2011). Populations with multiple resistances to both glyphosate and the ALS-inhibiting herbicides have been reported (Bond et al. 2010; Sosnoskie et al. 2011). Glufosinate is an additional tool for controlling Palmer amaranth populations that are resistant to glyphosate or glyphosate and pyrithiobac. However, it is only effective when it is applied to extremely small (< 10 cm) weed seedlings and only in cotton that is resistant to glufosinate (Culpepper et al. 2008).

The initiation of biological invasions often is attributed to the movement of progagules or organisms into uncolonized habitats outside of their native range (Novak 2007). In the case of plant invasions, the emigration/immigration of seeds and vegetative materials are considered crucial for the expansion of a species range (Hu and He 2006; Novack 2007). Although Palmer amaranth seeds are not adapted for wind dispersal, they can be spread by way of water, animals, and common agricultural activities such as plowing and harvesting (Costea et al. 2004, 2005). Another, although less commonly explored, aspect of biological invasions is the introduction of alien alleles into a new area (Petit 2004). For allogamous species, the dispersal of herbicide-resistance traits via pollen facilitates the introgression of a foreign, but advantageous, allele at one gene with locally-adapted alleles at others (Petit 2004). Although most pollen grains settle close to the source plant, long-distance dispersal events can and do occur (Alibert et al. 2005; Hanson et al. 2005; Matus-Cádiz et al. 2004; Rieger et al. 2002; Saeglitz et al. 2000). Because Palmer amaranth is dioecious and wind-pollinated, the GR trait can be highly mobile. Palmer amaranth pollen grains are small, fairly smooth, settle slowly (Sosnoskie et al. 2009), and are more likely to be transported away from the paternal plant than larger or more highly ornamented pollen grains (Ackerman 2000; Primack 1978). Additionally, the surfaces of Palmer amaranth pollen are covered with shallow pores that create a layer of turbulent air around the grain, thereby reducing pressure drag and increasing flight time (Borsch 1998; Franssen et al. 2001). The objective of this study was to determine if the GR trait is spread from resistant Palmer amaranth source plants via wind-mediated pollen dispersal to glyphosate-susceptible (GS) receptor plants under field conditions.

Materials and Methods

The study was conducted in a 30-ha field in Macon County, Georgia in 2006 and 2007. Soil at the site was a Dothan loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudults) with 1.9 to 2.1% organic matter and pH 6.2 to 6.4. The site is surrounded by pine plantations to the south, grass pastures to the east and west, and a wooded residential lot and pastures to the north. The nearest agricultural fields are located approximately 400 to 600 m north of the experimental site. The field size is representative of agricultural production acres in the state of Georgia; 75% of the farms are smaller than 72 ha.

The field was planted to cotton (DP555 BG/RR, Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63167) on May 15, 2006 and soybean (NK S76-L9, Syngenta, 3411 Silverside Road, Wilmington, DE 19810) on May 9, 2007. Cotton and soybean were planted at rates of 10 and 30 seed m^{-1} of row, respectively, in rows spaced 0.9 m apart. In 2006, the trial area received fomesafen (Reflex, Syngenta, 3411 Silverside Road, Wilmington, DE 19810) (280 g ai ha^{-1}) and pendimethalin (Prowl, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709) (920 g ai ha^{-1}) PRE, glyphosate (Roundup Weathermax, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167) (870 g ha^{-1}) and *S*-metolachlor (Dual Magnum, Syngenta, 3411 Silverside Road, Wilmington, DE 19810) (1,070 g ai ha^{-1}) POST, and diuron (Direx, E. I. du Pont de Nemours and Company, 1007 Market Street, Wilmington, DE, 19898) (1,120 g ai ha^{-1}) and MSMA (Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN 38017) (2,240 g ai ha^{-1}) postdirected (PD). In 2007, the site received pendimethalin (920 g ha^{-1}) preplant incorporated (PPI), pendimethalin (460 g ha^{-1}) and flumioxazin (Valor, Valent U.S.A. Corporation, P.O. Box 8025, Walnut Creek, CA 94596) (70 g ai ha^{-1}) PRE, and fomesafen (280 g ha^{-1}) POST. The field interior, as well as the surrounding acreage (within 300 to 600 m of the field edge), was hand-weeded regularly throughout the growing season to ensure that the nearest GR pollen source was the block of GR Palmer amaranth male plants transplanted into the center of the study site. Adjacent pastureland was also treated with 2,4-D (2,4-D Amine, Helena Chemical Company, 225 Schilling Boulevard, Suite 300, Collierville, TN 38017), biweekly. Additional crop production practices followed University of Georgia recommendations.

