The Effect of Foliar and Soil Application of Flufenacet and Prosulfocarb on Italian Ryegrass (Lolium Multiflorum L.) Control

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Abstract: Italian ryegrass (Lolium multiflorum Lam.) can be a troublesome weed that may causes high yield losses to several crops. Ryegrass resistance to the typically used acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase) inhibiting herbicides complicates the control. As an alternative, we evaluated and compared the effects of two soil-acting herbicides, flufenacet and prosulfocarb, on susceptible L. multiflorum. The herbicides were applied in two doses in three different methods of applications: (1) soil and foliar application (2) foliar application, and (3) soil application only. Two greenhouse experiments separated in time showed that both herbicides reduced root and foliar biomass significantly as compared to the nontreated plants. In experiment 1, both herbicides resulted in lower efficacy when they only were applied to the leaves compared to the nontreated plants. Especially the foliar effect of flufenacet was small. The highest dose of prosulfocarb (4200 g ai ha⁻¹) reduced the fresh foliar weight by 61% in experiment 1 and by 95% in experiment 2. The lowest dose of prosulfocarb (2100 g ai ha⁻¹) reduced the weights by 73% (experiment 1) and 98% (experiment 2), respectively. For both herbicides the soil and foliar application applied postemergence were effective in reducing growth of L. multiflorum significantly in both experiments. Foliar application showed inconsistent results, showing that soil absorption plays an important role on herbicide efficacy even when the herbicides are applied postemergence. Postemergence application of prosulfocarb and flufenacet were effective to reduce L. multiflorum growth having apparently good root and leaf absorption.

Keywords: herbicide efficacy; herbicide resistance management; herbicide uptake; weed resistance

1. Introduction

Italian ryegrass (Lolium multiflorum Lam.) is a competitive and troublesome grass weed with high adaptability to new environments, high tillering, and seeds that persist in the soil for up to three years [1,2]. For every 8–10 plants m⁻² a 5% yield reduction in wheat has been registered, and in heavy L. multiflorum-infested wheat fields, the yield loss may be up to more than 90% [2–4].

In cereals, acetyl-CoA carboxylase (ACCase) and acetolactate synthase (ALS) inhibitors have been the two preferred postemergence selective groups of herbicides used against grass weeds in Europe [5,6]. However, extended use of a few active ingredients leads to an increased occurrence of weed resistance to herbicide. This is true for grassy weeds such as Lolium rigidum Gaud. and L. multiflorum to these herbicides [7–9]. Worldwide, more than 165 weed species have been registered as resistant to ALS inhibitors and more than 49 weed species to ACCase inhibitors including L. multiflorum in 2019 [9,10].

Even though alternatives to chemical weed control exist, it is likely that herbicides will remain the most used weed control method in the near future due to high efficacy and cost-efficiency [7,11].
Use of herbicides with alternative modes of actions such as inhibitors of the biosynthesis of very long chain fatty acids (VLCFAs) could slow down the evolution of herbicide-resistant weeds [11]. Upright leaves are more susceptible to run-off of foliar applied herbicides and can sometimes result in reduced efficacy for controlling grass weeds if the herbicides do not provide soil activity [12]. Therefore, there has been an increasing interest for soil-acting herbicides, which have become the backbone in managing grass weed infestation. Among the alternatives for ryegrass control in winter cereals in Europe are flufenacet and prosulfocarb. The two soil acting herbicides may replace ALS- and ACCase-herbicides where weed resistant grass occur.

Flufenacet is an oxyacetamide used as a selective pre- and early postemergence herbicide with systemic properties and meristematic activity [13,14]. Flufenacet inhibits the biosynthesis of VLCFAs, which are essential for a wide range of processes within plants. VLCFAs are involved in cell division and the synthesis of cuticular waxes and suberin. Cuticular waxes and suberin serve as the primary protecting barrier on the leaf surface against external attacks (e.g., pests and diseases), herbicide penetration and dehydration [15]. Flufenacet is used to control a broad spectrum of grassy weeds such as Alopecurus myosuroides Huds., Apera spica-venti (L.) P. Beauv, Bromus diandrus Roth. and Lolium ssp. [10,13,16,17], and broad leaf species such as Phalaris minor Retz. and Rumex retroflex L. [16].

