Novel Use of Trinexapac-Ethyl to Study Weed Seed Germination

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Understanding seed biology and onset of germination requirements is a key point for designing effective weed management programs. Gibberellins (GAs) are known to play a role in onset of germination of several species. Onset of germination often requires an increase in de novo GA content or an increase in sensitivity to GAs. Reduced germination of seeds incubated in solutions containing compounds that inhibit GA synthesis provide evidence that GAs are required to trigger germination. Trinexapac-ethyl (TE), a GA synthesis inhibitor, is frequently used as a plant growth regulator in crop production. However, to the best of our knowledge, TE has not been used to study the requirement of GAs in onset of germination. Germination studies were conducted using seeds of artichoke thistle and common teasel under a range of TE concentrations (0 to 500 μM TE), a combined solution of 125 μM TE + 100 μM GA3 for artichoke, and 250 μM TE + 100 μM GA3 for common teasel. Germination tests were conducted at 20/10 C (12-h thermoperiod) in darkness for artichoke and at constant 15 C with 12 h of light for common teasel. Germination of artichoke in 125 μM TE was reduced to 47% when compared with the check (95%), but the combined TE + GA3 solution maintained germination at 84%. Germination of common teasel in 250 μM TE was reduced to 10% when compared with the check (91%), and the combined TE + GA3 solution increased germination to 63%. These results demonstrate the utility of TE to study the role of GAs in onset of germination. This novel use of TE is a valuable option to study germination requirements of weed species, and therefore contributes to the design of weed management programs.

Nomenclature: Gibberellins; trinexapac-ethyl; prohexadione-calcium; artichoke thistle, *Cynara cardunculus* L. CYCA; common teasel, *Dipsacus fullonum* L. DIFU2.

Key words: Dormancy, germination, plant growth regulators, seed biology, weed biology, weed management.

Designing a weed management program requires knowledge of seed biology and onset of germination requirements of the weed species involved.

However, knowledge about seed dormancy and germination requirements is limited or nonexistent for many weed species. Every tool that contributes to the study of weed seed germination is a useful tool for weed management.

Dormancy can be defined as the lack of capacity of a seed to germinate in a specified period of time under any combination of normal physical envi-
GAs are plant hormones synthesized in higher plants that are essential for many processes of plant growth and development, including breaking seed dormancy and seed germination (Yamaguchi 2008). GAs occur in different types of plants with different levels of activity. In general, GA synthesis involves three stages: ent-kaurene synthesis from geranylgeranyl diphosphate, GA12 and GA53 synthesis from ent-kaurene, and finally, the conversion of these compounds into GA4 and GA1 (Yamaguchi 2008). GA4 and GA1 are the main active GAs in higher plants (Sawada et al. 2008).

Plant growth regulators (PGRs) like trinexapac-ethyl (TE) and prohexadione-calcium (prohexadione-Ca) are effective in reducing active GA synthesis (Rademacher 2000). These PGRs interfere with later steps of GA synthesis (Yamaguchi et al. 2007). Both TE and prohexadione-Ca are frequently used as growth regulators in grass seed and cereal crop production, for growth control in turf grasses, and for reduction of vegetative growth in fruit trees (Qian and Engelke 1999; Rademacher 1995; Rajala and Peltonen-Sainio 2001; Zapiola et al. 2006). Incubation of seeds in prohexadione-Ca and other PGRs with different sites of action like paclobutrazol has been frequently used to study the involvement of de novo GAs in onset of germination (Arana et al. 2006; Yang et al. 1995; Zehhar et al. 2002). Nevertheless, to the best of our knowledge, TE has not been used to study involvement of GAs in onset of germination. Various PGRs are commercially available in several countries. In some countries, TE, but not prohexadione-Ca, can be found as ai in commercial growth regulators. Therefore, the potential use of TE in this type of study is a valuable alternative to studies of weed germination requirements when PGRs normally used are not commercially available. Having the ability to use the ai formulated as a commercial product increases the chances of obtaining the compound and reduces the cost of the experiment.

Artichoke thistle and common teasel are two weeds that have invaded thousands of hectares of Argentinean right-of-way and grasslands (Marzoca 1976). Since 1963, artichoke thistle was declared a national pest. In contrast, common teasel turned into a highly problematic weed species just in recent years, and still no official records certified this fact. Artichoke thistle is a herbaceous perennial plant native to the Mediterranean basin that produces several thousands of wind-dispersed seeds each year (Potts et al. 2008; White and Holt 2005). On the other hand, common teasel is a herbaceous biennial plant native to Europe and North Africa that disperses more than 3,000 seeds each year (Werner 1975). Local populations of both species are known to have seed dormancy (Huoarte and Benech-Arnold 2005; La Greca 2010). Artichoke thistle seeds require fluctuating temperatures for germination (Huoarte and Benech-Arnold 2005). On the other hand, common teasel seeds require either fluctuating temperatures or light for germination (Huoarte and Zapiola 2011). To design a weed management program, the possibility of reduced germination and emergence processes using non-chemical methods is important. To do these, knowledge about weed seed germination behavior is required.

