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Growth stage, temperature, and time of year affects the control of glyphosate-resistant and glyphosate-paraquat resistant *Conyza bonariensis* with saflufenacil

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ABSTRACT

Conyza bonariensis (L.) Cronq. is a problematic weed in crop and non-crop areas of California. This problem has been further aggravated by the discovery of herbicide-resistant biotypes. Experiments were conducted in 2012 and 2013 in fall and spring, respectively, to determine the efficacy of glyphosate, saflufenacil, and a tank-mixture of saflufenacil + glyphosate on glyphosate-susceptible (GS), glyphosate-resistant (GR), and glyphosate-paraquat-resistant (GPR) biotypes of *C. bonariensis*. Efficacy of the herbicides were evaluated at three growth stages (5- to 8-leaf seedling, rosette, and bolting); and three day/night temperature regimes (15/10 °C, 25/20 °C, 35/30 °C). Results differed between experiments conducted in the fall and spring. Saflufenacil-alone was more effective in the fall than in spring. All the GS, GR and GPR plants were controlled by saflufenacil-alone at the 5-to 8-leaf stage and rosette stage, but level of control declined at the bolting stage. Better control with saflufenacil-alone and glyphosate-alone was obtained at the 15/10 °C and 25/20 °C than at the 35/30 °C temperature regime. However, a tank-mixture of saflufenacil + glyphosate provided good control of the plants at 35/30 °C. Efficacy of saflufenacil-alone was inconsistent in spring and varied between the biotypes, but the control with saflufenacil + glyphosate was excellent and consistent between seasons. Glyphosate-alone provided good control of all three biotypes at the 5- to 8-leaf stage in the fall, but the control was poor in spring. Therefore, saflufenacil-alone can provide excellent control of *C. bonariensis* plants prior to the bolting stage in the fall; but in spring, it will be more effective when applied with glyphosate.

1. Introduction

Conyza bonariensis (L.) Cronq. is a problematic weed in California. In the past decade, its prevalence has increased in perennial and annual cropping systems, and non-crop systems such as roadsides and canal banks (Shrestha et al., 2008a, 2014). There are two possible reasons for this phenomenon. The first is that this species prefers areas with less soil disturbance and the other possibility is the evolution of herbicide-resistant populations, particularly glyphosate-resistant (GR) and glyphosate + paraquat-resistant (GPR) biotypes (Shrestha et al., 2008a; Moretti et al., 2013). Minimum soil disturbance associated with fallow, perennial cropping systems, and reduced tillage management practices of annual crops has provided a favorable niche for the ecological adaptation of *Conyzas* (Murphy and Lemerle, 2006).

Glyphosate-resistant *C. bonariensis* was first documented in the Central Valley of California in 2008 (Shrestha et al., 2008b). An additional GPR biotype was discovered in the same region in 2010 and glufosinate was identified as an immediate alternative for successful control of these herbicide-resistant biotypes (Moretti et al., 2015). However, in order to prevent the onset of the evolution of glufosinate-resistant populations, alternative herbicides and strategies are needed to combat this weed. Therefore, mode of action (MOA) other than glyphosate, paraquat, and glufosinate are required for a good resistance management plan. For immediate management of herbicide-resistant weed biotypes, alternative herbicides need to be identified as a short-term solution. However, *C. bonariensis* has evolved resistance to bipyridilium, glycine, sulfonylurea, and triazine herbicides in at least 11 countries worldwide (Heap, 2015). One herbicide introduced fairly recently in

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California is saflufenacil (Treevix ™), a protoporphyrinogen oxidase (PPO) inhibiting herbicide. Although there are several PPO inhibiting herbicides such saflufenacil, flumioxazin, carfentrazone, pyraflufen, and oxyfluorfen registered for use in permanent crops in California with the same MOA, their main chemical compositions differ (Vencill, 2002). Of the PPO herbicides listed above, saflufenacil is currently the only one that includes postemergence control of *C. bonariensis* on its label, while pyraflufen (Venue®) mentions control of this species in a supplemental label when tank-mixed with glyphosate.

The active ingredient saflufenacil has been registered for postemergence control of broadleaf weeds in citrus, nuts, and pome fruits. Eubank et al. (2013) reported that saflufenacil was effective against GR *Conyza canadensis*, which is another problematic weed in the Central Valley (Shrestha et al., 2008a). Although in 2000 there were no known cases of resistance to PPO inhibitors worldwide (Prather et al., 2000), by 2015 there are 7 species in 6 countries that have documented cases of resistance to PPO inhibitors, including several cases of multiple herbicide resistance in these species (Heap, 2015). However, as of yet, there is no documented case of PPO-resistant *C. bonariensis*.

It is a common practice for growers to apply tank-mixtures of herbicides with different MOAs as a resistance management strategy. There has been some work done to explore effects of PPO inhibitors when applied as a tank-mix with glyphosate. Some studies have suggested that combining saflufenacil and glyphosate as a single treatment can create a change in the efficacy of each herbicide. For example, Eubank et al. (2013) reported an additive effect when using a combination of glyphosate with saflufenacil on *C. canadensis*. Absorption and translocation effects of the combination were mixed with increased absorption of glyphosate in glyphosate-susceptible (GS) biotypes, reduced absorption in GR biotypes, and reduced glyphosate translocation in both. In a separate study comparing the efficacy of a saflufenacil-glyphosate combination on GS and GR *Brassica napus* L. varieties, it was found that the combination reduced translocation of saflufenacil in GS varieties. However, in GR varieties, the combination did not affect the translocation of saflufenacil (Ashigh and Hall, 2010). Additionally, Waggoner et al. (2011) showed a greater benefit to using a mix of saflufenacil and glyphosate on *C. canadensis* versus saflufenacil-alone in no-till *Gossypium* sp. Shrestha and Moretti (2011) showed no additional benefit for control of GR *C. canadensis* with a tank-mix of saflufenacil and glyphosate compared to saflufenacil-alone.