Prosulfocarb belongs to the group of thiocarbamates. It is a broad-spectrum systemic herbicide, which blocks the lipid synthesis in another way than ACCase inhibitors. While ACCase herbicides inhibit the enzyme acetyl-CoA carboxylase, which catalyzes the first step in the fatty acid synthesis, the specific target site(s) of prosulfocarb is unknown, but it is considered, to some extent, a ‘multisite’ herbicide. However, the largest family of elongase, which are proteins that act as biological catalysts accelerating the chemical reactions prolonging the chain of fatty acids, is located in the endoplasmic reticulum, and prosulfocarb is believed to inhibit the synthesis of very long chain of fatty acids [18,19]. Resistance to prosulfocarb in Lolium species from farm sites and glasshouse selection has been reported [18]. Flufenacet, in particular, has become a key herbicide for the control of multiple-resistant A. myosuroides [20]. However, in some of those populations, reduced flufenacet efficacy has been observed [20]. Flufenacet resistance has been reported in L. multiflorum populations from the northwest USA [21]. Field relevant levels of flufenacet resistance have also been observed in Lolium spp. populations in France, UK and Australia. It is important to reduce selection pressure and prevent any possible loss of flufenacet efficacy, as flufenacet has, especially in Europe, become a valuable tool for the management of Lolium spp. [22].

Both flufenacet and prosulfocarb have their predominated route into the weed plant via uptake from the soil [14,19,21]. Both herbicides are applied postemergence and foliar uptake should not be ignored. Prosulfocarb controls seedlings as a postemergent herbicide [9,18], but flufenacet is mainly used as a preplant incorporated (PPI) and pre-emergence (PRE) herbicide [14]. Prosulfocarb half-life is inversely affected by soil organic matter [23]. The high affinity for organic matter due to its high n-octanol/water partition coefficient (K\text{ow}) (log K\text{ow} = 4.48), and low solubility in water (13.2 mg l\text{−1}) [23]. Flufenacet has a log K\text{ow} of 3.2 and a solubility in water of 56 mg l\text{−1} at 20 °C [14]. This contrasting differences may play a role on herbicide absorption when applied on leaf and soil surfaces.

The objective of this study was to quantify and compare the effects of foliar and soil application of flufenacet and prosulfocarb on L. multiflorum under controlled conditions in a greenhouse. The effect of soil-applied herbicides varies considerably depending on the growing conditions [24,25]. Therefore, we studied the herbicide uptake in a controlled environment in two independent experiments to be able to separate the effects of foliar and root uptake of the herbicides. We hypothesized that soil application of the two herbicides would control L. multiflorum efficiently with the chosen dosages, while the foliar application would not be efficient. We also expected that the combination of soil and foliar application would result in the same effect as soil applications of the two herbicides.
2. Materials and Methods

2.1. Experiments

Two independent experiments were conducted in a greenhouse at the University of Copenhagen, Hoejbakkegaard, Taastrup, Denmark (55°38’ N, 12°17’ E). Experiment 1 was sown on 3 January, the treatments were applied on 24 January, and harvested on 22 February 2018. Experiment 2 was sown on 13 March, the treatments were applied on 4 April, and harvested on 2 May 2018.

The minimum temperature in the greenhouse was 20 °C and the maximum was 25 °C. Seeds of Lolium multiflorum (variety: Majesty) (Deutsche Saatveredelung AG (DSV), Lippstadt, Germany) were bought from a seed company and used to produce the model plants. Five to eight seeds of L. multiflorum were sown in 106 plastic pots (Ø = 10 cm). Vermiculite was used as growth substrate. Vermiculite is a commercial product and well defined which makes it possible to reduce the variation when the experiments are repeated. Vermiculite is a hydrous phyllosilicate mineral [26]. On the one leaf stage, plants were thinned to one plant per pot. Trays containing ten pots each were placed on tables inside the greenhouse. The trays had holes in the bottom to allow to irrigate the pots by capillarity.

