WEED SCIENCE

Vegetative Growth and Competitiveness of Common Cocklebur Resistant and Susceptible to Acetolactate Synthase-inhibiting Herbicides

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ABSTRACT

Herbicide-resistant biotypes of weeds are an increasing threat to crop production. An understanding of the basic biology of resistant biotypes, including their growth and development relative to susceptible biotypes, may yield information helpful in the management of resistant biotypes. Greenhouse and growth chamber experiments were conducted to compare growth characteristics of biotypes of common cocklebur (Xanthium strumarium L.) that are resistant or susceptible to acetolactate synthase (ALS)-inhibiting herbicides. Averaged over two temperature regimes (24/18 °C and 34/24 °C day/night) under noncompetitive conditions, an ALS herbicide-susceptible (S) biotype produced more leaves, greater leaf area, and greater shoot dry biomass at 20 and 30 d after planting (DAP) than an ALS herbicide-resistant (R) biotype. By 40 DAP, leaf number, leaf area, leaf area ratio (LAR), leaf dry biomass, and shoot dry biomass were similar for R and S biotypes. Susceptible plants produced a greater root biomass, but R plants were taller at 40 DAP under noncompetitive conditions. Averaged across biotypes, relative growth rate (RGR) and photosynthetic net assimilation rate (NAR) were greater at the 24/18 °C temperature regime. Averaged across temperature regimes, RGR and NAR were 11 and 17% greater for the R biotype than the S biotype under noncompetitive conditions. Under intertypic competitive conditions, the biotypes were similar in height, but the S biotype had a greater leaf number per plant. Evaluation of replacement series diagrams and relative crowding coefficient (RCC) estimates suggest that the two biotypes did not differ competitively in leaf production or plant height. The R biotype demonstrated a statistically significant, but probably trivial, competitive disadvantage in leaf dry biomass ($t_{\text{lof}} = -4.55, P = 0.01; \text{RCC}_{\text{R:S}} = 0.73 \pm 0.07$) and shoot dry biomass production ($t_{\text{lof}} = -3.73, P = 0.02; \text{RCC}_{\text{R:S}} = 0.73 \pm 0.08$).

Common cocklebur is a very competitive weed in cotton (Gossypium hirsutum L.) (Byrd and Coble, 1991; Snipes et al., 1982), peanut (Arachis hypogaea L.) (Royal et al., 1997), soybean [Glycine max (L.) Merr.] (Rushing and Oliver, 1998), and other crops (Neary and Majek, 1990). Common cocklebur at densities ranging from 2 to 32 plants per 15 m of row reduced cotton yield 17 to 66% (Snipes et al., 1982). Full-season interference from common cocklebur at one weed per 1.8, 0.9, and 0.3 m of crop row in soybean planted in 50-cm rows reduced yield by 16, 33, and 65%, respectively (Rushing and Oliver, 1998). Peanut yield was reduced 88% by 32 common cocklebur plants per 8 m of row (Royal et al., 1997). The competitive ability of this weed has been attributed to an extensive root system that provides an advantage in water and nutrient uptake (Davis et al., 1967; Geddes et al., 1979) and a tall growth habit and large canopy that shades the crop (Davis et al., 1967; Regnier et al., 1989).

Common cocklebur biotypes resistant to two herbicide groups have been identified in the United States. A biotype resistant to the organoarsenical herbicides MSMA and DSMA was discovered in South Carolina in 1985 (Haigler et al., 1988), and resistance to these herbicides has now been documented in seven states (Heap, 2005). Resistance to the ALS-inhibiting herbicide imazaquin was first confirmed in a biotype of common cocklebur in Mississippi by W. L. Barrentine in 1991 (Schmitzer et al., 1993). Since that initial discovery, other common cocklebur biotypes resistant to ALS-inhibiting herbicides have been verified in 10 states (Heap, 2005).