Inflorescences from a known GS Palmer amaranth population were collected at the University of Georgia Attapulgus Research and Education Center in Attapulgus, GA, in the autumns of 2005 and 2006. Palmer amaranth seedheads were air dried in an unheated greenhouse, mechanically abraded to separate the seed from the floral tissue, and the seed stored at 4 C until planting. Glyphosate-resistant seed were derived from a GR population, as described in Culpepper et al. (2006), which had been advanced through two additional generations in a greenhouse. Seed of both biotypes were planted in April of 2006 and 2007 in 2,650 cm^3 plastic pots filled with a Tifton sandy loam and grown outdoors in an open hoop house located at the University of Georgia Tifton Campus in Tifton, GA. The pots were irrigated and the plants fertilized as needed to ensure vigorous growth. The GR plants were sprayed with glyphosate at a rate of 870 g ha^{-1} when the plants were 5 to 10 cm tall using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles to verify the resistance phenotype. Plants that displayed glyphosate injury were discarded from the study.

Pollen-mediated gene flow was evaluated using a source/receptor experimental design that has been employed in the study of pollen-mediated gene flow for other species, (Alibert et al. 2005; Massinga et al. 2003; Saeglitz et al. 2000). Three hundred of the hoop house-grown GR Palmer amaranth plants were transplanted into a 0.01 ha area in the center of the field in both years. Of the 300 transplants, a total of 179 (in 2006) and 162 (in 2007) were males capable of producing pollen; female plants were not removed from the pollination block. In 2006, nine hoop house-grown GS Palmer amaranth plants were transplanted into plots (182 cm by 200 cm)

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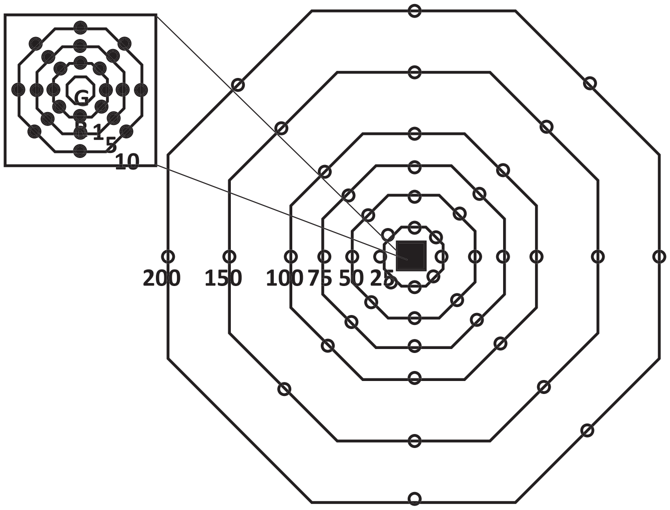

Figure 1. Field trial design of the 2006 study demonstrating the planting arrangement of plots of glyphosate-susceptible (GS) Palmer amaranth relative to the glyphosate-resistant (GR) pollen source. The GR source consisted of 300 plants in a 0.01 ha block in the center of the field; each GS plot (1.8 m by 4 m) consisted of nine plants each.

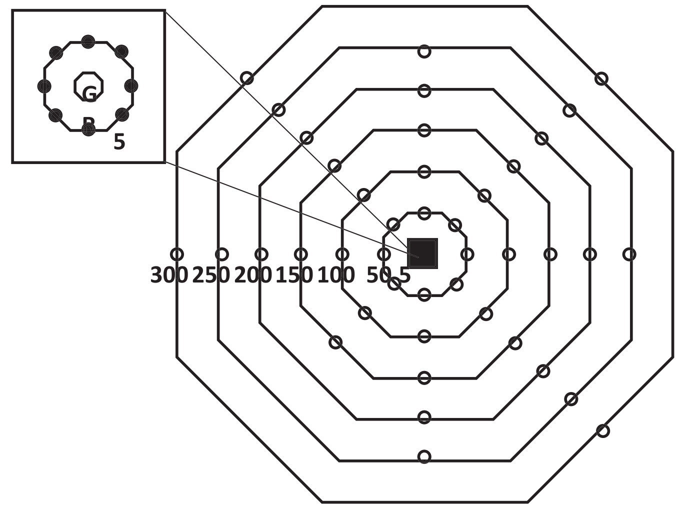

Figure 2. Field trial design of the 2007 study demonstrating the planting arrangement of plots of glyphosate-susceptible (GS) Palmer amaranth at distances of 5 to 300 m from the glyphosate-resistant (GR) source. The GR source consisted of 300 plants in a 0.01 ha block in the center of the field; each GS plot (1.8 m by 4 m) consisted of 15 plants each.

