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Differential sensitivity of locally naturalized *Panicum* species to HPPD- and ALS-inhibiting herbicides

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Abstract

Panicum schinzii (Transvaal millet), *P. dichotomiflorum* (Fall panicum) and *P. capillare* (Witchgrass) are alien panicoid grasses that have gradually spread and are now locally naturalized in corn fields in Belgium. One of the possible reasons for their expansion in corn fields might be a lower sensitivity to post-emergence herbicides acting against panicoid grasses, in particular those inhibiting 4-hydroxyphenyl pyruvate dioxygenase (HPPD) and acetolactate synthase (ALS). Dose-response pot experiments were conducted in the greenhouse to evaluate the effectiveness of five HPPD-inhibiting herbicides (sulcotrione, mesotrione, isoxaflutole, topramezone, tembotrione) and two ALS-inhibiting herbicides (nicosulfuron, foramsulfuron) for controlling populations of *P. schinzii*, *P. dichotomiflorum* and *P. capillare* (all naturalized Belgian populations except for *P. capillare)*. In another dose-response pot experiment, sensitivity of five local *P. dichotomiflorum* populations to HPPD-inhibitors and nicosulfuron was investigated. Finally, the influence of growth stage at time of herbicide application on efficacy of topramezone and nicosulfuron for *Panicum* control was evaluated. Large interspecific differences in sensitivity to HPPD-inhibiting herbicides were observed. *Panicum schinzii* was sensitive to tembotrione but moderately sensitive to topramezone and poorly sensitive to mesotrione and sulcotrione. However, *P. dichotomiflorum* was sensitive to mesotrione and topramezone but moderately sensitive to tembotrione. All *Panicum* species were sensitive to low doses of nicosulfuron and foramsulfuron. Naturalized *P. dichotomiflorum* populations exhibited differential herbicide sensitivity profiles. All species tested showed a progressive decrease in sensitivity to topramezone and nicosulfuron with seedling age. A satisfactory post-emergence control of *Panicum* species in the field will require appropriate choice of herbicide and dose, as well as a more timely application (i.e. before weeds reach the four leaves stage).

Key words: Growth stage, herbicide sensitivity, panicoid grasses, sulfonylurea herbicides, triketone herbicides

Introduction

The rapid increase of corn (*Zea mays*) cultivation in Flanders (Belgium) that started *ca*. four decades ago created optimal conditions for the establishment of self-perpetuating populations of many panicoid weed grasses (e.g. *Panicum* spp., *Echinochloa* spp., *Setaria* spp., *Digitaria* spp.) in and around corn fields (Vanderhoeven et al. 2006). Although the toolbox for weed control in corn contains an impressive variety of herbicides with different molecular modes of action (Santel 2009), newly introduced and naturalizing panicoid grasses continuously complicate appropriate choice of herbicides and their dosages.

Until recently the *Panicum* species *P. schinzii* (Transvaal millet or Land grass, native to South Africa), *P. dichotomiflorum* (Fall panicum, native to North and South America) and *P. capillare* (Witchgrass, native to North America) were completely overlooked in Belgium. Since 1970, these species have gradually spread and are now locally naturalized and abundant in and along corn fields, particularly on sandy soils (Hoste & Verloove 2001, Verloove 2001, Van Landuyt et al. 2006). Less than 3% of their populations are found outside corn fields (Vanderhoeven et al. 2006). Another *Panicum* species sporadically found in corn fields is *P. miliaceum* subsp. *miliaceum* (Proso millet, native to central Asia) but this subspecies rarely persists because of the retention of the florets on the plant and poor seed survival over winter (Freckmann & Lelong 2003).

