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Acetolactate Synthase–Inhibitor–Resistant Yellow Nutsedge (*Cyperus esculentus*): I—Phenotypic Differences

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Acetolactate synthase (ALS) –inhibitor resistance has been recently documented in a yellow nutsedge biotype in Arkansas rice production, with a target-site mutation resulting in an amino acid substitution from Trp574 to Leu. Preliminary observations have indicated that the resistant biotype showed distinct phenotypic characteristics. Two greenhouse experiments were conducted on the resistant biotype in comparison with three susceptible standards (1) to understand differential growth habit and spatial distribution, and (2) to characterize shoot emergence pattern and seedling vigor. The resistant biotype exhibited a drastically different growth habit with secondary and tertiary basal bulbs emerging away from the parent shoot, resulting in a wider spatial distribution and ground coverage compared to the very compact growth habit of susceptible biotypes. Unlike the susceptible biotypes, the rhizomes developing into tubers were not often connected to the primary basal bulb, but were originating randomly from daughter shoots. The resistant biotype produced an extensive subterranean network of rhizomes and basal bulbs, with wider root spread and distribution compared to the susceptible biotypes. The growth habit of the resistant biotype appeared to be intermediate between yellow and purple nutsedges. Further, the resistant biotype showed a considerably delayed emergence pattern with relatively high levels of tuber dormancy. Although the resistant plants exhibited low early-growth seedling vigor and biomass production compared to the susceptible biotypes (perhaps because of smaller tubers), final aboveground biomass production was greater than that of susceptible biotypes. The overall growth habit and phenotype of the resistant biotype may provide a competitive advantage over adjacent species through the ability to occupy niches and gain improved access to critical resources. The distinct growth pattern may also mean that tillage should not be relied upon for control because it can assist further spread by disconnecting and displacing the chains of rhizomes.

Nomenclature: Yellow nutsedge, *Cyperus esculentus* L.; purple nutsedge, *Cyperus rotundus* L.; rice, *Oryza sativa* L.

Key words: ALS-inhibitor resistance, herbicide-resistant weed, vegetative propagation, target-site resistance, resistant phenotype, Trp574 to Leu.

Yellow nutsedge is one of the most problematic perennial weeds infesting agricultural and horticultural crops across the world (Bendixen and Nandihalli 1987; Holm et al. 1977), including areas in mainland United States and Canada (Mulligan and Junkins 1976; Reed and Hughes 1970). In rice–soybean [*Glycine max* (L.) Merr.] production systems of the Mississippi Delta region, yellow nutsedge is often regarded as a difficult-to-control weed, with practitioners frequently requesting improved management options (Norsworthy et al. 2013). The dominance of yellow nutsedge can be attributed to its biology. It is an invasive and aggressive C_4 species with considerable allelopathic effect on other species (Drost and Doll 1980). It reproduces predominantly by tubers (Horak and Holt 1986) that are typically dispersed by tillage equipment (Schippers et al. 1993), but viable seed production has also been observed (Thullen and Keeley 1979).

Yellow nutsedge proliferates through the production of extensive underground system of rhizomes, tubers, and basal bulbs (Stoller and Sweet 1987; Wills et al. 1980). Rhizomes are weak thread-like structures that may differentiate into tubers or basal bulbs (Garg et al. 1967; Jansen 1971). Tubers are characterized by a vascular system, hard epidermis, roots, and well-developed lateral and apical buds (Bendixen 1973; Wills et al. 1980). Freshly produced tubers are dormant, but dormancy breaks over time and most tubers usually sprout in the next growing season (Stoller and Wax 1973). The rhizomes that develop into basal bulbs usually grow toward the soil surface. The basal bulbs contain meristem for shoot, root, rhizome, and

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flower stalks (Jansen 1971). The rhizomes produced by the primary basal bulb (i.e., from mother tuber) may further differentiate into secondary basal bulbs and tubers (Mulligan and Junkins 1976).

