WEED CONTROL EFFICACY AND WINTER WHEAT RESPONSE TO SAFLUFENACIL

by

JOHN CARL FRIHAUF

B.S., University of Wyoming, 2001
M.S., University of Wyoming, 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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Abstract

Saflufenacil is an experimental herbicide for control of broadleaf weeds in various crops including several herbicide resistant weed biotypes. Wheat is highly tolerant to preplant and preemergence applications of saflufenacil, but winter wheat growers prefer to apply herbicides postemergence (POST) in early spring. Objectives of this research were to (1) evaluate winter wheat and four common broadleaf weed species response to POST treatments of saflufenacil applied alone and in combination with bentazon or auxin herbicides at various rates both with and without adjuvants, and to (2) determine the possible mechanism(s) responsible for crop safening observed when saflufenacil is applied with 2,4-D amine or bentazon in winter wheat. Growth chamber, greenhouse, and field studies showed saflufenacil at a minimum rate of 25 g/ha controlled blue mustard and flixweed >85% when saflufenacil was applied alone or mixed with dicamba, 2,4-D amine, 2,4-D ester, or MCPA ester. Also, mixtures of bentazon with 13 g/ha of saflufenacil resulted in death of kochia, but increasingly higher rates of 2,4-D amine were needed to achieve 90% growth reduction when saflufenacil rates were decreased from 50 to 25 to 13 g/ha. In general, most of the saflufenacil combinations tested controlled henbit <85%. Leaf necrosis and stunting of winter wheat were reduced by tank mixing saflufenacil with dicamba, 2,4-D amine, or bentazon, but not with MCPA ester or 2,4-D ester. Including nonionic surfactant (NIS) in mixtures of saflufenacil plus 2,4-D amine resulted in significant wheat injury similar or greater than injury caused by saflufenacil plus NIS. Finally, 2,4-D amine enhanced saflufenacil absorption into winter wheat plants, whereas bentazon reduced absorption of saflufenacil. No more than 11% of applied saflufenacil translocated out of treated leaves to other plant parts when applied alone or when saflufenacil was mixed with 2,4-D amine or bentazon. Metabolism of saflufenacil by wheat plants was not affected by tank mixing with bentazon, but saflufenacil metabolism was slowed by mixing with 2,4-D amine. Overall, these studies indicate saflufenacil can potentially be used POST in wheat at an optimum rate of 25 g/ha plus 2,4-D amine or dicamba to effectively control blue mustard and flixweed.

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Phillip W. Stahlman

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I would also like to thank my wife and daughter, Jen and Lauren, for their support, patience, and encouragement. Also thanks to my parents, Glen and Barb, and my father- and mother-law, Jim and Judy, for their support and encouragement.

Dedication

This dissertation is dedicated to my wife, Jen, and daughters, Lauren and Emma, thank you for all your love, support, and patience. Jen, you are the most generous and remarkable person I know. I look forward to our next adventures together.

CHAPTER 1 - Winter Wheat and Weed Response to Postemergence Saflufenacil Alone and in Mixtures

ABSTRACT

Growth chamber experiments were conducted in the fall of 2006 and spring of 2007 to determine winter wheat, flixweed, and henbit response to POST treatments of saflufenacil (BAS 800H) at 13, 25, and 50 g ai/ha applied alone and in combinations with bentazon at 560 g ai/ha or 2,4-D amine at 533 g ae/ha and nonionic surfactant (NIS) at 0.25%. Mixtures of saflufenacil and 2,4-D amine also were applied without NIS. Necrosis was observed on wheat leaves within 1 day after treatment (DAT) and peaked at 7 DAT. Saflufenacil at 13, 25, or 50 g ai/ha applied alone or in combination with 533 g ae/ha of 2,4-D amine plus NIS caused 19 to 38% and 24 to 40% wheat foliar necrosis, respectively. Foliar necrosis was 14% or less when saflufenacil at any rate was mixed with bentazon or 2,4-D amine without NIS. Combinations of saflufenacil at any of the rates tested plus bentazon and NIS did not reduce wheat dry weight. Saflufenacil plus 2,4-D amine without adjuvant resulted in similar wheat dry weights as 2,4-D amine. Saflufenacil plus 2,4-D amine without NIS provided 99% control of flixweed at 21 DAT, but henbit control ranged from 81 to 88%. In comparison, saflufenacil at 50 g/ha mixed with bentazon and NIS controlled flixweed 92% and henbit 63% at 21 DAT. Wheat tolerance and weed efficacy data from these three experiments indicate saflufenacil has potential for POST application in winter wheat to control winter annual broadleaf weeds when tank mixed with 2,4-D amine without NIS.

Nomenclature: bentazon; saflufenacil; 2,4-D amine; flixweed, *Descurainia sophia* L. Webb. ex Prantl, DESSO; henbit, *Lamium amplexicaule* L., LAMAM; winter wheat, *Triticum aestivum* L. 'KS03HW6-1'.

Key words: winter annual broadleaf weeds, BAS 800H, crop response

INTRODUCTION

Saflufenacil is a new herbicide being developed for PRE and POST control of broadleaf weeds. Development of saflufenacil is targeted to preplant burndown and PRE application in various crops such as corn, cereals, sorghum, and soybeans. Wheat is highly tolerant to preplant- or PRE-applied saflufenacil at rates up to 400 g/ha (Jenks et al. 2008; Knezevic et al. 2008), but POST applications of saflufenacil injures wheat (Knezevic et al. 2008; Sikkema et al. 2008).

Susceptible broadleaf species are controlled by saflufenacil through inhibition of protoporphyrinogen oxidase (protox) (Anonymous 2008). Protox is the last enzymatic step before chlorophyll and heme synthesis pathways branch from the tetrapyrrole biosynthesis pathway (Matringe et al. 1992). Inhibition of protox results in the accumulation of protoporphyrinogen IX (protogen) in plastids (Jacobs et al. 1991; Witkowski and Halling 1989). The accumulated protogen leaks from the plastids and is converted to protoporphyrin IX (proto) by enzymes in the cytoplasm (Jacobs et al. 1991; Jacobs and Jacobs 1993; Lee et al. 1993). Proto reacts with light and oxygen to form radical singlet oxygen and results in the destruction of cellular membranes (Becerril and Duke 1989; Haworth and Hess 1988; Jacobs et al. 1991).

Although saflufenacil has been shown to be generally safe in wheat prior to emergence, many wheat producers are reluctant to use an herbicide at that time, preferring instead to apply herbicides POST in spring (D. E. Peterson and P. W. Stahlman, personal communication). The preference for POST herbicide application in spring is linked to concerns of wheat winter-kill, disease and insect infestations, and drought conditions, all of which could result in poor stands and crop failure. For these reasons, many winter wheat producers delay herbicide application until after the condition of the winter wheat crop following green-up in the spring is assessed. Furthermore, in order to reduce application costs, many growers choose to apply herbicides at the same time as top-dressing nitrogen in the spring.

Control of ALS-resistant weeds in winter wheat production systems is a problem for many producers. In the United States, 39 ALS-resistant biotypes of weed species have been documented (Heap 2009). Integrated weed management strategies that combine cultural, mechanical, and chemical crop techniques such as crop rotation, sanitation, rotation of herbicide modes-of-action, and tank mix partners with a different mode of action can minimize the development of herbicide resistant weeds (Lee et al. 2000; Mallory-Smith et al. 1993; Regehr and Morishita 1989). However, winter wheat producers trying to control ALS-resistant weed populations have a limited selection of herbicides with different modes-of-action to use in tank mixtures or to establish herbicide rotations. Few protox herbicides are labeled for use in wheat and saflufenacil could potentially be used to control many broadleaf weed species including ALS-resistant biotypes in this crop. However, because few winter wheat growers are likely to apply preplant or PRE treatments of saflufenacil, the objective of this research was to evaluate the response of winter wheat and winter annual broadleaf weed species to POST treatments of saflufenacil applied alone or mixed with bentazon or 2,4-D amine.

MATERIALS AND METHODS

Two winter wheat response experiments and one winter annual broadleaf efficacy experiment were conducted in growth chambers set for 20/15 C day/night temperatures, 12 hr photoperiod, and photosynthetic photon flux density of 250µmol/m^2/s. Space limitation prevented the experiments from being conducted simultaneously, thus they are reported separately. 'KS03HW6-1' winter wheat, flixweed, and henbit seeds were sown separately in 11-cm diam by 16-cm tall plastic pots containing a mixture of sand and Morrill loam (mesic Typic Argiudolls) (1:2 by vol). The mixed growth medium had a pH of 8.0 and 1.2% organic matter. Wheat seedlings were thinned to two plants per pot and henbit and flixweed seedlings were thinned to 10 plants per pot. Plants in each experiment were watered and fertilized[1] as needed. All herbicide treatments were applied using a bench-type sprayer[2] equipped with a single moving 80015LP spray tip[3] delivering 187 L/ha at 207 kPa and 3 km/hr.

Winter Wheat Response

Both winter wheat response experiments were designed as completely randomized blocks with each treatment replicated four times. Leaf necrosis and stunting (growth reduction) were estimated visually using a percentage scale from 0 (no response) to 100 (death) at 3, 7, and 14 days after treatment (DAT), and aboveground plant dry weight was determined at 14 DAT. Two wheat plants in each pot were cut at soil level, dried at 60 C for 48 h, and weighed.

Experiment 1

Winter wheat seedlings were treated POST with saflufenacil at 13, 25, or 50 g/ha alone and in combination with bentazon at 560 kg/ha or 2,4-D amine at 533 g/ha. Bentazon and 2,4-D amine were each applied alone at those rates and an untreated control was included for comparison. Non-ionic surfactant[4] was included at 0.25% v/v in all herbicide treatments. Herbicide treatments were applied to 15- to 25-cm tall wheat plants with 1 to 2 fully expanded leaves. The experiment was repeated.

Experiment 2

Winter wheat seedlings were treated POST with 13, 25, or 50 g/ha of saflufenacil with NIS at 0.25% v/v and in combination with 2,4-D amine at 533 g/ha both with and without NIS. Treatments of 2,4-D amine at 533 g/ha with and without NIS and an untreated control were included for comparison. Herbicide treatments were applied to wheat measuring 15 to 23 cm tall with 1 to 2 fully expanded leaves. The experiment was repeated three times.

Weed Efficacy

The experiment was a completely randomized design arranged as a factorial of weed species (flixweed and henbit) and herbicide treatments with each treatment combination replicated three times. The experiment was repeated. Flixweed and henbit seedlings were treated POST with saflufenacil at 13, 25, or 50 g/ha plus NIS at 0.25% v/v and in combination with bentazon at 560 g/ha or 2,4-D amine at 533 g/ha. Also, saflufenacil at each rate was applied with 2,4-D amine at 533 g/ha without NIS. Bentazon at 560 g/ha with 0.25% v/v NIS, and 2,4-D amine at 533 g/ha with and without NIS at 0.25% v/v. An untreated control was included for comparison. Herbicide treatments were applied to henbit and flixweed plants measuring 1 to 2.5 cm in diam.

Visual weed control was estimated on a percentage scale from 0 (no control) to 100 (death) at 3, 7, 14, and 21 DAT. Aboveground plant dry weight at 21 DAT was determined by cutting all living plants in each pot at soil level, drying the plant samples at 60 C for 48 hr, and weighing.

Statistical Analysis

Data were checked for normality and homogeneity of variances. Arcsine transformations were performed to correct non-homogenous variances when necessary; however, untransformed means are presented for clarity. Data for individual experiments were subjected to ANOVA using PROC MIXED in SAS[5] with herbicide treatment as a fixed effect. Repeated experiments (runs) and the run-by-treatment interaction were considered random effects. Treatments means were separated using least square means at $P \leq 0.05$.

Untreated control treatments were omitted from analyses of flixweed and henbit control in the weed efficacy experiment. Prior to analysis, plant dry weights for all experiments were converted to a percentage of the untreated control. Orthogonal contrasts were conducted for all response variables in all experiments. In both winter wheat response experiments, correlation analysis of leaf necrosis at 14 DAT and plant dry weight reduction data were performed using PROC CORR in SAS. Correlation analysis also was performed between visually estimated henbit and flixweed control at 21 DAT and plant dry weight reductions.

RESULTS AND DISCUSSION

Wheat Response Experiment 1

Leaf necrosis was observed within one day after herbicide application. Leaf necrosis at 3, 7, and 14 DAT increased as the rate of saflufenacil with NIS increased from 13 up to 50 g/ha, except when mixed with bentazon (Table 1.1). Mixing 533 g/ha of 2,4-D amine with saflufenacil at 13 or 25 g/ha increased leaf necrosis compared to saflufenacil alone at those rates at 3 and 14 DAT. A similar, non-significant trend was observed at 7 DAT. Necrosis was similar between treatments of 50 g/ha saflufenacil mixed with

2,4-D amine and the same saflufenacil rate applied alone at 3, 7, and 14 DAT. At each evaluation time, greatest leaf necrosis resulted from application of 50 g/ha saflufenacil alone or saflufenacil at 25 or 50 g/ha plus 2,4-D amine (39 to 41% at 3 DAT; 35 to 40% at7 DAT; 27 to 32% at 14 DAT). In comparison, within each evaluation time foliar necrosis was intermediate for treatments of saflufenacil alone at 13 or 25 g/ha and saflufenacil at 13 g/ha mixed with 2,4-D amine. However, the mixture of 13 g/ha saflufenacil plus 2,4-D amine caused similar necrosis as saflufenacil alone at 25 g/ha.

Opposite the effect of 2,4-D amine, bentazon at 560 g/ha mixed with saflufenacil at each rate substantially reduced leaf necrosis compared to saflufenacil alone, and nearly eliminated leaf necrosis at the 13 g/ha saflufenacil rate (Table 1.1). Neither 2,4-D amine nor bentazon caused any leaf necrosis when applied alone. Averaged over saflufenacil rates, contrasts indicted significant differences in leaf necrosis at 3, 7 and 14 DAT between treatments of saflufenacil alone versus saflufenacil plus 2,4-D amine or bentazon, and between saflufenacil plus 2,4-D amine versus saflufenacil plus bentazon.

Saflufenacil at 50 g/ha applied alone or in mixture with 2,4-D amine caused similar and greater stunting at 7 and 14 DAT (13 and 20% at 7 DAT; 13 and 15% at 14 DAT) than lower rates of saflufenacil alone or in combination with 2,4-D amine (≤8% at 7 and 14 DAT) (Table 1.1). No saflufenacil plus bentazon treatment or bentazon or 2,4-D amine alone stunted wheat.

Wheat plant dry weight varied greatly among herbicide treatments (Table 1.1). Most treatments of saflufenacil alone or in combination with 2,4-D amine reduced plant dry weight by 30 to 33% compared to non-treated wheat. Saflufenacil at 13 g/ha alone did not reduce plant dry weight, whereas saflufenacil at 50 g/ha plus 2,4-D amine reduced plant dry weight by 47%. Conversely, wheat treated with bentazon alone or in mixture with saflufenacil produced 27% and 13 to 23% more dry plant matter, respectively, than non-treated wheat. In addition, plant dry weights were similar among saflufenacil treatments and saflufenacil mixed with 2,4-D averaged over saflufenacil rates (Table 1.1). Plant dry weights were reduced most by applications of saflufenacil at 25 or 50 g/ha alone and saflufenacil at 13, 25 or 50 g/ha in combination with 2,4-D amine (33, 32, 30, 33, and 47%, respectively). Plant dry weights of the remaining treatments were similar or greater than the untreated control. Plant dry weight reductions were negatively correlated to leaf necrosis at 14 DAT ($r = -0.65663$, P-value = <0.0001), indicating a relationship between visible leaf necrosis and plant dry weight. Although leaf necrosis at 14 DAT was negatively correlated to wheat dry weights, necrosis ratings generally underestimated treatment effects on wheat dry weights. Leaf necrosis was one component of injury symptoms observed in wheat with stunting as the other response. An overall evaluation of injury that took into account leaf necrosis and stunting may have better estimated the effect of injury on plant dry weights.

Wheat Response Experiment 2

Similar to results in wheat response experiment 1, leaf necrosis was observed soon after herbicide application. Saflufenacil plus 2,4-D amine without NIS caused <15% leaf necrosis at 3, 7, and 14 DAT, regardless of saflufenacil rate (Table 1.2). Adding NIS to saflufenacil plus 2,4-D tank mixtures increased leaf necrosis 3- to 4- times, to as high as 36% at the 50 g/ha rate of saflufenacil. At each evaluation time, wheat response to saflufenacil alone and saflufenacil plus 2,4-D amine in the presence of NIS was similar within saflufenacil rates. In the absence of 2,4-D amine, increasing saflufenacil rate from 13 to 25 g/ha increased leaf necrosis from 18 to 28% at 3 DAT and from 21 to 32% at 7 DAT, but increasing saflufenacil rate from 25 to 50 g/ha did not further increase leaf necrosis compared to the 25 g/ha rate. Differences in necrosis between saflufenacil plus NIS and saflufenacil plus 2,4-D amine and NIS at 14 DAT were not significant. For treatments of saflufenacil plus 2,4-D amine without NIS, leaf necrosis trended higher with increasing saflufenacil rate at each evaluation time; however, only the 13 and 50 g/ha rates at 3 DAT differed significantly.

Maximum leaf necrosis was observed at 7 DAT with application of 25 or 50 g/ha of saflufenacil plus NIS, and 2,4-D amine plus NIS with 13, 25, or 50 g/ha of saflufenacil (27 to 36%) (Table 1.2). At 14 DAT, new wheat growth had reduced the percentage of necrotic tissue compared to evaluations at 7 DAT. Averaged over saflufenacil rates, contrasts indicated leaf necrosis caused by saflufenacil plus NIS was similar to saflufenacil plus 2,4-D amine plus NIS at 7 and 14 DAT. In addition, leaf necrosis was significantly different in comparisons of saflufenacil plus 2,4-D amine and saflufenacil plus 2,4-D amine plus NIS or saflufenacil plus NIS.

Herbicide induced patterns of wheat stunting were similar to response patterns expressed as leaf necrosis (Table 1.2). Plant stunting at 7 and 14 DAT generally increased as the rate of saflufenacil with NIS was increased from 13 to 25 g/ha when applied alone or in combination with 2,4-D amine. At both evaluation times, 5% or less stunting was observed following application of saflufenacil plus 2,4-D amine without NIS, regardless of saflufenacil rate, or following application of 2,4-D amine with or without NIS.

Wheat plant dry weights were reduced by all herbicide treatments compared to the untreated control (Table 1.2). Greatest reductions in plant dry weight of 42 to 56% resulted from applications of saflufenacil at 25 and 50 g/ha plus NIS and all saflufenacil rates tested mixed with 2,4-D amine and NIS. Despite a range of 14%, differences were not significant. Treatments of saflufenacil plus 2,4-D amine without NIS, and applications of 2,4-D amine with or without NIS reduced plant dry weights by 20 to 38%; neither were these differences significant. Contrasts indicated dry weights of plants treated with saflufenacil plus 2,4-D amine differed significantly from those of saflufenacil with NIS or saflufenacil mixed with 2,4-D amine and NIS. Plant dry weight reductions were negatively correlated with leaf necrosis recorded 14 DAT ($r = -0.59689$, P-value = <0.0001), indicating herbicide treatments causing the greatest necrosis at 14 DAT also reduced dry plant biomass the most. Similar to results in wheat response experiment 1, leaf necrosis at 14 DAT was negatively correlated to wheat dry weights and visual necrosis ratings generally underestimated treatment effects on wheat dry weights. Overall injury estimates encompassing both leaf necrosis and stunting likely would have better estimated treatment effect on wheat dry weights.

Weed Efficacy

Generally, flixweed was more sensitive than henbit to most saflufenacil treatments (Table 1.3). There was no saflufenacil rate response for flixweed control at any evaluation time when saflufenacil was applied alone or mixed with 2,4-D amine, but control increased with increasing saflufenacil rate when mixed with bentazon. Also, there was a general trend of increasing henbit control as saflufenacil rate was increased from 13 up to 50 g/ha, except at 14 and 21 DAT for treatments of saflufenacil plus 2,4-D amine applied either with or without NIS. However, henbit control differences between saflufenacil rates within groups of the same herbicides were not always significant.

Bentazon plus NIS controlled henbit and flixweed poorly (≤32%) throughout the course of the experiment and inhibited control of both species when tank mixed with saflufenacil, especially at the two lower saflufenacil rates (Table 1.3). Conversely, tank mixing 2,4-D amine with saflufenacil increased the speed of flixweed control and improved henbit control overall compared to saflufenacil alone. Adding NIS to mixtures of saflufenacil and 2,4-D amine did not enhance flixweed control but there were consistent trends of improved henbit control with NIS use; however, control differences between NIS absence and presence were seldom significant. In addition, significant differences in flixweed or henbit control were not indicated by contrasts of saflufenacil mixed with 2,4-D amine and saflufenacil in combination with 2,4-D amine and NIS at 3, 7, 14, or 21 DAT (Table 1.3).

Most herbicide treatments containing saflufenacil controlled both weed species rapidly; 60% or greater control at 3 DAT. Treatment combinations of saflufenacil and bentazon were slower acting than saflufenacil alone or saflufenacil plus 2,4-D amine combinations. Saflufenacil at 50 g/ha mixed with 2,4-D amine and NIS provided the greatest henbit control (84%) at 3 DAT.

At 7 DAT, flixweed control by most herbicide treatments had increased considerably compared to control at 3 DAT, but henbit control did not dramatically improve (Table 1.3). Treatments of saflufenacil with NIS, and saflufenacil plus 2,4-D amine with and without NIS controlled flixweed ≥92% at 7 DAT and ≥95% at 14 DAT. Tank mixing bentazon with saflufenacil at 13 or 25 g/ha reduced flixweed control by 14 and 12%, respectively, compared to the same rates of saflufenacil alone at 7 DAT. Generally, henbit control by most herbicide treatments did not improve from 3 DAT to 7 DAT. Improved henbit control was not observed until 14 DAT.