located at distances between 1 and 250 m from the edge of the source in the four cardinal (N, S, E, and W) and four ordinal (NE, SE, SW, and NW) directions (Figure 1). In 2007, 15 GS plants were transplanted into plots (1.8 m by 4 m) located at distances up to 300 m from the GR source in the same eight directions (Figure 2). Because of the shape of the field, plots were not able to be located at each distance in every direction (Figures 1, 2). All plants were between 0.3 and 0.5 m in height at the time of transplanting; plants were transplanted directly into the crop rows and were spaced 1 m apart. The transplants were irrigated and fertilized one to three times after planting to promote establishment and growth. The susceptible plots were surveyed one to two times weekly for the presence of male plants, which were then removed to prevent them from pollinating nearby females.

The inflorescences from the GS mother plants were hand-harvested in October of 2006 and 2007 when it was determined that ≥ 75% of the seeds possessed coats that were dark violet to black in color. This measure was taken to balance the need for seed maturity, while preventing dehiscence and minimizing seed loss. Palmer amaranth seed were cleaned and stored as described previously. Seed from individual susceptible mother plants were maintained separately and the total seed weight produced by each plant was recorded.

The glyphosate-resistance trait was used as a selective marker to evaluate the movement of pollen between the source and receptor plots. Seeds from mother plants were planted in 128-cell seedling trays (one tray per parent) and thinned to one plant per cell following emergence. Glyphosate was applied to 5- to 7-cm-tall plants at 530 g ha^{-1} following a protocol established by Culpepper et al. (2006) to account for increased sensitivity or Palmer amaranth to glyphosate under greenhouse conditions. The proportion of seedlings surviving the glyphosate application was recorded 7 and 14 d after treatment (DAT). An individual plant was considered to have survived the glyphosate application if healthy leaf or bud tissue remained, or if the plant showed signs of regrowth, following the herbicide application. Glyphosate-resistant and -susceptible standards also were screened at the same time; averaged over years, the GR and GS controls were controlled by glyphosate 4% and 100%, respectively. The resistance percentages from all plants located at each distance by direction combination were averaged together to generate a grand mean for each receptor plot. Mean percent resistant offspring and mean seed weight served as response variables in a mixed model analyses of variance with direction and distance from the pollen source serving as fixed effects. Distances varied between years; therefore, the interaction between distance and direction was not replicated and could not be examined. Data from the 250 and 300 m distances for years 2006 and 2007, respectively, were not included in the analyses due to insufficient replication within each given year. Data from the NW transect for 2006 also were insufficiently replicated and were not used in the statistical analyses. The Tukey–Kramer method was used to determine which means were significantly different from each other. If MANOVA indicated that distance from the GR pollen source was a significant factor in the model, data were subjected to additional regression analyses. Years were analyzed separately.

Hourly surface meteorological data, including wind direction and speed, were collected daily from June 1 to October 31 for both years. The weather station in Americus, GA, is managed by the National Oceanic and Atmospheric Administration (NOAA) as part of the National Climatic Data Center; the data is regarded as being representative of the region. Data collection was restricted to between the hours of 7:00 A.M. and 3:00 P.M. when pollen shedding was most likely to occur (L. M. Sosnoskie, personal observation). Wind speed and direction (blowing from) data were graphically presented using a wind rose. The data are presented in a circular format; wind direction is converted from compass degrees to discrete spokes corresponding to the four cardinal and four ordinal directions. The length of each spoke reflects the relative amount of time winds blow from a given direction. Individual spokes are further divided into frequency categories describing the proportion of time that winds blow at certain speed ranges. Statistical analyses were not conducted on wind speed and direction data.

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Table 1. Mean percent resistant offspring plant^{-1} of Palmer amaranth for plots at each distance by direction combination for both 2006 and 2007. NA indicates either that no female plants were planted at the site or that no female plants survived to reproductive maturity.