Yellow nutsedge is often confused with closely related purple nutsedge. However, yellow nutsedge could be differentiated from purple nutsedge and other *Cyperus* species based on the presence of conspicuous scales on the rhizomes (Mulligan and Junkins 1976). Yellow nutsedge produces solitary tubers at the end of the rhizome (terminal tubers), whereas purple nutsedge produces chains of tubers along the rhizome. Yellow nutsedge plants exhibit a compact growth habit with tubers in close proximity to the mother plant, whereas purple nutsedge produces a network of basal bulbs and tubers away from the parent shoot, creating a much wider distribution of individual plants within a local scale (Webster 2003; Wills 1987). At maturity, the rhizomes of purple nutsedge become wiry and produce bitter-tasting, dark-colored tubers, whereas the tubers of yellow nutsedge are yellow–beige in color with a pleasant almond-like taste (Webster 2003; Wills and Briscoe 1970). The leaves of purple nutsedge are boat shaped with rounded tips, whereas yellow nutsedge has pointed leaf tips (Wills 1987). Further, the inflorescence of yellow nutsedge is golden yellow in color, unlike the reddish-purple–colored inflorescence of purple nutsedge.

Herbicide resistance is an emerging problem in a number of arable weeds in herbicide-dominant production systems. Although rare, it is not unlikely that resistance could evolve in a predominantly asexually reproducing species such as yellow nutsedge. A yellow nutsedge biotype with resistance to halosulfuron has been documented in Arkansas rice production (Tehranchian et al. 2014). Studies have revealed that the resistant biotype had > 2,714-fold resistance to halosulfuron compared to a susceptible standard and also showed cross resistance to other acetolactate synthase (ALS) –inhibiting herbicides including imazamox, imazethapyr, bispyribac-sodium, pyrithiobac, bensulfuron, and penoxsulam (Tehranchian et al. 2014). Subsequent molecular characterization revealed the presence of a target-site mutation resulting in an amino acid substitution from Trp574 to Leu (Tehranchian et al. 2014).

Preliminary observations have indicated differential phenotypic traits in the resistant yellow nutsedge biotype, especially for emergence pattern, early growth vigor, and growth habit. The objective of this study was to characterize and document the differential phenotypic traits of the halosulfuron-resistant yellow nutsedge biotype compared to susceptible standards.

Materials and Methods

Plant Material. The halosulfuron-resistant (hereafter *Res*) yellow nutsedge biotype was originally collected from a rice production field in Lawrence County (near Hoxie), AR in 2012. Three susceptible (hereafter *Sus*) biotypes were included as standards for comparison. Two of the *Sus* biotypes were collected from crop fields in Stuttgart and Fayetteville, AR. The third *Sus* biotype included in the study was sourced from a commercial distributor in the region (Azlin Seed Company, Leland, MS). Previous evaluations have confirmed the susceptibility of the *Sus* biotypes to the ALS herbicide chemistry (data not shown). These three geographically isolated *Sus* biotypes were included in this study to represent the variability in growth characteristics expected within this species and to ensure that the differences observed, if any, are beyond the natural range for this species. Hereafter the Azlin, Stuttgart, and Fayetteville biotypes will be referred to as *Sus-1*, *Sus-2*, and *Sus-3*, respectively.

Growth Habit. The goal of this experiment was to document differences in growth habit between the *Res* and *Sus* biotypes. This greenhouse experiment was conducted during spring/fall 2013 under 30/20 C day/night temperature regime and a 14-h photoperiod. The *Res* and *Sus* tubers were multiplied under greenhouse conditions during the fall of 2012. Tubers of individual biotypes were planted in plastic pots (30-cm diameter by 20-cm height) filled with potting-soil mix (LC1, Sun Gro Horticulture, AB, Canada) and allowed to sprout. At one-leaf stage, one healthy seedling pertaining to a given biotype was transplanted to the center of a large plastic flat (54 cm by 40 cm by 6.5 cm) containing the potting-soil mix. A total of 16 flats (four biotypes and four replications) were arranged in a completely randomized design. The plants were watered and fertilized as required.

Observations on number of aerial shoots produced plant^{-1}, distance from the parent shoot, and ground cover (%) were carried out at 15, 30, and 50 d after transplanting (DAT). Emerged shoots within each observation were marked with colored toothpicks. Ground cover was estimated visually based on the percent area occupied within each flat (total surface area of 2,160 cm^2) by a given biotype. After the observations at 50 DAT, plants were harvested from the flats for estimation of root distribution

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(%) and aboveground biomass production. Root distribution was estimated based on percent root area coverage within each flat. Aboveground biomass was weighed after drying the harvested shoots at 45 C for 72 h. The experiment was repeated in time (i.e., two experimental runs).