Although considerable work has been done on the genetics and physiology of herbicide resistance, the causes of resistance within weed species is not well understood. Therefore, a knowledge-based approach is needed to deal with the continuing evolution of the plant-herbicide relationship including the physiological and environmental factors that affect this relationship.

Growth stage at the time of herbicide application has been shown to be a major factor in the level of control of *C. canadensis* by glyphosate (Shrestha et al., 2007; VanGessel et al., 2009). Studies by Gonzalez-Torralva et al. (2010) and Puricelli et al. (2015) on *C. bonariensis* also showed differences in susceptibility when treated with glyphosate at three developmental stages (rosette, bolting, and flowering), with early developmental stages being much more sensitive. Although the MOA of glyphosate and saflufenacil are not similar, it should be ascertained if similar effects of plant growth stage can be expected with saflufenacil or a tank-mix of glyphosate and saflufenacil.

An environmental factor of interest influencing herbicide efficacy, in recent years, is temperature as some GR weed populations have been reported to be susceptible when glyphosate was sprayed during cooler parts of the year (Moretti et al., 2013). For example, a study in Israel compared the response of several populations of *C. bonariensis* and *C. canadensis* to glyphosate at day/night thermoperiods of 16/10 °C, 22/16 °C, 28/22 °C, and 34/28 °C and found a significant negative linear correlation between rising temperature and plant response to glyphosate in terms of effective dose (ED_{50}) values (Rubin et al., 2011). Plants grown at higher temperatures were 2- to 10-fold more tolerant to glyphosate than at lower temperatures. However, it is unknown if there will be an effect of temperature on control of GR and GPR *C. bonariensis* biotypes of the Central Valley with glyphosate or saflufenacil. This must be ascertained because *C. bonariensis* emerges at different times of the year, primarily in spring and fall months, but also in late spring and early summer.

To increase herbicide efficacy, our knowledge should involve both biology of the pest and interactions with environmental factors. A knowledge-based approach to weed management will reduce the overall cost to the grower, increase efficacy of the pest management system, and reduce the impact on the environment. To ascertain the biological and environmental factors necessary to achieve the greatest benefit from the use of saflufenacil as an effective herbicide against all biotypes of *C. bonariensis*, it is essential to explore the effect of factors such as plant growth stage and temperature at the time of herbicide application. Therefore, the objectives of this study were to evaluate the: efficacy of saflufenacil on GR, GPR, and glyphosate-susceptible (GS) *C. bonariensis* biotypes at three different growth stages of the plants: 5- to 8-leaf stage, rosette stage (15- to 20-leaf stage), and initial bolting stage; and effect of temperature on the efficacy of saflufenacil and other herbicides on GR, GPR, and GS *C. bonariensis* biotypes.

2. Materials and methods

2.1. Growth stage study

The experiment was conducted in an open field near the Ornamental Horticulture Unit at California State University, Fresno, CA, USA (36.816335 N, −119.734500 W). Seeds of *C. bonariensis* were obtained from various locations of the Central Valley of California, GR population (36°29′15.00″N; 119°24′10.00″ W), GPR population (36°35′48.33″N; 119°30′50.45″W), and GS population (36°47′58.00 N; 119°57′16 W). These locations are within 50 km of each other in Fresno County, CA. The biotypes were previously verified as GR, GPR, and GS by Moretti et al. (2013). Seeds were planted in seedling trays of 100 separate cells each (52 cm by 25 cm by 6 cm) that had been prefilled with a moist potting mix (Sunshine Mix #3, Sun Gro ® Horticulture, Sacramento, CA 95814, USA). Plants were placed in a no-hole catchment tray and water was added to the catchment tray for sub-irrigation. The trays were kept in a greenhouse set at 25°/18 °C day/night temperature with ambient lighting for germination. Once a 2- to 3-leaf seedling was established, plants of the same size were selected for transplanting. Some of the seedlings were transplanted into 5.7 cm by 5.7 cm by 8.25 cm plastic pots and some were transplanted into 8.9 cm by 8.9 cm by 12.7 cm plastic pots containing the same commercial potting mix used for germination. The former pot size was assigned to plants to be sprayed at the 5- to 8-leaf stage and the latter pot size was assigned to plants to be sprayed at the rosette or the bolting stage. The seeding and transplanting dates for the targeted growth stages are shown in Table 1. The plants were kept in the greenhouse and watered until they reached the following three growth stages: 5- to 8-leaf stage, rosette stage, and initial bolting.

The experimental design was a split-split-plot with growth stage (5- to 8-leaf stage, rosette stage, bolting stage) as the main plot; the biotype (GR, GPR, GS) as sub-plot; and the herbicide treatments as the sub-sub-plot. The herbicide treatments were