Distance	N	NE	E	SE	S	SW	W	NW
m				%				
2006								
1	68	51	54	75	48	72	73	28
5	72	57	44	64	38	58	70	NA
10	63	51	52	74	24	53	71	NA
25	58	49	30	36	81	53	64	NA
50	NA	43	27	49	84	NA	46	NA
75	34	39	52	37	81	48	51	NA
100	38	27	43	35	36	43	NA	NA
150	31	38	46	66	50	19	24	NA
200	28	43	36	44	30	NA	30	47
250	18	NA	NA	NA	NA	NA	NA	NA
2007								
5	51	32	59	41	72	41	70	NA
50	35	32	44	36	38	43	51	47
100	28	24	42	37	28	31	49	29
150	30	24	44	26	37	28	13	27
200	25	25	37	30	37	NA	31	11
250	50	36	NA	28	NA	NA	24	NA
300	NA	20	NA	22	NA	NA	NA	NA

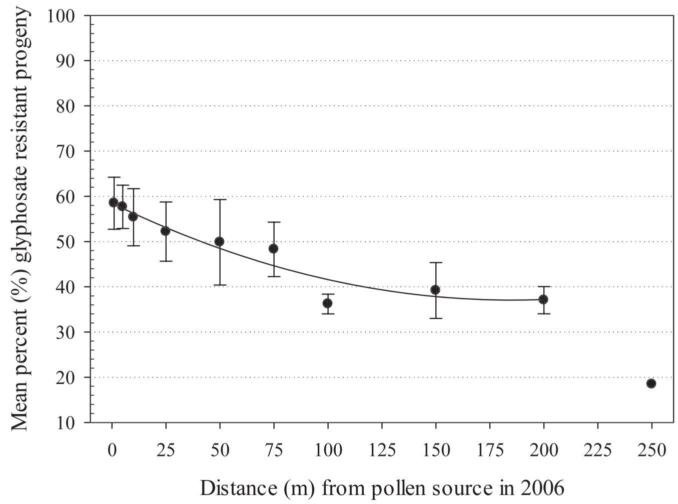

Figure 3. Mean percent resistant offspring (and standard errors) produced by glyphosate-susceptible female Palmer amaranth plants growing at varying distances from the glyphosate-resistant pollen source in 2006. Means were averaged across all directions. Error bars represent one standard error of the mean. Because statistical analyses indicated a significant effect of distance on the occurrence of resistance, data were subjected to regression using a second-order polynomial model: $Y = 0.0006x^2 - 0.2360x + 58.5680$, $R^2 = 0.93$.

Results and Discussion

The GR males and GS females commenced flowering at approximately the same time each year (mid- to late July); flowering continued through September and into early October, although pollen shed decreased as the growing season advanced (L. M. Sosnoskie, personal observation). In 2006, a total of 14,037 progeny from 249 GS females were screened for resistance to glyphosate; in 2007, 13,685 seedlings from 292 GS females were evaluated. Glyphosate-resistant offspring were recovered from every plot in which female receptor plants survived to reproductive maturity (Table 1).

Analyses of variance showed that the incidence of glyphosate resistance was significantly ($P \leq 0.05$) affected by distance for both years (Figures 3 and 4). Averaged across direction, 52 to 58% of the of the offspring produced by GS mother plants located 1 to 25 m away from the pollen source were resistant to glyphosate in 2006 (Figure 3). Approximately 49% of the progeny produced by GS Palmer amaranth at plots 50 and 75 m distant from the GR block were glyphosate-resistant. At distances of greater than 100 m, 36 to 39% of the offspring were resistant (Figure 3). Eighteen percent of the offspring from the plot at 250 m were resistant to glyphosate (Table 1). Tukey–Kramer multiple comparison procedures indicated that the plots closest to the pollen source (1 to 10 m) differed significantly ($P \leq 0.05$) with respect to percent resistant progeny from the furthermost sites (> 100 m). The plots located at intermediate distances (25 to 75 m) did not differ statistically ($P \geq 0.05$) from either the nearest or the furthest distances. There were no differences ($P \geq 0.05$) in resistance levels among the most distant plots (100 to 200 m).

In 2007, 52 and 41% of the offspring produced from GS receptor plants located 5 and 50 m away from the pollen source, respectively, survived a normally lethal dose of glyphosate, when averaged over direction (Figure 4). At the furthermost distances (100 to 250 m) from the GR Palmer amaranth block, 28 to 34% of the offspring of GS mothers were resistant to glyphosate (Figure 4). Twenty-one percent of the offspring from the plot at 300 m were resistant to glyphosate (Table 1). Results from Tukey–Kramer comparison procedures indicated that the levels of resistance observed in the progeny derived from GS mothers located 5 m from the resistant pollen source were significantly ($P \leq 0.05$) different from the levels of resistance observed in the progeny from 50 to 250 m. There were no differences ($P \geq 0.05$) in resistance levels among the furthermost plots (100 to 250 m) included in the analysis. Similar results were reported by Alibert et al. (2005), Hanson et al. (2005), Massinga et al. (2003), Matus-Cádizet et al. (2004), Rieger et al. (2002), and Saeglitz et al.