Emergence Pattern and Early-Growth Vigor. Based on observations of differential emergence and seedling vigor in the above experiment, a separate experiment was designed to characterize the emergence pattern and early-growth vigor of the *Res* biotype compared to the three *Sus* biotypes. This experiment was conducted in spring/summer 2014 in the greenhouse under the conditions described above. The experiment was arranged in a completely randomized design with four replications and two experimental runs. Tubers harvested in fall 2013 (all test biotypes were grown simultaneously under the same greenhouse conditions) were used in this experiment. Ten mature and uniform tubers pertaining to a given biotype were planted at a depth of 5 cm in plastic containers (15-cm diameter by 12-cm height) containing the LC1 potting soil. Seedling emergence was recorded once every 2 d for up to 30 d (peak emergence ceased by this time). Nonsprouted tubers were retrieved and examined for any desiccation to determine the proportion of dormant tubers at 30 DAT.

At each observation time, seedlings with a fully opened leaf were considered as emerged and transplanted individually to pots (10-cm diameter by 7.5-cm depth) containing LC1 potting soil. Plants were watered as necessary, but were not fertilized. At 30 DAT for each cohort, plant vigor estimates were carried out visually (based on overall appearance and growth) on a scale of 1 to 5, with 5 representing the greatest shoot vigor. Subsequently, plants were harvested individually, along with any secondary shoots emerging in the pot. Harvested shoot tissues were dried at 45 C for 72 h prior to weighing aboveground biomass production.

Data Analyses. Data were analyzed with the use of SAS (Version 9.4, SAS Institute, Cary, NC). Data from the first experiment pertaining to number of shoots produced plant^{-1}, distance of shoot emergence from parent shoot, and biomass, as well as proportion of nonsprouted tuber and biomass production in the second experiment were analyzed with the use of a generalized linear model, following the PROC GLM of SAS. Prior to ANOVA, normality of the data set was checked based on the Shapiro-Wilk test with the use of the PROC UNIVARIATE of SAS. Data transformations were necessary for number of shoots produced plant^{-1} (square root) and proportion of dormant nonsprouted tubers (arcsine), but nontransformed means are presented.

Data pertaining to ground cover, root distribution, and plant-vigor scores were analyzed with the use of nonparametric Kruskal-Wallis test (Kruskal and Wallis 1952), following the PROC NPAR1-WAY of SAS. The Nemenyi (for equal sample sizes—ground cover and root distribution) and the Dunn's tests (for unequal sample sizes—vigor scores) were carried out as post hoc tests following the Kruskal-Wallis analysis for multiple comparison and group mean separation at an alpha value of 0.05. These tests were conducted with the use of a specialized SAS Macro developed by Elliott and Hynan (2011).

Seedling emergence data collected over the 30-d period were fitted to a three-parameter sigmoidal model, which took the following form:

$$Y = \hat{\mathrm{E}}a \Big/ \left\{1 + e^{-[(x-x_0)/b]}\right\}$$

where a is the upper asymptote, x_0 is the time (days) taken for 50% emergence, and b is the slope of the curve at x_0. All analyses were performed on the data pooled across the two experimental runs as no significant differences were observed between the two runs, based on a sum-of-squares reduction test (Schabenberger et al. 1999). Root mean square error (RMSE) values were calculated as

$$\mathrm{RMSE} = \sqrt{\mathrm{SSE}/(n-2)},$$

where SSE is the sum of squared errors and n is the error degrees of freedom.

Results and Discussion

Growth Habit. The *Res* biotype exhibited a distinct growth habit, drastically different than what is typical for yellow nutsedge. The differences between the *Res* and *Sus* biotypes were striking even with the limited sample size used in the evaluations. Specifically, the secondary and tertiary aerial shoots were produced further away from the parent shoot, creating a wider distribution of the plant (Figures 1A and 1B), whereas in a typical yellow nutsedge (wild type), the secondary shoots are produced much closer to the parent shoot, leading to a compact growth habit (Figures 1C and 1D). With respect to the

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Figure 1. Differential aboveground growth habit of the acetolactate synthase-inhibitor–resistant (*Res*) and –susceptible (*Sus*) yellow nutsedge biotypes. (A) *Res* biotype at 20 d after transplanting (DAT), with the primary shoot seen at the center of the flat. (B) *Res* biotype at 50 DAT. (C) *Sus* biotype at 20 DAT, and (D) *Sus* biotype at 50 DAT.

underground plant assembly, the *Res* biotype forms an underground network of rhizomes and basal bulbs, wherein the secondary and tertiary basal bulbs are produced from the rhizomes arising from the parent and daughter shoots, respectively (Figures 2A and 3). Instead, the *Sus* rhizomes that develop into secondary basal bulbs and tubers are often connected to the primary basal bulb (Figures 2B and 3).