Henbit and flixweed control peaked at 14 DAT (Table 1.3). Flixweed control was similar at ≥89% when saflufenacil was applied alone, applied in combination with 2,4-D amine with or without NIS, or mixed with bentazon and NIS at 14 DAT. Tank mixing bentazon with saflufenacil did not improve henbit control compared to the same rates of saflufenacil alone at 14 DAT.

At 21 DAT, henbit treated with saflufenacil alone and saflufenacil plus bentazon exhibited new growth indicating recovery; however, new growth of flixweed plants was not evident. Saflufenacil in combination with 2,4-D amine and NIS controlled henbit 92 to 94% depending on saflufenacil rate, compared to 81 to 83% for the same herbicide combinations without NIS. In general, trends in henbit and flixweed control at 21 DAT were similar to those observed at 14 DAT for treatments that included saflufenacil (Table 1.3). However, flixweed control with 2,4-D amine plus NIS improved from 77% at 14 DAT to 93% at 21 DAT. Improved henbit and flixweed control was also observed with application of 2,4-D amine without NIS at 21 DAT compared to control with this treatment at 14 DAT.

There were strong, negative correlations between visual control estimates at 21 DAT and plant dry weight reductions for both species (henbit, $r = -0.92960$, P-value = <0.0001; flixweed, -0.92889, P-value = <0.0001). Visual control ratings generally underestimated most herbicide treatment effects on henbit plant dry weight reduction. Henbit control and plant dry weight reduction percentages were most similar (<5% difference) for treatments of saflufenacil plus 2,4-D amine and NIS (Table 1.3). Visual control ratings were 13 to 15% lower than plant dry weight reductions for applications of saflufenacil with NIS, 5 to 6% lower for applications of saflufenacil plus 2,4-D amine without NIS, and 10 to 21% lower for treatments of saflufenacil plus bentazon with NIS. Visual flixweed control ratings were in close agreement with plant weight reductions for most treatments of saflufenacil alone or in combination with 2,4-D amine. However, visual control percentages underestimated flixweed plant dry weight reductions by more than 5% for saflufenacil plus bentazon, 2,4-D amine without NIS and bentazon with NIS.

This research indicates saflufenacil has potential for POST use to control winter annual broadleaf weeds in winter wheat but additional research is needed to discover ways to improve crop safety without reducing weed control. Minimal foliar necrosis and plant stunting were observed when saflufenacil at rates of 13, 25, or 50 g/ha was tank mixed with bentazon and NIS or 2,4-D amine without adjuvant. However, the combination of saflufenacil plus bentazon and NIS reduced the speed and completeness of weed control at the lowest saflufenacil rate tested. Conversely, tank mixing 2,4-D amine with saflufenacil improved henbit control and provided similar flixweed control compared to saflufenacil alone. Saflufenacil mixed with 2,4-D amine reduced leaf necrosis and stunting compared to saflufenacil plus NIS and saflufenacil mixed with 2,4-D amine and NIS. However, dry weights were lower than the untreated control yet similar to plants treated with only 2,4-D amine. The foliar necrosis observed was considered a temporary effect without longer term negative effects though the study was terminated before recovery from stunting could be assessed. New wheat growth was unaffected by the herbicide treatments. Additional research is needed to investigate crop response and weed control efficacy of saflufenacil applied POST in winter wheat under field conditions and to investigate ways to improve crop safety. It would also be of interest to identify the mechanism for the reduced phytotoxicity of saflufenacil when it is applied POST in winter wheat with 2,4-D amine or bentazon.

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SOURCES OF MATERIALS

[1] Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 14111 Scottslawn Road, Marysville, OH 43041.

[2] Research Track Sprayer SB-8, Devries Manufacturing, 28081 870th Ave., Hollandale, MN, 56045.

[3] Spraying Systems Co., N. Avenue at Schmale Road, P.O. Box 7900, Wheaton, IL 60189-7900.

[4] Nonionic surfactant, Activator 90, Loveland Products, Inc., 7251 W. 4th St., P.O. Box 1286, Greeley, CO 80632-1286

[5] SAS version 9.1, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

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Table 1.1. Winter wheat response to POST saflufenacil-based treatments in a controlled environment[a].

Treatment [b, c, d]	Rate[e]	Necrosis, DAT 3	Necrosis, DAT 7	Necrosis, DAT 14	Stunting, DAT 7	Stunting, DAT 14	Plant dry weight
	g /ha	%					% of control
Saflufenacil + NIS	13	19 c[d]	19 d	13 de	1 c	0 b	99 ab
Saflufenacil + NIS	25	26 bc	27 bc	17 cd	3 c	4 b	67 c
Saflufenacil + NIS	50	40 a	38 a	29 a	13 ab	13 a	68 c
Saflufenacil + 2,4-D + NIS	13 + 533	28 b	24 cd	20 bc	1 c	1 b	70 bc
Saflufenacil + 2,4-D + NIS	25 + 533	39 a	35 ab	27 ab	8 bc	6 b	67 c
Saflufenacil + 2,4-D + NIS	50 + 533	41 a	40 a	32 a	20 a	15 a	53 c
Saflufenacil + bentazon + NIS	13 + 560	4 e	1 f	1 f	0 c	0 b	121 a
Saflufenacil + bentazon + NIS	25 + 560	10 d	7 e	6 f	0 c	0 b	123 a
Saflufenacil + bentazon +NIS	50 + 560	11 d	9 e	7 ef	0 c	0 b	113 a
2,4-D + NIS	533	0 f	0 f	0 f	0 c	0 b	99 ab
Bentazon + NIS	560	0 f	0 f	0 f	0 c	0 b	127 a
Untreated[f]	—	0 f	0 f	0 f	0 c	0 b	—
Contrasts[g]							
Safl + NIS vs. safl + 2,4-D + NIS		**[h]	*	**	NS	NS	NS
Safl + NIS vs. safl + bent + NIS		***	***	***	NS	**	***
Safl + 2,4-D + NIS vs. safl + bent + NIS		***	***	***	**	***	***

[a] Means followed by the same letter within columns are not significantly different based on least square means at $P \leq 0.05$
[b] Abbreviations: bent, bentazon; NIS, nonionic surfactant; safl, saflufenacil.
[c] Amine formulation of 2,4-D.
[d] NIS applied at 0.25% v/v.
[e] Units for 2,4-D rate are g ae/ha.
[f] Dry weight for the untreated control was 0.38 grams.
[g] Contrasts are averaged over saflufenacil rates.
[h] Levels of significance represented by * = < 0.05, ** = <0.01, and *** = <0.001.

Table 1.2. Effects of non-ionic surfactant on winter wheat response to POST saflufenacil-based herbicide treatments in a controlled environment[a].

Treatment[b, c, d]	Rate[e]	Necrosis, DAT			Stunting, DAT		Plant dry weight
		3	7	14	7	14	
	g /ha	%					% of control
Saflufenacil + NIS	13	18 cd[e]	21 bc	15 b	13 cd	5 c	69 abc
Saflufenacil + NIS	25	28 ab	32 a	22 a	27 ab	16 ab	58 cde
Saflufenacil + NIS	50	29 ab	34 a	23 a	30 a	17 ab	55 cde
Saflufenacil + 2,4-D + NIS	13+ 533	23 bc	27 ab	20 ab	21 bc	11 b	58 cde
Saflufenacil + 2,4-D + NIS	25+ 533	32 a	34 a	22 a	27 ab	19 a	46 de
Saflufenacil + 2,4-D + NIS	50+533	34 a	36 a	23 a	31 a	18 a	44 e
Saflufenacil + 2,4-D	13+ 533	7 ef	8 de	4 cde	3 e	0 c	80 a
Saflufenacil + 2,4-D	25+ 533	11 e	12 cd	6 cd	4 de	1 c	70 abc
Saflufenacil + 2,4-D	50+ 533	13 de	14 cd	9 c	5 de	1 c	62 bcd
2,4-D + NIS	533	1 f	1 e	1 de	1 e	2 c	70 abc
2,4-D	533	0 f	0 e	0 e	1 e	1 c	78 ab
Untreated[f]	———	0 f	0 e	0 e	0 e	0 c	———
Contrasts[g]							
Safl + NIS vs. Safl + 2,4-D		***[h]	**	***	***	***	*
Safl + NIS vs. Safl + 2,4-D+NIS		*	NS	NS	NS	*	*
Safl + 2,4-D vs. Safl +2,4-D+NIS		***	***	***	***	***	***

[a] Means followed by the same letter within columns are not significantly different based on least square means at $P \leq 0.05$

[b] Abbreviations: NIS, nonionic surfactant; safl, saflufenacil.

[c] Amine formulation of 2,4-D was used in experiment.

[d] NIS applied at 0.25% v/v.

[e] Units for 2,4-D rate are g ae/ha.

[f] Dry weight for the untreated control was 0.48 grams

[g] Contrasts are averaged over saflufenacil rates.

[h] Levels of significance represented by * = <0.05, ** = <0.01, and *** = <0.001.

Table 1.3. Henbit and flixweed control at 3, 7, 14, and 21 DAT with saflufenacil-based herbicide treatments in a controlled environment[a].

Treatment[a, b, c]	Rate[d]	DESSO 3 DAT	LAMAM 3 DAT	DESSO 7 DAT	LAMAM 7 DAT	DESSO 14 DAT	LAMAM 14 DAT	DESSO 21 DAT	LAMAM 21 DAT	Dry weight[f] DESSO	Dry weight[f] LAMAM
	g ai/ha	%								% of control	
Safl + NIS	13	58 b	59 bcd	92 ab	55 cd	95 a	43 gh	94 abc	33 hi	2 bc	54 ab
Safl + NIS	25	60 b	63 bc	96 a	66 bcd	98 a	59 d-g	95 ab	51 e-h	2 bc	36 bc
Safl + NIS	50	60 b	67 bc	96 a	70 bc	99 a	69 b-f	99 a	58 d-h	0 c	27 cd
Safl + 2,4-D + NIS	13 + 533	68 a	72 b	95 a	80 ab	99 a	92 ab	99 a	93 ab	0 c	3 ef
Safl + 2,4-D + NIS	25 + 533	70 a	73 ab	96 a	78 ab	99 a	93 a	99 a	94 ab	0 c	3 ef
Safl + 2,4-D + NIS	50 + 533	70 a	84 a	96 a	90 a	99 a	94 a	99 a	95 a	0 c	2 f
Safl + 2,4-D	13 + 533	67 a	63 bc	95 a	72 bc	99 a	82 a-d	99 a	88 abc	0 c	7 def
Safl + 2,4-D	25 + 533	68 a	72 b	96 a	78 ab	99 a	81 a-e	99 a	86 abc	0 c	9 def
Safl + 2,4-D	50 + 533	70 a	73 ab	95 a	78 ab	99 a	83 abc	99 a	81 a-d	0 c	13 def
Safl + bent + NIS	13 + 560	47 c	47 d	78 c	51 d	90 a	48 fgh	83 c	41 gh	4 bc	38 abc
Safl + bent + NIS	25 + 560	53 bc	55 cd	84 bc	57 cd	89 ab	54 fg	85 bc	48 fgh	6 b	42 abc
Safl + bent + NIS	50 + 560	60 b	67 bc	93 a	67 bcd	95 a	67 c-f	92 abc	63 c-g	1 bc	25 c-f
2,4-D + NIS	533	60 b	60 bcd	65 d	60 cd	77 b	55 fg	93 abc	69 b-f	3 bc	26 cde
2,4-D	533	60 b	60 bcd	60 d	59 cd	63 c	58 efg	83 c	75 a-e	5 b	27 cd
Bentazon + NIS	560	25 d	15 e	32 e	20 e	30 d	27 h	25 d	15 i	44 a	61 a
Untreated[f]	———	0	0	0	0	0	0	0	0	———	———
Contrasts[g]											
Safl vs. safl + 2,4-D+NIS		***[h]	**	NS	**	NS	***	NS	***	NS	***
Safl vs. safl + 2,4-D		***	NS	NS	*	NS	**	NS	***	NS	***
Safl vs. safl + bent + NIS		**	NS	***	NS	NS	NS	*	NS	NS	NS
Safl+2,4-D+NIS vs safl+2,4-D		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Safl+2,4-D+NIS vs. safl+bent+NIS		***	***	***	***	*	***	**	***	*	***
Safl+2,4-D vs. safl+bent+NIS		***	**	***	**	*	**	**	***	*	**

[a] Means followed by the same letter within columns are not significantly different based on least square means at P ≤ 0.05.
[b] Abbreviations: DESSO, flixweed; LAMAM, henbit; bent, bentazon; NIS, nonionic surfactant; safl, saflufenacil.
[c] Amine formulation of 2,4-D was used in experiment.
[d] NIS applied at 0.25% v/v.
[e] Units for 2,4-D rate are g ae/ha.
[f] Dry weight yields for henbit and flixweed were 1.76 and 2.10 grams, respectively.
[g] Contrasts are averaged over saflufenacil rates.
[h] Levels of significance represented by * = < 0.05, ** = <0.01, and *** = <0.001.

CHAPTER 2 - Response of Weeds and Winter Wheat to Saflufenacil Mixed with 2,4-D Amine or Bentazon at Various Rates

ABSTRACT

Two greenhouse experiments were conducted to evaluate the response of winter wheat, kochia, henbit, and flixweed to POST treatments of saflufenacil mixed with various rates of 2,4-D amine or bentazon. In the first experiment, saflufenacil was applied to wheat, henbit, kochia, and flixweed at 0, 13, 25, and 50 g ai/ha in combination with 2,4-D amine at 0, 67, 133, 267, and 533 g ae/ha. Adjuvant was not included in any treatment. In the second experiment, saflufenacil was applied to the same plant species at the same saflufenacil rates, but mixed with bentazon at 0, 70, 140, 280, and 560 g ai/ha. Crop oil concentrate (COC) was included at 1% v/v with all saflufenacil and bentazon combinations except the untreated control (0 g/ha combination of saflufenacil and bentazon). Flixweed plant dry weights were reduced ≥99% when saflufenacil at 13, 25, or 50 g/ha was mixed with at least 67 g/ha of 2,4-D amine compared to the untreated control. Saflufenacil rates at 13 or more g/ha completely controlled flixweed, regardless of bentazon rate. Flixweed also was completely controlled by bentazon at 533 g/ha without saflufenacil. Less 2,4-D amine was needed to achieve 90% growth reduction of kochia as saflufenacil rate increased from 13 to 25 to 50 g/ha (GR_{90} = 373, 220, and 110 g/ha of 2,4-D amine, respectively). Kochia plant dry weights were ≤10% of the untreated control when 13, 25, and 50 g/ha of saflufenacil were mixed with any rate of bentazon. Overall, henbit was less susceptible to herbicide treatments compared to flixweed and kochia in both experiments. Saflufenacil rates of at least 25 g/ha or greater and 2,4-D amine at 267 and 533 g/ha reduced henbit plant dry weights by >70% compared to the untreated control, whereas henbit plant dry weights were reduced 89% when 560 g/ha of bentazon was mixed with at least 25 g/ha of saflufenacil. No herbicide combination in either study completely controlled henbit. Wheat plant dry weights were reduced <10% by 50 and 533 g/ha of saflufenacil and 2,4-D amine, respectively. Averaged over bentazon rates, winter wheat plant dry weights generally decreased as saflufenacil rate increased. Also, wheat plant dry weights were reduced 23% when saflufenacil was applied without bentazon averaged over saflufenacil rates. Overall these studies indicate saflufenacil can potentially be POST-applied in winter at 25 g/ha without severe dry weight reductions. Saflufenacil and companion herbicide rates required for weed control were weed species dependent, but herbicide treatments including saflufenacil were more effective in controlling flixweed and kochia than solo applied 2,4-D amine or bentazon.

Nomenclature: bentazon; saflufenacil; 2,4-D amine, *Descurainia sophia* L. Webb. ex Prantl, DESSO; henbit, *Lamium amplexicaule* L., LAMAM; kochia, *Kochia scoparia* (L.) Schrad., KCHSC; winter wheat, *Triticum aestivum* L. 'Danby'.

Key words: winter annual broadleaf weeds, BAS 800H, crop response, wheat

INTRODUCTION

Winter annual broadleaf weeds such as flixweed, henbit, field pennycress (*Thlaspi arvense* L.), bushy wallflower (*Erysimum repandum* L.), blue mustard [*Chorispora tenella* (Pallas) DC.], sheperd's purse [*Capsella bursa-pastoris* (L.) Medik.], and pinnate tansymustard [*Descurainia pinnata* (Walt.) Britt.] are common in winter wheat fields in the United States (Dowler 1994; Klein 2005; Peel et al 1997; Peterson 1997). Left uncontrolled, these weeds can significantly reduce wheat grain yield. In Kansas, wheat grain yield was reduced 25% by an uncontrolled population 272 bushy wallflower plants/m^2 (Peterson 1997). In Washington, season-long competition of 11, 33, and 98 blue mustard plants/m^2 reduced wheat grain yields by 28, 42, and 51%, respectively (Swan 1971). Even when blue mustard was controlled with herbicides in spring infestations at those densities still reduced wheat yields by 13, 21, and 29%, respectively (Swan 1971). Other studies reported henbit and flixweed reduced wheat yields 38 to 48% and 13 to 34%, respectively (Conley and Bradley 2005; Northam et al. 1993).

Acetolactate synthase (ALS)-inhibiting herbicides have been used to control many winter annual broadleaf weed species. However the continuous and often exclusive use of ALS-inhibiting herbicides in wheat has led to the development ALS-resistant weed biotypes. Recently, populations of bushy wallflower (*Erysimum repandum* L.) and flixweed have been documented as resistant to ALS-inhibiting herbicides in central Kansas (Peterson et al. 2006; Peterson et al. 2009). Acetolactate synthase-resistant biotypes of other troublesome weeds in wheat such as kochia, Russian thistle (*Salsola tragus* L., and prickly lettuce (*Lactuca serriola* L.) have also been documented in wheat growing regions of the United States (Peterson 1999; Primiani et al. 1990; Mallory-Smith et al. 1990). Currently, 39 weed species have been documented to be resistant to ALS-inhibiting herbicides in the United States (Heap 2009).

The presence of ALS-resistant weeds in wheat severely reduces the number of herbicide options available to growers. Auxin herbicides are the second most utilized herbicide family for weed control in wheat; 39% of treated wheat in the U.S. in 2006 received an auxin herbicide (USDA 2007). However, some winter annual broadleaf weeds such as blue mustard, flixweed, field pennycress, and henbit can be difficult to control with only auxin herbicides (Bernards et al. 2009; Thompson et al. 2009). Few herbicide options are available for weed control in wheat other than ALS-inhibiting and auxin herbicides.

Saflufenacil is an experimental herbicide being developed for burndown and preemergence control of broadleaf weeds in many crops. This herbicide controls susceptible plants through inhibition of protoporphyrinogen oxidase (protox) (Anonymous 2008) and may be a useful tool to control herbicide resistant weeds in winter wheat and other crops. However, few PRE herbicide applications are made by winter wheat growers because most prefer to apply herbicides POST in spring (D. E. Peterson and P. W. Stahlman, personal communication).

Few studies have been conducted to evaluate POST saflufenacil treatments in winter wheat. Saflufenacil applied POST with an adjuvant caused unacceptable injury and reduced yield in barley, oats, spring wheat, and corn (Frihauf et al. 2008a; Frihauf et al. 2008b; Sikkema et al. 2008; Soltani et al 2008). A wheat dose response study of POST-applied saflufenacil in fall found 54, 38, and 10 g/ha of saflufenacil applied alone and mixed with NIS or COC reduced grain yield by 5% (Knezevic et al. 2008). In addition, grain yields were reduced by lower rates of saflufenacil applied-POST in wheat during the spring (24, 7, and 4 g ai/ha of saflufenacil applied alone and tank-mixed with NIS, or COC, respectively) (Knezevic et al. 2008). A growth chamber study found saflufenacil applied at 13, 25, and 50 g/ha with adjuvant caused 19 to 40% leaf necrosis at 3 DAT, but mixtures with 2,4-D amine without adjuvant or bentazon plus NIS minimized leaf necrosis and resulted in wheat dry biomass similar to or greater than plants treated with 2,4-D amine (unpublished data).

Other studies have also reported reduced levels of saflufenacil injury in POST applications of saflufenacil with 2,4-D amine or bentazon plus COC. Field studies in Kansas found leaf necrosis and stunting were minimized by tank mixing 2,4-D amine with water dispersible granule (WG) or emulsifiable concentrate (EC) saflufenacil formulations (Frihauf et al. 2008a). In addition, solo application of these formulations and tank mixes of EC and WG formulations with 2,4-D amine provided >90% blue mustard control. Other studies found POST-applied saflufenacil at 25 g/ha mixed with dicamba, or 2,4-D amine caused little leaf necrosis, effectively controlled blue mustard and flixweed, and did not negatively impact grain yield under conditions of low weed density (Frihauf et al. 2008b). Mixtures of saflufenacil at 13 and 25 g/ha with bentazon and COC also reduced leaf necrosis, but control of blue mustard and flixweed was reduced by 13 g/ha of saflufenacil mixed with bentazon and COC compared to the same saflufenacil rate with COC. Blue mustard control was not reduced and similar to 25 g/ha of saflufenacil with COC when the high rate of saflufenacil was mixed with bentazon and COC, but flixweed control was inconsistent among experiments.

Research has shown that 2,4-D amine and bentazon may reduce saflufenacil injury in winter wheat, but it is not known what 2,4-D amine or bentazon rates are needed to reduce wheat injury and maintain control of broadleaf weeds. The objectives of this research were evaluate the response of winter wheat, kochia, henbit, and flixweed to POST treatments of saflufenacil mixed with various rates of 2,4-D amine or bentazon.

MATERIALS AND METHODS

Two greenhouse experiments were conducted during the spring of 2008. Winter wheat, henbit, flixweed, and kochia were grown under greenhouse conditions with 25/20 C day/night temperatures, 16 hr photoperiod, and supplemental light at a photosynthetic photon flux density of 84μmol/m^2/s. Plant species were sown separately in 11-cm diam by 16-cm tall plastic pots containing a mixture of sand and Morril loarm (mesic Typic Argiudolls) (1:2 by vol). The mixed growth medium had a pH of 8.0 and 1.2% organic matter. Wheat seedlings were thinned to two plants per pot and each weed species was thinned to a final population of four plants per pot. Plants in each experiment were watered and fertilized[1] as needed.

