RESPONSES OF TWO NATIVE AND TWO NON-NATIVE GRASSES
TO IMAZAPIC HERBICIDE ON PHOSPHATE MINED LANDS IN FLORIDA

by

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Abstract: Successful reclamation of upland habitats on phosphate mined lands in Florida requires control of invasive, non-native weed species without detrimental effects on the revegetation of native plant species. To address this problem we have researched the post emergence application of the new selective herbicide imazapic (Plateau®) on 2 native and 2 invasive grasses. For example, imazapic tolerance (0.05 to 0.21 kg a.i./ha) was tested on the native species wiregrass (Aristida beyrichiana Trinius & Ruprecht) and lopsided indiangrass (Sorghastrum secundum [Ell.] Nash) in the greenhouse and in the field on reclaimed overburden soil. We also evaluated the rate effects (0.07 to 0.21 kg a.i./ha) on the invasive non-native species natalgrass (Rhynechelytrum repens [Wild.] C.E. Hubb) and bahiagrass (Paspalum notatum Fluegge) on reclaimed sandtailings soil. In the greenhouse, both lopsided indiangrass and wiregrass showed sensitivity to imazapic damage, but the reduction in foliar growth of indiangrass occurred at lower rates and was longer lasting. Under more stressful growing conditions in the field, however, wiregrass showed tolerance to imazapic, and only lopsided indiangrass showed sensitivity to the same imazapic rates. The effects on indiangrass included reduced survival and percentage of green foliage. Interestingly, older indiangrass plants (31 months) were more susceptible than young plants (3 months). Imazapic reduced cover and plant vigor of both natalgrass and bahiagrass in the field, but the effects on natalgrass were evident at lower doses and were longer lasting than on bahiagrass. Suppression of seedheads was observed on both weed species, and for natalgrass this effect resulted in lower seedling densities in the following spring. Imazapic has excellent potential for selective control of natalgrass in stands of wiregrass on reclaimed lands in Florida. Bahiagrass control will likely require higher and perhaps repeated doses of imazapic, which may damage some native species. Caution is indicated when using imazapic on sites containing lopsided indiangrass.

Additional Key Words: reclamation, uplands, Plateau®, wiregrass, lopsided indiangrass, natalgrass, bahiagrass, weed control

Introduction

The Florida Institute of Phosphate Research (FIPR) is a state agency whose mission is to conduct research that will accurately assess and help resolve significant phosphate issues affecting the environment and the health and safety of the citizens of Florida. A priority area of the FIPR research program is the reclamation of phosphate mine lands.

Because of the highly regulated nature of wetlands, much attention has been given to wetland restoration on phosphate mined lands in Florida, including weed management (Richardson and Johnson 1998). The ecological value of uplands has become better appreciated, and thus upland restoration is now receiving more attention. Xeric (dry) and mesic uplands are important because they represent critical habitats for indigenous animal and plant species in Florida, including species of special concern such as the gopher tortoise and scrub jay. Upland ecosystems in Florida have been dramatically reduced in area (Christman 1988) due to a variety of causes, including development, and agriculture, as well as mining. Reclamation of upland habitats, therefore, represents a significant opportunity to conserve the biodiversity of Florida.

Successful reclamation of upland habitats requires weed control (Randall et al. 1997). For example, invasive, non-native species are commonly found on roadsides, fallow agricultural lands, and older mined and reclaimed lands in Florida, which then serve as weed seed sources for newer reclamation. Reclaimed upland habitats in Florida have modified soil properties compared to unmined soils (Segal et al. in press). Overburden has higher clay content, water-holding capacity and P and K content, than the native sandy soils, which may give aggressive weeds a competitive advantage over slower-growing natives. Although sand tailings have

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higher P and K contents and slightly coarser sand grain sizes than native soils, sand tailings are more similar to native soils than overburden. Examples of highly competitive non-native species on reclaimed uplands in Florida include cogongrass (*Imperata cylindrica* [L.] Beauv), natalgrass (*Rhynchelytrum repens* [Wild.] C.E. Hubb), bahiagrass (*Paspalum notatum* Fluegge), bermudagrass (*Cynodon dactylon* [L.] Pers.), and crabgrass (*Digitaria* spp).