Plants were grown under 16 h of light and 8 h of dark per day. Natural light was supplemented by artificial light from two SON-T high-pressure sodium lamps, 400 W (48,000 lm each) (Phillips lighting, Copenhagen, Denmark). Every 4–5 days the trays with pots were rearranged to avoid the influence of variable growth conditions such as colder places along the greenhouse wall and differences in irradiance from the artificial lights or the sun.

Eight pots from each tray were sprayed with the herbicides in three different methods of applications: (1) soil and foliar application (substrate + foliar) (2) foliar application only (foliar-only), and (3) soil application only (substrate-only). The two remaining pots from each tray were moved to another tray to avoid contamination from sprayed plants and were used as controls. Eight replicates were used for each herbicide application. Additionally, ten plants were used as nontreated control plants. Altogether, each experiment consisted of 8 (replicates) × 3 (types of application) × 2 (doses) × 2 (herbicides) + 10 controls = 106 pots.

2.2. Chemicals and Treatments

The two herbicides, Flufenacet 500 g/L SC™ (flufenacet SC (Suspension Concentraten), 500 g ai L⁻¹, FMC, Hørsholm, Denmark) and Boxer™ (prosulfocarb EC (Emulsifiable Concentrate), 800 g ai L⁻¹, Syngenta, Copenhagen, Denmark) were used.

The pots were sprayed with the herbicides when the plants had developed two to three leaves. One to two days before foliar-only application, the pots were covered with a layer of active charcoal (<5 mm) preventing the herbicides leaching to the roots [27] (Figure 1a). A filter paper was placed between the charcoal and the vermiculite to minimize the risk of mixing vermiculite and charcoal.

At the day of spraying, a wooden stick was placed in the pots with substrate-only application to stabilize a glass test tube covering all green parts of the plant (Figure 1b). The substrate + foliar pots were not modified before application. Flufenacet and prosulfocarb were applied in a spraying cabin, which was calibrated before use. Two different dosages of each herbicide were applied, the recommended dose (1.0 × dose) and half the recommended dose (0.5 × dose). The applied active ingredient were for flufenacet 250 g ai ha⁻¹ (1.0 × dose) and 125 g ai ha⁻¹ (0.5 × dose), and for prosulfocarb 4200 g ai ha⁻¹ (1.0 × dose) and 2100 g ai ha⁻¹ (0.5 × dose). We used a two-nozzle sprayer delivering 135.1 L of spraying solution ha⁻¹ with a pressure of 400 kPa bars and a speed of 7.5 km h⁻¹. Two HARDI Low Drift Nozzles (Yellow)—ISO LD-02-110 (Hardi, Nørre Alslev, Denmark) were used delivering a medium spray-droplet pattern ensuring that leaves and soil was covered. The pots were moved to the greenhouse 30 min after spraying. The pots were irrigated daily by flooding the table with water containing fertilizer avoiding drought and nutrition stress. Pots were subirrigated to avoid herbicide being sprayed on the charcoal and leaching down in the soil, which might happen if they were irrigated from above.
Figure 1. General overview of the experimental unity and the preparation for herbicide application. (a) A pot (Ø = 10 cm) with vermiculate and an emerging L. multiflorum plant after charcoal had been added on the top of the vermiculite preventing the herbicide from leaching to the growth substrate and the roots, to account only for leaf absorption. (b) A pot (Ø = 10 cm) with vermiculite and an emerging L. multiflorum plant covered with a glass tube supported by a wood stick ready to be sprayed, to account only for soil absorption of the herbicide.

Three weeks after spraying, the plants were harvested by gently removing the vermiculite from the roots in a water bath. Fresh weights of shoots and roots were recorded.