Panicum dichotomiflorum and *P. schinzii* are morphologically hard to distinguish, particularly at early growth stages. Correct identification requires a careful study of the lower florets with the aid of a hand lens. The lower florets of *P. schinzii* are staminate whereas in *P. dichotomiflorum* the lower florets are neuter (Van Der Meijden 2005). Moreover, *P. schinzii* has blunter spikelets. One of the possible reasons for its expansion into corn fields, besides e.g. the lack of crop rotation (Vanderhoeven et al. 2006), might be a lower sensitivity to post-emergence (POST) corn herbicides used to control panicoid grasses, in particular 4-hydroxyphenyl pyruvate dioxygenase (HPPD)-inhibiting herbicides and acetolactate synthase (ALS)-inhibiting sulfonylureas.

Abovementioned *Panicum* species are highly competitive and prolific weeds in cereal grains and need to be controlled soon after emergence to prevent yield loss (Baker & Terry 1991, Clements et al. 2004). Indeed, despite being relatively late emerging species, they grow fast and tiller profusely (Vengris & Damon 1976). *Panicum dichotomiflorum* is one of the most important grassy weeds in American and Asian corn and sorghum (*Sorghum bicolor*) fields. According to Benson (1982) a season-long competition by *P. dichotomi-*

florum at a density of 12–20 plants m^{-2} reduced corn grain yield by 15 to 77%. *Panicum schinzii* is a major weed in tropical cereals in Africa (Gibbs-Russell et al. 1990). Wax et al. (1981) listed *P. capillare* as one of the seven most common annual grasses found in North American corn and soybean fields. In small grain crops, *P. capillare* was among the three most common summer annual grass weeds in Nebraska where *P. capillare* and *Echinochloa crus-galli* were the most difficult to control (Wicks et al. 1995). Unfortunately, the actual impact of *P. schinzii* and *P. capillare* infestations on crop yield is not well documented.

Recently, in Belgium there have been many complaints about unsatisfactory *Panicum* control in corn. Unfortunately, in sharp contrast to other naturalized panicoid grasses belonging to the genera *Echinochloa*, *Setaria* and *Digitaria*, scientific literature detailing the herbicide sensitivity is lacking for abovementioned naturalized *Panicum* species. Therefore, in the present study the following research questions were addressed: (1) Do *P. capillare*, *P. dichotomiflorum* and *P. schinzii* show a difference in sensitivity to corn herbicides acting against panicoid grasses, in particular HPPD- and ALS-inhibitors? (2) Do locally naturalized *P. dichotomiflorum* populations vary in herbicide sensitivity? (3) What is the optimal growth stage for *Panicum* control?

Materials and methods

Experiments

During the summer of 2011, three dose-response pot experiments were conducted in the greenhouse. In experiment 1, the effectiveness of five foliar-applied HPPD-inhibiting herbicides [topramezone (ARIETTA®), mesotrione (CALLISTO®), tembotrione (LAUDIS®), sulcotrione (MIKADO®), isoxaflutole (Experimental product)] and two ALS-inhibiting herbicides [nicosulfuron (KELVIN®), foramsulfuron (EQUIP®)] for controlling populations of *P. capillare*, *P. dichotomiflorum* and *P. schinzii* was evaluated. Selected *P. capillare* population was 'Herbiseed' (a well-known reference population purchased from the seed company Herbiseed, Twyford, UK). Reference populations of *P. dichotomiflorum* and *P. schinzii* were not available and therefore we used locally naturalized populations from Belgian corn fields, namely 'Bellem' and 'Ursel', respectively. Although abovementioned HPPD-inhibitors are solely applied post-emergence in Belgian corn fields, except for isoxaflutole (MERLIN®) which is only applied pre-emergence to avoid crop injury, they also have residual soil activity (Bulcke et al. 1996, Rouchaud et al. 2000, Schönhammer et al. 2006, Schulte & Köcher 2009). For this reason, experiment 1 was also designed to investigate the relative contribution from soil activity to weed control resulting from post-emergence applications of HPPD- and ALS inhibitor herbicides. For this purpose, half of all pots were covered with a herbicide adsorbing film (1 mm) of activated charcoal (Aktivkole, Roth, Karlsruhe, Germany) shortly after sowing. The charcoal-treated pots were used to evaluate foliar activity of the applied herbicides, whereas pots without charcoal were used to evaluate total activity. Experiment 2 was designed to evaluate the importance of intraspecific variability in herbicide sensitivity. Since *P. dichotomiflorum* is the most widespread species amongst naturalized *Panicum* species, in Belgium (Van Landuyt et al. 2006), experiment 2 was focused on *P. dichotomiflorum*. Five local *P. dichotomiflorum* populations (Belgian populations 'Bellem', 'Adegem', 'Lembeke', 'Ursel' and 'Damme'), located at least 14 km apart, were screened for their sensitivity to four HPPD-inhibitors (mesotrione, tembotrione, sulcotrione and topramezone) and one ALS-inhibitor (nicosulfuron). Seeds of these populations were each collected in 2010 on at least 50 plants scattered over the whole corn field. In experiment 3, the relation between weed growth stage and herbicide sensitivity was investigated by subjecting *P. capillare*, *P. dichotomiflorum* and *P. schinzii* plants, differing in growth stage, to foliar-applied topramezone and nicosulfuron. Topramezone was selected since it is a frequently and widely applied HPPD-inhibitor in corn in Flanders. Nicosulfuron is the most important ALS-inhibitor in corn. The same populations as in experiment 1 were used.