The experimental design of experiment 1 was a randomized complete block with factorial arrangement of saflufenacil at 0, 13, 25, and 50 g ai/ha, 2,4-D amine at 0, 67, 133, 267, and 533 g ae/ha, and the four previously mentioned plant species. The 25 g/ha rate of saflufenacil is the proposed field use rate for burndown control of broadleaf weeds. The 1x rate of 2,4-D amine at 533 g/ha is the high labeled use rate for winter wheat. Design of experiment 2 was similar to experiment 1 except bentazon was substituted for 2,4-D amine. Saflufenacil was applied at the same rates with bentazon at 0, 70, 140, 280, and 560 g ai/ha to winter wheat, henbit, flixweed, and kochia. All herbicide treatments in experiment 2 included crop oil concentrate[2] (COC) at 1.0% v/v; the untreated control (0 g/ha combination of saflufenacil and bentazon) did not. Herbicide treatments were applied using a bench-type sprayer[3] equipped with a single 80015 LP nozzle[4] delivering 187 L/ha at 207 kPa and 3 km/hr. Herbicide treatments in both experiments were applied to wheat measuring 20 to 30-cm tall with 2 to 4 tillers. Henbit and flixweed were 2 to 10-cm in diam, and kochia were 2 to 10-cm tall at application. At 21 DAT, plant dry weights were determined by cutting aboveground biomass even with the soil surface. The samples were then dried at 60 C for 48 hr and weighed.

Data were checked for normality and homogeneity of variances. Data of each plant species was subjected to ANOVA using PROC MIXED in SAS[5] with saflufenacil rates, 2,4-D amine rates, and all possible interactions as fixed effects and run and replicates within runs as random effects. Means were separated using least square means at $P \leq 0.05$. Prior to analysis plant dry weight for all experiments were converted to a percentage of the untreated control.

Relative dry weight data of each species were subjected to regression analysis using SigmaPlot10[6]. Dry weight data were regressed against saflufenacil, 2,4-D amine rate or bentazon rate using the equation:

$$Y = B0+B1*e^{(-B2*x)}$$

where Y is relative dry weight, B0 is the lower asymptote, B1is the reduction in Y from the upper to the lower asymptote, B2 is the rate at which the lower asymptote is reached, and x is 2,4-D amine or bentazon rate (Chism et al. 1992). Simple linear or quadratic regression was fit to data when the previously described model did not fit the data. A lack of fit test of each model was performed by partitioning sums of squares into lack of fit error and pure experimental error (Draper and Smith 1981). Models were considered appropriate if an F-test value for lack of fit sums of squares was not significant at $\alpha = 0.05$. Herbicide rates needed to reduce plant dry weight by 90% (GR90) were determined from regression equations.

RESULTS AND DISCUSSION

Saflufenacil & 2,4-D Amine Combinations

Flixweed plant dry weight decreased with increasing 2,4-D amine rate when saflufenacil was not included (Figure 2.1). Solo application of 2,4-D amine at 67 g/ha reduced flixweed dry weight by 71%, but near complete control was not achieved until 2,4-D amine rate reached 533 g/ha. Conversely, saflufenacil at 13 and 25 g/ha plus any rate of 2,4-D amine tested resulted in plant death. The 50 g/ha rate of saflufenacil applied alone completely controlled flixweed regardless of 2,4-D amine rate. It was not beneficial to increase the 2,4-D rate above 67 g/ha when applied with 13 or 25 g/ha of saflufenacil.

Kochia plant dry weight decreased as 2,4-D amine rate increased within each saflufenacil rate (Figure 2.2). Reduction in kochia dry weight did not exceed 36% when 2,4-D amine was applied without saflufenacil (B0 = 64). Increasing the rate of saflufenacil alone from 13 or 25 to 50 g/ha reduced plant dry weight, thus indicating increased control of kochia; differences between 13 and 25 g/ha rates were not significant. Plant dry weights for each rate of saflufenacil were reduced further with incremental additions of 133 and 267 g/ha of 2,4-D amine. However at 2,4-D amine rates higher than 267 g/ha, increasing the saflufenacil rate from 13 to 50 g/ha was less beneficial. Less 2,4-D amine was needed to reduced kochia dry weight by 90% as saflufenacil rate increased from 13 to 25 to 50 g/ha (GR_{90} = 373, 220, and 110 g/ha of 2,4-D amine, respectively). Complete control of kochia was only achieved when saflufenacil at 13, 25, and 50 g/ha was mixed with 533 g/ha of 2,4-D amine.

The relationship between henbit dry weight and saflufenacil or 2,4-D amine rates were weak (R^2 = 0.19 and 0.23, respectively) (Figures 2.3 and 2.4). Averaged over runs and 2,4-D amine rates, henbit dry weights decreased as saflufenacil rates increased from 0 up to 50 g/ha. Also, henbit dry weights decreased as 2,4-D amine rates increased from 0 to 533 g/ha averaged over runs and saflufenacil rates. Complete control of henbit was not achieved with any saflufenacil and 2,4-D amine combination tested.

Averaged over 2,4-D amine rates and runs, wheat dry weight was reduced 9% by the 50 g/ha rate of saflufenacil, but wheat dry weights were not affected by lower rates of saflufenacil (Figure 2.5). Similarly, wheat dry weights averaged over saflufenacil rates were reduced by 10% when treated with 533 g/ha of 2,4-D amine, but were not affected by lower rates of 2,4-D amine (Table 2.6).

Saflufenacil & Bentazon Combinations

Solo applications of saflufenacil at 13, 25, and 50 g/ha reduced dry weights of flixweed (Figure 2.7) and kochia (Figure 2.8) by $\geq$90%. Mixing bentazon with saflufenacil did not affect control of either species. Dry weights of flixweed and kochia decreased with increasing rates of solo bentazon treatments. A higher bentazon rate was required to reduce the dry weight of kochia to 90% compared to flixweed (GR_{90} = 533 and 362 g/ha of bentazon, respectively). Complete control of flixweed with bentazon alone required a rate of 560 g/ha, whereas, kochia was not completely controlled with any bentazon rate tested. Saflufenacil at 13, 25, and 50 g/ha applied alone completely controlled flixweed and kochia and the addition of bentazon to these saflufenacil rates was not beneficial.

Bentazon alone generally was less effective in controlling henbit than most treatments containing saflufenacil (Figure 2.9). However, saflufenacil generally was not as effective on henbit when applied without bentazon, but solo applications of saflufenacil at 13, 25, and 50 g/ha were more effective than solo bentazon applications at 70 and 140 g/ha. Henbit dry weights declined as bentazon rates increased from 0 up to 560 g/ha when applied without saflufenacil. Bentazon at 455 g/ha was needed to reduced henbit dry weight by 90% when bentazon was applied alone. Also, dry weights of henbit decreased linearly as bentazon rates increased when applied with 13 g/ha of saflufenacil, but the relationship between henbit dry weights and bentazon rates within the 13 g/h rate of saflufenacil was weak ($R^2 = 0.29$). In general, dry weights were lowest when saflufenacil was applied at 50 g/ha with any rate of bentazon and when 560 g/ha of bentazon was applied with any saflufenacil rate (>80% reduction). Saflufenacil and bentazon combinations tested did not completely control henbit.

Averaged over bentazon rates, winter wheat dry weights generally decreased as saflufenacil rate increased (Figure 2.10). Saflufenacil at 50 g/ha reduced wheat plant dry weight by 25%, compared to reductions of 15% for saflufenacil rates of 13 and 25 g/ha. Dry weight reduction was 3% when bentazon was applied without saflufenacil.

Averaged over saflufenacil rates, winter wheat plant dry weights were reduced 17 to 23% when bentazon was applied at 0 and 70 g/ha (Figure 2.11). However, saflufenacil plus bentazon at 140, 280, and 560 g/ha resulted in similar dry weight reductions of 15, 10, 15, and 11%, respectively. Dry weight reductions caused by these treatments were significantly less compared to when bentazon was not applied.

These studies show it may be possible to use saflufenacil in winter wheat POST to control annual broadleaf weeds without negatively affecting wheat. However, further research is needed to determine efficacy of saflufenacil in combination with various rates of 2,4-D amine or bentazon plus COC when applied to broadleaf weeds at different growth stages under field conditions. Additional research is also needed to identify the mechanism for the reduced phytotoxicity of saflufenacil when saflufenacil is POST applied in combination with 2,4-D amine or bentazon in winter wheat.

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SOURCES OF MATERIALS

[1] Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 14111 Scottslawn Road, Marysville, OH 43041.

[2] Crop oil concentrate, Agri-dex, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

[3] Research Track Sprayer SB-8, Devries Manufacturing, 28081 870th Ave., Hollandale, MN, 56045.

[4] Spraying Systems Co., N. Avenue at Schmale Road, P.O. Box 7900, Wheaton, IL 60189-7900.

[5] SAS version 9.1, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

[6] SigmaPlot10, Systat Software, Inc., 1735 Technology Dr. Ste 430, San Jose, CA 95110.

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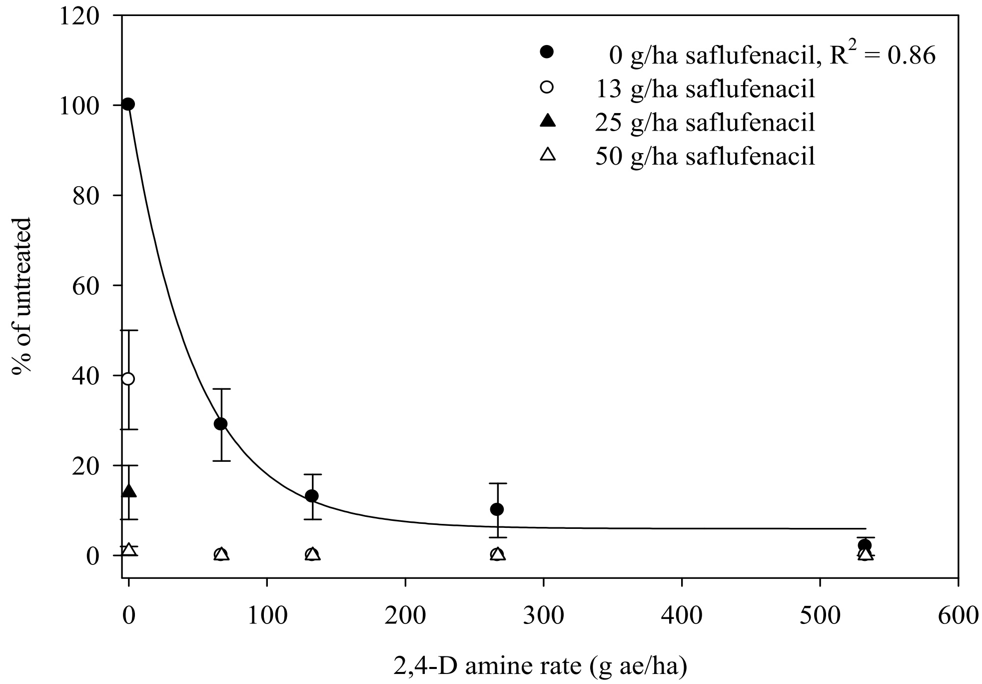

Figure 2.1. Effect of saflufenacil and 2,4-D amine mixtures on flixweed dry weight. Data are means plus or minus the standard error. The regression equation for 0 g/ha of saflufenacil is percent of untreated = $5.72+93.17*e^{(-0.02*x)}$. A regression equation could not be fitted to data from flixweed plants treated with saflufenacil at 13, 25, or 50 g/ha in combination with 2,4-D amine rates.

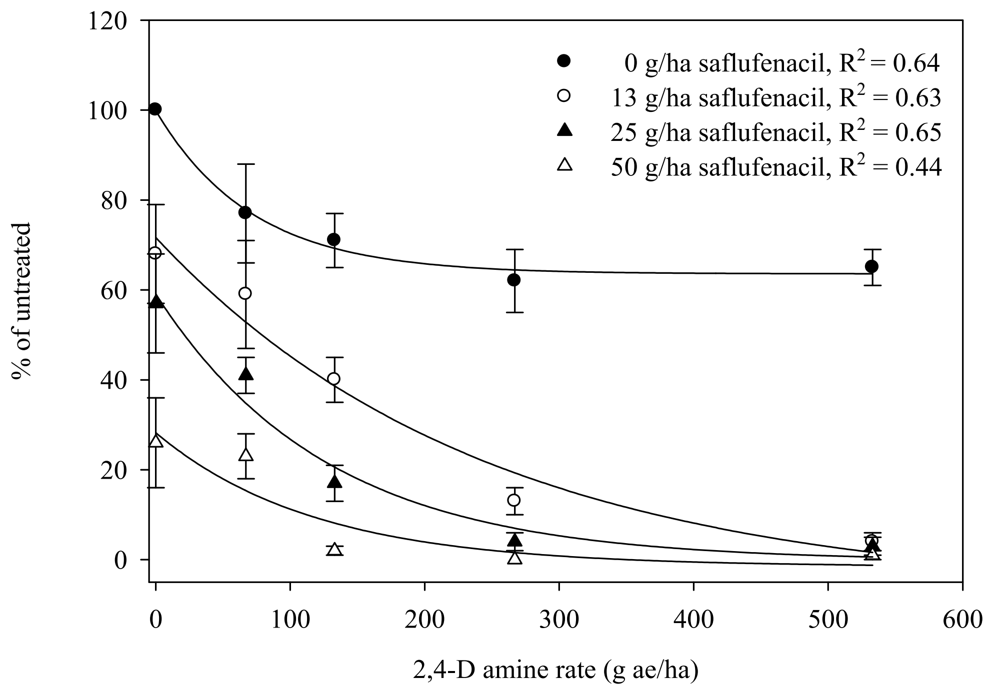

Figure 2.2. Effect of saflufenacil and 2,4-D amine mixtures on kochia dry weight. Data are means plus or minus the standard error. The regression equations for 0, 13, 25, and 50 g/ha saflufenacil are percent of untreated = $63.78+36.19*e^{(-0.01*x)}$, percent of untreated = $-4.93+80.89*e^{(-0.005*x)}$, percent of untreated = $-0.79+60.02*e^{(-0.008*x)}$, and percent of untreated = $-1.18+30.03*e^{(-0.009*rate)}$, respectively.

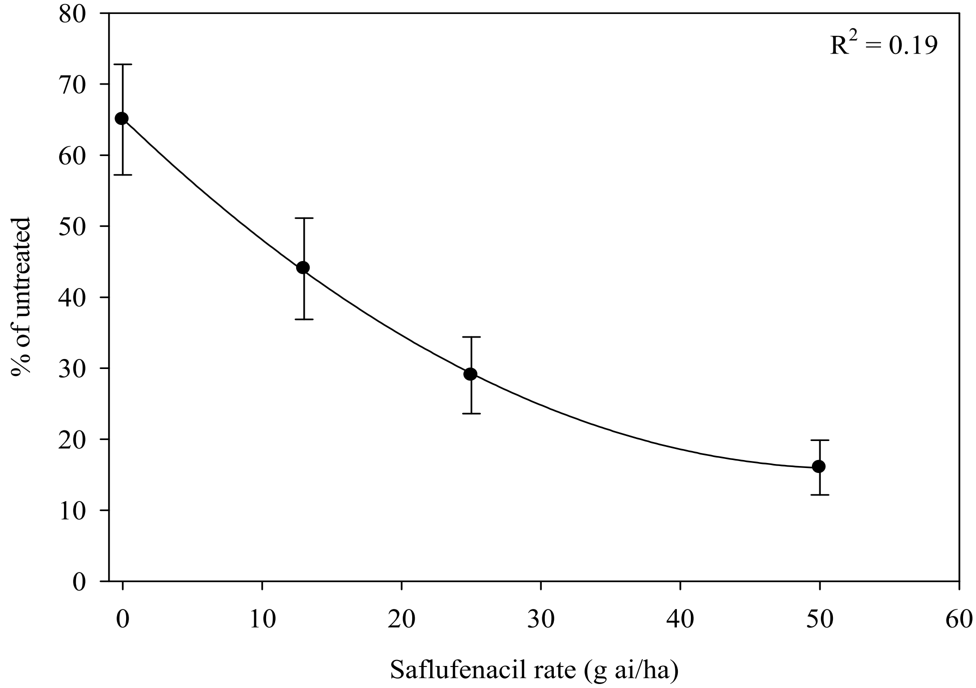

Figure 2.3. Effect of saflufenacil on henbit dry weight averaged over 2.4-D amine rates. Data are means plus or minus the standard error. The regression equation is percent of untreated = 65.63 – 1.93x + 0.02x^2.

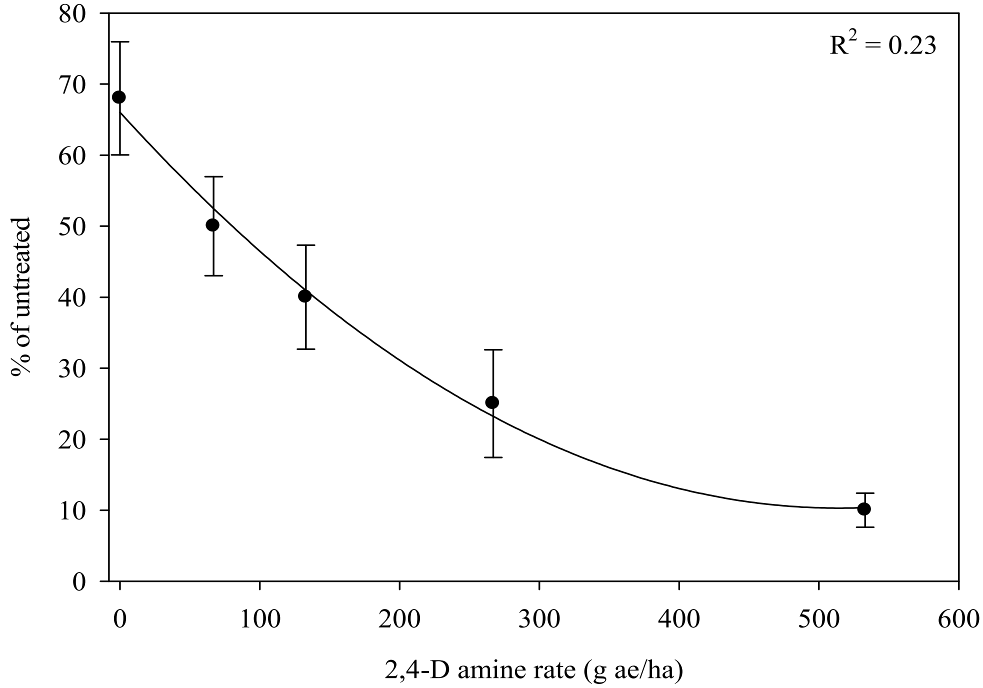

Figure 2.4. Effect of 2,4-D amine on henbit dry weight averaged over saflufenacil rates. Data are means plus or minus the standard error. The regression equation is percent of untreated = 66.000 - 0.217x + $0.002x^2$.

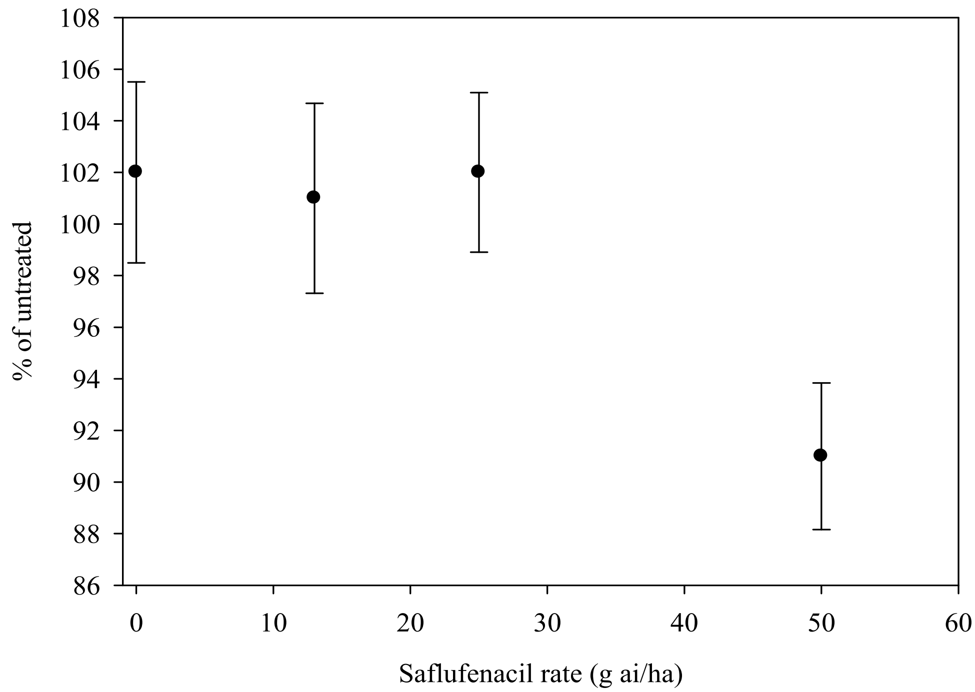

Figure 2.5. Effect of saflufenacil on winter wheat dry weight averaged over 2,4-D amine rates. Data are means plus or minus the standard error. A regression equation could not be fitted to data.