The tubers of both *Sus* and *Res* biotypes formed individually at the tip of the rhizomes (solitary and terminal), yet the rhizomes that terminate into tubers in the *Res* biotype were not necessarily connected to the parent shoot as in the *Sus* biotypes and were randomly originating from daughter shoots as well (Figure 3). This subterranean proliferation creates a chain-like network of aerial shoots in the *Res* biotype (Figures 1A and 1B), favoring its spread for wider distances. As early as 15 DAT, one *Res* individual reached the farthest distance of 27 cm from the primary basal bulb in

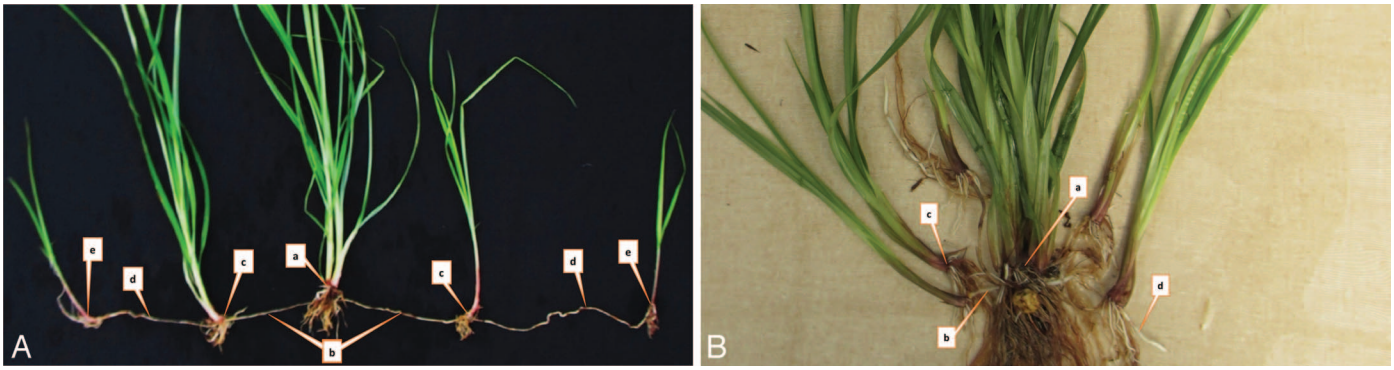

Figure 2. Differential subterranean growth habit of (A) acetolactate synthase-inhibitor–resistant and (B) –susceptible yellow nutsedge biotypes. The resistant biotype shows (a) primary basal bulb originated from parent tuber, (b) rhizomes produced by primary basal bulb developing into (c) secondary basal bulbs that give rise to daughter shoots, and (d) rhizomes produced by secondary basal bulbs developing into (e) tertiary basal bulbs. The network of daughter shoots appear to be similar to purple nutsedge. The susceptible biotype shows (a) primary basal bulb, (b) rhizome produced by primary basal bulb that develop into (c) secondary basal bulb, and (d) rhizome produced by secondary basal bulb. The susceptible biotype produces a compact growth habit.