FIPR is actively engaged in developing cost effective, integrated weed control strategies, including chemical herbicides, in reclamation of upland habitats in Florida (Shilling et al. 1997). The potential of imazapic herbicide is based on its demonstrated selectivity of controlling annual and perennial broadleaves and grasses in prairie restoration projects in the Midwest USA (Beran et al. 1999; Washburn et al. 1999; Masters et al. 1996). It is registered for the USDA Conservation Reserve Program under the tradename Plateau® (Anonymous 1997a), and under the tradename Cadre®, it is applied at early post emergence on peanuts (Padgett et al. 1996).

A critical aspect of our imazapic research is the evaluation of its phytotoxicity on Florida native plants used in the reclamation of upland habitats. Different tolerances among Florida plant species can be hypothesized due to reported phytotoxic effects on the germination and growth of some native wildflower species in prairie restoration projects (Beran et al. 1999; Washburn et al. 1999; Masters et al. 1996), and the manufacturer’s literature on imazapic effects (Anonymous 1997b). Reported studies of imazapic phytotoxicity for species and ecotypes of Florida native wildflowers are very limited. One comparison of local and non-local ecotypes of 4 Florida dicots grown under greenhouse conditions showed imazapic injuries ranging from stunting to necrosis and differences among species and between ecotypes (Aldrich et al. 1998).

The potential susceptibility of Florida native plants to imazapic may likely be critical from an ecosystem perspective too. Florida upland ecosystems are pyrogenic and, therefore, are dependent on periodic fires for maintenance of their ecological functioning and structure (Myer and Ewel 1990). These fires occur because of the presence of fire adapted native grasses, such as wiregrass (*Aristida beyrichiana* Trinius & Ruprecht) and lopsided indiangrass (*Sorghastrum secundum* [Ell.] Nash), which provide the fuel. Considering that imazapic phytotoxicity has been reported on wiregrass grown as tubelings under greenhouse conditions (Norcini et al. 1997), it is especially important to evaluate any effects on these keystone species in the field.

The objectives of this study were to evaluate the effects of different rates of the herbicide imazapic on the growth and survival of 2 native (wiregrass and lopsided indiangrass) and 2 non-native grasses (bahiagrass and natalgrass). These species are commonly found at reclaimed sites of native uplands in Florida. We also compared the responses of the native grasses to imazapic in both greenhouse and field evaluations.

**Materials and Methods**

For our imazapic studies, we applied imazapic as Plateau® herbicide in the liquid formulation containing 23.6% imazapic. The adjuvant Activate Plus® was added to all the imazapic dosages at the rate of 0.25% (v/v). In all experiments, our application method was a CO₂ backpack sprayer (R&D Sprayers, model T), using a 4 nozzle boom sprayer (nozzle type XR0002; 48.3 cm spacing) calibrated using a flow rate of 374 L/ha at 280 kPa pressure. Our boom sprayer was set at approximately 1m height. For the herbicide applications, the time of day was between 7:45 -10 AM in the field experiments and 2-4 PM in the greenhouse experiments. The weather conditions of the field experiments ranged from clear to partly cloudy skies, 8 or less kph wind speed, 85-92% relative humidity, and 21-27 °C air temperature.

Parameters from the experiments were statistically analyzed by the MSTAT (Nissen 1993) package. Treatment effects were analyzed with parametric (ANOVA) statistics. Tests for violations of assumptions of analysis of variance (ANOVA) were performed by the STATISTICA (STATSOFT 1995) package. Data as percentages were calculated with the arcsine transformation to meet the assumptions of ANOVA. Means separations with ANOVA were done with Duncan's Multiple Range Test. Orthogonal comparisons of the means (excluding the adjuvant control) were calculated for response trends (e.g., linear, quadratic and cubic) to the herbicide rates. In figures of this article all significant mean differences are reported at the P = 0.05 level of significance. In tables of this article the ANOVA are reported for the orthogonal trend comparisons, including levels of significance.
Wiregrass and Lopsided Indiangrass Greenhouse Experiments.