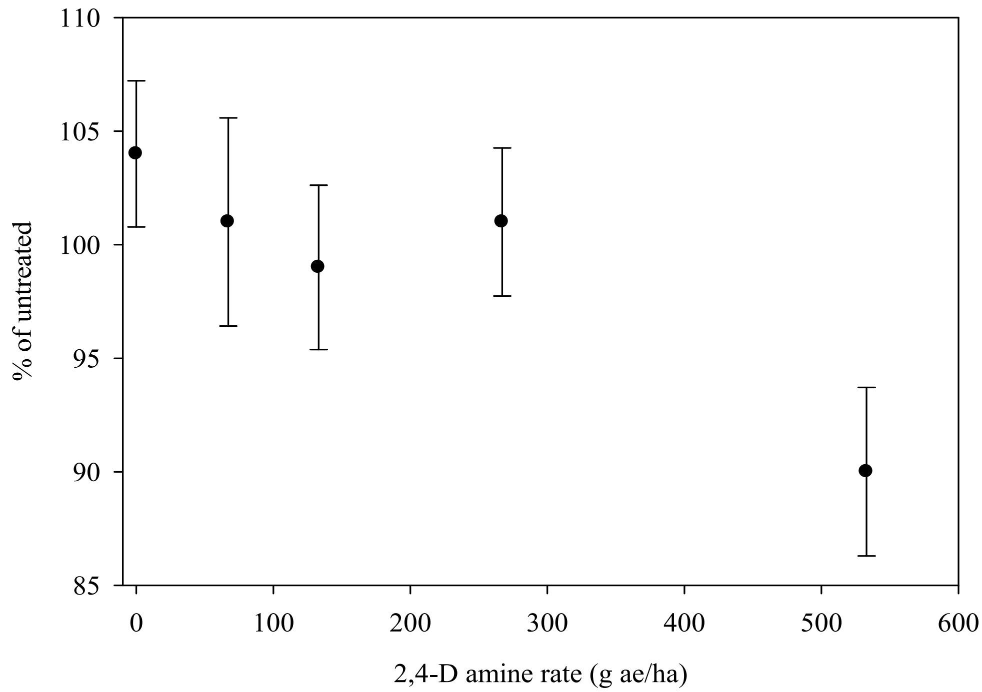

Figure 2.6. Effect of 2,4-D amine on winter wheat dry weight averaged over saflufenacil rates. Data are means plus or minus the standard error. A regression equation could not be fitted to data.

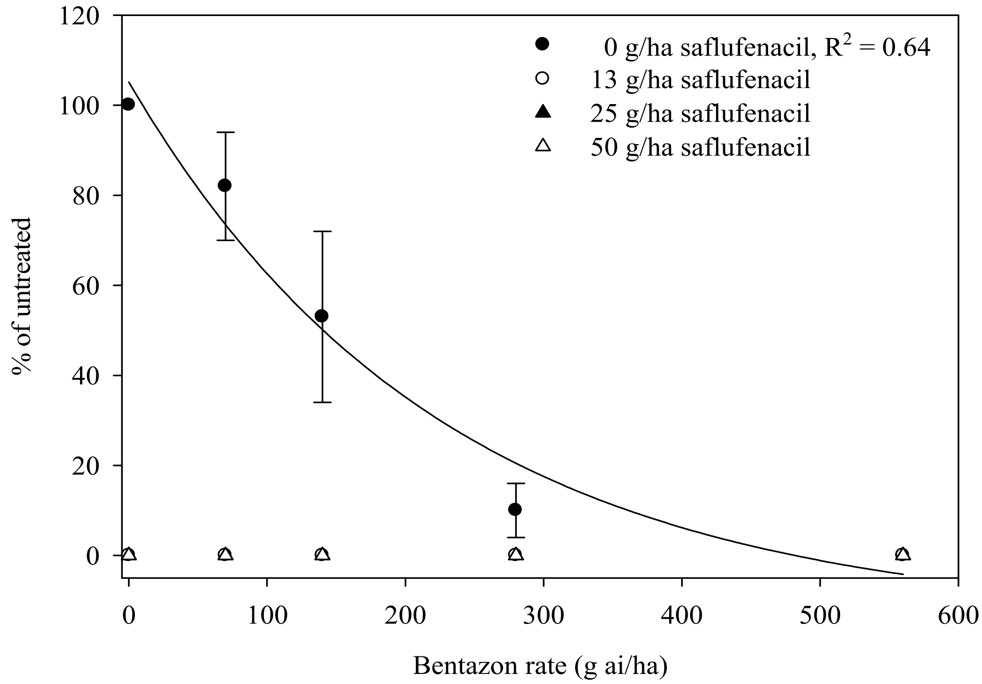

Figure 2.7. Effects of saflufenacil and bentazon rate combinations on flixweed dry weight. Data are means plus or minus the standard error. The regression equation for 0 g/ha of saflufenacil is percent of untreated = $-15.18+120.37*e^{(-0.004*x)}$. A regression equation could not be fitted to data from flixweed plants treated with saflufenacil at 13, 25, or 50 g/ha in combination with bentazon rates.

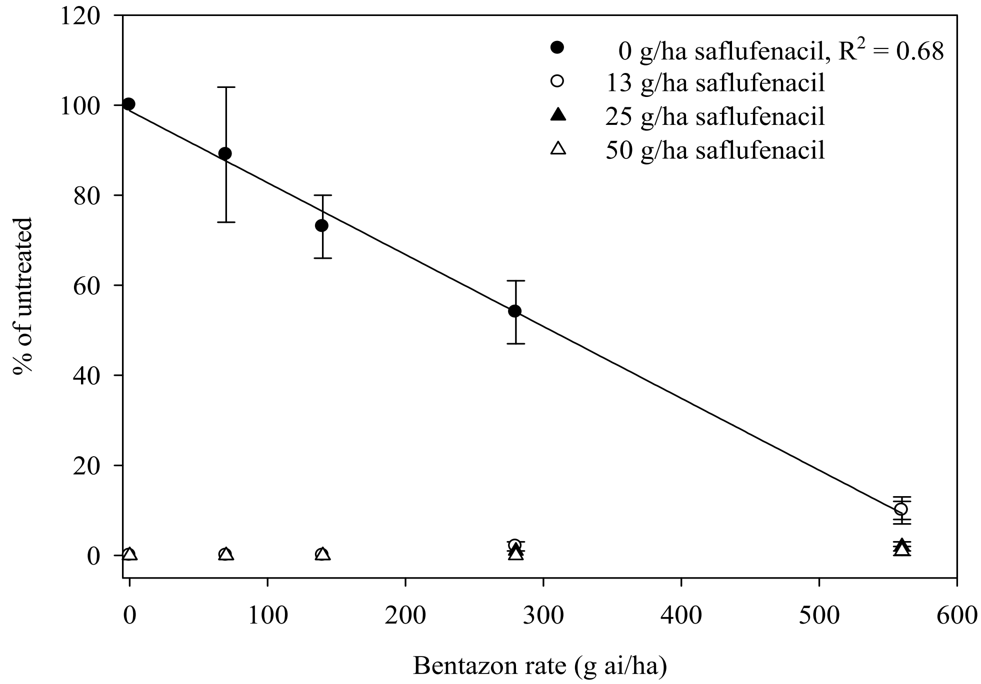

Figure 2.8. Effects of saflufenacil and bentazon rate combinations on kochia dry weight. Data are means plus or minus the standard error. The regression equation for 0 g/ha of saflufenacil is percent of untreated = 98.99 - 0.16(x). A regression equation could not be fitted to data from kochia plants treated with saflufenacil at 13, 25, or 50 g/ha in combination with 2,4-D amine rates.

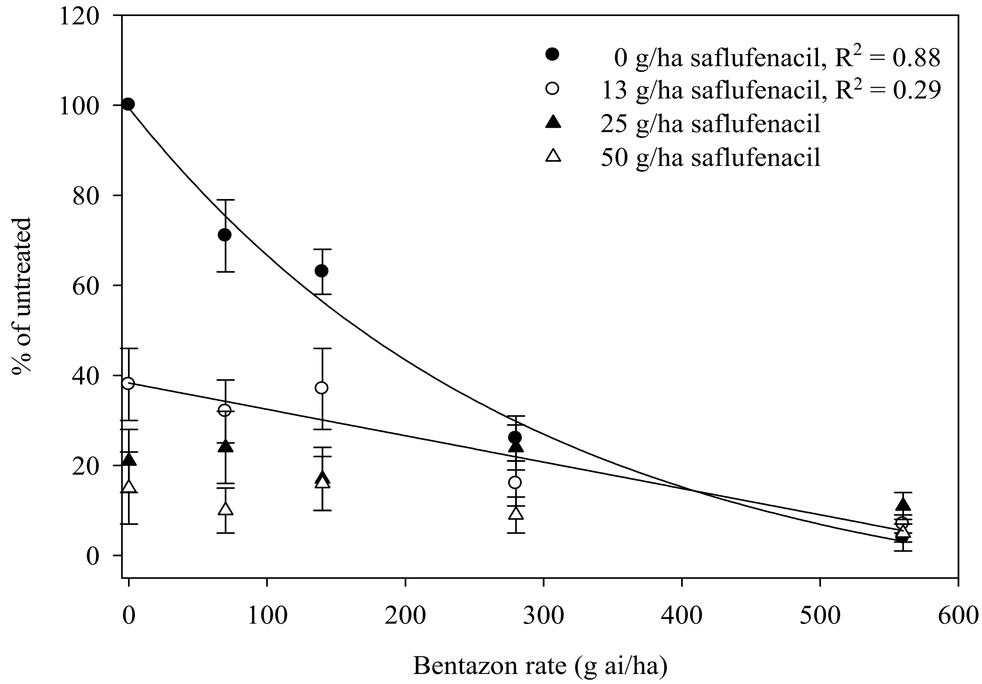

Figure 2.9. Effect of saflufenacil and bentazon rate combinations on henbit dry weight. The regression equations for 0 and 13 g/ha saflufenacil are percent of untreated = $-14.65+114.41*e^{(-0.003*x)}$ and percent of untreated = 38.14 - 0.06(x), respectively. A regression equation could not be fitted to data from henbit plants treated with saflufenacil at 25 or 50 g/ha in combination with bentazon rates.

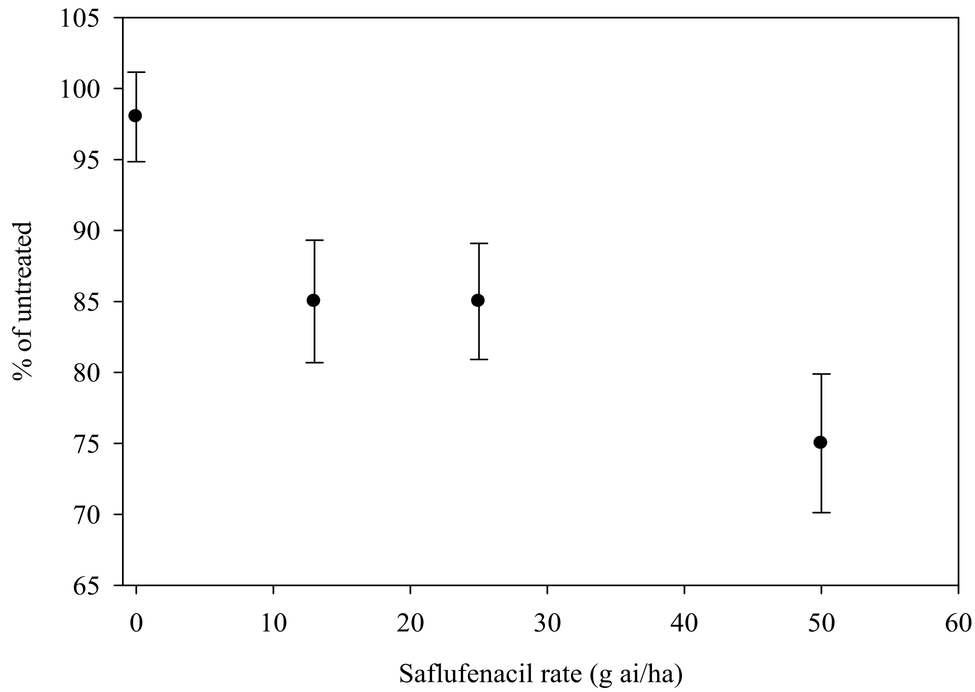

Figure 2.10. Effect of saflufenacil on winter wheat dry weight averaged over bentazon rates. Data are means plus or minus the standard error. A regression equation could not be fitted to data.

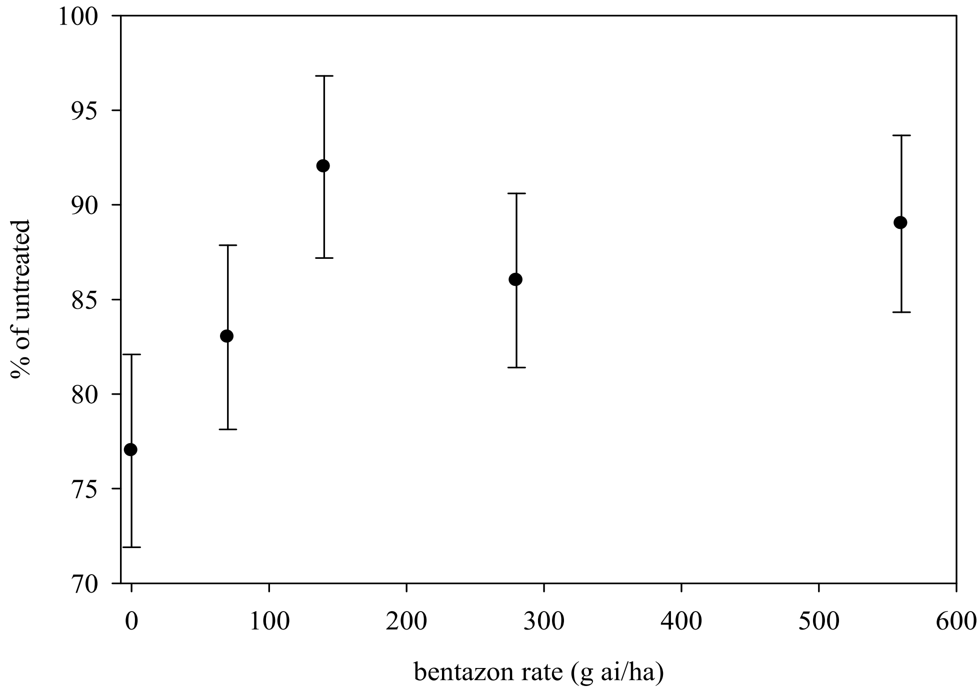

Figure 2.11. Effect of bentazon on winter wheat dry weight averaged over saflufenacil rates. Data are means plus or minus the standard error. A regression equation could not be fitted to data.

CHAPTER 3 - Winter Annual Broadleaf Weeds and Winter Wheat Response to Two Saflufenacil Formulations

ABSTRACT

Field experiments were conducted at two locations during the 2006-2007 and 2007-2008 winter wheat growing seasons to evaluate winter annual broadleaf weeds and winter wheat response to POST applications of two saflufenacil formulations alone and in combination with 2,4-D amine. Emulsifiable concentrate (EC) and water dispersible granule (WG) formulations of saflufenacil at 13, 25, and 50 g ai/ha were applied with 1.0% v/v crop oil concentrate (COC) and in combination with 2,4-D amine at 533 g ae/ha without adjuvant. Regardless of rate, saflufenacil plus COC and saflufenacil plus 2,4-D amine controlled blue mustard ≥91% at 17 to 20 DAT compared to ≤50% control with 2,4-D amine alone. Blue mustard control was similar between saflufenacil formulations within and between most saflufenacil and saflufenacil plus 2,4-D amine treatments; 2,4-D amine mixed with 13 g/ha of the WG saflufenacil formulation was less efficacious than the WG formulation alone in one of four experiments. At least 25 g/ha of saflufenacil was needed to control flixweed >90%, which was much greater control than achieved with 2,4-D amine. Flixweed control was decreased by mixing 2,4-D amine with saflufenacil compared to saflufenacil plus COC. The adverse effect of 2,4-D amine on flixweed control with saflufenacil was greater with the WG than the EC formulation of saflufenacil. Most saflufenacil treatments did not control henbit satisfactorily, especially at 13 g/ha saflufenacil (≤80% control). Wheat foliar necrosis increased with increasing saflufenacil rate to as high as 30% at 3 to 6 DAT, but declined to <15% at 15 ± 5 DAT and disappeared completely at 30 DAT. Saflufenacil rate, formulation, and mixing with 2,4-D amine also influenced wheat stunting, but to a lesser extent than foliar necrosis. However, the period of recovery from stunting was longer than the time required for plants to recover from leaf necrosis. The EC formulation consistently caused greater foliar necrosis and stunting than the WG formulation. Leaf necrosis and stunting were reduced by tank mixing either saflufenacil formulation with 2,4-D amine. Grain yields of most saflufenacil treatments were similar to 2,4-D amine under weedy conditions and herbicide treatments had no affect on grain yield in weed-free experiments. This research showed that POST saflufenacil effectively controls blue mustard and flixweed without reducing wheat grain yields despite foliar crop injury. Injury severity can be reduced by applying the WG formulation of saflufenacil instead of the EC formulation and by tank mixing saflufenacil with 2,4-D amine.

Nomenclature: saflufenacil; 2,4-D amine; blue mustard, *Chorispora tenella* (Pallas) DC., COBTE; flixweed, *Descurainia sophia* L. Webb. ex Prantl, DESSO; henbit, *Lamium amplexicaule* L., LAMAM; winter wheat, *Triticum aestivum* L., ‘AP502-CL’, ‘Danby’, and ‘KS03HW6-1’.

Key words: winter annual broadleaf weeds, BAS 800H, crop response

INTRODUCTION

Weed management is necessary for maximum winter wheat production in most areas of the United States. A chemical use survey revealed that synthetic auxins and acetolactase synthase (ALS)-inhibiting herbicides accounted for 81% of all herbicides used in wheat in 2004 (USDA 2005). By 2006, auxins and ALS-inhibitor were applied to 95% of treated winter wheat hectares with ALS-inhibitor accounting for 56% of the total (USDA 2007).

The extensive use of ALS-inhibiting herbicides in winter wheat has resulted in the development of ALS-resistant weed species. Kochia [*Kochia scoporia* (L.) Shrad.] populations resistant to chlorsulfuron were first reported in Kansas in 1987, and now occur throughout the Great Plains, Intermountain and Pacific Northwest regions of the United States (Heap 2009; Primiani et al. 1990). Also in 1987, ALS-resistant populations of prickly lettuce (*Lactuca serriola* L.) were confirmed in Idaho and subsequently in Oregon and Washington (Heap 2009; Mallory-Smith, et al. 1990). More recently, ALS-resistant populations of bushy wallflower (*Erysimum repandum* L.), flixweed, Russian thistle (*Salsola tragus* L.), and Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] have been documented in winter wheat production areas of the United States (Ellis et al. 2008; Heap 2009; Peterson et al 2006; Peterson et al. 2009).

Integrated weed management practices such as crop rotation, sanitation, rotation of herbicide modes-of-action, and tank mix partners with different modes of action can delay or prevent the development of herbicide resistant weeds (Lee et al. 2000; Mallory-Smith et al. 1993; Regehr and Morishita 1989). However, there are few herbicides for use in winter wheat with different modes-of-action to use in tank mixtures or to establish herbicide rotations.

Saflufenacil is a new herbicide being developed for burndown and preemergence control of broadleaf weeds. This herbicide controls susceptible species through inhibition of protoporphyrinogen oxidase (protox) (Anonymous 2008) and may be a useful tool to control herbicide resistant weeds in winter wheat. However, saflufenacil must be applied to winter wheat POST to be useful because many winter wheat growers prefer to apply herbicides after green-up in spring (D. E. Peterson and P. W. Stahlman, personal communication).

Few studies have evaluated POST saflufenacil treatments in winter wheat. However, in Canada POST application of the water-dispersible granule (WG) formulation of saflufenacil with an adjuvant caused unacceptable levels of injury and yield loss in barley, oats, spring wheat, and corn (Sikkema et al. 2008; Soltani et al. 2008). Growth chamber experiments found that mixing 2,4-D amine or bentazon with saflufenacil lessened leaf necrosis and resulted in wheat plant dry weights similar or greater than plants treated with 2,4-D amine (unpublished data). At 21 DAT, saflufenacil at 13, 25 or 50 g/ha mixed with 2,4-D amine or 50 g/ha of saflufenacil mixed with bentazon controlled flixweed 99 and 92%, respectively, but henbit control with these treatments was ≤88%.

Three formulations of saflufenacil are planned for commercial development including WG, suspension concentrate (SC), and emulsifiable concentrate (EC) formulations (Anonymous 2008). Research has found that crop tolerance can differ between formulations of the same herbicide active ingredient. Broccoli (Gast et al. 2004), cabbage (Hatterman-Valenti and Auwarter 2007), and onions (Richardson et al. 2006) were injured less when sprayed POST with the SC formulation of oxyflurofen, a protox-inhibiting herbicide, compared to the EC formulation at equivalent rates. Weller and Carpenter (1983) reported cranberry cotoneaster (*Cotoneaster apiculatus* Rehder & E.H. Wilson), white creeper [*Euonymus fortunei* (Turcz.) Hand.-Maz.], and creeping juniper (*Juniperus horizontalis* Moench) were injured more by granular and EC formulations of oxyflurofen than by a wettable powder formulation. Other examples include differences between formulations in creeping bentgrass response to POST dithiopyr (Bevard and Watschke 1999); glyphosate-resistant soybean response to different glyphosate formulations (Reddy and Zablotowicz 2003); and rice response to propanil (Baltazar and Smith 1994).

Differences in weed response between formulations of the same herbicide active ingredient have also been documented. The EC and SC formulations of oxyfluorfen provided similar weed control in onions when applied at the first true leaf stage of onion, but the SC formulation was less effective than the EC formulation when applied at the second true leaf stage (Richardson et al. 2006). Another study found broadleaf weed control with oxyfluorfen was greater with POST application of EC formulation than the SC formulation at equivalent rates (Gast et al. 2004). Barnyardgrass control in Louisiana sometimes was controlled better by the EC formulation of propanil than the dry flowable formulation (Jordan et al. 1997). Molin and Hirase (2004) reported that a glyphosate formulation containing 1-aminomethanamide dihydrogen tetraozsultate was 2 to 3-times more active than two isopropylamine salt formulations of glyphosate on prickly sida (*Sida spinosa* L.), sicklepod (*Senna obtusifolia* L.), morningglory (*Ipomoea hederacea* var. *integriuscula* Gray), and purple nutsedge (*Cyperus rotundus* L.).

Research comparing winter wheat and/or weed response to different formulations of saflufenacil is limited. The objectives of this research were to (1) evaluate winter wheat and winter annual broadleaf weed species response to POST treatments of two saflufenacil formulations applied alone and mixed with 2,4-D amine and (2) to determine winter wheat response to these treatments under weed-free conditions.