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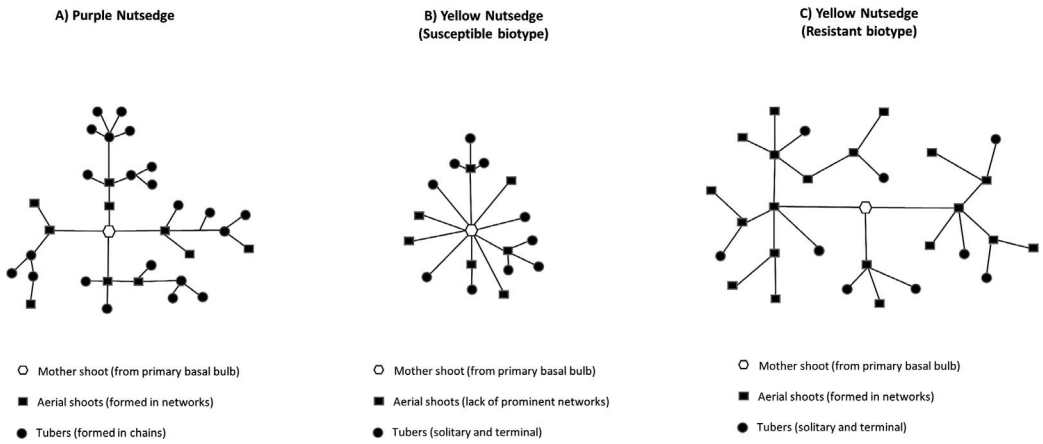

Figure 3. Shoot and tuber production patterns compared among (A) purple nutsedge, (B) susceptible yellow nutsedge, and (C) acetolactate synthase-inhibitor–resistant yellow nutsedge. Graphical model adapted from Webster (2014).

the center of the plot and at 30 DAT, all *Res* individuals produced shoots at this distance (Table 1).

As a result of shoots emerging away from the primary basal bulb, the *Res* biotype achieves high ground coverage within a short time period. The difference in ground cover was significant as early as 15 DAT (Table 1). At 50 DAT, the ground cover achieved by the *Res* plants within the plot area (2,160 cm^2) reached about 95%, which only ranged from 29 to 39% in the *Sus* biotypes (Table 1). Consequently, the root system of the *Res* plants was widely proliferated in the plots (99% of area occupied) compared to that of the *Sus* biotypes (range from 35 to 46%) (Table 1). This difference could have been much greater if unlimited space was available.

The *Res* biotype produced the greatest shoot biomass (70 g $plant^{-1}$), which greatly differed from the *Sus* biotypes (45 to 55 g $plant^{-1}$) (Table 1). The *Res* biotype also exhibited high shoot production (number of shoots $plant^{-1}$) (Table 1). Although shoot production in the *Sus-1* biotype was on par with the *Res* biotype, total biomass production was the greatest with the latter. This could be explained by the occurrence of greater intraplant competition among the shoots of the *Sus* plants because of the compact growth habit and confined root distribution. The *Res* biotype perhaps avoided intraplant competition by the extended network of shoots and roots, leading to more area coverage, greater access to resources, and subsequently greater overall biomass production. Plant species that have an ability to spread their roots and shoots are more competitive and effective in resource acquisition (Cole and Holch 1941).

In a production field, the *Res* biotype will have the ability to occupy the niches (e.g., interrow spacing) effectively and gain access to resources that may not be available to the crop, especially in its early stages of growth. Moreover, yellow nutsedge has been shown to be highly sensitive to shade (Keeley and Thullen 1978; Patterson 1982), particularly because of its C_4 nature. The ability of the *Res* biotype to spread and occupy less-shaded areas may be a highly beneficial trait. In addition to improving resource utilization, the chain-like subterranean growth may help enhance the spread of the *Res* biotype through tillage implements. Thus, tillage should not be relied upon for controlling the *Res* biotype. The differential growth habit could be used to identify the *Res* biotype under field conditions.

Emergence Pattern and Early Vigor. There was a considerable difference in emergence pattern (days taken for 50% emergence) between the biotypes (Figure 4). The difference was almost 6 d between the *Sus-2* and *Res* biotypes. Further, there were also significant differences among the biotypes for the proportion of dormant tubers, based on the number of nonsprouted tubers at the end of the 30-d period. The dormant tuber proportion was significantly greater in the *Res* biotype (19%) compared to the *Sus-2* (1%) and *Sus-3* (5%) biotypes, yet it was comparable to the *Sus-1* biotype (17%) (data not shown). The observation that the proportion of dormant tubers was comparable between the *Res* and *Sus-1* biotypes indicate that the *Res* biotype is within the range expected for this trait among different yellow nutsedge ecotypes. However, differences between the *Res* biotype and *Sus-2* as well as *Sus-3* biotypes mean that the *Res* biotype represents a phenotype with a relatively high proportion of tuber dormancy.