Experimental setup

All dose-response experiments were conducted in greenhouses using plastic pots filled with steamed sandy loam soil containing 2.2% organic matter, 51.6% silt (2–50 μm), 39.9% sand (> 50 μm) and 8.6% clay with a pH-KCl of 5.7. The greenhouse was a rain-shelter plastic greenhouse, with sides left open up to 1 m high for natural ventilation. Daytime and nighttime mean temperature and humidity values, and mean light intensity during the experimental periods are given in Table 1.

Table 1: Daytime and nighttime mean temperature, relative humidity and mean daytime light intensity during the bioassay pot experiments

	Experimental period	Daytime/nighttime mean temperature °C	Daytime/nighttime mean humidity %	Mean daytime light intensity lux
Experiment 1	11/07/2011–23/08/2011	23/16	70/90	7030
Experiment 2	24/08/2011–03/10/2011	23/14	66/90	7555
Experiment 3	28/06/2011–09/08/2011	23/16	64/84	5055

In all experiments, pots were seeded with 25 seeds per pot at 2 mm depth. As soon as seedlings had one fully developed true leaf (BBCH stage 11), they were thinned to five uniform plants per pot. Pots were irrigated by overhead sprinklers as needed. The experimental design was always a randomized block with three replicates. The experimental unit was one pot of five seedlings. All herbicides were applied with TeeJet XR11002 flat fan nozzles (TeeJet Technologies, Wheaton, USA) at a spray pressure of 180 kPa and a spray volume of 300 l ha^{-1}. Each herbicide was tested in eight doses and compared to a control as enumerated in Table 2. According to preliminary experiments, dose ranges mentioned in Table 2 were optimal for the construction of dose-response curves. In experiment 1 and 2, herbicides were applied in the three (for *P. dichotomiflorum* and *P. capillare*) to four (for *P. schinzii*) true leaves stage. The four true leaves stage is the stage at which POST herbicides are most commonly applied in Flemish corn fields. In experiment 3, topramezone and nicosulfuron were applied at four different weed growth stages: BBCH11, one true leaf; BBCH12, two true leaves, BBCH13, three true leaves; BBCH14, four true leaves. These weed growth stages were achieved by staggered sowing times.

Measurements

In all bioassay experiments, foliage fresh biomass was harvested (clipped at the soil surface) and weighed 20 days after treatment (DAT).

Data analysis

Data obtained from single POST bioassays were analyzed with the Open Source language and environment R (version R2.11.1; R Development Core Team 2010) and its dose-response curves extension package drc (Ritz & Streibig 2005) based on Knezevic et al. (2007). The block effect of foliage fresh biomass was checked by analysis of variance and was in all instances non-significant. Hence, data from the three blocks were pooled before analysis.