Herbicides that inhibit ALS are considered desirable because of excellent crop tolerance, low mammalian toxicity, and low use rates (Brown,
Unfortunately, extensive use of these herbicides, combined with their high level of efficacy and environmental persistence, has resulted in selection for resistant biotypes in 90 weed species worldwide (Heap, 2005). Resistance is usually attributed to an altered, less sensitive ALS enzyme (Schmitzer et al., 1993; Sprague et al., 1997). Cross-resistance of common cocklebur biotypes to two or more classes of herbicide chemistry has been identified in Mississippi (Kendig and Barrentine, 1995; Schmitzer et al., 1993) and North Carolina (Batts et al., 2003).

A consequence of herbicide resistance in weed biotypes may be reduced “fitness” (i.e., a less “fit” biotype produces fewer progeny) compared to susceptible biotypes (Gressel and Segel, 1982). Many reports in the literature compare vegetative growth characteristics (e.g., competitiveness, biomass, height, leaf area) of resistant and susceptible biotypes rather than fecundity. Although these characteristics are often highly correlated (Watkinson and White, 1985), they are not synonymous. The magnitude of differences in vegetative growth and competitiveness, if any, varies with herbicide class and plant species. Triazine-resistant common groundsel (Senecio vulgaris L.) (Holt, 1988), common lambsquarters (Chenopodium album L.) (Elliot and Peirson, 1983), jimsonweed (Datura stramonium L.) (Williams and Jordan, 1994), and common waterhemp (Amaranthus rudis Sauer) (Anderson et al., 1996) biotypes were less competitive and produced less biomass than triazine-susceptible biotypes. MSMA-susceptible common cocklebur was taller, had greater leaf area, and was more efficient in water utilization than MSMA-resistant biotypes (Akanda et al., 1996). Herbicide resistance, however, may not have a detectable effect on growth characteristics. For some species, canopy height, biomass, and seed yield were similar for weed biotypes with resistance and susceptibility to cyclohexanedione, dinitroaniline, organoarsenical, and sulfonylurea herbicides (Harris et al., 1995; Holt and Thill, 1994; Park et al., 2004; Wiederholt and Stoltenberg, 1996). Moreover, weed biotypes resistant to ALS-inhibiting herbicides may exhibit similar vegetative growth characteristics and competitiveness relative to susceptible biotypes of the same species (Alcocer-Ruthling et al., 1992; Christoforetti et al., 1997; Marshall et al., 2001; Massinga et al., 2005; Park et al., 2004; Thompson et al., 1994).

A common cocklebur biotype with resistance to the ALS-inhibiting herbicides, chlorimuron, imazethapyr, pyrithiobac, primisulfuron, and cloransulam, at rates that are at least 32 times normal use rates was identified in a grower’s field in North Carolina in 1999 (Batts et al., 2003). In greenhouse experiments investigating response to other herbicides, initial growth of the R biotype was slower than the S biotype. Two separate experiments were conducted to compare growth characteristics and competitiveness of the R and S common cocklebur biotypes.

MATERIALS AND METHODS

Growth of S and R common cocklebur under noncompetitive conditions. Fruit from the previously mentioned R common cocklebur biotype in Warren County, NC, and from a known S biotype in Guilford County, NC, (collection sites separated by 100 km) (Batts et al., 2003) were collected in the fall of 1999 and maintained in a freezer at -10 °C until used for these experiments. The experiment was conducted in growth chambers at the North Carolina State University Southeastern Plant Environmental Laboratory, Raleigh, NC, from September to November in 2002. Two identical 3 m² chambers with a height of 2.1 m, were used to grow common cocklebur under high (HT) and low temperature (LT) day/night regimes of 34/24 ± 2 °C and 24/18 ± 2 °C, respectively. Lighting was provided by a combination of fluorescent and incandescent lamps (450 µmol m⁻² s⁻¹ photosynthetic photon flux), and both chambers were set for a 14-hr photoperiod with a 30-min night interruption to prevent flowering. Each chamber contained 24 pots (25.4-cm diameter). Fruit of each biotype were clipped on both ends and planted at a depth of 2.5 cm in a substrate of steam-sterilized, washed, #16 gravel and a proprietary blend of peat and vermiculite (Redi-Earth; Scotts-Sierra Horticultural Products Co.; Marysville, OH). Five fruit were planted in each pot (one biotype per pot), and seedlings were thinned to one plant per pot 7 DAP. To ensure consistent germination, both chambers were initially set at the HT regime, and one of the chambers was reset to the LT regime after thinning. All pots were watered with a standard nutrient solution (Thomas et al., 2004) three times daily throughout the study.