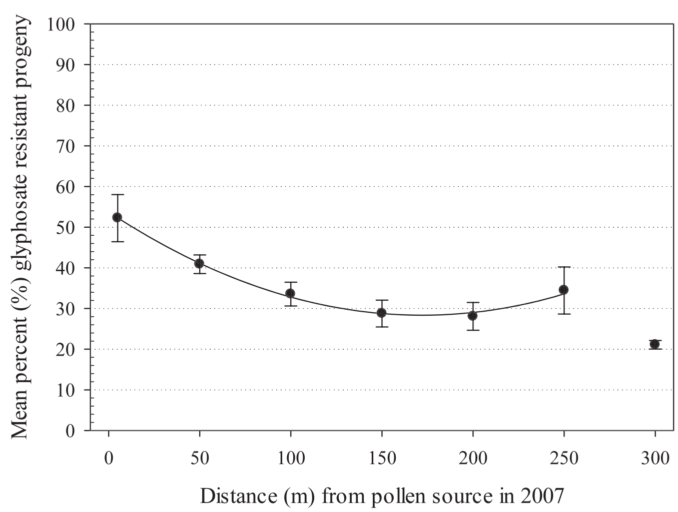

Figure 4. Mean percent (and standard errors) resistant offspring produced by glyphosate-susceptible female Palmer amaranth plants growing at varying distances from the glyphosate-resistant pollen source in 2007. Means were averaged across all directions. Error bars represent one standard error or the mean. Because statistical analyses indicated a significant effect of distance on the occurrence of resistance, data were subjected to regression using a second-order polynomial model: $Y = 0.0009x^2 - 0.2986x + 53.8150$, $R^2 = 0.99$.

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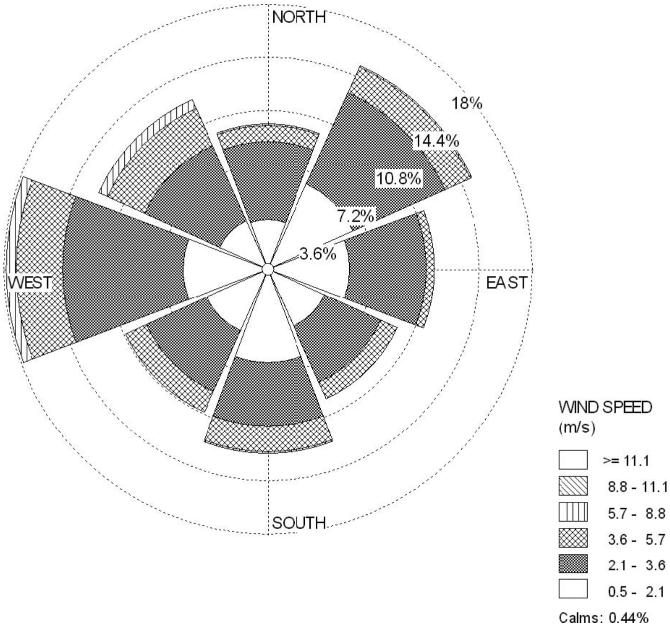

Figure 5. Distribution and frequency of wind speeds and directions (blowing from) between June 1 and October 31, 2006 at a weather station managed by the National Oceanic and Atmospheric Administration. The length of each spoke describes the frequency with which winds blow from a particular bearing; a longer spoke indicates that a greater number of winds originated from that particular direction. The concentric circles within each spoke describe the proportion of time that winds blow at certain speed ranges.