It was unclear what specific physical characteristics of tuber and/or physiological mechanism(s) favored

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Table 1. Growth characteristics of the acetolactate synthase-inhibitor–resistant (*Res*) yellow nutsedge biotype compared with three susceptible (*Sus*) biotypes at 15, 30, and 50 d after transplanting (DAT).

Observation time	Biotype	Shoot production[a]			Farthest distance[a,c]			Ground cover[b]			Root distribution[b,d]			Biomass[a,d]		
		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
		No. plant^{-1}			cm			%			%			g		
15 DAT	*Sus-1*	4	0.53	A	4	0.40	B	4	0.25	B	–	–		–	–	
	Sus-2	3	0.16	B	3	0.18	BC	3	0.21	B	–	–		–	–	
	Sus-3	3	0.25	AB	3	0.27	C	3	0.28	B	–	–		–	–	
	Res	4	0.40	A	20	1.93	A	24	1.00	A	–	–		–	–	
30 DAT	*Sus-1*	24	2.74	AB	5	0.45	C	10	0.48	B	–	–		–	–	
	Sus-2	16	0.98	C	8	0.33	B	9	0.50	B	–	–		–	–	
	Sus-3	20	1.19	B	6	0.53	C	10	0.45	B	–	–		–	–	
	Res	28	3.88	A	27	0.00	A	71	1.67	A	–	–		–	–	
50 DAT	*Sus-1*	98	6.63	A	6	0.42	C	29	1.89	C	34	1.92	C	51	6.73	B
	Sus-2	57	4.03	B	10	0.50	B	35	1.93	B	41	2.31	B	45	5.06	B
	Sus-3	72	8.06	B	9	0.61	B	39	1.85	B	46	2.40	B	55	6.73	B
	Res	111	10.58	A	27	0.00	A	95	0.73	A	99	0.38	A	70	6.43	A

[a] For each variable and for each observation time, mean values followed by different letters are significantly different, based on the Fisher's protected LSD test ($\alpha = 0.05$).

[b] For each variable and for each observation time, mean values followed by different letters are significantly different, based on the Nemenyi test ($\alpha = 0.05$).

[c] Seedlings were transplanted at the center of 54-cm-long flats, with an available distance of 27 cm to reach the boundary.

[d] Root distribution and biomass measured only at the time of harvest at 50 DAT.

delayed emergence, but dormancy is likely to favor staggered emergence and prolonged persistence in the soil. Although the presence of tuber dormancy and delayed emergence can act as a bet-hedging strategy that is particularly advantageous under unpredictable environments (Venable and Brown 1988), delayed emergence can also represent a competitive disadvantage with crops, because early emergence and establishment can be highly beneficial (Ross and Harper 1972). The potential long-term demographic costs of tuber dormancy is not known, but a delay in emergence could help a proportion of the individuals escape control interventions.

The early-growth vigor scorings (30 DAT) were significantly lower for the *Res* biotype compared to all *Sus* biotypes used in the experiment (Figure 5). The early growth of the *Res* biotype usually appeared to be less aggressive compared to the *Sus* biotypes. Moreover, the shoot weights measured at 30 DAT were also lower for the *Res* biotype compared to any of the *Sus* biotypes investigated (Figure 5). The early growth vigor and shoot biomass followed the order of *Sus-2*> *Sus-3*> *Sus-1*> *Res*. The tubers of the *Res* biotype were smaller than that of the *Sus* biotypes. Tuber diameter values (average of 20 random tubers) were 0.62, 0.71, 1.51, and 1.35 mm, respectively for the *Res*, *Sus-1*, *Sus-2*, and *Sus-3* biotypes. The reduction in early-growth vigor and biomass production can be explained in part by the relatively smaller tubers produced by the *Res* biotype. These observations corroborate Stoller et al. (1972), who found a positive correlation between tuber size and seedling biomass production. In a subsequent research, Stoller and Wax (1973) observed that seedlings produced by larger tubers were more vigorous than those produced by smaller tubers. Tuber sprouting, however, was not influenced by tuber size (Stoller et al. 1972).