Our greenhouse studies of wiregrass and lopsided indiangrass were completed at the reclamation research facilities of FIPR in Bartow, FL. From February to April 1999, we evaluated the effects of imazapic as a post emergence application (February 5, 1999) on foliar regrowth of 18-month-old plants of wiregrass and lopsided indiangrass in the greenhouse. These plants had been propagated in tubing trays (5.7 x 15.2 cm cell size), using a potting mix of peat:sand:tailings:perlite in a ratio of 2:2:1. Fertilization of all plants was done 7 weeks before the experiment, and 2.5, 5.0, and 6.5 weeks after treatment (WAT) at the rate of 25 mL/cell of Miracle Grow (15-30-15) solution (12 g/11.4 L). Our experimental units for each separate species consisted of 8 plants in 28 x 25 cm tubing trays. The plants were arranged in alternating cells to minimize any shading from neighboring plants. Just prior to the imazapic application, the foliage of these plants was clipped to a height of 12.5 cm above the soil surface in order to standardize growth responses after the treatments. The foliage regrowth was measured as the longest leaf length per plant at 2, 4, 6 and 8 WAT. Growing conditions in the greenhouse included temperature maintained between 16 and 32 °C, daily overhead irrigation of approximately 2 cm, and the natural photoperiod of 11 to 12.5 hours.

We applied 4 rates of imazapic at 0.05, 0.10, 0.16, and 0.21 kg a.i./ha (or 3, 6, 9 and 12 oz/ac of Plateau®), as well as 2 controls (water only and water + adjuvant). There were 6 replications in separate experiments for each species. The spraying procedure consisted of placing the tubing trays outside on the ground in order to use the CO₂ backpack sprayer. After spraying, the experimental units for each separate species were arranged on the greenhouse benches in a randomized complete block (RCB) design, using position under the overhead irrigation as the blocking factor.

Wiregrass and Lopsided Indiangrass Field Experiments.

This study was conducted at the Hookers Prairie mine (overburden-capped sand tailings) of Cargill, Inc., in Polk County, FL. We evaluated the effects of a post emergence application of imazapic (August 13, 1999) on 2 ages of wiregrass and lopsided indiangrass plantings, i.e., 31 months (planted January 1997) and 3 months (planted May 1999). Plants were from plots established as seeding trials of native grasses by USDA Natural Resource Conservation Service, Brooksville, FL. Plantings of January 1997 were monocultures of each grass while plantings of May 1999 were mixtures of the 2 grasses. We used 4 rates of imazapic at 0.04, 0.07, 0.14 and 0.21 kg a.i./ha (or 2, 4, 8 and 12 oz/ac of Plateau®), as well as a water only control. There were 4 replications, using plots of 0.9 x 2.4 m size in a randomized complete block (RCB) design, using depth of overburden cap as our blocking factor. At 13 WAT six plants per plot (which were tagged at application) were individually monitored for survival and plant vigor. Plant vigor was defined as the percentage of green foliage present (not chlorotic or necrotic) and was estimated visually on a per plant basis. None of the plants were fertilized or watered during the course of the experiments.

Natalgrass Field Experiment.

From November 1998 to June 1999 we evaluated the effects of a post emergence application of imazapic on a naturally established natalgrass-dominated grassland. This study was conducted on a sand tailings site at the Tenoroc Fish Management Area near Lakeland, FL. Natalgrass was treated with imazapic on November 13, 1998, and was monitored for plant vigor and foliar cover at 7 and 26 WAT, as well as on the treatment date (0 WAT). We used 3 rates of imazapic at 0.07, 0.14 and 0.21 kg a.i./ha (or 4, 8 and 12 oz/ac of Plateau®), as well as 2 controls (water only and water + adjuvant). Each treatment contained a dye marker (Terramark® SPI) at a rate of 1.25 mL/L. There were 4 replications, using plots of 1.9 x 6.1 m size in a RCB experimental design, using slope position (apparent effects on drainage and soil moisture) as our blocking factor. Plant vigor was defined as percentage of green foliage (not chlorotic or necrotic) and was estimated visually only for natalgrass plants on a plot basis. There were natalgrass seedlings at 26 WAT (but not 0 and 7 WAT), and plant vigor then was given a combined rating for established and seedling plants. We sampled the density of natalgrass seedlings at 26 WAT, using three 0.3 x 0.3 m quadrats per plot. Cover was estimated visually on a per plot basis for natalgrass using a 10 point scale.