MATERIALS AND METHODS

Two weed control and two weed-free crop tolerance experiments were conducted near Hays (H) and Manhattan (M), KS during the 2006-2007 (season 1) and 2007-2008 (season 2) winter wheat growing seasons. Weed-free (wf) and weed control (wc) experiments from each location and season are designated H1-wf, M1-wf, H2-wf, M2-wf, H1-wc, M1-wc, H2-wc, and M2-wc. Soil characteristics for each experiment are shown in Table 3.1.

Tillage practices before planting consisted of disking and field cultivation at Hays, and disking, subsurface tillage, and field cultivation at Manhattan. Flixweed, blue mustard, and henbit seed were mixed and broadcast using a hand-crank spreader prior to seeding the weed control experiments at Manhattan in both years. At Hays, those species were metered through a second drill box and dropped onto the soil surface at the same time wheat was seeded in the fall of 2006. The experimental area in 2007-2008 was naturally infested with those species and was not over seeded. Weed-free experiments were established in areas free of weed infestations and no weed control measures were needed to maintain plots in each weed-free experiment.

All experiments were randomized complete blocks with four replications. Treatments consisted of two saflufenacil formulations (EC and WG) each applied at 13, 25, and 50 g ai/ha alone or in combination with 2,4-D amine at 533 g ae/ha. Also, 2,4-D amine alone and untreated control treatments were included. Crop oil concentrate[1] (COC) was included at 1% v/v in solo saflufenacil treatments.

Winter wheat cultivars were seeded in rows spaced 25 cm apart in all experiments. Cultivars, seeding rates and dates, and application dates for each experiment are shown in Table 3.2. Herbicide treatments were applied with a tractor-mounted, compressed-air sprayer or CO_2-knapsack sprayer equipped with TT110015 nozzles[2] delivering 122 L/ha at 207 kPa and 5 km/hr. At both locations in 2007, herbicides were applied to winter wheat that was 15- to 20-cm tall. Wheat plants at Hays had 5 to 15 tillers and plants at Manhattan had 7 to 8 tillers per plant. Despite overseeding the weed control experiments, weeds species were sparse at both locations in 2007. In 2008, wheat measured 8- to 15-cm tall with 3 to 8 tillers at each location at time of herbicide application. Blue mustard was the predominant weed species in H2-wc (>100 plants/m^2) along with flixweed and henbit at densities of <10 plants/m^2. Blue mustard plants were 10- to 13-cm diam, flixweed plants were 5- to 13-cm diam, and henbit plants were 3- to 5-cm diam at time of herbicide application. In experiment M2-wc blue mustard, flixweed, and henbit were predominant weed species at densities of 50, 20, and 10 plants/m^2, respectively. Blue mustard plants were 10- to 15-cm diam, flixweed plants were 10- to 15-cm diam, and henbit plants were 3- to 5-cm diam at herbicide application.

Winter wheat foliar necrosis and growth reduction (stunting) were estimated visually on a percentage scale of 0 (no injury) to 100 (plant death) in weed control experiments at 3 to 6 and 14 to 20 days after treatment (DAT) and at 4 to 6 and 10 to 16 DAT in weed-free experiments. Weed control was also visually estimated using the same scale in experiments at Hays and Manhattan in 2008. Weed densities were too low to estimate weed control in 2007.

Wheat height to the top of the awns was determined based on three measurements per plot taken within 10 days prior to harvest in experiment H1-wf and in all experiments in 2008. Height measurements were not taken in other experiments in 2007 because of severe, late season freeze damage at Manhattan and hail damage at Hays. Grain yields, adjusted to 12.5% moisture content, were determined in weed control and weed-free experiments by harvesting the center 1.5 m of each plot with a plot combine. Harvest dates for each experiment are shown in Table 3.2.

Data were subjected to ANOVA using PROC GLM in SAS[3] and means were separated using LSD at $P \leq 0.05$. Untreated control treatments were omitted from statistical analysis of weed control data. Data were arcsine-square-root transformed before analysis. However, transformation did not improve variance homogeneity; therefore, nontransformed data were used for analysis and presentation. Each site-year was considered an environment. Data analysis indicated an interaction between environment and treatments for foliar necrosis and stunting of wheat in all experiments and grain yield in weed control experiments. Necrosis, stunting, and grain yield data were separated by environment and reanalyzed. The environment-by-treatment interaction was not significant for grain yield data from the weed-free experiments. Orthogonal contrasts were conducted for all response variables.

RESULTS AND DISCUSSION

Weed Control

Saflufenacil at 13 g/ha or more plus COC controlled blue mustard 95% or greater at Hays, whereas at Manhattan all saflufenacil treatments controlled blue mustard 100% at 17 to 20 DAT (Table 3.3). Increasing the rate of either saflufenacil formulation from 13 to 25 g/ha increased blue mustard control at Hays from 95 to 98%. No benefit was gained by increasing the saflufenacil rate further to 50 g/ha. Differences between formulations at equivalent rates were not significant. The WG formulation of saflufenacil at 13 g/ha mixed with 2,4-D amine without COC did not control blue mustard as well as the same rate and formulation mixed with COC instead of 2,4-D amine. However, control was similar between those treatment combinations at the two higher saflufenacil rates. Applications of 2,4-D amine alone controlled blue mustard ≤50% at both locations.

Flixweed control by both formulations of saflufenacil plus COC increased as saflufenacil rate was increased from 13 to 25 g/ha, but control was not further improved by increasing rate to 50 g/ha (Table 3.3). However, when saflufenacil was mixed with 2,4-D amine, flixweed control was improved with each saflufenacil rate increase at Manhattan. Furthermore, there was a similar trend of increasing control with the EC but not the WG formulation at Hays. At 17 to 20 DAT, saflufenacil at 25 or 50 g/ha with COC, regardless of formulation, and the EC formulation at 25 or 50 g/ha tank-mixed with 2,4-D amine provided 91 to 100% flixweed control at both locations. Most saflufenacil treatments at 13 g/ha, regardless of formulation or tank mix partner, controlled flixweed 73 to 85%. However, at Manhattan the WG formulation at 13 g/ha plus 2,4-D amine controlled flixweed 55%. In comparison, 2,4-D amine controlled flixweed 55 and 40% at Hays and Manhattan, respectively. Flixweed control was similar between EC and WG formulations applied with COC at both locations. Furthermore, contrast comparison (averaged over rates) between the two formulations applied alone also was not significant. Mixing 2,4-D amine with the EC formulation reduced flixweed control compared to the EC formulation applied with COC at Manhattan but not Hays. Conversely, flixweed efficacy was reduced at both locations when 2,4-D amine was mixed with the WG formulation compared to the WG formulation applied with COC.

Henbit control was generally poor at ≤55% for most herbicide treatments at Manhattan (Table 3.3). A consistent rate response at Hays was not evident and none of the contrast comparisons were significant. Henbit control at 17 to 20 DAT was greater at Hays (68 to 88%) than at Manhattan for all herbicide treatments. At Manhattan, henbit control increased with increasing saflufenacil rate, regardless of formulation, especially when applied with 2,4-D amine. Application of 2,4-D amine resulted in the poorest henbit control at 17 to 20 DAT in both experiments.

Wheat Tolerance

In six of eight experiments, wheat foliar necrosis at 3 to 6 DAT increased to 30% as the rate of EC-formulated saflufenacil was increased from 13 to 25 to 50 g/ha (Tables 3.4 and 3.5). A similar trend also occurred in experiment H2-wc, except necrosis ratings for the low and mid rate treatments were not significantly different. There was no rate response in experiment H1-wc. Unlike with the EC formulation, foliar necrosis increased with increasing rate of the WG formulation in three of eight experiments to a maximum of 18%. Response differences were significant only between the low and high saflufenacil rates. Contrasts comparing the EC and WG formulations indicated leaf necrosis at 3 to 6 DAT was greater with the EC formulation compared to the WG formulation in each experiment (Tables 3.4 and 3.5). Averaged over environments, application of the EC and WG formulations with COC caused leaf necrosis ranging from 12 to 24% and 9 to 13%, respectively. Mixing 2,4-D amine with either formulation reduced leaf necrosis to <15% in all experiments. A saflufenacil rate response was not evident in most experiments when the WG formulation was applied with COC or when either saflufenacil formulation was applied with 2,4-D amine. At 15 ± 5 DAT, leaf necrosis had decreased to ≤15% for most herbicide treatments in all experiments with greatest necrosis observed in treatments of the EC and WG saflufenacil formulations plus COC. Plants completely recovered in experiments M1-wf, M1-wc and M2-wc when either saflufenacil formulation was tank-mixed with 2,4-D amine.

Wheat was stunted <15% in all experiments (Tables 3.4 and 3.5). In 6 of 8 experiments, the EC formation at 50 g/ha plus COC stunted wheat 14% at 15 ± 5 DAT. Stunting was reduced to ≤6% when either formulation of saflufenacil was tank-mixed with 2,4-D amine in the same experiments. Contrasts indicated there were no differences in stunting when comparing the WG formulation with COC and the WG formulation mixed with 2,4-D amine in 6 of 8 experiments. However, less stunting occurred with application of EC formulation in mixture with 2,4-D amine compared to the EC formulation applied with COC (Tables 3.3 and 3.5). No stunting was observed in experiment H2-wf at 10 to 16 DAT and no significant differences among treatments for stunting was detected in experiment H2-wc.

There was no treatment by environment interaction for wheat height. In weed control experiments, herbicide treatments did not affect wheat height (P = 0.6022), indicating wheat recovered from stunting (data not shown). However, in weed-free experiments 25 g/ha of saflufenacil plus COC or the EC formulation at 50 g/ha with COC reduced wheat height 3 and 4%, respectively, compared to the untreated control (P = 0.0454).

Grain yield differences among herbicide treatments were not significant in weed-free experiments, and mean grain yield was greater at Hays (3950 kg/ha) compared to Manhattan (2550 kg/ha) due to rain-delayed harvest, which reduced test weights (data not shown). Although grain yields did not differ among herbicide treatments, contrast comparison (averaged over rates and mixtures) between the EC and WG saflufenacil formulations was significant (P = 0.0402) due to 4.5% greater grain yield for the WG formulation.

All herbicide treatments increased grain yields compared to the untreated control in weed control experiments (Table 3.4). Grain yields were similar among herbicide treatments in experiment H2-wc. In experiment M2-wc, most herbicide treatments yielded similar to 2,4-D amine. However, the EC and WG saflufenacil formulations applied at 13 g/ha plus COC, and the WG saflufenacil formulation at 13 g/ha mixed with 2,4-D amine reduced yields 13, 11, and 10% compared to 2,4-D amine alone. There were no differences in grain yield when comparing the two formulations. However, mixtures of the EC formulation with 2,4-D amine resulted in 6% higher grain yields than the EC formulation applied with COC. This trend did not hold in comparison of the WG formulation vs. WG formulation mixed with 2,4-D amine as grain yields were similar.

Data from weed control and weed-free experiments indicate EC and WG formulations of saflufenacil may potentially be used POST in winter wheat. Despite early-season leaf necrosis, data generally shows saflufenacil, regardless of formulation, at a minimum of 25 g/ha applied alone or tank-mixed with 2,4-D amine can adequately control blue mustard and flixweed without negatively impacting grain yield. Nevertheless, most winter wheat growers would likely not apply the EC saflufenacil formulation to winter wheat because of severe early season injury despite knowledge that wheat plants will recover without grain yield loss. Also, winter wheat growers would likely be more inclined to utilize the WG saflufenacil formulation alone or mixed with 2,4-D amine because winter wheat injury caused by these treatments were generally <15% at all saflufenacil rates tested which is likely an acceptable level of injury to many growers.

Winter wheat injury caused by saflufenacil WG is similar another protox herbicide, carfentrazone-ethyl. Durgan et al. (1997) reported 0.026 and 0.035 g/ha of carfentrazone-ethyl POST applied with non-ionic surfactant caused 10 and 18% injury at 4 to 5 DAT. This study indicated necrosis due to saflufenacil WG ranged from 5 to 18% in eight experiments when applied at 50 g/ha and necrosis was further reduced when this saflufenacil formulation at any rate tested was mixed with 2,4-D amine. Despite injury to wheat, carfentrazone-ethyl is labeled for use in winter wheat, but this herbicide is only labeled for POST applications with non-ionic surfactant.

Saflufenacil is a useful tool to control herbicide resistant weed species in winter wheat because few protox inhibiting herbicides are labeled for use in winter wheat. However, additional research on crop injury and weed control is needed before saflufenacil can be a POST herbicide option for winter wheat growers. Future research should explore weed control efficacy and crop response of saflufenacil in combination with different adjuvant systems and tank mixes of saflufenacil with other auxin herbicides. Research is needed to further define the spectrum of weed control provided by POST applications of saflufenacil, and determine the rate of 2,4-D amine required in mixtures with saflufenacil to reduce necrosis of wheat, but maintain weed control.

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SOURCES OF MATERIALS

[1] Crop oil concentrate, Agri-dex, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

[2] Spraying Systems Co., N. Avenue at Schmale Road, P.O. Box 7900, Wheaton, IL 60189-7900.

[3] SAS version 9.1, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

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Table 3.1. Soil characteristics for each experiment.

Experiment[a]	Soil type	Soil classification	pH	Organic matter
				%
H1-wc	Crete silty clay loam	Fine, smectitic, mesic Pachic Argiustolls	6.2	1.9
M1-wc	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
H1-wf	Crete silty clay loam	Fine, smectitic, mesic Pachic Argiustolls	6.5	2.3
M1-wf	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
H2-wc	Roxbury silt loam	Fine-silty, mixed, superactive, mesic Cumulic Haplustolls	7.7	2.5
M2-wc	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
H2-wf	Crete silty clay loam	Fine, smectitic, mesic Pachic Argiustolls	6.4	1.9
M2-wf	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3

[a] Abbrevations: H1-wc, Hays, season 1, weed control; M1-wc, Manhattan, season 1, weed control; H1-wf, Hays,season 1, weed-free; M1-wf, Manhattan, season 1, weed-free; H2-wc, Hays, season 2, weed control; M2-wc, Manhattan, season 2, weed control; H2-wf, Hays, season 2, weed-free; M2-wf, Manhattan, season 2, weed-free.

Table 3.2. Wheat cultivars, seeding rate and dates, and harvest dates for each experiment.

Experiment[a]	Cultivars	Seeding rates	Planting dates	Application dates	Harvest dates
		kg/ha			
H1-wc	Danby	67	October 2, 2006	March 23, 2007	——
M1-wc	KS03HW6-1	84	October 5, 2006	March 22, 2007	——
H1-wf	AP502-CL	67	September 27, 2006	March 23, 2007	——
M1-wf	KS03HW6-1	84	October 5, 2006	March 22, 2007	——
H2-wc	Danby	73	October 2, 2007	March 26, 2008	July 2, 2008
M2-wc	Danby	101	October 11, 2007	March 25, 2008	July 10, 2008
H2-wf	Danby	63	October 2, 2007	March 28, 2008	June 29, 2008
M2-wf	Danby	101	October 11, 2007	March 25, 2008	July 10, 2008

[a] Abbrevations: H1-wc, Hays, season 1, weed control; M1-wc, Manhattan, season 1,weed control; H1-wf, Hays, season 1, weed-free; M1-wf, Manhattan, season 1, weed-free; H2-wc, Hays, season 2, weed control; M2-wc, Manhattan, season 2, weed control; H2-wf, Hays, season 2, weed-free; M2-wf, Manhattan, season 2, weed-free.

Table 3.3. Weed control at 17 to 20 DAT at Hays and Manhattan, KS in 2008[a, b].

		COTBE		DESSO		LAMAM	
Treatments[c]	Rate[d]	H2-wc	M2-wc	H2-wc	M2-wc	H2-wc	M2-wc
	g/ha	%					
Safl EC + COC	13	95 cd	100 a	73 d	84 d	75 cde	30 g
Safl EC + COC	25	98 abc	100 a	94 a	96 ab	85 ab	40 ef
Safl EC + COC	50	100 a	100 a	94 a	100 a	85 ab	45 de
Safl WG + COC	13	96 bcd	100 a	80 cd	84 d	80 a-d	30 g
Safl WG + COC	25	99 ab	100 a	93 a	94 b	78 bcd	35 fg
Safl WG + COC	50	99 ab	100 a	94 a	96 ab	88 a	40 ef
Safl EC + 2,4-D amine	13+533	94 de	100 a	85 bc	75 e	83 abc	40 ef
Safl EC + 2,4-D amine	25+533	98 abc	100 a	91 ab	91 bc	78 bcd	53 bc
Safl EC + 2,4-D amine	50+533	100 a	100 a	94 a	100 a	85 ab	68 a
Safl WG + 2,4-D amine	13+533	91 e	100 a	80 cd	55 f	73 de	38 f
Safl WG + 2,4-D amine	25+533	98 abc	100 a	78 cd	73 e	83 abc	48 cd
Safl WG + 2,4-D amine	50+533	99 ab	100 a	83 c	88 cd	83 abc	55 b
2,4-D amine	533	50 f	30 b	55 e	40 g	68 e	20 h
Untreated	—	—	—	—	—	—	—
LSD 0.05		3	1	7	5	8	5
Contrast[e]							
EC alone vs. WG alone		NS[f]	NS	NS	NS	NS	*
EC alone vs. EC + 2,4-D amine		NS	NS	NS	**	NS	***
WG alone vs. WG + 2,4-D amine		*	NS	***	***	NS	***
EC+2,4-D amine vs. WG+2,4-D amine		NS	NS	***	***	NS	***

[a] Abbrevations: COC, crop oil concentrate; COTBE, blue mustard; DAT, days after treatment; DESSO, flixweed; EC, emulsifiable concentrate, H2-wc, Hays, season 2, weed control; LAMAM, henbit; M2-wc, Manhattan, season 2, weed control; safl, saflufenacil; WG, water dispersible granule.
[b] Means followed by the same letter within columns are not significantly different.
[c] COC applied at 1% v/v.
[d] Units for 2,4-D amine are g ae/ha.
[e] Contrasts are averaged over saflufenacil rates.
[f] Levels of significance represented by * = < 0.05, ** = <0.01, and *** = <0.001.

Table 3.4. Winter wheat leaf necrosis and stunting in weed control experiments[a, b].

		Necrosis 3 to 6 DAT				Necrosis 14 to 20 DAT				Stunting 14 to 20 DAT				Grain yield	
Treatments[c]	Rate[d]	H1-wc	M1-wc	H2-wc	M2-wc	H1-wc	M1-wc	H2-wc	M2-wc	H1-wc	M1-wc	H2-wc	M2-wc	H2-wc	M2-wc
	g/ha	%												kg/ha	
Safl EC + COC	13	19 a	10 c	14 bc	13 cde	10 a	0 b	10 b	4 b	0 c	0 c	3	3 bcd	4700 a	2710 d
Safl EC + COC	25	19 a	15 b	16 b	23 b	10 a	5 a	11 b	5 b	11 a	7 b	3	6 b	4780 a	2870 cd
Safl EC + COC	50	21 a	20 a	23 a	30 a	10 a	5 a	15 a	10 a	11 a	14 a	3	11 a	4670 a	2890 bcd
Safl WG + COC	13	13 bcd	5 d	10 c	11 de	10 a	0 b	5 c	0 c	0 c	0 c	0	0 d	4840 a	2760 d
Safl WG + COC	25	15 b	5 d	11 c	14 cd	10 a	0 b	9 b	1 c	7 b	1 c	1	1 cd	4740 a	2870 cd
Safl WG + COC	50	15 b	6 d	13 bc	16 c	10 a	0 b	10 b	5 b	9 ab	0 c	1	3 bcd	4650 a	3165 a
Safl EC + 2,4-D amine	13+533	10 d	0 e	10 c	6 fg	10 a	0 b	5 c	0 c	0 c	0 c	0	1 cd	4800 a	3160 ab
Safl EC + 2,4-D amine	25+533	11 cd	5 d	10 c	9 ef	7 b	0 b	4 cd	0 c	0 c	0 c	1	0 d	4780 a	2980 a-d
Safl EC + 2,4-D amine	50+533	14 bc	5 d	11 c	11 de	10 a	0 b	4 cd	0 c	1 c	1 c	0	4 bc	4640 a	2880 cd
Safl WG + 2,4-D amine	13+533	5 e	5 d	0 e	1 hi	0 c	0 b	1 de	0 c	0 c	0 c	0	0 d	4850 a	2800 d
Safl WG + 2,4-D amine	25+533	5 e	0 e	5 d	5 fgh	0 c	0 b	0 e	0 c	6 b	0 c	0	0 d	4680 a	2920 a-d
Safl WG + 2,4-D amine	50+533	3 ef	0 e	5 d	4 ghi	0 c	0 b	0 e	0 c	0 c	0 c	1	0 d	4790 a	2970 a-d
2,4-D amine	533	0 f	0 e	0 e	0 i	0 c	0 b	0 e	0 c	0 c	0 c	0	0 d	4520 a	3100 abc
Untreated	——	0 f	0 e	0 e	0 i	0 c	0 b	0 e	0 c	0 c	0 c	0	0 d	2630 b	1940 e
LSD 0.05		3	1	4	4	2	4	3	1	3	4	NS	3	420	270
Contrast[e]															
EC alone vs. WG alone		***[f]	***	***	***	NS	***	***	***	*	***	*	***	NS	NS
EC alone vs. EC + 2,4-D amine		***	***	***	***	NS	***	***	***	***	***	**	***	NS	*
WG alone vs. WG + 2,4-D amine		***	***	***	***	***	NS	***	***	***	NS	NS	NS	NS	NS
EC+2,4-D amine vs. WG+2,4-D amine		***	***	***	***	***	NS	***	NS	*	NS	NS	*	NS	NS

[a] Abbrevations: COC, crop oil concentrate; DAT, days after treatment; EC, emulsifiable concentrate; H1-wc, Hays, season 1, weed control; M1-wc, Manhattan, season 1, weed control; Hays, season 2, weed control; M2-wc, Manhattan, season 2, weed control; safl, saflufenacil; WG, water dispersible granule.
[b] Means followed by the same letter within columns are not significantly different.
[c] COC applied at 1% v/v.
[d] Units for 2,4-D amine are g ae/ha.
[e] Contrasts are averaged over saflufenacil rates.
[f] Levels of significance represented by * = < 0.05, ** = <0.01, and *** = <0.001.