Early-growth vigor is an important trait that can help a plant species compete effectively with its

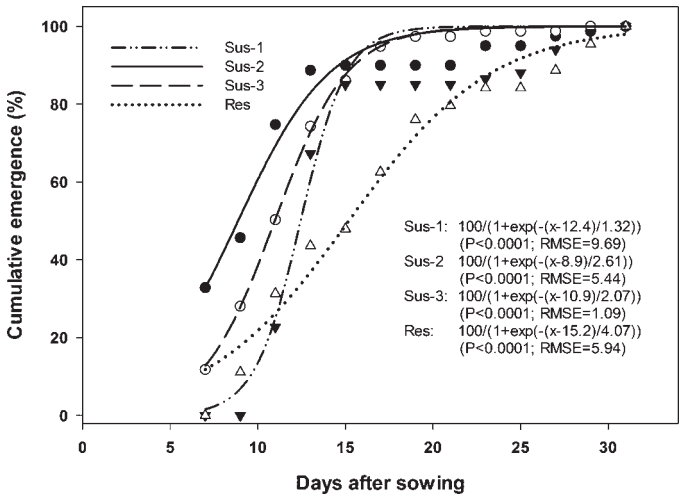

Figure 4. Comparison of emergence patterns between the susceptible (*Sus-1* to *Sus-3*) and resistant (*Res*) yellow nutsedge biotypes. The emergence data were fit to a three-parameter sigmoidal curve [$Y = a/(1+\exp(-(x - x_0)/b))$], where a is the upper asymptote, x_0 is the time (days) taken for 50% emergence, and b is the slope of the curve at x_0.

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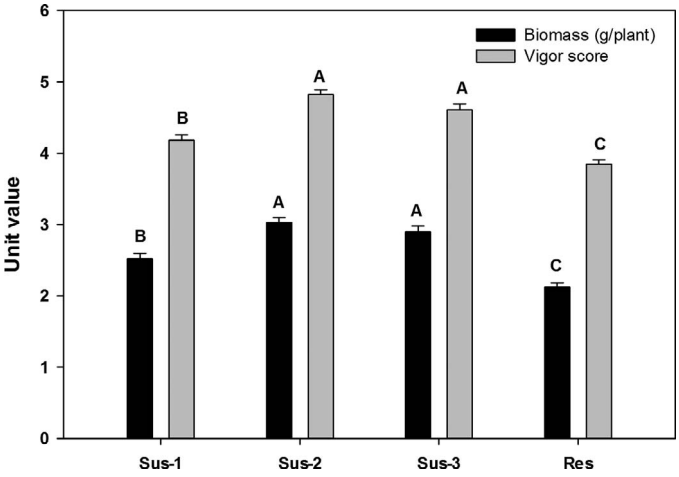

Figure 5. Comparison of early-growth vigor and biomass production (30 d after transplanting) between the acetolactate synthase-inhibitor–resistant and –susceptible yellow nutsedge biotypes. Lines above the bars indicate standard errors of the mean. For each response variable, bars topped by different letters indicate significant differences at $\alpha = 0.05$. Treatment means were separated either with the Fisher's protected LSD method (biomass) or following the Dunn's test (vigor scores).

neighbor, and a lack of early-growth vigor can be disadvantageous (Smith 1995). It is likely that the reduced early-growth vigor observed in the *Res* biotype could have an undesirable impact on its demography. However, when the phenotypic characteristics of the *Res* biotype are considered as a whole, it is possible that the reduced early-growth vigor is negated by its ability to spread the roots and shoots and utilize resources effectively.

It was unclear as to how the *Res* biotype acquired the differential growth habit and other characteristics noted above and whether these characteristics are directly associated with herbicide resistance. The target-site mutation Trp574 to Leu found in the *Res* biotype is also common in other ALS-inhibitor–resistant weed species (e.g., Warwick et al. 2010; Yu et al. 2008). However, there is no known evidence of any pleiotropic effects on vegetative growth caused by this mutation (e.g., Li et al. 2013). It is very likely that the differential growth habit already existed in this biotype prior to the evolution of resistance.