Treatments consisted of a factorial arrangement of two biotypes (R and S), two temperature regimes (HT and LT), and three destructive sampling dates. Photosynthetic net assimilation rate, leaf area, leaf number, plant height, above-ground dry biomass, and root dry biomass were measured 20, 30, and 40 DAP. Photosynthetic net assimilation rate was measured on the youngest fully expanded leaf using a LI-COR LI-
6400 portable photosynthesis system (LI-COR Inc.; Lincoln, NE), and total leaf area was measured with a LI-COR 3100 area meter (LI-COR Inc.; Lincoln, NE). Dry biomass of above-ground tissue and roots were determined after drying at 65 °C for 5 d. Leaf area ratio (LAR), RGR, and NAR were determined as described by Radford (1967).

The experimental design was a split-plot with temperature regimes as the whole-plot factor and biotypes and sample dates as subplot factors. Within each chamber (whole plot), biotypes and sampling dates were replicated four times, and seedlings were blocked according to size at the beginning of the experiment. The experiment was conducted twice (with a total of 48 pots per run). Data were square-root transformed because of variance heterogeneity before being subjected to analysis of variance using the PROC MiXeD procedure of the Statistical Analysis System (version 8.0; SAS Institute, Inc.; Cary, NC) with partitioning appropriate for a two (temperature regimes) by two (biotypes) by three (sampling dates) factorial treatment arrangement. Transformed means were separated using Fisher’s Protected LSD at $P = 0.05$. Non-transformed means are presented for clarity.

**Intertypic competition of S and R common cocklebur biotypes.** The experiment was conducted in a greenhouse at North Carolina State University from July through September in 2002. Fruit of each biotype were clipped and planted in separate 6x26x52-cm trays containing a commercial potting medium (Metro Mix 200; Scotts-Sierra Horticultural Products Co.; Marysville, OH). At 7 DAP, seedlings of similar size, approximately 5 cm in height, were transplanted into 30-cm diameter pots filled with 12 L of potting medium. All pots received 20 ml of a 15 g L$^{-1}$ commercial greenhouse fertilizer (Peters Professional All Purpose 20-20-20 fertilizer; Scotts-Sierra Horticultural Products Co.; Marysville, OH) solution 14 and 21 DAP. Plants were grown with approximate day/night temperatures of 30/20 °C and watered daily. Natural light was supplemented for a 12-h photoperiod by metal halide lamps (giving an additional 200 μmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux at soil level).

Each pot contained six plants, which gave a density of 85 plants m$^{-2}$ in the following R:S mixtures: 6:0, 5:1, 4:2, 3:3, 2:4, 1:5, and 0:6. Pots were planted with one or both biotypes to give a conventional replacement series experiment arranged in a randomized complete block design (Cousens, 1991; deWit, 1960; Radosevich, 1987). Plants of each biotype were identified with labeled stakes. Plant height and leaf number were determined 14, 21, 28, 35, 42, and 48 DAP, leaf area was determined 48 DAP, and leaf and stem matter were harvested 48 DAP and dried for 5 d at 65 °C. Treatments were replicated three times and the study was conducted twice.