Table 3.5. Winter wheat leaf necrosis and stunting in weed-free experiments[a, b].

Treatments[c]	Rate[d]	Necrosis 4 to 6 DAT				Necrosis 10 to 16 DAT				Stunting 10 to 16 DAT			
		H1-wf	M1-wf	H2-wf	M2-wf	H1-wf	M1-wf	H2-wf	M2-wf	H1-wf	M1-wf	H2-wf	M2-wf
	g/ha	%											
Safl EC + COC	13	11 c	9 c	5 cd	14 cd	10 ab	0	5 c	13 de	0 b	0 c	0	1 cd
Safl EC + COC	25	15 b	15 b	14 b	21 b	10 ab	5	10 b	19 b	0 b	9 b	0	5 b
Safl EC + COC	50	18 a	20 a	30 a	30 a	11 a	5	15 a	23 a	6 a	13 a	0	10 a
Safl WG + COC	13	10 c	5 d	8 c	11 de	0 d	0	10 b	10 e	0 b	0 c	0	1 cd
Safl WG + COC	25	10 c	6 d	6 cd	18 bc	8 b	0	10 b	15 cd	0 b	0 c	0	4 bc
Safl WG + COC	50	11 c	5 d	16 b	18 bc	10 ab	0	10 b	16 bc	0 b	1 c	0	3 cd
Safl EC + 2,4-D amine	13+533	5 d	5 d	0 e	5 fg	5 c	0	0 e	1 g	0 b	0 c	0	0 d
Safl EC + 2,4-D amine	25+533	5 d	5 d	3 d	9 ef	5 c	0	0 e	5 f	0 b	0 c	0	0 d
Safl EC + 2,4-D amine	50+533	9 c	5 d	8 c	10 de	5 c	0	0 e	6 f	0 b	0 c	0	0 d
Safl WG + 2,4-D amine	13+533	0 f	1 e	0 e	0 h	5 c	0	0 e	0 g	0 b	0 c	0	0 d
Safl WG + 2,4-D amine	25+533	1 ef	0 e	0 e	1 gh	0 d	0	0 e	0 g	0 b	0 c	0	0 d
Safl WG + 2,4-D amine	50+533	3 de	0 e	0 e	5 fg	1 d	0	3 d	0 g	0 b	0 c	0	0 d
2,4-D amine	533	0 f	0 e	0 e	0 h	0 d	0	0 e	0 g	0 b	0 c	0	0 d
Untreated	———	0 f	0 e	0 e	0 h	0 d	0	0 e	0 g	0 b	0 c	0	0 d
LSD 0.05		2	2	4	4	2	NS	1	3	1	2	NS	3
Contrast[e]													
EC alone vs. WG alone		***[f]	***	***	***	***	***	NS	***	***	***	NS	***
EC alone vs. EC + 2,4-D amine		***	***	***	***	***	***	***	***	***	***	NS	***
WG alone vs. WG + 2,4-D amine		***	***	***	***	***	NS	***	***	NS	NS	NS	*
EC+2,4-D amine vs. WG+2,4-D amine		***	***	***	***	***	NS	*	***	NS	NS	NS	NS

[a] Abbrevations: COC, crop oil concentrate, DAT, days after treatments; EC, emulsifiable concentrate; H1-wf, Hays, season 1, weed-free; M1-wf, Manhattan, season 1, weed-free; H2-wf, Hays, season 2, weed-free; M2-wf, Manhattan, season 2, weed-free; safl, saflufenacil; WG, water dispersible granule.
[b] Means followed by the same letter within columns are not significantly different.
[c] COC applied at 1% v/v.
[d] Units for 2,4-D amine are g ae/ha.
[e] Contrasts are averaged over saflufenacil rates.
[f] Levels of significance represented by * = < 0.05, ** = <0.01, and *** = <0.001.

CHAPTER 4 - Efficacy of Foliar-Applied Saflufenacil Mixed with Auxin or Bentazon Herbicides in Winter Wheat

ABSTRACT

Field experiments were conducted at two locations in Kansas during the 2006-2007 and 2007-2008 winter wheat growing seasons to evaluate winter annual broadleaf weeds and winter wheat response to POST treatments of saflufenacil applied alone or mixed with 2,4-D amine, 2,4-D ester, MCPA ester, dicamba, or bentazon. Saflufenacil was applied at 13 or 25 g ai/ha. Dicamba, 2,4-D amine, 2,4-D ester, MCPA ester, and bentazon were applied alone or in combination with each rate of saflufenacil at 140, 533, 533, 520, and 560 g/ha, respectively. Crop oil concentrate (COC) was included at 1% v/v with solo applied saflufenacil and bentazon treatments and when saflufenacil was mixed with bentazon. Treatments of saflufenacil at 13 or 25 g/ha mixed with dicamba or 2,4-D amine caused 13% or less wheat necrosis and controlled blue mustard greater than 90%. The 25 g/ha rate of saflufenacil was required in tank mixtures with dicamba or 2,4-D amine to achieve 88% or greater control of flixweed. Saflufenacil at 13 g/ha with COC controlled blue mustard 93% and flixweed 100% but wheat injury was twice as great compared to saflufenacil at the same rate in combination with 2,4-D amine or dicamba. Saflufenacil at 25 g/ha mixed with COC, MCPA ester, or 2,4-D ester controlled blue mustard and flixweed 100%, but these treatments injured wheat more than all other herbicide treatments. No herbicide treatment controlled henbit by as much as 60%. In weed-infested experiments, herbicide treatments that included saflufenacil resulted in similar or higher grain yields than companion herbicide treatments alone. All herbicide treatments improved grain yield compared to the weedy check in weed control experiments. Averaged over environments, saflufenacil at 13 or 25 g/ha mixed with MCPA ester or 2,4-D ester, and 2,4-D ester alone reduce grain yield of weed-free wheat 6 to 9% compared to the untreated control. Grain yields of the remaining herbicide treatments were similar to the untreated control. In general, saflufenacil treatments did not negatively impact yield parameters or grain yields. These experiments showed that tank mixing dicamba or 2,4-D amine with saflufenacil at 25 g/ha effectively controlled blue mustard and flixweed without reducing grain yield.

Nomenclature: bentazon; dicamba; MCPA; saflufenacil; 2,4-D amine; 2-4-D ester; blue mustard, *Chorispora tenella* (Pallas) DC., COBTE; flixweed, *Desurainia sophia* L. Webb. ex Prantl, DESSO; henbit, *Lamium amplexicaule* L., LAMAM; winter wheat, *Triticum aestivum* L., ‘Danby’, & ‘KS03HW6-1’

Key words: winter annual broadleaf weeds, BAS 800H, crop response, tank mixes

INTRODUCTION

Saflufenacil is a new herbicide that controls broadleaf weeds through inhibition of protoporphyrinogen oxidase (protox) (Anonymous 2008). This herbicide is being developed for burndown and PRE weed control and may be used to control herbicide resistant weeds in many crops including corn, grain sorghum, small grains, legumes, and tree nut and fruits (Anonymous 2008). It may be particularly useful in grain sorghum and wheat because few herbicides with this mode-of-action are registered for use in these crops (Regehr et al. 2008). However, use of saflufenacil in winter wheat could be limited since many winter wheat growers prefer to apply herbicides POST early spring (D. E. Peterson and P. W. Stahlman, personal communication).

Few studies have evaluated POST saflufenacil treatments in winter wheat. Researchers in Canada found saflufenacil applied POST with an adjuvant caused unacceptable levels of injury and yield loss in cereal grain crops and corn (Sikkema et al. 2008; Soltani et al 2008). In a growth chamber study, saflufenacil applied at 13, 25, and 50 g/ha with nonionic surfactant (NIS) caused 19 to 40% wheat leaf necrosis at 3 DAT. However, mixing 2,4-D amine without NIS or bentazon plus NIS with saflufenacil reduced leaf necrosis compared to saflufenacil with NIS and resulted in wheat dry biomass similar to or greater than plants treated with 2,4-D amine (unpublished data).

Numerous studies have shown that including 2,4-D or bentazon in tank mixes can reduce injury caused by certain herbicides. Brown et al. (2004) found grain sorghum was partially safened from metsulfuron injury when tank-mixed with 2,4-D amine or dicamba. The addition of 2,4-D with nicosulfuron applied after terbufos insecticide treatment at planting reduced corn injury by more than 50% (Simpson et al. 1994). B entazon reduced soybean injury when mixed with thifensulfuron compared to thifensulfuron alone (Hart and Roskamp 1998; Lycan and Hart 1999), and tank mixes of bentazon and imazethapyr injured dry beans less than imazethapyr alone (Bauer et al. 1995a; Soltani et al. 2008). Also, mixing bentazon with paraquat reduced injury in peanuts and green peas compared to paraquat alone (Wehtje et al. 1992; Bellinder et al. 1997).

Tank mixes with 2,4-D or bentazon can improve crop safety but can also reduce weed control. Tank mixing 2,4-D with glyphosate (Nalewaja and Matysiak 1992; Thelen et al. 1995), haloxyfop (Mueller et al. 1990), clethodim (Blackshaw et al. 2006), or quizolofop-P (Blackshaw et al. 2006) reduced control of certain grass species. Tank mixing with bentazon has also been shown to inhibit the performance of several grass and broadleaf herbicides (Bauer et al. 1995b; Gerwick et al. 1988; Hart 1997; Sorensen et al. 1987; Wanamarta et al. 1989; Wehtje et al. 1992).

Field observations indicated that 2,4-D amine and bentazon may reduce saflufenacil injury in winter wheat, but it is not known whether tank mixtures with these herbicides affect weed control or whether other auxin herbicides such as dicamba and MCPA in mixture with saflufenacil will impact crop safety and weed control in winter wheat. The objectives of this research were to (1) evaluate winter annual broadleaf weed species and winter wheat to POST treatments of saflufenacil applied with COC or mixed with bentazon or auxin herbicides and to (2) determine winter wheat response to these treatments under weed-free conditions.

MATERIALS AND METHODS

Weed-free (wf) and weed control (wc) experiments were conducted near Hays (Hs) and Manhattan (Mn), KS (250 km distance) during the 2006-2007 (season 1) and 2007-2008 (season 2) winter wheat growing seasons. Individual experiments are designated Hs1-wf, Mn1-wf, Hs2-wf, Mn2-wf, Hs1-wc, Mn1-wc, Hs2-wc, and Mn2-wc. Soil characteristics for each experiment are shown in Table 4.1.

Seedbed preparation included disking and field cultivation prior to seeding at Hays, and disking, subsurface tillage, and field cultivation at Manhattan. Flixweed, blue mustard, and henbit seed were broadcast before seeding using a hand-crank spreader in experiments Mn1-wc and Mn2-wc, and seeded with a Great Plains 1520 drill at planting in experiment Hs1-wc. Experiment Hs2-wc was conducted on a site with a dense population of blue mustard. Weed-free experiments were conducted in areas free of weed infestations and weed control measures were not needed to maintain plots.

The experimental design of all experiments was randomized complete block design with four replications of each treatment. Treatments consisted of saflufenacil at 13 or 25 g ai/ha applied alone and in combination with dicamba, MCPA ester, 2,4-D ester, 2,4-D amine, or bentazon at 140, 520, 533, 533, and 560 g/ha, respectively. Crop oil concentrate[1] was included only in saflufenacil and bentazon solo treatments and bentazon tank mixes. An untreated control was included for comparison.

Winter wheat cultivars were seeded in rows spaced 25 cm apart in all experiments. Cultivar selection, seeding rates and dates, and herbicide application dates for each experiment are shown in Table 4.2. Treatments were applied with a tractor–mounted, compressed-air sprayer or CO_2 knapsack sprayer equipped with TT110015 nozzles[2] delivering 122 L/ha at 207 kPa and 5 km/hr. At the time of application winter wheat was 15- to 23-cm tall with 7 to 12 tillers in 2006-2007 experiments at both locations. In experiment Hs1-wc, flixweed density was <10 plants/m^2 and plants were 5- to 10-cm diam at application. Conversely, few weeds were present at time of application in experiment Mn1-wc. In experiments conducted in 2007-2008, herbicides were applied to wheat with 3 to 8 tillers when plants were 8- to 15-cm tall in experiments at both locations. Blue mustard was 10- to 13-cm diam at application and was the predominant weed species at >100 plants/m^2 in experiment Hs2-wc. Blue mustard, flixweed, and henbit were present at application at densities of 10, 30, and 20 plants/m^2 and were 10 to 15, 10 to 15, and 3 to 5-cm diam, respectively.

Winter wheat leaf necrosis and growth reduction (stunting) were estimated visually on a percentage scale of 0 (no injury) to 100 (plant death) in weed control experiments at 3 to 5 and 16 to 20 days after treatment (DAT). Necrosis and stunting were rated using the same scale at 4 to 5 and 16 to 20

DAT in weed-free experiments. Weed control was also rated on a percentage scale of (no control) to 100 (plant death) in experiments Hs2-wc and Mn2-wc at 18 to 20 DAT. Weed infestations were too low to rate weed control in experiments Hs1-wc and Mn1-wc.

Wheat height to the top of the awns was determined based on three measurements per plot taken within 10 days prior to harvest. Grain yields were determined by harvesting the center 1.5 m of each plot with a plot combine and grain yields were adjusted to 12.5% moisture content. Harvest dates for each experiment are shown in Table 4.2. Wheat heights were not determined in experiments Mn1-wc or Mn1-wf because of freeze damage and grain yields were not recorded in experiments Hs1-wc, Mn1-wc, and Mn1-wf because of hail and freeze damage at Hays and Manhattan, respectively.

Data were subjected to ANOVA using PROC GLM in SAS[3] and means were separated using LSD at $P \leq 0.05$. Each site-year was considered an environment. Data analysis indicated environment by treatment interactions for wheat necrosis and stunting in weed-free and weed control experiments and grain yield in weed control experiments. Necrosis, stunting, and grain yield data were separated by environment and reanalyzed. The treatment-by-environment interaction was not significant for grain yield in weed-free experiments. Prior to ANOVA analysis, data were tested for non-homogenous variances. Untreated control treatments were omitted from statistical analyses of weed control data. Data were arcsine transformed when appropriate before ANOVA analysis because of non-homogenous variances. However, transformation did not affect results therefore nontransformed data are presented.

RESULTS AND DISCUSSION

Weed Control

Saflufenacil at 13 or 25 g/ha plus COC and mixtures of saflufenacil at those rates plus dicamba, MPCA ester, 2,4-D amine, or 2,4-D ester without COC controlled blue mustard 91% or greater in Hays, whereas at Manhattan the same treatments provided complete control of blue mustard at 18 to 20 DAT (Table 4.3). At Hays, blue mustard control increased as saflufenacil rate increased from 13 to 25 g/ha when mixed with dicamba and bentazon, but not when mixed with MCPA ester or either formulation of 2,4-D. Blue mustard control responded similarly to increasing saflufenacil rates at Manhattan when saflufenacil was mixed with bentazon, but not with other herbicides tested. Tank mixing bentazon with saflufenacil at 13 g/ha reduced blue mustard control from 94 to 81% at Hays and from 99 to 55% at Manhattan. Furthermore, saflufenacil at 25 g/ha with COC controlled blue mustard 98%, but mixing bentazon with the same rate of saflufenacil reduced control to 91% at Hays. Treatments including saflufenacil provided greater blue mustard control than applications of dicamba, 2,4-D amine, 2,4-D ester, or bentazon alone at both locations.

Flixweed control differed among treatments within experiments at Hays and Manhattan (Table 4.3). Most saflufenacil treatments provided 100% flixweed control at Hays in 2007, except when mixed with dicamba at either saflufenacil rate or when mixed with bentazon at the low saflufenacil rate. Mixing dicamba with saflufenacil at 13 or 25 g/ha compared to saflufenacil at the same rates plus COC reduced flixweed control from 100 to 83 and 88%, respectively. Also, mixing bentazon with 13 g/ha of saflufenacil reduced flixweed control from 100 to 90%, compared to the same rate of saflufenacil with COC.

At Manhattan in 2008, saflufenacil plus COC and saflufenacil plus MCPA ester or 2,4-D ester provided ≥93% control of flixweed at 18 to 20 DAT, regardless of saflufenacil rate. Saflufenacil at 25 g/ha with 2,4-D amine resulted in 100% flixweed control, whereas control was only 81% when 2,4-D amine was mixed with 13 g/ha of saflufenacil. Flixweed control was also reduced by tank mixing dicamba or bentazon with saflufenacil. Flixweed control was reduced 5% when dicamba was tank-mixed with 25 g/ha of saflufenacil compared to the same rate of saflufenacil plus COC. However, much greater reduction in flixweed control resulted when saflufenacil at 13 g/ha was mixed with dicamba (-35%) or bentazon (-61%). Furthermore, saflufenacil at 25 g/ha plus bentazon controlled flixweed 53% compared to complete control for the same rate of saflufenacil plus COC. Applications of companion herbicides alone controlled flixweed ≤40% at Manhattan in 2008, whereas at Hays in 2007, both dicamba and bentazon controlled flixweed 79%. Complete flixweed control was achieved with 2,4-D amine, 2,4-D ester, and MPCA ester.

No herbicide treatment controlled henbit as well as blue mustard or flixweed at Manhattan (Table 4.3). Henbit was controlled <40% by saflufenacil plus COC. Tank mixing saflufenacil with MCPA ester, 2,4-D amine, or 2,4-D ester increased henbit control compared to saflufenacil with COC, but no treatment exceeded 58% control at 19 DAT. Henbit control improved with increased saflufenacil rate when applied with COC or in combination with 2,4-D amine, 2,4-D ester, or bentazon. However, control differences between saflufenacil rates were not significant when saflufenacil was mixed with dicamba or MPCA ester. Conversely, henbit control was reduced when bentazon was mixed with saflufenacil compared to saflufenacil plus COC, regardless of saflufenacil rate.

Wheat Tolerance

In six of eight experiments, leaf necrosis at 3 to 5 DAT increased to as high as 24% as saflufenacil rate was increased from 13 to 25 g/ha when applied with COC (Tables 4.4 and 4.5). A rate response was evident in most experiments when saflufenacil was mixed with companion herbicides. Generally, greatest necrosis occurred with application of 25 g/ha of saflufenacil with COC, MPCA ester, or 2,4-D ester. In five of eight experiments, necrosis was similar to or greater than saflufenacil at 25 g/ha applied with COC when the higher rate of saflufenacil was mixed with MCPA ester or 2,4-D ester. Conversely, saflufenacil with dicamba, 2,4-D amine, or bentazon reduced necrosis to <15% in all experiments compared to saflufenacil with COC, regardless of saflufenacil rate. At 16 to 20 DAT, necrosis had declined to <15% for all herbicide treatments in all experiments. Greatest necrosis generally was observed in treatments of saflufenacil at 25 g/ha with COC, MCPA ester, and 2,4-D ester. Wheat had completely recovered from necrosis in three of eight experiments by 16 to 20 DAT.

Stunting (≤15%) was evident in seven of eight experiments at 16 to 20 DAT (Tables 4.4 and 4.5). In four of eight experiments, saflufenacil at 25 g/ha with COC, MPCA ester, and 2,4-D ester stunted wheat 6 to 11%, which was greater than any other treatment within those experiments. However, stunting was greatest (13 to 15%) in two other experiments when saflufenacil at 25 g/ha was mixed with MCPA ester or 2,4-D ester. Wheat stunting was reduced by tank mixing 25 g/ha of saflufenacil with dicamba, 2,4-D amine, or bentazon in most experiments. Solo companion herbicides caused ≤5% stunting in all experiments and saflufenacil at 13 g/ha with COC stunted wheat ≤6% in seven of eight experiments. No stunting was observed in experiment Hs2-wf.

Averaged over weed control experiments, herbicide treatments did not affect wheat height (P = 0.2246) indicating wheat plants had recovered from stunting by harvest. Conversely, herbicide treatments significantly influenced wheat height in weed-free experiments (data not shown). Averaged over environments, wheat height ranged from 89 to 92 cm for all herbicide treatments with wheat heights similar among most herbicide treatments. However, wheat tended to be shorter when treatments contained 2,4-D ester, and when saflufenacil at 25 g/ha was mixed with MCPA ester or 2,4-D amine.

Averaged over environments wheat treated with saflufenacil at 13 or 25 g/ha mixed with MCPA ester or 2,4-D ester and of 2,4-D ester alone yielded 6 to 9% less grain than untreated weed-free wheat (Table 4.5). The reduced grain yields indicate that severe early season necrosis and stunting caused by those treatments affects yield parameters. Grain yields of the remaining herbicide treatments were similar to the untreated control.

In both weed control experiments, herbicide treatment increased wheat grain yields compared to untreated wheat (Table 4.4). In most cases at Hays in 2008, grain yields were greatest for treatments that included saflufenacil and lowest for solo companion herbicide treatments. However, most herbicide treatments at Manhattan in 2008 resulted in similar grain yields. Saflufenacil at 13 or 25 g/ha mixed with dicamba resulted in the highest grain yields at 2750 and 2800 kg/ha respectively, but yields of these treatments were similar to most other herbicide treatments.

These experiments indicate POST-applied saflufenacil at 25 g/ha mixed with dicamba, or 2,4-D amine caused little leaf necrosis and effectively controlled blue mustard and flixweed, but not henbit. Few herbicide options are available to growers with ALS-resistant weed problems in winter wheat. The addition of saflufenacil for use in wheat would diversify herbicide rotation options and increase the number of herbicides available to control ALS-resistant weeds. However, further research is needed to address control of other broadleaf weeds in winter wheat with POST application of saflufenacil. Studies are also needed to further explore tank mixes of saflufenacil and dicamba or 2,4-D amine including determination of dicamba and 2,4-D amine rates required to reduce leaf necrosis of wheat but maintain weed control.

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SOURCES OF MATERIALS

[1] Crop oil concentrate, Agri-dex, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

[2] Spraying Systems Co., N. Avenue at Schmale Road, P.O. Box 7900, Wheaton, IL 60189-7900.