Bahiagrass Field Experiment.

At the same location and time as the natalgrass field study, we evaluated the effects of a post emergence application of imazapic on a naturally established bahiagrass-dominated grassland. Bahiagrass was treated on November 17, 1998 with the same rates of imazapic and experimental procedures as the natalgrass experiment and...
monitored for plant vigor and cover. However, here the monitoring times for plant vigor and coverage were at 12 and 25 WAT, as well as 0 WAT. Plant vigor was recorded as the percent green tissue of the entire sward of bahiagrass. Seedlings of bahiagrass were not recorded because it was too difficult to distinguish seedlings in the dense bahiagrass swards.

Results

Wiregrass and Lopsided Indiangrass Greenhouse Study.

In the greenhouse, all the wiregrass and lopsided indiangrass plants survived all imazapic rates. Foliar regrowth of the control plants of lopsided indiangrass was much greater than that of wiregrass, but wiregrass was more tolerant of imazapic and more resilient in recovering from negative effects (Figures 1 and 2). For example, wiregrass growth at 4, 6 and 8 WAT compared to the water control was significantly reduced at rates of 0.10 kg a.i./ha and above, and at 2 WAT only by the two highest dosages (Figure 1). On the other hand, lopsided indiangrass foliar regrowth was strongly inhibited at all imazapic rates and sampling dates (Figure 2).

Both wiregrass and lopsided indiangrass demonstrated significant response trends of less growth with increasing imazapic rates at every sampling period. For example, the trends were linear for wiregrass, and linear, quadratic and cubic for lopsided indiangrass (Table 1). There was no effect of the adjuvant control on either native species (Figures 1 and 2).

Wiregrass and Lopsided Indiangrass Field Experiments.

In the field, percent survival of wiregrass was not affected by imazapic, whereas indiangrass percent survival was (Figure 3). Survival of lopsided indiangrass from both planting dates was significantly reduced at the 0.21 kg a.i./ha rate, and the older lopsided indiangrass (31 month) appeared to be more susceptible, with only 34% survival from the August 1999 application compared to younger plants (3 month) with 55% survival. Reduced survival response trends with increasing imazapic rates were significant only for lopsided indiangrass (Table 1), and were linear for the older plants, and linear and quadratic for the younger plants.

Similarly, imazapic had no effect on the plant vigor of wiregrass, but indiangrass did tend to decrease in percent green tissue with greater imazapic rates (Figure 4). Older indiangrass plants showed greater vigor damage than younger plants, and vigor response trends were significant only for lopsided indiangrass (Table 1). For example, there were significant linear and quadratic trends for 31 month-old plants and a significant linear trend for 3 month-old plants for decreased vigor with increased imazapic rates. Older lopsided indiangrass also had a significant positive correlation between survival and plant vigor ($r = 0.46$).
Table 1. ANOVA orthogonal contrasts for response trends by sampling date, and variable of wiregrass and lopsided indiangrass to increasing rates of imazapic from greenhouse and field experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plant species</th>
<th>Age (mo.)</th>
<th>Sampling Date(^b) (WAT)</th>
<th>Variable(^m)</th>
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<td>Foliar Growth</td>
<td>Survival</td>
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<td></td>
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<td>Lopsided indiangrass</td>
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<td>Lopsided indiangrass</td>
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<td>31</td>
<td>13</td>
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</table>

\(^*\), \(^**\), \(^***\) Significant at the 0.05, 0.01, 0.001 levels of probability, respectively. NS is non significant.

\(^b\) Table summarizes separate experiments reported in the text. Omitted results indicate variables not measured.

\(^m\) Sampling date shown as weeks after treatments (WAT).

\(^w\) Arcsine transformation of means used in ANOVA for variables measured as percent (survival, plant vigor).

\(^v\) Response trends of means: L= linear, Q= quadratic, C= cubic.