Plant height and leaf number for each biotype were averaged over the mixed culture ratio, and means were subjected to linear regression. Replacement series diagrams were constructed for each sampling period for leaf number. Similarly, replacement series diagrams were constructed for leaf dry biomass and total above-ground (shoot) dry biomass at 48 DAP. Homogeneity of variance between the experimental runs was confirmed using analysis of variance. A single $t$-test, comparing observed and expected yields combined over all plant proportions except monocultures, was performed. Data were converted to relative values (with monoculture leaf dry biomass or shoot dry biomass set equal to 1 separately for each run), and relative values were averaged over replicates for each proportion within each run. Two-way analysis of variance was conducted for each biotype on the mean leaf dry biomass or shoot dry biomass for all proportions except monocultures using the PROC MiXeD procedure of the Statistical Analysis System with experimental runs treated as replicates and proportion as a fixed effect. Means were summed over proportions and compared with the sum of the expected relative values using the appropriate standard error and degrees of freedom from PROC MiXeD to carry out a $t$-test.

The relative crowding coefficient (RCC) is another method that has been used to quantify the competitiveness of one weed biotype with another when grown in mixed culture (Harper, 1977). This statistic was calculated as:

$$\frac{(X_{R}^{X_{S}}/X_{S}^{X_{R}}+X_{R}^{X_{S}}/X_{R}^{X_{S}}+X_{R}^{X_{S}}/X_{R}^{X_{S}}+X_{R}^{X_{S}}/X_{R}^{X_{S}}+X_{R}^{X_{S}}/X_{R}^{X_{S}})/N}{(X_{R}^{X_{S}}/X_{S}^{X_{R}})}$$

where $X_{R}^{X_{S}}$ is average plant height, leaf number, leaf dry biomass, or shoot dry biomass for the R biotype of common cocklebur at a ratio of r:s; $X_{S}^{X_{R}}$ is the average plant height, leaf number, leaf dry biomass, or shoot dry biomass for the S biotype of common cocklebur at a ratio of r:s [corrected from Novak et al., 1993 (erratum Environ. Entomol. 1994. 23(6): ii) and Marshall et al., 2001], and N is equal to the number of mixed species comparisons (in this case, N = 5). Note that this corrected equation uses mean values for each biotype, rather than sums, for each growth characteristic. Therefore, coefficients used by others to balance this equation were deleted. As
written, RCC values greater than 1 indicate that the R biotype is more competitive than the S biotype; RCC values less than 1 indicate that the S biotype is more competitive; and an RCC = 1 indicates that the biotypes are equally competitive.

RESULTS AND DISCUSSION

Growth of S and R common cocklebur under noncompetitive conditions. Two temperature regimes were used to determine if differences in competitiveness might occur between biotypes under cool or warm spring climates (McCloskey and Holt, 1991). Temperature regime by biotype interactions were not significant for NAR, RGR, LAR, leaf number, plant height, leaf dry biomass, shoot dry biomass, and root dry biomass (data not shown), so the main effect of biotypes was averaged over temperature regimes at each sampling period. The S biotype had an early advantage in leaf production as more leaves were observed on these plants compared to the R biotype 20 and 30 DAP (Fig. 1A). The S biotype also had greater leaf area and leaf dry biomass at 20 and 30 DAP (Fig. 1B and 1C). By 40 DAP, leaf number per plant, leaf area, and leaf dry biomass was not different between biotypes. Similar results were noted with shoot dry biomass (Fig. 2A). Shoot dry biomass was greater for the S biotype than the R biotype at 30 DAP, but both biotypes were of similar biomass at 40 DAP (Fig. 2B). The biotypes were similar in height at 20 and 30 DAP, but the R biotype was taller at 40 DAP (Fig. 2C). Averaged over biotypes, RGR and NAR at 40 DAP were 19 and 24% greater, respectively, for plants grown at the cooler 24/18 °C temperature regime than at 34/24 °C (Table 1), which might indicate the 34 °C temperature exceeds the temperature optima for these common cocklebur biotypes (and resulted in photorespiration losses). There was no effect of temperature regime on LAR. Averaged over temperature regimes, RGR and NAR were 11 and 17%, respectively, greater for the R biotype than for the S biotype (Table 2), while LAR was similar across biotypes. Photosynthetic net assimilation rate was similar across sampling dates within biotypes (data not shown).