The objective was to evaluate the potential use of TE to confirm the requirement of de novo GA synthesis in onset of germination of artichoke thistle and common teasel, and therefore identify an option for use in germination and seed dormancy studies in countries where traditionally used compounds are not available or are too expensive. We hypothesized that TE would reduce germination in cases where onset of germination depends on de novo GA synthesis.

Materials and Methods

Seeds. Heads of artichoke thistle were collected at an infested roadside in Saldungaray (38°11'S, 61°46'W), province of Buenos Aires, Argentina, in January 2009 and at an infested research plot at the
School of Agricultural Sciences, Universidad Católica Argentina (UCA) (34°35′S, 58°27′W), city of Buenos Aires, in January 2013. Common teasel seeds were collected at an infested research plot at the School of Agricultural Sciences, UCA in January 2009, 2011, and 2013. Seeds were hand threshed and kept in paper bags at −18°C until use for germination experiments.

**Germination Experiments.** The effect of TE on seed germination was evaluated. TE treatment levels evaluated for artichoke thistle were 0, 10, 25, 50, 100, 125, 250, and 500 μM TE using Moddus® 250 EC (Syngenta, Switzerland). An additional treatment consisted of 125 μM TE + 100 μM GA3 (Sigma Chemical Company, St. Louis, MO). Three replicates of 25 seeds each per treatment level were placed on blotting paper in 9-cm petri dishes with 6 ml of each corresponding solution. Petri dishes were placed in germination chambers at fluctuating temperatures (10/20°C, 12 h) in darkness. Germination was scored daily for 14 d.

TE treatment levels evaluated for common teasel were 0, 62.5, 125, 250, and 500 μM TE. An additional treatment consisted of 250 μM TE + 500 μM GA3. Three replicates of 30 seeds each per treatment level were placed on blotting paper in 9-cm petri dishes with 8 ml of each corresponding solution. Petri dishes were placed in germination chambers at constant temperature (15°C) and 12-h light. Germination was scored daily for 24 d.

For both species, seeds with visible radicle protrusion were considered germinated and were removed. All germination experiments were repeated. The data presented were averaged across two and three experimental runs for artichoke and common teasel, respectively, because there was no significant experiment-by-treatment interaction (P = 0.86 and 0.67) for artichoke and common teasel, respectively. Germination was expressed as percentage of total seeds germinated by the end of the experiment per petri dish. Data were analyzed by ANOVA using Statistix 8.0 Analytical Software (Tallahassee, FL). Tukey’s test at 5% level of probability was used for mean comparisons.

**Results and Discussion**

There was an effect of TE on germination of artichoke thistle (Figure 1) (P < 0.05). The 125, 250, and 500 μM TE treatments reduced germination of artichoke to 47, 33, and 25%, respectively, when compared with the control (95%). Incubation in a solution of 125 μM TE + 100 μM gibberellin3 (GA3) maintained germination at a level comparable with that of the control (84%).

Common teasel germination was also affected by TE (Figure 2) (P < 0.05). The 125, 250, and 500 μM TE reduced germination to 44, 10, and 1%, respectively, when compared with the control (91%). Incubation in a solution of 250 μM TE + 500 μM GA3 only increased germination to 63%, 31% below that of the control.

The fact that incubation of seeds in TE reduced germination in both species confirms their requirement of de novo GA synthesis for onset of germination. However, greater TE concentrations were needed to reduce germination in common teasel than in artichoke thistle, suggesting that the effective TE concentration to prevent germination is species specific. The effect of TE was suppressed by coincubation with GA3 in both species, demonstrating that the reduction in germination found with TE was a result of de novo GA synthesis inhibition and not of other causes. These results provide evidence of the efficacy of using TE to study the requirement of GAs in onset of germination. Consequently, TE is a valuable option to study
onset of germination requirements in weed species in countries where traditionally used compounds such as paclobutrazol or prohexadione-Ca are not commercially available. In addition, TE could be used in combination with other GA synthesis inhibitors and molecular techniques to identify the step of GA biosynthesis vital for onset of germination. A greater understanding of onset of germination requirements in weeds can contribute to the development of comprehensive weed management programs.

Acknowledgments

Funding for this study was provided by UCA, Project CAEJG (2009-10). The authors thank Syngenta Argentina for providing Moddus 250 EC used in these experiments.

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Received December 3, 2013, and approved April 15, 2014.