Within experiments 1 and 2, dose-response curves for all *Panicum* populations were fitted simultaneously for each tested herbicide. Within experiment 3, dose-response curves for all growth stages of a particular *Panicum* population were fitted simultaneously for each tested herbicide. The initial regression model was the log-logistic model with four parameters (Streibig et al. 1993):

$$Y = c + \frac{d - c}{1 + \exp[b(\log x - \log e)]}$$

where Y represents biomass, i.e. foliar fresh weight per pot (g), at herbicide dose x. The dose required for 50% biomass reduction is given by e, also referred to as the ED_{50} (g ha^{-1}). The parameter b denotes the relative slope around this value and the upper and lower limit of the curve are d and c respectively. For all herbicides tested in experiment 1, except tembotrione and nicosulfuron, a likelihood ratio test indicated that the initial model could be reduced to the three parameter log-logistic model, with c being zero. A Box-Cox transformation was applied on the data of meso-

Table 2: Herbicides and their doses examined in post-emergence (POST) dose-response bioassays

Herbicide (formulated product)	Herbicide dose
HPPD-inhibitors (HRAC group F2)	g ai ha^{-1}
Isoxaflutole[1]	0 – 3.125 – 6.25 – 12.5 – 25 – 50 – 100 – 200 – 400
(SP102000016788, 240 g ai l^{-1}, SC, Bayer CropScience, 1831, Belgium)	
Mesotrione	0 – 2.5 – 5 – 10 – 20 – 40 – 80 – 160 – 320
(Callisto, 100 g ai l^{-1}, SC, Syngenta Crop protection, 7180 Seneffe, Belgium)	
Sulcotrione	0 – 15 – 30 – 60 – 120 – 240 – 480 – 960–1 920
(Mikado, 300 g ai l^{-1}, SC, Bayer CropScience, 1831 Diegem, Belgium)	
Tembotrione[2]	0 – 2.75 – 5.5 – 11 – 22 – 44 – 88 – 176–352
(Laudis, 44 g ai l^{-1}, OD, Bayer CropScience, 1831 Diegem, Belgium)	
Topramezone[3]	0 – 1.6–3.2 – 6.3 – 12.6 – 25.2 – 50.4 – 100.8 – 201.6
(Arietta, 336 g ai l^{-1}, SC, Basf Belgium, 1170 Brussel, Belgium)	
ALS-inhibitors (HRAC-group B)	
Foramsulfuron[4]	0 – 1.875 – 3.75 – 7.5 – 15 – 30 – 60 – 120–240
(Equip, 22.5 g ai l^{-1}, SC, Bayer CropScience, 1831 Diegem, Belgium)	
Nicosulfuron	0 – 0.63 – 1.25 – 2.5 – 5 – 10 – 20 – 40 – 80
(Kelvin, 40 g ai l^{-1}, SC, DuPont De Nemours, 410 Waterloo, Belgium)	

[1] The experimental product combines isoxaflutole and the safener cyprosulfamide (1:1 ratio).
[2] Laudis combines tembotrione and the safener isoxadifen-ethyl (2:1 ratio) with an adjuvant system in an oil dispersion (OD) formulation.
[3] 1 l ha^{-1} triglyceride oil (Actirob B, 812 g ai l^{-1}, EC, Novance, F-60206 Compiegne, France), a methylated seed oil, was added to the herbicide spray solution to enhance foliar uptake and distribution within the shoot.
[4] Equip combines foramsulfuron and the safener isoxadifen-ethyl (1:1 ratio).