Table 1. Growth parameters of acetolactate synthase-resistant and -susceptible common cocklebur 40 d after planting under noncompetitive conditions as affected by growth chamber temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Growth parameter&lt;sup&gt;2&lt;/sup&gt;</th>
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</thead>
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<tr>
<td></td>
<td>RGR (g g&lt;sup&gt;-1&lt;/sup&gt;d&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>24/18</td>
<td>0.145 a</td>
</tr>
<tr>
<td>34/24</td>
<td>0.122 b</td>
</tr>
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<sup>2</sup>RGR = shoot relative growth rate; LAR = leaf area ratio; NAR = net assimilation rate. Data averaged over biotypes and two runs. Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD at \( P \leq 0.05 \).
These results indicate that under noncompetitive conditions, early season growth (<40 DAP) was similar for R and S biotypes, despite higher RGR and NAR rates for the R biotype. The S biotype appeared to have an advantage in leaf production initially; however, leaf number, leaf area, or leaf dry biomass at 40 DAP were not different. The S biotype did produce more root biomass, which indicates that under drought conditions the S biotype might have a competitive advantage over the R biotypes. Wassom et al. (2003) reported differences in shoot/root biomass (ratios ranged from 3.5 to 4.3) among greenhouse-grown common cocklebur accessions. The RGR and NAR values suggest that the R biotype may be capable of more vigorous growth over time, but variability has been observed in NAR among populations of common cocklebur, and high NAR was not a reliable indicator of biomass accumulation (Wassom et al., 2003).

**Intertypic competition of S and R common cocklebur biotypes.** Plant height and leaf number data were averaged over biotype ratios in the mixed culture because the biotype by ratio interaction was not significant. Regression analysis indicated that under intertypic competitive conditions, R and S common cocklebur heights were similar at each sample date (Fig. 3A). As in the previous experiment, the S biotype produced more leaves per plant by 21 DAP and later (Fig. 3B). The lack of departure from the expected lines for the R or S biotype in the replacement series diagrams indicates that neither biotype has a competitive advantage in terms of leaf production (Fig. 4). Total leaf number of the S biotype was at least 30% greater than was observed for the R biotype 28 and 42 DAP.

Visual evaluation of the diagrams for actual values of final leaf dry biomass and shoot dry biomass indicated additional analyses were warranted. Where visual evaluation of replacement series diagrams has been

![Figure 2. Average shoot dry biomass (A), root dry biomass (B), and height (C) of ALS-resistant and -susceptible common cocklebur grown under noncompetitive conditions. Bars represent ± 1 standard error of the mean.](image-url)
unsatisfactory or undesirable, traditional approaches of statistical analysis have included regression to test for non-linearity of the response, or a series of t-tests comparing observed and expected relative yields separately at each plant ratio (Roush et al., 1989; Wiederholt and Stoltenberg, 1996). Because the separate t-tests approach can lack power or lead to ambiguous results (Roush et al., 1989), a single t-test comparing observed and expected yields combined over all plant proportions except monocultures was performed in this experiment. The data were converted to relative values and re-plotted, and each biotype was compared with theoretical equal competition values using a combined t-test. Visual evaluation of the re-plotted data reveals that the intersection point of the R and S lines for relative leaf dry biomass (Fig. 5A) and shoot dry biomass (Fig. 5B) is to the right of the 3:3 ratio. The slightly concave shape of the R line for leaf dry biomass and shoot dry biomass suggests that the R biotype may be at a small competitive disadvantage, but visual interpretation of the S biotype response for both leaf and shoot dry biomass was ambiguous. Results of the combined t-tests indicate that the S biotype did not differ from the theoretical equal competition line for leaf or shoot dry biomass ($l_{eq} = 1.00, 1.40; P = 0.38, 0.23$, respectively), while the R biotype was statistically less competitive for leaf and shoot dry biomass than would be expected under equal competition ($l_{eq} = -4.55, -3.73; P = 0.01, 0.02$, respectively). In a study on sulfonylurea-resistant and -susceptible prickly lettuce (Lactuca serriola L.), Alcocer-Ruthling et al. (1992) observed equal competitiveness between biotypes despite greater biomass production and growth rate of the S biotype. Others have observed similar biomass accumulation in competitive and non-competitive conditions (Park et al., 2004).