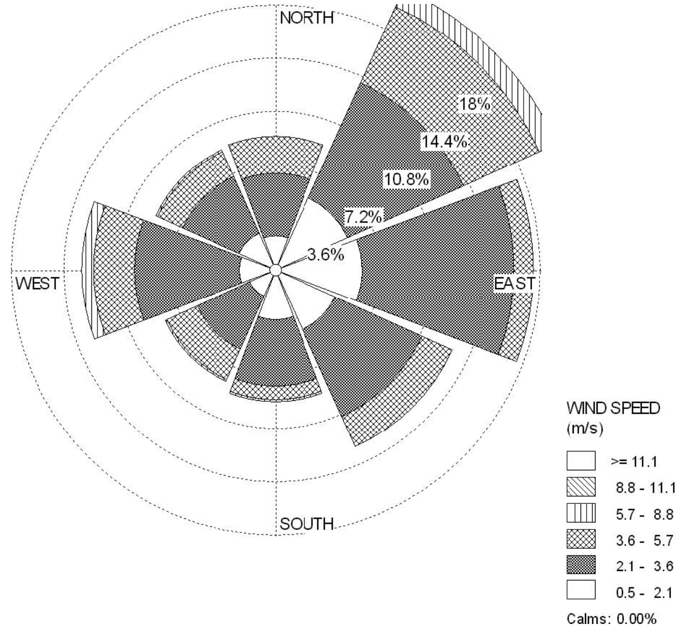

Figure 6. Distribution and frequency of wind speeds and directions (blowing from) between June 1 and October 31 , 2007 at a weather station managed by the National Oceanic and Atmospheric Administration. The length of each spoke describes the frequency with which winds blow from a particular bearing; a longer spoke indicates that a greater number of winds originated from that particular direction. The concentric circles within each spoke describe the proportion of time that winds blow at certain speed ranges.

(2000), who found that pollen-mediated gene flow was reduced in plots furthest away from the pollinator block, as compared to the nearest experimental units. Observed maximum dispersal distances in these studies ranged from 30 to 3,000 m and were dependent on species biology (plant height and the timing of anther dehiscence), environmental conditions (temperature, humidity, and prevailing winds), and experimental design (study size and sampling effort).

Results from regression analyses also showed that the mean number of resistant individuals, when averaged across directions, generally decreased with increased distance from the pollen source for both years (Figures 3 and 4). In the current study, data from both years were best described using a second-order polynomial model. This is because numerically higher mean resistance percentages were observed for the 150- and 200-m distances, as compared to 100 m (in 2006) (Figure 3), and for 250 m plots, as compared to the 100- to 200-m plots (in 2007) (Figure 4). Although the second-order polynomial best describes the information that we were able to collect, it might not accurately portray the true dispersal functions for both pollen and gene flow. Previously published reports have demonstrated that wind-mediated pollen dispersal is usually leptokutic, wherein the vast majority of pollen deposition occurs close to the parental source with relatively few events occurring further away (Busi et al. 2008). However, Tonsor (1985) showed that pollen- and gene-flow are not necessarily equivalent. Although pollen dispersal in narrowleaf plantain (*Plantago lanceolata* L.) exhibited a leptokurtic distribution, actual gene-flow was platykurtic, meaning that peak mating occurs at intermediate distances (Tonsor 1985). Because of inadequate replication, data from the furthermost sites, 250 m in 2006 and 300 m in 2007, were not included in the analyses. The ability to replicate these distances in more directions, and to expand the sampling effort further away from the pollen source, would have improved our ability to better estimate the dispersal potential of the GR trait under these study conditions.

Analyses of variance indicated that the occurrence of resistant offspring was not affected by wind direction either year; winds originated from every cardinal and ordinal direction in both 2006 and 2007 (Figures 5 and 6). In 2006, 18% of the observed hourly winds ($n = 912$) were westerly in origin (Figure 5). Fifteen percent of the wind vectors originated from the NE, and 13% from both the S and NW (Figure 5). Nine to 11% of the recorded winds originated from the N, E, SE, or SW (Figure 5). The majority of observed wind speeds ranged from 0.5 to 3.6 m s^{-1}; less than 17% of recorded winds exceed 3.7 m s^{-1} (Figure 5). In 2007, 21% of the recorded winds ($n = 760$) originated from the NE (Figure 6). Eighteen percent of the total number of observed hourly winds originated from the E, and 13% from both the SE and W (Figure 6). Winds blew from the N, S, SW, or NW 8 to 9% of the time (Figure 6). Similar to 2006, the greatest proportion (78%) of winds in 2007 blew at speeds of 0.5 to 3.6 m s^{-1} (Figure 6). An evaluation of mean daily wind direction and speed data collected from five regional weather stations (Byromville, Ft. Valley, Plains, Unadilla, and Vienna, GA) operated by the University of Georgia as part of the Georgia Automated Environmental Monitoring Network (GAEMN) also showed changeable wind patterns (data not shown). Considering that winds were variable with respect to origin and speed for both 2006 and 2007, it is not unreasonable to expect that GR offspring would be produced by receptor plants located in plots at every direction by distance combination. Assuming a terminal settling velocity of 2 to 5 cm s^{-1} (Sosnoskie et al. 2009) and using a simple ballistic model, wind speeds ranging from 0.5 to 3.6 m s^{-1} are