The growth habit of the *Res* biotype appears to be intermediate between yellow nutsedge and purple nutsedge. The *Res* biotype produces brown-colored tubers, which is intermediate in shade between yellow nutsedge (yellow beige) and purple nutsedge (black) (Figure 6). The shoot emergence pattern in networks and away from parent tuber is a characteristic similar to purple nutsedge (Figure 3). However, most other characteristics of the *Res* biotype are similar to yellow nutsedge. Although purple nutsedge produces tubers in long chains, the *Res* biotype produces solitary, terminal tubers typical to that of yellow nutsedge. The rhizome morphology (presence of conspicuous scales), leaf shape (sharp tip), and inflorescence color (golden yellow) of the *Res* biotype are also similar to that of yellow nutsedge. In fact, the *Res* biotype was identified as yellow nutsedge by Dr. Charles Bryson, a sedge taxonomist, based on individual plant phenotype. The genetic relationship between the ALS-inhibitor–resistant yellow nutsedge biotype, susceptible yellow nutsedge, and purple nutsedge is yet to be determined.

Future Research. Given the preliminary evidence of intermediary growth habit of the *Res* biotype, detailed studies are necessary to establish the genetic relationship of the *Res* biotype with other nutsedges. Natural hybridization between yellow and purple nutsedge has not been reported (Mulligan and Junkins 1976), but there are cases of biotypes resulting from putative hybridization (Tayyar et al. 2003). Additional phylogenetic studies on the *Res* biotype may shed new lights on its origin. Based on tuber production within the 50 d of the growth

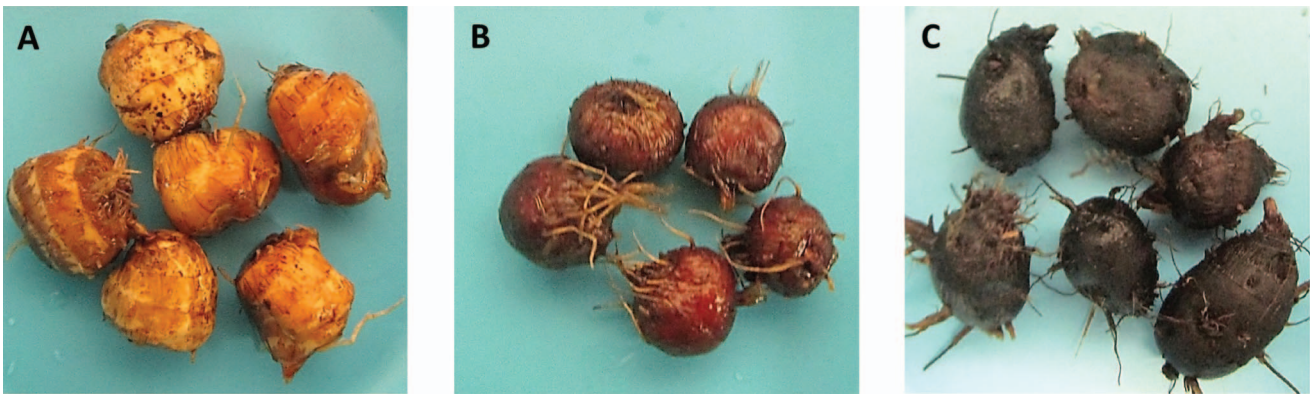

Figure 6. Comparison of tuber color among (A) susceptible yellow nutsedge, (B) acetolactate synthase-inhibitor–resistant yellow nutsedge, and (C) purple nutsedge. (Color for this figure is available in the online version of this article.)

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habit experiment, there were indications that the *Res* biotype produced fewer tubers compared to the *Sus* biotypes (data not shown). However, elaborate studies are necessary, especially by allowing a complete growing season for tuber production.

Flowering was consistently observed in the *Res* biotype under greenhouse growing conditions (30/20 C day/night temperature and a 14-h photoperiod), but none of the *Sus* biotypes flowered under these conditions. This indicates that the *Res* biotype has different photoperiodic/temperature requirements for flowering. The influence of differential flowering habit on the population dynamics of the *Res* biotype needs to be understood. Although yellow nutsedge reproduces predominantly by tubers (Horak and Holt 1986; Mulligan and Junkins 1976), it is also likely that seed production plays a role in population establishment and spread (Thullen and Keeley 1979). Whether seed production, seed viability, and seedling establishment of the *Res* biotype differ from the *Sus* biotypes is not known and needs to be investigated. Additionally, studies need to be conducted to understand the likelihood of resistance transfer from *Res* to *Sus* biotypes through pollen-mediated gene flow and introgression. Such knowledge will help us understand the importance of seed production on the evolution and spread of herbicide resistance in the *Res* biotype.

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