Based on the R/S RCC values for plant height and leaf number, R and S biotypes were equally competitive at each sampling period (Table 3), although trivial differences are indicated for plant height at

<table>
<thead>
<tr>
<th>Days after planting</th>
<th>Relative crowding coefficients</th>
<th>Dry biomass</th>
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<tr>
<td></td>
<td>Height</td>
<td>Leaf</td>
</tr>
<tr>
<td></td>
<td>Leaf number</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.12 ± 0.06</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td>21</td>
<td>1.09 ± 0.12</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>28</td>
<td>0.96 ± 0.10</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td>35</td>
<td>0.91 ± 0.09</td>
<td>1.06 ± 0.14</td>
</tr>
<tr>
<td>42</td>
<td>0.94 ± 0.06</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td>48</td>
<td>0.96 ± 0.02</td>
<td>1.02 ± 0.13</td>
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*Values greater than 1 indicate the R exceeded the S biotype; values less than 1 indicate the S exceeded the R biotype. Values are listed with standard error.*
14 and 48 DAP. Leaf and shoot dry biomass values of R and S biotypes in monoculture were not different (data not shown), and RCC values for leaf and shoot dry biomass indicate that the S biotype had a competitive advantage over the R biotype 48 DAP (Table 3). Biomass measurements were determined at the termination of the experiment; therefore, data for earlier comparisons were not available.

These results suggest that, by 48 DAP, small differences may exist in height and shoot dry biomass as measures of competitiveness between the R and S common cocklebur biotypes; however, results of regression analysis indicate heights were not different.

No difference in leaf area between biotypes was observed. In previous research, other weeds resistant to the ALS-inhibiting herbicides did not exhibit reduced vegetative growth characteristics or competitiveness compared with susceptible biotypes (Alcocer-Ruthling et al., 1992; Christoffoleti et al., 1997; Mallory-Smith and Eberlein, 1996; Marshall et al., 2001; Park et al., 2004; Thompson et al., 1994).

Variability in common cocklebur accessions has been documented elsewhere (Wasson et al., 2002; 2003). The differences that were detected in the present study between R and S biotypes in intertypic competition may be nominal in terms of interspecific competition with crops during vegetative growth. Cotton is most susceptible to weed competition early in the growing season (Culpepper and York, 1999; Papamichail et al., 2002; Rogers et al., 1996). Our results suggest competition with cotton would be similar with the R and S biotypes of common cocklebur. Additional work is necessary to determine whether differences in late-season vegetative growth and fecundity exist between R and S biotypes before predictions can be made regarding the likelihood of persistence and prevalence of the R biotype in the absence of selection pressure from ALS-inhibiting herbicides (Maxwell et al., 1990).

Figure 4. Replacement series diagrams for leaf number of competing ALS-resistant and ALS-susceptible common cocklebur at 14, 28 and 42 d after planting. Bars represent ± 1 standard error of the mean.

Figure 5. Replacement series diagrams for relative leaf dry biomass (A) and shoot dry biomass (B) of competing ALS-resistant and ALS-susceptible common cocklebur 48 d after planting.
REFERENCES


Byrd, J. D., Jr., and H. D. Coble. 1991. Interference of common cocklebur (Xanthium strumarium) and cotton (Gossypium hirsutum). Weed Technol. 5:270-278.


Geddies, R. D., H. D. Scott, and L. R. Oliver. 1979. Growth and water use by common cocklebur (Xanthium pensylvanicum) and soybeans (Glycine max) under field conditions. Weed Sci. 27:206-212.