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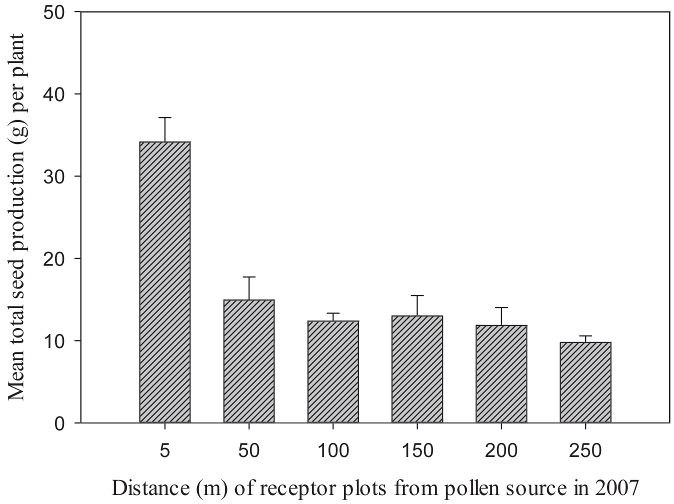

Figure 7. Mean total seed weight produced by glyphosate-susceptible female Palmer amaranth plants in 2007 on a per plant basis with respect to distance from pollen source averaged across all directions. Error bars represent one standard error of the mean. The Tukey–Kramer comparison procedure indicated that glyphosate-susceptible females located at a distance of 5 m from the pollen source produced significantly ($P \leq 0.05$) more seed than females at all other distances.

sufficient to disperse GR Palmer amaranth pollen hundreds of meters away from the source (Sosnoskie et al. 2007).

Per plant seed production was significantly ($P \leq 0.05$) affected by direction in 2006, although the disparities observed among transects were not related to prevailing wind patterns. In 2006, deer heavily browsed the Palmer amaranth transplants in both the SW and the W, resulting in a significant reduction in mean per plant seed production (13 to 21 g $plant^{-1}$) relative to the other directions (28 to 43 g $plant^{-1}$). Seed production in 2006 was unaffected ($P \geq 0.05$) by distance from the pollen source. Statistical analyses indicated that mean total seed production per receptor plant was a function of distance in 2007. In 2007, significantly ($P \leq 0.05$) greater amounts of seed were produced by plants growing 5 m from the in-field source of herbicide resistance (34 g $plant^{-1}$) than at distances greater than 50 m (8 to 15 g $plant^{-1}$) (Figure 7). Busi et al. (2008) reported that annual ryegrass (*Lolium rigidum* Gaudin) bait plants located nearest (0 to 400 m) to the herbicide-resistant pollen donors produced more seed per plant as compared to more distant (500 to 3,000 m) receptors and suggested that a dilution in the concentration of airborne pollen contributed to the reduction in seed set. The degree to which GR Palmer amaranth pollen was restricted or limited at the furthermost plots for both years is unknown. Because most pollen grains, especially those that form clumps, are expected to disperse relatively short distances from the parent plants, it would be expected that a significant proportion of the pollen in this study was intercepted by the GR female plants, which were not removed from the pollinator block (Sosnoskie et al. 2009; Tonsor 1985). However, amaranths are capable of producing prodigious amounts of pollen, which could mitigate load limitations; Reddi and Reddi (1986) reported that several *Amaranthus* species, including spiny (*Amaranthus spinosus* L.) and slim (*Amaranthus hybridus* L.) amaranths, were capable of producing between 1,100 and 5,500 pollen grains per anther. Palmer amaranth produces similarly sized anthers and pollen, so it is not unreasonable to expect that total pollen production per flower should be comparable. The pollen to ovule ratio, which can significantly impact reproductive success, for Palmer amaranth is yet unknown, although it is expected to be high for an anemophilous species (Michalski and Durka 2009).