trione to obtain variance homogeneity (Streibig et al. 1993). For tembotrione and nicosulfuron, the four parameter Weibull model was used (Streibig et al. 1993):

$$Y = c + (d - c)\exp\{-\exp[b(\log x - e)]\}$$

For biomass data of distinct *P. dichotomiflorum* populations (Experiment 2), the three parameter log-logistic model was used except for the response to mesotrione and topramezone, for which the initial four-parameter Weibull model could be reduced to the three parameter Weibull model, with *c* being zero. The four parameter log-logistic model was used in experiment 3, for biomass data of *Panicum* species treated with topramezone. Data of *P. capillare* were Box-Cox transformed to obtain variance homogeneity. For the response to nicosulfuron the three parameter log-logistic model was used except for the response of *P. schinzii* for which the four parameter log-logistic model could not be reduced.

Effective dosages (ED) and selectivity indices (SI) are commonly used to compare different dose-response curves. Effective dosage ED_{90} (dose required for 90% biomass reduction) and selectivity indices (SI) as relative potencies between two dose-response curves were derived from the regression model utilizing the delta method (Van der Vaart 1998). SI_{90} (i.e. the ratio between ED_{90} for one dose response curve and ED_{90} for another dose-response curve) and SI_{50} (i.e. the ratio between ED_{50} for one dose-response curve and ED_{50} for another dose-response curve) were used to compare the relative differences of ED_{90} and ED_{50} among curves, respectively.

Results and discussion

Experiment 1

Large interspecific differences in sensitivity to HPPD-inhibiting herbicides were observed (Table 3). *Panicum schinzii* was sensitive (i.e., required a dose lower than the maximum authorized field dose to achieve 90% reduction in biomass) to tembotrione but moderately sensitive (i.e. required maximum field dose) to topramezone and poorly sensitive (i.e. required three-fold higher dose than maximum field dose) to mesotrione and sulcotrione. However, *P. dichotomiflorum*, a species that morphologically closely resembles *P. schinzii*, was sensitive to mesotrione (ED_{90} of 60.9 g ha^{-1}) and topramezone (ED_{90} of 7.5 g ha^{-1}) but moderately sensitive to tembotrione. However, according to Soltani et al. (2012), mesotrione at 150 g ha^{-1} and topramezone at 12.5 g ha^{-1}, both with oil concentrate at 1.25% v/v, provided moderate control (70–77% biomass reduction) of 2–3 leaf stage seedlings of *P. dichotomiflorum*. *Panicum capillare* was sensitive to sulcotrione and topramezone, moderately sensitive to tembotrione and poorly sensitive to mesotrione. Similarly, in the study of Soltani et al. (2012), 2–3 leaf stage seedlings of *P. capillare* were adequately controlled (> 90% biomass reduction) by topramezone but not by mesotrione. *Panicum capillare* and *P. schinzii* were poorly sensitive to foliar-applied isoxaflutole: doses two- to three-fold higher than maximum field dose (i.e. 75 g isoxaflutole ha^{-1} for pre-emergence commercial corn herbicide Merlin which contains 75% isoxaflutole, without safener) were required for 90% control.

Table 3: ED_{50} and ED_{90} responses with standard errors of *P. dichotomiflorum*, *P. capillare* and *P. schinzii* to post-emergence HPPD- and ALS-inhibitors applied at the three to four true leaves stage (Experiment 1)