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**Figure 3.** Imazapic dosage effects on survival at 13 WAT of lopsided indiangrass and wiregrass from different planting dates in field experiment (Means of one species/date with the same letter are not different at 0.05 level).

**Figure 4.** Imazapic dosage effects at 13 WAT on plant vigor of surviving lopsided indiangrass and wiregrass from different planting dates in field experiment (Means of one species/date with the same letter are not different at P=0.05 level).
Natalgrass Field Experiment.

Imazapic, applied in the field in November, greatly reduced natalgrass cover (Figure 5) and natalgrass seedling density (Figure 6). For example, significant cover reductions were seen with .14 kg a.i./ha at 7 WAT and with all rates at 26 WAT samplings (Figure 5). The reduction of natalgrass cover over time was herbicide based because there was no change in natalgrass cover in the control plots throughout the same period. There was no effect due to adjuvant. At 26 WAT there was a significant linear response trend, and at both 7 and 26 WAT there were significant quadratic response trends for reduced natalgrass cover with increased imazapic rates (Table 2).

Density of natalgrass seedlings at 26 WAT was significantly decreased with imazapic compared to the water only control (Figure 6). There were significant linear and quadratic response trends for reduced seedling density with increased imazapic rates (Table 2). There was a significant positive correlation between natalgrass seedling density and cover (r=0.85).

Plant vigor (percent green tissue) in natalgrass was seasonally low (about 20 percent) in November when imazapic was applied (Figure 7). At 7 WAT, green tissue in the controls had increased to about 30 percent, but imazapic had reduced green tissue to 4 to 8 percent. At 26 WAT, the surviving plants had recovered (green tissue was about 80 percent in all treatments), but there were fewer plants.

Figure 5. Imazapic rate effects on natalgrass cover over time (Means at one sampling with the same letter are not different at P=0.05 level).

Figure 6. Imazapic rate effects on density of natalgrass seedlings at 26 WAT (Means with the same letter are not different at P=0.05 level).

| Table 2. ANOVA orthogonal contrasts for response trends by sampling date and variable of natalgrass and bahiagrass to increasing rates of imazapic from field experiments. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Plant species   | Age (mo.)       | Sampling Date   | Plant Vigor     | Foliar Cover    | Density         |
|                 |                 | (WAT)           | L | Q | C | L | Q | C | L | Q | C |
| Natalgrass      | n/a             | 7               | *** | *** | NS | NS | * | NS | NS | NS | NS |
| Natalgrass      | n/a             | 26              | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Natalgrass      | seedling        | 26              | NS | NS | NS | *** | ** | NS | NS | NS | NS |
| Bahiagrass      | n/a             | 12              | *** | ** | NS | NS | NS | NS | NS | NS | NS |
| Bahiagrass      | n/a             | 25              | NS | NS | NS | NS | NS | NS | NS | NS | NS |

* *, **, *** Significant at the 0.05, 0.01, 0.001 levels of probability, respectively. NS is non significant.

i Table summarizes separate experiments reported in the text. Omitted results indicate variables not measured.

ii Sampling date shown as weeks after treatments (WAT).

iii Arcsine transformation of means used in ANOVA for variables measured as percent (plant vigor, cover).

iv Response trends of means: L= linear, Q= quadratic, C= cubic.
in the herbicide treated plots. There was no effect due to adjuvant. At 7 WAT the imazapic effects were evident at the lowest rate, and there were significant linear and quadratic trends for reduced vigor with increased imazapic rates (Table 2). Also at 7 WAT there was a significant positive correlation between natalgrass vigor and cover (r=0.57). At 7 WAT, observations of natalgrass symptoms for imazapic herbicide damage included seedhead suppression and hormone-like effects of leaf bud stimulation at nodes on the culms. At 26 WAT these herbicide damage symptoms were not observed.

**Figure 7.** Imazapic rate effects on plant vigor of surviving natalgrass over time (Means at one sampling with the same letter are not different at P=0.05 level).

**Figure 8.** Imazapic rate effects on plant vigor of bahiagrass over time (Means at one sampling with the same letter are not different at P=0.05 level).