[3] SAS version 9.1, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

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Table 4.1. Soil characteristics for each experiment.

Experiment[a]	Soil type	Soil classification	pH	Organic matter
				%
Hs1-wc	Crete silty clay loam	Fine, smectitic, mesic Pachic Argiustolls	6.2	1.9
Mn1-wc	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
Hs1-wf	Roxbury silt loam	Fine-silty, mixed, superactive, mesic Cumulic Haplustolls	7.8	1.6
Mn1-wf	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
Hs2-wc	Roxbury silt loam	Fine-silty, mixed, superactive, mesic Cumulic Haplustolls	7.7	2.5
Mn2-wc	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3
Hs2-wf	Crete silty clay loam	Fine, smectitic, mesic Pachic Argiustolls	6.4	1.9
Mn2-wf	Reading silt loam	Fine-silty, mixed, superactive, mesic Pachic Agriudolls	6.3	2.3

[a] Abbrevations: Hs1-wc, Hays, season 1, weed control; Mn1-wc, Manhattan, season 1, weed control; Hs1-wf, Hays,season 1, weed-free; Mn1-wf, Manhattan, season 1, weed-free; Hs2-wc, Hays, season 2, weed control; Mn2-wc, Manhattan, season 2, weed control; Hs2-wf, Hays, season 2, weed-free; Mn2-wf, Manhattan, season 2, weed-free.

Table 4.2. Wheat cultivars, seeding rates and dates, and harvest dates for each experiment.

Experiment[a]	Cultivars	Seeding rates	Planting dates	Application dates	Harvest dates
		kg/ha			
Hs1-wc	Danby	67	October 2, 2006	March 23, 2007	———
Mn1-wc	KS03HW6-1	84	October 5, 2006	March 20, 2007	———
Hs1-wf	KS03HW6-1	56	October 5, 2006	March 23, 2007	June 22, 2007
Mn1-wf	KS03HW6-1	84	October 5, 2006	March 20, 2007	———
Hs2-wc	Danby	73	October 2, 2007	March 26, 2008	July 2, 2008
Mn2-wc	Danby	101	October 11, 2007	March 25, 2008	July 10, 2008
Hs2-wf	Danby	63	October 2, 2007	March 28, 2008	June 29, 2008
Mn2-wf	Danby	101	October 11, 2007	March 25, 2008	July 10, 2008

[a] Abbrevations: Hs1-wc, Hays, season 1, weed control; Mn1-wc, Manhattan, season 1, weed control; Hs1-wf, Hays, season 1, weed-free; Mn1-wf, Manhattan, season 1, weed-free; Hs2-wc, Hays, season 2, weed control; Mn2-wc, Manhattan, season 2, weed control; Hs2-wf, Hays, season 2, weed-free; Mn2-wf, Manhattan, season 2, weed-free.

Table 4.3. Weed control at 18 to 20 DAT and winter wheat grain yield in weed control experiments[a, b].

Treatments[c]	Rate[d]	COTBE		DESSO		LAMAM	Grain yield	
		Hs2-wc	Mn2-wc	Hs1-wc	Mn2-wc	Mn2-wc	Hs2-wc	Mn2-wc
	g ai/ha	%					kg/ha	
Safl + COC	13	94 cd	99 a	100 a	93 b	25 gh	4660 abc	2600 abc
Safl + COC	25	98 abc	100 a	100 a	100 a	38 de	4450 abc	2560 abc
Safl + dicamba	13+140	91 d	100 a	83 bc	58 d	28 fg	4920 ab	2750 ab
Safl + dicamba	25+140	97 abc	100 a	88 bc	95 b	33 ef	4360 bc	2800 a
Dicamba	140	20 h	0 d	79 c	30 g	0 j	3560 d	2350 abc
Safl + MCPA ester	13+520	97 abc	100 a	100 a	100 a	50 bc	4930 ab	2450 abc
Safl + MCPA ester	25+520	100 a	100 a	100 a	100 a	55 ab	4470 abc	2340 abc
MCPA ester	520	55 f	28 c	99 a	40 f	20 h	4100 cd	2420 abc
Safl + 2,4-D amine	13+533	95 bcd	99 a	100 a	81 c	40 de	5050 a	2490 abc
Safl + 2,4-D amine	25+533	96 abc	100 a	100 a	100 a	50 bc	5020 ab	2580 abc
2,4-D amine	533	43 g	30 c	100 a	40 f	20 h	4030 cd	2600 abc
Safl + 2,4-D ester	13+533	97 abc	100 a	100 a	100 a	45 cd	4920 ab	2280 c
Safl + 2,4-D ester	25+533	99 ab	100 a	100 a	100 a	58 a	4440 abc	2300 bc
2,4-D ester	533	55 f	30 c	100 a	40 f	20 h	3470 d	2500 abc
Safl + bentazon	13+560	81 e	55 b	90 b	32 g	10 i	5050 a	2490 abc
Safl + bentazon	25+560	91 d	100 a	100 a	53 e	23 gh	4890 ab	2600 abc
Bentazon	560	16 h	0 d	79 c	32 g	0 j	3460 d	2270 c
Untreated	—	—	—	—	—	—	2590 e	1660 d
LSD 0.05		4	3	9	4	7	670	460

[a] Abbrevations: COC, crop oil concentrate; COTBE, blue mustard; DAT, days after treatment; DESSO, flixweed; Hs1-wc, Hays, season 1, weed control;Mn1-wc, Manhattan, season 1, weed control; Hs2-wc, Hays, season 2, weed control; LAMAM, henbit; Mn2-wc, Manhattan, season 2, weed control; safl, saflufenacil.
[b] Means followed by the same letter within columns are not significantly different.
[c] COC applied at 1% v/v.
[d] Units for dicamba, MCPA ester, 2,4-D ester, and 2,4-D amine are g ae/ha.

Table 4.4. Winter wheat leaf necrosis and stunting in weed control experiments[a, b]

Treatments[c]	Rate[d]	Necrosis 3 to 5 DAT				Necrosis 16 to 20 DAT				Stunting 16 to 20 DAT			
		Hs1-wc	Mn1-wc	Hs2-wc	Mn2-wc	Hs1-wc	Mn1-wc	Hs2-wc	Mn2-wc	Hs1-wc	Mn1-wc	Hs2-wc	Mn2-wc
	g ai/ha	%											
Safl + COC	13	16 b	10 c	16 c	10 c	10 a	0	6 b	5 a	5 bc	0 d	12 abc	3 cd
Safl + COC	25	19 ab	20 a	24 ab	20 b	10 a	0	14 a	5 a	5 bc	9 a	16 a	8 ab
Safl + dicamba	13+140	6 d	0 e	1 gh	0 e	5 cd	0	0 d	0 b	1 cd	5 b	5 efg	0 d
Safl + dicamba	25+140	10 c	5 d	11 d	5 d	9 ab	0	0 d	0 b	4 bcd	5 b	4 e-h	0 d
Dicamba	140	2 e	0 e	3 fgh	0 e	1 ef	0	0 d	0 b	0 d	5 b	0 h	0 d
Safl + MCPA ester	13+520	20 a	10 c	21 b	21 b	10 a	0	15 a	5 a	8 b	0 d	10 bcd	8 ab
Safl + MCPA ester	25+520	20 a	20 a	28 a	33 a	10 a	0	14 a	6 a	13 a	8 a	13 ab	11 a
MCPA ester	520	6 d	0 e	0 h	0 e	6 bcd	0	0 d	0 b	0 d	0 d	6 de	0 d
Safl + 2,4-D amine	13+533	8 cd	5 d	5 efg	5 d	8 abc	0	1 d	0 b	1 cd	0 d	3 fgh	1 d
Safl + 2,4-D amine	25+533	11 c	13 b	11 d	8 c	9 ab	0	4 c	0 b	0 d	1 cd	3 fgh	0 d
2,4-D amine	533	1 e	0 e	3 fgh	0 e	1 ef	0	0 d	0 b	0 d	0 d	4 e-h	0 d
Safl + 2,4-D ester	13+533	17 ab	10 c	26 a	19 b	10 a	0	14 a	5 a	5 bc	3 bc	4 e-h	5 bc
Safl + 2,4-D ester	25+533	19 ab	20 a	28 a	31 a	9 ab	0	15 a	6 a	13 a	9 a	6 def	11 a
2,4-D ester	533	10 c	0 e	0 h	0 e	4 de	0	0 d	0 b	1 cd	0 d	8 cde	0 d
Safl + bentazon	13+560	2 e	4 d	8 de	0 e	1 ef	0	0 d	0 b	0 d	0 d	1 gh	0 d
Safl + bentazon	25+560	6 d	9 c	6 ef	5 d	0 f	0	0 d	0 b	3 cd	0 d	1 gh	0 d
Bentazon	560	1 e	0 e	0 h	0 e	1 ef	0	0 d	0 b	1 cd	0 d	0 h	0 d
Untreated	——	0 e	0 e	0 h	0 e	0 f	0	0 d	0 b	0 d	0 d	0 h	0 d
LSD 0.05		3	1	4	2	3	NS	2	1	4	2	4	3

[a] Abbrevations: COC, crop oil concentrate; DAT, days after treatment; Hs1-wc, Hays, season 1, weed control; Mn1-wc, Manhattan, season 1, weed control; Hs2-wc, Hays, season 2, weed control; Mn2-wc, Manhattan, season 2, weed control; safl, saflufenacil.
[b] Means followed by the same letter within columns are not significantly different.
[c] COC applied at 1% v/v.
[d] Units for dicamba, MCPA ester, 2,4-D ester, and 2,4-D amine are g ae/ha.

Table 4.5. Leaf necrosis, stunting, and grain yield of weed-free winter wheat[a, b].

Treatments[c]	Rate[d]	Necrosis 4 to 5 DAT				Necrosis 16 to 20 DAT				Stunting 16 to 20 DAT				Grain yield[e]
		Hs1-wf	Mn1-wf	Hs2-wf	Mn2-wf	Hs1-wf	Mn1-wf	Hs2-wf	Mn2-wf	Hs1-wf	Mn1-wf	Hs2-wf	Mn2-wf	
	g ai/ha	%												kg/ha
Safl + COC	13	10 b	10 b	18 ab	13 d	0	0	9 b	5 b	0 d	0 c	0	6 b	3060 b-e
Safl + COC	25	18 a	19 a	18 ab	20 c	0	0	11 ab	5 b	8 a	6 ab	0	0 c	3060 b-e
Safl + dicamba	13+140	6 bcd	0 d	0 d	1 g	0	0	0 c	0 c	3 c	5 b	0	0 c	3220 abc
Safl + dicamba	25+140	9 bc	5 c	0 d	5 f	0	0	0 c	0 c	0 d	5 b	0	1 c	3230 ab
Dicamba	140	4 de	0 d	0 d	0 g	0	0	0 c	0 c	0 d	5 b	0	0 c	3120 a-e
Safl + MCPA ester	13+520	16 a	10 b	18 ab	25 b	0	0	11 ab	5 b	8 a	0 c	0	6 b	3030 de
Safl + MCPA ester	25+520	20 a	20 a	19 a	35 a	0	0	13 a	10 a	10 a	8 a	0	15 a	3040 cde
MCPA ester	520	3 de	0 d	0 d	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	3250 a
Safl + 2,4-D amine	13+533	6 bcd	4 c	0 d	5 f	0	0	0 c	0 c	3 c	0 c	0	0 c	3120 a-e
Safl + 2,4-D amine	25+533	6 bcd	8 b	0 d	8 e	0	0	0 c	0 c	3 c	0 c	0	0 c	3100 a-e
2,4-D amine	533	0 e	0 d	0 d	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	3180 a-d
Safl + 2,4-D ester	13+533	9 bc	10 b	14 b	21 c	0	0	13 a	5 b	4 c	1 c	0	8 b	2950 e
Safl + 2,4-D ester	25+533	18 a	20 a	19 a	33 a	0	0	13 a	10 a	10 a	8 a	0	14 a	3040 cde
2,4-D ester	533	5 cd	0 d	5 c	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	2990 e
Safl + bentazon	13+560	6 bcd	5 c	0 d	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	3260 a
Safl + bentazon	25+560	5 cd	10 b	0 d	5 f	0	0	0 c	0 c	0 d	0 c	0	0 c	3200 a-d
Bentazon	560	4 ef	0 d	0 d	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	3180 a-d
Untreated	——	0 e	0 d	0 d	0 g	0	0	0 c	0 c	0 d	0 c	0	0 c	3240 ab
LSD 0.05		4	2	4	2	NS	NS	2	1	2	2	NS	2	180

[a] Abbrevations: COC, crop oil concentrate, DAT, days after treatments; H1-wf, Hays, season 1, weed-free; M1-wf, Manhattan, season 1, weed-free; H2-wf, Hays, season 2, weed-free; M2-wf, Manhattan, season 2, weed-free; safl, saflufenacil.

[b] Means followed by the same letter within columns are not significantly different.

[c] COC applied at 1% v/v.

[d] Units for dicamba, MCPA ester, 2,4-D ester, and 2,4-D amine are g ae/ha.

[e] Averaged over experiments.

CHAPTER 5 - Saflufenacil Absorption, Translocation, and Metabolism in Winter Wheat

ABSTRACT

Field research in Kansas has shown POST-applied mixtures of saflufenacil and 2,4-D amine or bentazon to be less injurious to winter wheat compared to saflufenacil applied alone. Saflufenacil absorption, translocation, and metabolism studies were conducted to determine possible mechanism(s) responsible for the crop safening effects observed when saflufenacil is applied with 2,4-D amine or bentazon in winter wheat. Winter wheat plants were treated with ^{14}C-saflufenacil at 25 g/ha alone and in combination with 533 g ae/ha of 2,4-D amine or 560 g ai/ha of bentazon and 0.25% v/v crop oil concentrate. Wheat plants were harvested at 1, 3, 7, and 14 DAT in absorption and translocation studies, and 3, 7, and 14 DAT in the metabolism study. Saflufenacil absorption increased over time except when applied with bentazon. Depending on harvest timing, 2.8- to 3.5-times more saflufenacil was absorbed by wheat plants when saflufenacil was applied with 2,4-D amine compared to saflufenacil alone. Less than 10% of saflufenacil was absorbed when mixed with bentazon. Saflufenacil absorption into wheat plants was intermediate when saflufenacil was applied alone compared to the other treatments. Regardless of herbicide treatment or harvest timing, ≤11% of absorbed saflufenacil was translocated from the treated leaf to other plant parts. Metabolism of saflufenacil by wheat plants was not affected by tank mixing with bentazon, but saflufenacil metabolism was slowed by mixing with 2,4-D amine. This study clearly indicates 2,4-D amine enhances saflufenacil absorption into winter wheat plants, whereas, bentazon reduces absorption of saflufenacil.

Nomenclature: bentazon; saflufenacil; 2,4-D amine; *Triticum aestivum* L. ‘Danby’.

Key words: BAS 800H, absorption, translocation, metabolism, autoradiography, wheat

INTRODUCTION

Saflufenacil is new herbicide developed by BASF Corporation that controls common annual broadleaf weeds such as kochia [*Kochia scoparia* (L.) Schrad.], *Amaranthus* spp., Russian thistle (*Salsola tragus* L.), flixweed [*Descurainia sophia* (L.) Webb. Ex Prantl], field pennycress (*Thlaspi arvense* L.), shepard's purse[*Capsella bursa-pastoris* (L.) Medik.], and several other weed species in wheat and other crops by inhibiting protoporphyrinogen oxidase (protox) (Anonymous 2008). Protox inhibiting herbicides prevent the biosynthesis of chlorophyll and heme by competitive inhibition of the protox enzyme (Camadro et al. 1991; Matringe et al. 1992). As a result of protox inhibition, protoporphyrinogen IX (protogen) is not converted to protoporphyrin IX (proto) in chloroplasts and protogen accumulates until it leaks from chloroplasts into the cytoplasm (Jacobs et al. 1991; Jacobs and Jacobs 1993; Lee et al. 1993; Witkowski and Halling 1989). In the cytoplasm protogen is converted to proto by enzymes in the cytoplasm (Jacobs et al. 1991; Jacobs and Jacobs 1993; Lee et al. 1993). Proto reacts with light and oxygen to form radical singlet oxygen that causes lipid peroxidation (Becerril and Duke 1989; Haworth and Hess 1988; Jacobs et al. 1991).

Saflufenacil may particularly be useful to control annual broadleaf weeds including herbicide resistant biotypes in winter wheat because few protox-inhibiting herbicides are registered for use in wheat (Thompson et al. 2009; Bernards et al. 2009). Research in Kansas, Oregon, and North Dakota found preplant and PRE treatments of saflufenacil up to 100 g/ha did not injure wheat (Jenks et al. 2008). Knezevic et al. (2008) reported similar findings in winter wheat in Nebraska at rates up to 400 g/ha of saflufenacil did not injure or reduce grain yields. However use of saflufenacil in winter wheat maybe limited because many winter wheat growers prefer to apply herbicides POST in the spring (D. E. Peterson and P. W. Stahlman, personal communication).

Research has shown POST-applied saflufenacil causes significant injury and yield loss in several small grain crops (Frihauf et al. 2008a; Frihauf et al. 2008b; Knezevic et al. 2008; Sikkema et al. 2008; Soltani et al. 2008). However, studies in Kansas found winter wheat leaf necrosis and stunting were reduced when saflufenacil was tank mixed with 2,4-D amine (Frihauf et al. 2008a; Frihauf et al. 2008b). Tank mixing bentazon with saflufenacil also reduced necrosis and stunting of wheat compared to saflufenacil with crop oil concentrate (COC) in field (Frihauf et al. 2008b).

The mechanism(s) responsible for safening of wheat to saflufenacil by mixing with 2,4-D amine or bentazon is not known. The objectives of this research were to determine absorption, translocation, and metabolism of saflufenacil in winter wheat when saflufenacil is applied alone and mixed with 2,4-D amine or bentazon.

MATERIALS AND METHODS

Plant Materials

Absorption, translocation, and metabolism studies were conducted in a growth chamber set for 20/15 C day/night temperatures, 12 hr photoperiod, and photosynthetic photon flux density of 250 µmol/m^2/s. Seeds of 'Danby' winter wheat were sown in 3.8-cm diam by 21-cm tall containers[1] with one seed sown per container. Soil was a mixture of sand and Morrill loam (mesic Typic Argiudolls) (1:1 by vol) with a pH of 8.0 and 1.2% organic matter. Plants in each experiment were watered and fertilized as needed with a commercial fertilizer[2] solution containing 1.2 g/L total nitrogen, 0.4 g/L phosphorus, and 0.8 g/L potassium.

Absorption and Translocation

Leaves of uniform 30-day old winter wheat plants were treated with 25 g/ha ^{14}C-saflufenacil alone and mixed with 533 g/ha of 2,4-D formulated as a dimethylammonium salt or 560 g/ha of bentazon. Crop oil concentrate[3] was included in all herbicide treatments at 0.25% v/v to facilitate placement of droplets with a microsyringe on the leaf surface. Ten 1-µl droplets containing 101 Bq of ^{14}C saflufenacil (specific activity 5540 Bq/µg, phenyl labeled ^{14}C-saflufenacil) were applied uniformly across the upper surface of the youngest fully expanded leaf of the main tiller when plants were 26 to 35-cm tall with 2 to 3 tillers. Unlabeled saflufenacil (emulsifiable concentrate formulation), 2,4-D amine, and bentazon were added to appropriate solutions to achieve desired rates for each herbicide. Radioactive herbicide solutions were mixed based on a carrier volume of 187 L/ha.

Wheat plants were harvested at 1, 3, 7, and 14 DAT and separated into treated leaf, treated tiller, untreated tillers, and root tissues. Treated leaves were rinsed in 15 ml of 50% methanol (by volume) to remove unabsorbed herbicide. Plant parts were oxidized with a biological oxidizer[4] after plant parts were dried at 45 C for 48 hr. Radioactivity in leaf rinsates and radioactivity recovered from each plant part were determined by using liquid scintillation spectrometry[5] (LSS). Herbicide absorption was calculated by dividing the radioactivity recovered from each plant by the radioactivity applied to each plant. Herbicide translocation was calculated by dividing the radioactivity recovered from each plant part by the total radioactivity in the plant.

In a separate study, the pattern of ^{14}C-saflufenacil translocation was determined by using autoradiography. One 5 µl droplet of ^{14}C-saflufenacil, ^{14}C-saflufenacil plus 2,4-D amine, or ^{14}C-saflufenacil plus bentazon was placed on the adaxial surface of the youngest fully expanded leaf of each wheat plant. One 5 µL droplet of each herbicide solution contained 4130 Bq of ^{14}C saflufenacil (phenyl labeled ^{14}C saflufenacil, specific activity 5540 Bq/µg). Plants were harvested at 1, 3, 7, and 14 DAT. At harvest, the treated leaf of each plant was rinsed with 15 ml of 50% methanol (by volume) and then plants were cut even with the soil surface. Wheat plants were pressed between paper towels, dried at 45 C for 48 hr, and then mounted on 28 by 35 cm white poster board with glue. After glue dried, mounts were placed against X-ray film[6] and then wrapped in aluminum foil in a darkroom. Plant mount and X-ray film packages were weighted with cinder block to maximize film to mount contact. X-ray film was developed using an automatic processor after a 6 wk exposure time.

Metabolism

Plants were treated with 25 g/ha of ^{14}C-saflufenacil alone and mixed with 533 g/ha of 2,4-D amine or 560 g/ha of bentazon as described for the absorption and translocation experiments. Four fully expanded leaves of wheat plants were treated with sixteen 1-µl droplets of ^{14}C-saflufenacil, ^{14}C-saflufenacil plus 2,4-D amine, or ^{14}C-saflufenacil plus bentazon when wheat was 27 to 30-cm tall with 2 to 3 tillers. Plants were harvested at 3, 7, and 14 DAT and treated leaves of wheat plants were rinsed in 15 ml of 50% methanol to remove unabsorbed herbicide. Plants were then cut at the soil surface, frozen in liquid nitrogen, ground with a mortar and pestle, and stored at -80 C until radioactivity was extracted. Before samples were stored, subsamples of wheat plants were weighed and oxidized with a biological oxidizer, and captured $^{14}CO_2$ was measured using LSS to determine the level of radioactivity in the plant tissue.