Although Darmency et al. (2009) found that the number of glufosinate-resistant beet seeds produced per fruit decreased with increased distance from a resistant pollen source, they also reported that the number of susceptible seeds per fruit remained unchanged with respect to location (Darmency et al. 2009). They concluded that an external pollen provider was generating "nonnegligible" amounts of glufosinate-susceptible pollen that resulted in the generation of the sensitive offspring. In our study, care was taken to ensure that the nearest pollen source was located greater than 300 to 600 m away from the edges of the experiment boundaries. If the presence of background pollen had been minimized by the elimination of all indigenous Palmer amaranth plants during the course of our studies, then the proportion of GR to GS offspring recovered from the donor plants should have remained constant, regardless of distance or direction. The incidence of increased numbers of susceptible offspring at the furthest distances from the GR pollen donor block are suggestive of the presence of a background pollen cloud derived from plants outside of the managed area. This study was conducted in Macon County, Georgia, the site of the first-reported occurrence of GR Palmer amaranth. Although the county is considered to be severely infested, an inestimable number of sensitive individuals do exist and are likely contributing to the overall pollen load. Depending on wind speed and the degree of convective mixing, Palmer amaranth pollen grains can be dispersed hundreds to thousands of meters from their source (Sosnoskie et al. 2007).

The production of a greater number of susceptible individuals at the furthest distances also could be due to the effects of either autopollination or agamospermy (Trucco et al. 2007). Autopollination could result from the production of infrequent male flowers distributed among the female blossoms in the inflorescences of the GS receptor plants (Trucco et al. 2007). Fertilization accomplished by the pollen produced by such flowers would result in the generation of GS offspring. Although we were careful to inspect all of the plots to ensure the gender of the GS receptor plants, it is possible that a low frequency of male flowers could have gone unnoticed. For both years of the study, only one plant was observed to have produced both types of floral structures. Agamospermy also can significantly influence outcrossing rates. Trucco et al. (2007) observed the production of nonhybrid progeny resulting from crosses between Palmer amaranth and common waterhemp (*Amaranthus rudis* Sauer). A characterization of the offspring revealed that they possessed DNA content values similar to those of the female parent and were all female in gender. If agamospermy is responsible for the increase in numbers of GS progeny at the furthest distances, then it would be expected that the same offspring should be predominantly female, because most will be clones of the GS mother plants. An evaluation of the male to female ratio of the offspring of randomly selected Palmer amaranth plants from plots located nearest (1 m in 2006, 5 m in 2007) and furthest (150 to 250 m in 2006, 150 to 300 m in 2007) from the pollen source in our studies showed that there were no statistical biases towards one gender or the other (data not shown).

Herbicide susceptibility is a resource that is held in common by growers; the continued maintenance of that

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condition is threatened by the presence of a mobile herbicide-resistance trait (Webster and Sosnoskie 2010). Agricultural practices aimed at either delaying or preventing the development of herbicide-resistance are not considered economical in the short term, and might not be readily adopted by all growers (Culpepper et al. 2006). Trust in the future availability of chemical technologies to combat herbicide-resistance and/or belief that resistance management strategies are futile could lead to the indiscriminate use of currently available products, which can result in a loss of weed susceptibility for an entire community of farmers (Webster and Sosnoskie 2010). This is called the tragedy of the commons and is defined as a situation in which the actions, or inactions, of individuals precipitate the collapse of a resource for which they are competing. The degree of mobility, or how quickly the resistant trait is dispersed, dictates the scale of ownership, i.e., the local vs. the regional level. Herbicide-resistance traits that can be spread long distances via wind-mediated pollen dispersal would require the concerted efforts of many individuals to ensure that susceptibility is maintained. The individual investment in the management of collective property is expected to diminish.

Palmer amaranth pollen grains are small and settle slowly out of the air and are, theoretically, capable of being moved long distances across the agricultural landscape. Results from this study demonstrated that GR Palmer amaranth was able to be dispersed up to 300 m under natural field conditions. Even if a competing pollen source affected our evaluation of gene flow, the donor parents were a minimum of 300 to 600 m distant from the study site. Pollen movement can increase the speed with which herbicide resistance develops in a weed population if the rate of outcrossing exceeds the rate of natural genetic mutation (Hidayat et al. 2006; Jasieniuk et al. 1996). In 2005, GR palmer amaranth was confirmed in only one county in Georgia; as of 2011, 65 counties in the state were infested to some degree with the GR biotype. We believe that the rapid development of glyphosate resistance in Palmer amaranth in Georgia was due, in part, to the movement of pollen between spatially segregated populations. Palmer amaranth also has developed resistance to additional herbicide classes (Heap 2011; Vencill et al. 2002; Wise et al. 2009), and there is concern that wind-mediated pollen flow has (Sosnoskie et al. 2011) and will continue to allow for multiple herbicide resistances to accumulate in a single population. Current University of Georgia Extension recommendations encourage growers to remove all Palmer amaranth plants from their agricultural field prior to them achieving reproductive maturity in order to mitigate the spread of herbicide-resistance traits.

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