2.3. Statistical Analysis

Shoot and root fresh weights were analyzed with a linear mixed model with interaction between experiments, doses and herbicides as fixed effect and trays as random effect. Model assumptions were checked through visual assessment of residual and QQ (quantile-quantile) plots. All measurements of weights were log-transformed to obtain homogeneity of variance. Estimates and 95% confidence intervals were back-transformed and presented on the original scale in the figures. Percentage reductions compared to the control treatment were estimated in an after-fitting step from the models based on raw data to avoid bias and loss of information [28]. Pairwise comparisons were made on the log-scale and adjusted for simultaneous inference with the single step procedure [29]. All analyses were made in the statistical programming software R version 3.4.2 [30] with the add-on package lme4 [31] and multcomp [29].

3. Results

Three-way interactions between experiment, application method and dose were significant for both leaf ($p = 0.007$) and root fresh weight ($p = 0.0017$). Accordingly, the results were presented for the two experiments separately. In the following text, 95% confidence intervals (95% CI) are shown in parentheses. The difference between experiments probably occurred because light intensities and temperatures varies between experiments resulting in different biomass productions.

3.1. Flufenacet (Experiment 1)

Flufenacet at 0.5 $\times$ dose applied as foliar-only did not result in any significant reduction of the biomass after three weeks for neither root nor foliar weight compared with the nontreated plants (Figures 2A and 3A). Substrate-only application resulted in a significant 75.7% (95% CI: 52.7–87.5%) reduction in the foliar weight and a smaller but still significant reduction in the root weight of 63.5% (95% CI: 38.3–78.4%) (Figures 2A and 3A) compared to nontreated plants. The substrate + foliar application resulted in a significant reduction in the weight of both foliar and root biomass of 98.3% (95% CI: 96.8–99.15%) and 92.7% (95% CI: 87.7–95.7%), respectively, compared to nontreated controls (Figures 2A and 3A).
Spraying with flufenacet at 1.0 × dose resulted in more than 97% reduction of the foliar fresh weight for both substrate + foliar and substrate-only application compared to the nontreated controls. Foliar-only application resulted in a reduction of 73.3% (95% CI: 48.2–86.3%) of the foliar weight compared to the nontreated plants (Figure 2B).

Flufenacet reduced the root weight significantly for foliar-only, substrate + foliar, and substrate-only with 63.1% (95% CI: 37.6–78.2%), 82.8% (95% CI: 71.0–89.8%) and 88.8% (95% CI: 81.0–93.4%) respectively, compared to the nontreated plants (Figure 3B).
3.2. Prosulfocarb (Experiment 1)

Prosulfocarb at 0.5 × dose reduced both foliar and root weight compared to the nontreated plants ($p < 0.05$) (Figures 2A and 3A). The reduction in foliar weight for foliar-only, substrate + foliar and substrate-only were 73.3% (95% CI: 48.2–86.26%), 97.3% (95% CI: 94.7–98.6%) and 97.7% (95% CI: 95.6–98.8%), respectively. The corresponding reductions in root biomass were 63.1% (95% CI: 37.6–78.2%), 81.8% (95% CI: 69.2–89.2%) and 88.8% (95% CI: 81.0–93.4%) compared to the nontreated plants, respectively.

Prosulfocarb reduced the root weight for substrate-only application more than flufenacet did ($p = 0.006$) (Figure 3A). However, the effect on the leaf weights was not significantly different for the two herbicides (Figure 2A).
For prosulfocarb at 1.0 $\times$ dose applied in substrate + foliar and substrate-only application reduced foliar fresh weights with 97.3% (95% CI: 94.7–98.6%) and 97.8% (95% CI: 95.7–98.9%) compared to the nontreated plants, respectively (Figure 2B). There were no significant differences in the reduction of neither leaf nor root biomass between substrate + foliar and substrate-only (Figures 2B and 3B). For foliar-only, there was no significant reduction in foliar weights with prosulfocarb but a reduction in root weight of 77.5% (95% CI: 61.9–86.7%) compared to the nontreated plants (Figures 2B and 3B).