**Figure 9.** Imazapic rate effects on bahiagrass foliar cover over time (Means at one sampling with the same letter are not different at P=0.05 level).

### Discussion

Successful chemical weed control in reclamation of upland habitats in Florida requires tolerance by the native species and phytotoxicity to the invasive weeds. The results of our research demonstrated that imazapic has a potential for selective weed control, but at the same time, we identified important constraints. Successful revegetation strategies using imazapic will need to balance the different effects on native plants and weeds. The native wiregrass exhibited good tolerance to imazapic, while the non-native natalgrass was quite susceptible. Unfortunately, the native lopsided indiangrass showed some sensitivity to imazapic, and the non-native bahiagrass was only suppressed and not killed.
Susceptibility to imazapic was exhibited by both wiregrass and lopsided indiangrass in the greenhouse experiments (Figures 1 and 2), but wiregrass exhibited greater tolerance than lopsided indiangrass. Lopsided indiangrass consistently had significantly reduced foliar growth compared to the controls. Wiregrass, on the other hand, only had significantly reduced growth, compared to the controls, at the two highest rates.

A comparison of the greenhouse and field experiments implies the importance of physiology and growth stage of these native grasses to imazapic injury plus differences in sensitivity of the response parameters that were measured or estimated. Wiregrass, for example, was not as susceptible to the same range of imazapic rates in the field as in the greenhouse. It is possible that foliar growth (our greenhouse experiment variable) is more sensitive to imazapic injury than survival, cover, or vigor (our field experiment variables). However, Norcini et al. (1997) also reported imazapic injury (as measured by a phytotoxicity rating scale which integrates growth and survival effects) to wiregrass in the greenhouse over 3 months at a rate of 0.14 kg a.i./ha. Compared to greenhouse growing conditions, the field environment was much more stressful. Because of an extended summer drought, the field application date, in fact, was delayed until the arrival of rainfall, and we observed leaf unrolling of indiangrass, indicating recovery from drought stress (Begg 1980). Greenhouse plants were watered daily with an overhead sprinkler and were fertilized periodically, and it is possible they were physiologically more active than the field plants.

In contrast to wiregrass, lopsided indiangrass demonstrated sensitivity to imazapic both in the greenhouse and the field experiments. Our results with lopsided indiangrass were dramatically different from results reported for yellow indiangrass, *Sorghastrum nutans* (L.) Nash, which is listed as tolerant of imazapic at the highest rates we tested (Anonymous 1997a). Nevertheless, less imazapic injury to plant vigor and survival of young lopsided indiangrass plants (3 months) in the field, compared to older plants (31 months), implicated the age factor (Figure 4). Plant vigor was defined in our study as the percentage of green foliage, and, therefore, suggests the capacity for photosynthesis and growth of the plant. These results give hope that lower imazapic rates might be successfully managed for weed control in newly established stands of native species with only modest damage to, and adequate survival and growth of, lopsided indiangrass. However, caution is warranted in using imazapic on lopsided indiangrass.

Our field application of imazapic to the invasive grasses, natalgrass and bahiagrass, was seasonally late (November) compared to the recommended timings for post-emergence applications (Anonymous 1997b). Our studies were intended to be preliminary to a future, more expanded research program with imazapic, and this was the first opportunity for field application. Nevertheless, this late application was still quite effective on natalgrass, and pre-emergence (reduced seedling emergence) as well as post-emergence responses are indicated.

Both of the non-native grasses were damaged by imazapic, but the effect occurred at lower doses and was longer lasting with natalgrass than with bahiagrass. Imazapic use with bahiagrass at our rates only produced the sod suppression that is listed in its registered labeling (Anonymous 1997b). More effective and longer lasting bahiagrass control may require either repeated applications or higher imazapic rates than reported in this paper. Of course, there is a risk that those higher rates may cause more damage to the desirable native plants as well.

Imazapic has excellent potential for selective control of natalgrass in stands of wiregrass on reclaimed lands in Florida. Bahiagrass control will likely require higher and perhaps repeated doses of imazapic, which may damage some native species. Caution is indicated when using imazapic on sites containing lopsided indiangrass.

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