Samples were homogenized with 15 ml of 75% acetone (by volume) with continuous shaking for 1 hr at room temperature. Samples were centrifuged[7] for 15 min at 23,700 g and 4 C. The supernatant was saved and the remaining plant tissue was re-suspended in 15 ml of 50% acetone (by volume) and shaken at room temperature for another hour. Samples were centrifuged again and the supernatant was added to the first supernatant. The remaining plant tissue was re-suspended in 100% acetone and shaken for an additional hour at room temperature. Tissue samples were then filtered with Whatman 4 filter paper[8] and the supernatant was added to supernatant collected after samples were centrifuged. The remaining plant tissue and filter paper was oxidized with a biological oxidizer to determine radioactivity not extracted into the supernatant and the radioactivity was counted using LSS. Averaged over all samples, 89% of ^{14}C-saflufenacil was extracted from wheat plants.

Supernatants were evaporated to 1 ml using a centrivap[9] at 45 C. Extracts were filtered using a 0.2 µm filter[10] and stored at -20 C until injected into a high-performance liquid chromatography. Extracts were injected into a Beckman high-performance liquid chromatograph[11] using a Zorbax ODS endcapped Sb-C18 column[12] (4.6 X 250 mm), operated at 25 C with a mobile phase of 0.1% formic acid in water and acetonitrile with a flow rate of 1 ml/min. The elution profile was as follows: 30 to 70% acetonitrile linear gradient for 15 min, 70 to 30% acetonitrile linear gradient for 1 min and then constant 30% acetonitrile for 6 minutes. Radioactivity was measured with an EG&G Berthold scintillation spectroscope[13]. A saflufenacil standard was included to determine when the herbicide eluted from the column.

Experimental Design and Statistical Analysis

The absorption, translocation, and metabolism experiments were designed as a randomized complete blocks with factorial arrangement of herbicide treatments and harvest timings replicated four times. The absorption and translocation experiment was repeated; however, the metabolism experiment was not repeated. The experimental design of the autoradiography experiment was the same as described in the absorption and translocation experiment except treatments were replicated twice and the experiment was not repeated.

Data from absorption, translocation, and metabolism studies were checked for normality and homogeneity of variances. Arcsine transformations were performed to stabilize variance in translocation data and untransformed means are presented for clarity. Data for individual experiments were subjected to ANOVA in PROC MIXED in SAS[14]. Treatment means from all experiments were separated using least square means at $\alpha = 0.05$.

Analysis of absorption data indicated runs-by-herbicide treatments-by-harvest timings interaction was significant, but the interaction was due to differences in magnitudes between runs. Mean separation tests revealed that the ranking of treatment means within each run was similar, thus data were reanalyzed across runs with treatments and harvest timings as fixed effects and runs and replications within runs as random effects.

Absorption and metabolism data were subjected to regression analysis using single rectangular hyperbola and exponential decay models, respectively. A lack of fit test of each model was performed by partitioning nonlinear sums of squares into lack of fit error and pure experimental error (Draper and Smith 1981). Models were considered appropriate if an F-test value for lack of fit sums of squares was not significant at $\alpha = 0.05$. Pair-wise differences in saflufenacil absorption and metabolism curves among herbicide treatments were tested using F-tests at $\alpha = 0.05$ as described by Seefeldt et al. (1995).

RESULTS AND DISCUSSION

Absorption and Translocation

Saflufenacil absorption increased over time when applied alone or in mixture with 2,4-D amine (Figure 5.1). However, absorption of saflufenacil was minimal and did not increase over time when saflufenacil was applied with bentazon. Depending on harvest timing, 2.8- to 3.5-times more saflufenacil was absorbed by wheat plants when saflufenacil was applied with 2,4-D amine compared to saflufenacil applied alone. Similar amounts of ^{14}C-saflufenacil (6±2%) were absorbed at 1 and 3 DAT when applied alone or in mixture with bentazon. Absorption of ^{14}C-saflufenacil increased to 13% at 7 DAT and to 16% at 14 DAT when saflufenacil was applied alone, but ^{14}C-saflufenacil absorption did not increase significantly beyond 3 DAT when applied in mixture with bentazon.

Averaged over harvest timings, 89% or more of absorbed ^{14}C-saflufenacil remained in the treated leaves (Figure 5.2). The amount of saflufenacil remaining in treated leaves was similar between saflufenacil applied alone and saflufenacil plus 2,4-D amine. Less saflufenacil was recovered from treated leaves when saflufenacil was applied with bentazon compared to the other two treatments. Regardless of herbicide treatments, minimal amounts of saflufenacil translocated from the treated leaf to other plant parts with ≤6% of the absorbed saflufenacil found in treated tillers. Within each herbicide treatment, the amount of ^{14}C-saflufenacil found in plant parts decreased as follows: treated leaf > treated tillers > untreated tillers > roots. Results of the autoradiograph study support these findings and show that little saflufenacil translocated out of treated leaves at any harvest timing regardless of herbicide treatment (Figure 5.3). The results of these experiments are similar to earlier research which also found little translocation of protox-inhibiting herbicides in various plants (Higgins et al. 1988; Ritter and Coble 1981; Shoup and Al-Khatib 2005; Unland et al. 1999; Vanstone and Stobbe 1978.

Metabolism

Eight distinct peaks of radioactivity, each representing a metabolite of saflufenacil, were separated from the parent compound (saflufenacil). These peaks were discovered at 4.6, 6.1, 7.6, 9.2, 10.5, 11.7, 13.7, and 14.6 minutes with saflufenacil eluting from the column at 16.5 minutes (Table 5.1). All metabolites were present in each treatment but varied according to harvest timings and herbicide treatment. However, the amount of the metabolite that eluted from the column at 11.7 minutes was significantly greater than all other metabolites for all treatments and harvest timing (Table 5.1).

Metabolism of saflufenacil did not differ between saflufenacil applied alone and saflufenacil mixed with bentazon ($F_{4, 23} = 1.52$, p-value = 0.23) (Figure 5.4). Saflufenacil declined over time in all treatments; however, metabolism was slower when saflufenacil was mixed with 2,4-D amine compared to saflufenacil alone or with bentazon. At 3 DAT, 49% of absorbed saflufenacil remained when wheat plants were treated with saflufenacil in mixture with 2,4-D amine. Less of the parent structure remained at each harvest timing when saflufenacil was applied alone or in combination with bentazon compared to saflufenacil plus 2,4-D amine. Greater saflufenacil absorption when saflufenacil was applied with 2,4-D amine likely resulted in more saflufenacil at 3 DAT. By 7 DAT, the saflufenacil concentration dropped to similar levels in all treatments (20 to 22%). Saflufenacil in all wheat plants continued to decline by 14 DAT, regardless of herbicide treatment. However, metabolism of saflufenacil was more complete when saflufenacil was applied alone or with bentazon.

This research clearly showed that in the presence of COC, absorption of saflufenacil was enhanced by 2,4-D amine but reduced by bentazon compared to saflufenacil applied alone. The effect of bentazon on saflufenacil absorption is consistent with numerous studies reporting bentazon limits absorption of several herbicides in various weed and crop species (Bauer et al. 1995a ; Gerwick 1988; Lycan and Hart 1999; Sorensen et al. 1987; Wehtje et al. 1992). Because of less saflufenacil absorption when saflufenacil was applied alone or mixed with bentazon, metabolism processes in wheat plants were likely not overwhelmed and saflufenacil was metabolized faster compared to saflufenacil in combination with 2,4-D amine. This research also found that saflufenacil metabolism by wheat plants was slowed when saflufenacil was mixed with 2,4-D amine. The combined effects of enhanced absorption and slowed metabolism of saflufenacil in the presence of 2,4-D amine and COC explain severe wheat necrosis caused by similar treatments in field and greenhouse studies. Although not addressed in this study it is probable less saflufenacil is absorbed by wheat plants when mixed with 2,4-D amine without COC, resulting in less visible wheat injury (Frihauf et al. 2008a; Frihauf et al. 2008b).

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SOURCES OF MATERIALS

[1] SC10 Super Cell Cone-tainer, Stuewe & Sons, 31933 Rolland Drive, Tangent, OR 97389.

[2] Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 14111 Scottslawn Road, Marysville, OH 43041.

[3] Crop oil concentrate, Agri-dex, Helena Chemical Co., 7664 Moore Road, Memphis, TN 38120.

[4] R. J. Harvey Biological Oxidizer, Model OX-600, R. J. Harvey Instrument Co., 123 Patterson Street, Hillsdale, NJ 07642.

[5] Tricarb 2100TR Liquid Scintillation Analyzer, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[6] X-ray film, X-Omat™ LS, Eastman Kodak Co., Rochester, NY 14650.

[7] J2-MC centrifuge, Beckman Coulter Inc, Life Science Research Division, 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100.

[8] Whatman International Ltd., Springfield Mill, James Whatman Way, Maidstone, Kent ME14 2LE, United Kingdom.

[9] Centrivap, Labconco, 8811 Prospect, Kansas City, MO 64132.

[10] 0.2-mm filter, Osmonics Inc., 5951 Clearwater Drive, Minnetonka, MN 55343.

[11] Beckman high-performance liquid chromatograph, Beckman Coulter Inc., Life Science Research Division, 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100.

[12] Zorbax ODS endcapped Sb-C18 column, Agilent Technologies, Chemical Analysis Group, 2850 Centerville Road, Wilmington, DE 19808.

[13] Scintillation spectroscope, EG&G Berthold, Postfach 100163, Bad Wilbad D-75312, Germany.

[14] SAS version 9.1, SAS Institute Inc., Box 8000, Cary, NC 27511-8000.

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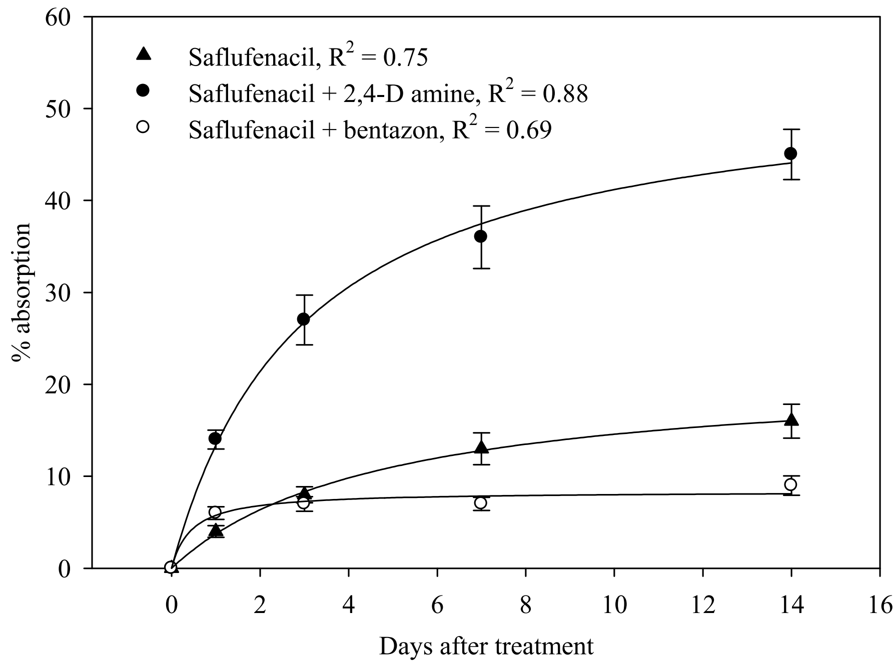

Figure 5.1. Saflufenacil absorption into wheat plants at 1, 3, 7, and 14 days after treatment. Data are means plus or minus the standard error. The regression equations for saflufenacil, saflufenacil plus 2,4-D amine, and saflufenacil plus bentazon are percent saflufenacil = 21.45*x/(4.80+x), percent saflufenacil = 54.78*x/(3.20+x), and percent saflufenacil = 8.14*x/(0.38+x), respectively.

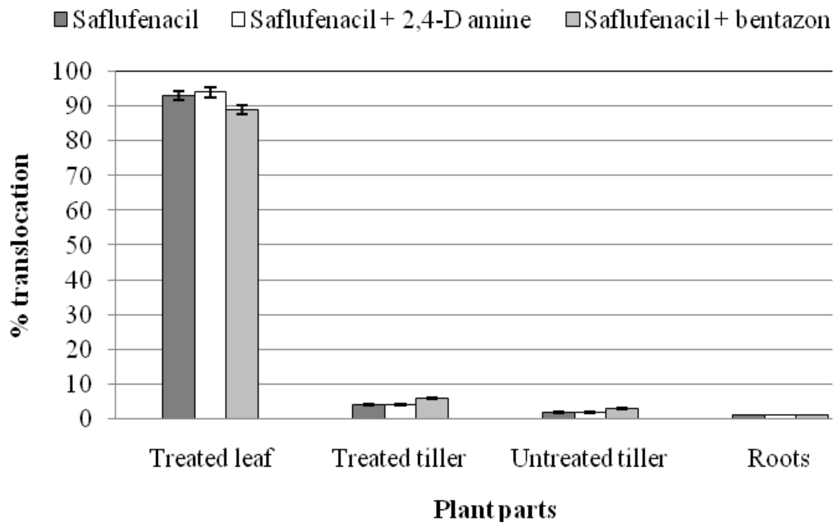

Figure 5.2. Translocation of saflufenacil applied alone and with 2,4-D amine or bentazon in wheat. Data are averaged over harvest timings.

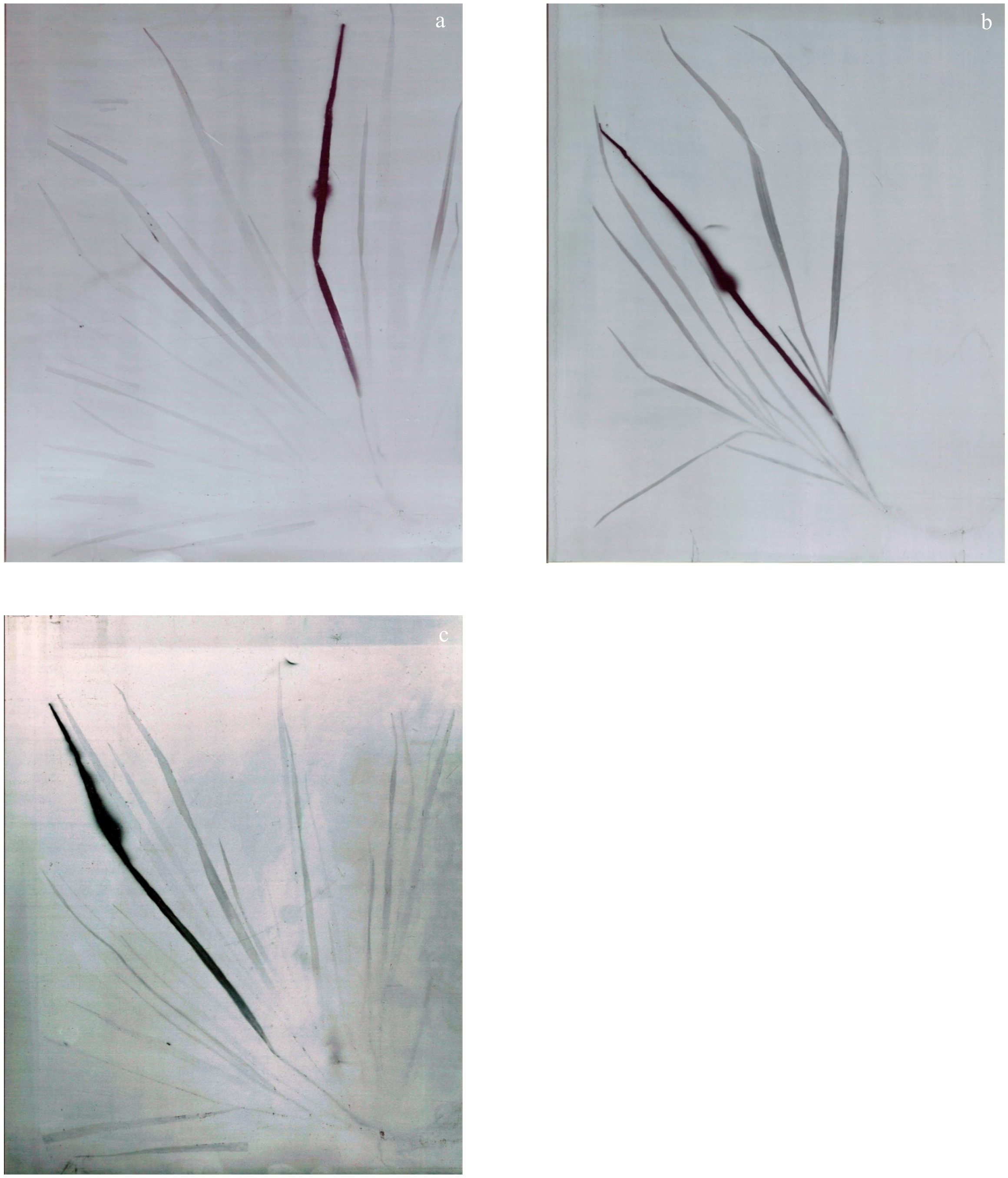

Figure 5.3. Autoradiographs of wheat plants treated with saflufenacil (a), saflufenacil plus 2,4-D amine (b), and saflufenacil plus bentazon (c) at 14 days after treatment.

Table 5.1. Saflufenacil and metabolites extracted from wheat treated with saflufenacil, saflufenacil plus 2,4-D amine, and saflufenacil plus bentazon at 3, 7, and 14 DAT[a].

		Saflufenacil[c]			Saflufenacil + 2,4-D amine[d]			Saflufenacil + bentazon[e]		
Compound	Retention time	3 DAT[b]	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT
	min	% of compound								
Metabolite 1	4.6	1 ± 1	3 ± 1	6 ± 2	2 ± 1	3 ± 1	6 ± 1	2 ± 1	2 ± 1	7 ± 2
Metabolite 2	6.1	2 ± 1	2 ± 1	14 ± 1	4 ± 1	4 ± 1	11 ± 1	4 ± 1	2 ± 1	9 ± 1
Metabolite 3	7.6	4 ± 1	4 ± 1	9± 2	6 ± 2	7 ± 1	10 ± 2	13 ± 1	13 ± 2	15 ± 2
Metabolite 4	9.2	5 ± 1	9 ± 1	8 ± 2	8 ± 2	10 ± 3	8 ± 1	8 ± 1	9 ± 1	9 ± 1
Metabolite 5	10.5	3 ± 1	6 ± 1	7 ± 1	3 ± 1	6 ± 1	7 ± 2	4 ± 1	6 ± 1	4 ± 1
Metabolite 6	11.7	21 ± 3	31 ± 1	36 ± 3	20 ± 3	29 ± 2	29 ± 2	27 ± 2	30 ± 2	31 ± 2
Metabolite 7	13.7	16 ± 4	15 ± 1	7 ± 1	7 ± 1	10 ± 1	4 ± 1	8 ± 1	12 ± 3	4 ± 1
Metabolite 8	14.6	9 ± 1	10 ± 2	10 ± 2	8 ± 1	11 ± 1	10 ± 1	6 ± 1	8 ± 1	7 ± 1
Saflufenacil	16.5	30 ± 2	22 ± 2	5 ± 1	49 ± 2	22 ± 3	17 ± 2	29 ± 3	18 ± 4	15 ± 4

[a] Abbreviation: DAT, days after treatment.
[b] Data are means ± standard error.
[c] 25 g/ha of ^{14}C-saflufenacil.
[d] 25 g/ha of ^{14}C-saflufenacil plus 533 g/ha 2,4-D amine.
[e] 25 g/ha of ^{14}C-saflufenacil plus 560 g/ha of 2,4-D amine.

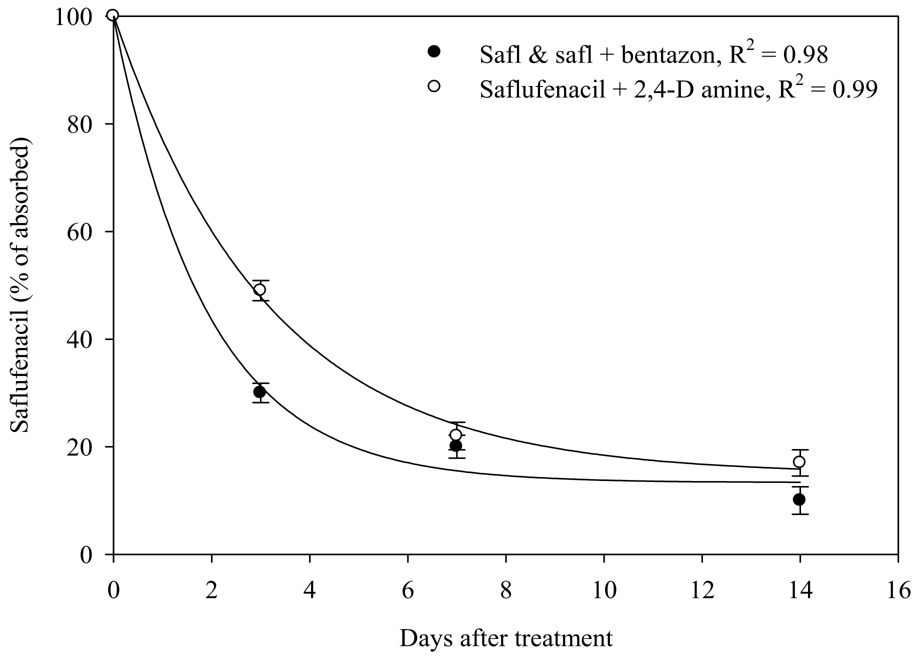

Figure 5.4. Metabolism of saflufenacil in wheat at 3, 7, and 14 days after treatment. Data are means plus or minus the standard error. The regression equations are percent saflufenacil = $13.18+86.73e^{(-0.53x)}$ (saflufenacil & saflufenacil + bentazon) and percent saflufenacil = $14.58+87.71e^{(-0.32x)}$ (saflufenacil + 2,4-D amine). Abbreviation: safl, saflufenacil.