3.3. Flufenacet (Experiment 2)

Spraying with flufenacet at 0.5 $\times$ dose in substrate + foliar application reduced the foliar and root weight with 99.2% (95% CI: 98.4–99.6%) and 98.2% (95% CI: 97.0–98.9%) compared to the nontreated plants, respectively, while a substrate-only application reduced the foliar and root weight with 97.7% (95% CI: 95.6–98.8%) and 92.0% (95% CI: 86.5–95.3%) compared to the nontreated plants, respectively (Figures 2C and 3C). Foliar-only did not significantly affect the foliar weight (Figure 2C) but did reduced the root weight by 29.0% compared to the nontreated plants, but it was not significant (Figure 3C).

Flufenacet at 1.0 $\times$ dose reduced the foliar and root weight significantly for substrate + foliar and substrate-only treatments, but not for foliar-only compared to the nontreated plants (Figures 2D and 3D). Both substrate + foliar and substrate-only reduced foliar and root weight with more than 98% (foliar: 99.4% (95% CI: 98.9–99.7%) and 99.3% (95% CI: 98.6–99.6%), root: 98.4% (95% CI: 97.4–99.1%) and 98.2% (95% CI: 97.0–98.93%) compared to the nontreated plants (Figures 2D and 3D).

3.4. Prosulfocarb (Experiment 2)

Prosulfocarb at 0.5 $\times$ dose reduced the foliar and root weights significantly in all treatments. The reduction in leaf weight was 97.8% (95% CI: 95.8–98.9%), 99.3% (95% CI: 98.6–99.6%) and 99.0% (95% CI: 98.1–99.5%) for foliar-only, substrate + foliar, and substrate-only compared to the nontreated plants, respectively. There was no significant difference between weight reductions for substrate + foliar and substrate-only application (Figures 2C and 3C).

Prosulfocarb at 0.1.0 $\times$ dose reduced foliar and root weight by 95.2% (95% CI: 90.7–97.5%) and 87.8% (95% CI: 79.4–92.8%), respectively, for foliar-only compared to the nontreated plants. Foliar and root weights were significantly reduced ($p < 0.005$) for substrate + foliar and substrate-only with more than 99% and 95% compared to the nontreated plants, respectively (Figures 2D and 3D). Both application methods reduced the leaf weights significantly more than foliar-only application (both $p < 0.001$). The flufenacet treatment resulted in a significant larger reduction in root weight for substrate + foliar than prosulfocarb did ($p < 0.02$) compared to the nontreated plants (Figures 2D and 3D).

4. Discussion

When the herbicides were applied as substrate + foliar, both herbicides had a high efficacy at both dosages (0.5 $\times$ dose and 1.0 $\times$ dose) on root and foliar development at postemergence (two to three leaf stage) application. The treatment reduced the fresh weight by more than 80% compared to the nontreated plants. However, the recommended dosages are based on ensuring a satisfactory weed control under variable weather and soil conditions in the field [13,18], and consequently the experiments should be studied under different conditions as we have done in a greenhouse. Experiment 1 was conducted in the winter time with low light intensity and short days resulting in a slow growth, while experiment 2 were conducted in the spring with high light intensities and long days resulting in rapid growth and significantly larger plants at the time of harvest. We investigated the effects under two very different conditions to see if the plants reacted the same way. Although the efficacy varied, it was consistent that foliar-substrate and substrate-only resulted in a high control of L. multiflorum. However, the effect of foliar application was consistent for prosulfocarb but not for flufenacet.

Also, soil and weather conditions profoundly influence soil-active herbicides, and often results from field experiments vary significantly. The high Kow and low solubility in water especially for prosulforcarb explain partly this variation. Good weed control with preplant incorporated and
pre-emergence herbicide application depends on many factors, including soil moisture, rainfall after application, soil type, and soil temperature, and weed species. Herbicides, which are incorporated into the soil surface, usually require less rainfall after application for effective weed control than unincorporated herbicides [32].

Pot and field experiments have shown that the effect of soil-applied herbicides can vary considerably depending on growing conditions [24,33]. Høgh [33] did a field experiment with flufenacet applied to L. multiflorum at several growth ages (BBCH 10–13), which resulted in large variations in the dry-weight biomass measurements and visual ratings three weeks after application. The variations were observed even though the field application was conducted in the autumn and on moist soil as recommended for soil acting herbicides [3,32]. Results with both flufenacet in a mixture with metribuzin [34] and alone [14,33] showed how variable the effect of postemergence applied flufenacet and other soil acting herbicide like prosulfocarb can be at variable environmental conditions [24].

Our results showed that flufenacet had a good efficacy on L. multiflorum. However, the efficacy of flufenacet and prosulfocarb in these experiments may not only be an effect of the high availability for root uptake of the herbicides but may also be affected by the ability of vermiculite to adsorb chemical substances [35]. We did not stir the herbicides into the vermiculite, and perhaps we would have obtained an even better effect if the herbicides were distributed better in the vermiculite. Menne et al. [13] found that soils irrigated from below kept a higher amount of herbicides at the soil surface compared to soils irrigated from above. This could increase the effect of the postemergence applied flufenacet and prosulfocarb by preventing the herbicide from leaching out of the pots. In the pots, where active charcoal was used as a filter (foliar-only), watering from the bottom probably have prevented the herbicides leaching to the roots, and thereby increasing the filtering effect of the charcoal.

The dominating effect of flufenacet came from the root uptake. This has been confirmed by FMC [14]. Flufenacet was formulated in a suspension concentrate. After application and complete droplet dry-down, only the crystals of the active ingredients are left on the leaf surface, which makes the herbicide unavailable for foliar uptake [3,36]. Prosulfocarb significantly reduced the foliar and root fresh weight in most of the treatments. Vera et al. [37] observed that after 72 h only 3% of the applied prosulfocarb had entered the leaves of L. multiflorum and Carlsen et al. [38] found that 80% of the applied prosulfocarb was evaporated within the first 24 h after application.

The effect of the foliar application could be caused by thiocarbamates in plants, which like prosulfocarb, are metabolized to the more potent sulfoxide [39], which may increase the efficacy. Prosulfocarb may enter the vermiculite because of its volatility even though the charcoal was not removed after application. Vermiculite includes the minerals with the largest cation exchange capacity (CEC) of the mineral fraction of soils, but the presence of hydroxyl-Al interlayers may considerably reduce the effective CEC [25]. However, we cannot exclude that vermiculite may have compromised the effect by adsorption of the herbicides. The extent of adsorption of e.g., prosulfocarb also increases with increasing organic matter and clay content in soils due to the high $K_{ow}$. This has been demonstrated in relation to the control of L. multiflorum with prosulfocarb in pot experiments [23]. In general, the difference between the effects of the herbicides were small except for foliar-application where prosulfocarb reduced the biomass significantly more than flufenacet (Figures 2 and 3).

In summary, both substrate + foliar application of flufenacet and prosulfocarb applied postemergence reduced the weight of L. multiflorum significantly in both experiments. Foliar application of prosulfocarb reduced both root and leaf biomass in most cases, but flufenacet showed also to have a significant foliar effect in some instance. Substrate-only application reduced the biomass significantly for both herbicides. Our results are promising for managing herbicide-resistant L. multiflorum as both herbicides provide farmers with an alternative mode of action than ALS and ACCase herbicides. We suggest that further studies should be done as field trials on different soil types and under different weather conditions, especially for flufenacet as only little information is available about the foliar effect of this herbicide on L. multiflorum.
5. Conclusions

For both herbicides applied postemergence the substrate + foliar application were effective in reducing growth of L. multiflorum significantly in both experiments. Foliar-only application showed inconsistent results. Soil absorption plays an important role on herbicide efficacy even when the herbicides are applied postemergence. The use of prosulfocarb and flufenacet in postemergence of L. multiflorum was effective to reduce weed growth having apparently good root and leaf absorption.

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