Herbicide	Effective Dose	*P. dichotomiflorum*	*P. capillare*	*P. schinzii*	Max. field dose in Belgium
		g ai ha^{-1}			
HPPD-inhibitors					
Isoxaflutole	ED_{50}		38.1 ± 9.32a	86.2 ± 15.97b	
	ED_{90}		231.2 ± 84.68a	2 450.5 ± 993.73b	
Mesotrione	ED_{50}	21.5 ± 7.44a	80.9 ± 22.36b	101.3 ± 14.36b	150
	ED_{90}	60.9 ± 39.18a	492.0 ± 237.86b	479.8 ± 138.71b	
Sulcotrione	ED_{50}		64.4 ± 16.79a	121.3 ± 15.70b	450
	ED_{90}		278.3 ± 126.09a	1 043.6 ± 217.59b	
Tembotrione	ED_{50}	16.1 ± 6.62a	71.5 ± 10.46b	19.3 ± 0.94a	99
	ED_{90}	66.0 ± 43.14ab	125.6 ± 34.18b	26.2 ± 1.65a	
Topramezone	ED_{50}	2.4 ± 0.74a	2.5 ± 0.42a	5.8 ± 0.70b	50.4
	ED_{90}	7.5 ± 4.93a	10.4 ± 2.71a	52.2 ± 10.66b	
ALS-inhibitors					
Foramsulfuron	ED_{50}		1.3 ± 0.37a	3.4 ± 0.25b	60
	ED_{90}		3.4 ± 0.97a	8.5 ± 1.01b	
Nicosulfuron	ED_{50}	1.4 ± 0.58a	1.1 ± 0.13a	4.6 ± 0.31b	60
	ED_{90}	5.6 ± 3.23b	2.5 ± 0.37a	9.4 ± 0.83b	

No significant differences (based on computed selectivity indices and corresponding *p*-values) between figures with the same letter, comparison within herbicide only (Experiment 1).

Provided no antagonism or decrease in crop selectivity is foreseen, tank mixtures of HPPD-inhibiting herbicides should be considered to avoid unacceptable *Panicum* control. Indeed, our results show that for *Panicum* species that were poorly suppressed by one of the HPPD-inhibitors, good control was obtained with other HPPD-inhibitors. These differential responses may be attributed to differences in foliar uptake. However, differential herbicide metabolism and/or differential sensitivity or activity of the HPPD enzyme cannot be completely ruled out. Moreover, the safeners isoxadifen-ethyl and cyprosulfamide, included in the formulation with tembotrione and with isoxaflutole, respectively, may have obscured potential interspecific differences in foliar uptake by a possible differential effect on herbicide detoxification in the plant. Further research on the fundamental biokinetics of herbicide metabolism and uptake of these HPPD-inhibitor herbicides in *Panicum* species is necessary to provide conclusive explanations for this differential herbicide sensitivity profile.

The abovementioned poor (sulcotrione and mesotrione) to moderate (topramezone) sensitivity of *P. schinzii* to some HPPD-inhibiting herbicides may partly explain the rapid expansion of *P. schinzii* into Flemish corn fields during the last two decades (Verloove 2001, Van Landuyt et al. 2006, Vanderhoeven et al. 2006). This is particularly true for the widely used sulcotrione, being the first triketone herbicide introduced in 1992 into the Belgian corn market. Sulcotrione was rapidly and widely adopted by corn growers due to its broad-spectrum weed control ability. Similar to sulcotrione, mesotrione and topramezone gradually became a standard herbicidal compound in many corn herbicide tank mix combinations since their introduction in 2003 and 2008, respectively.

Contrary to HPPD-inhibiting herbicides, small interspecific differences in sensitivity to ALS inhibiting herbicides were observed (Table 3). *Panicum schinzii* and *P. dichotomiflorum* were equally sensitive to nicosulfuron. Moreover, *P. capillare*, P. *schinzii* and *P. dichotomiflorum* were very sensitive to doses that were at least six-fold lower than maximum authorized field doses. So, the addition of nicosulfuron or foramsulfuron to POST tank mixes containing HPPD-inhibitors may potentially improve control of mixed *Panicum* populations in the field provided no incompatibility is foreseen. As shown by Schuster (2007) mesotrione antagonized several ALS-inhibiting herbicides by reducing the efficacy of controlling the panicoid grasses *Setaria viridis* (green foxtail) and *Setaria pumila* (yellow foxtail). In case of antagonism, nicosulfuron and foramsulfuron may be applied in sequence. By adding one of these ALS-inhibitors selective pressure exerted by some HPPD-inhibiting herbicides, particularly when applied repeatedly, might be lowered thus slowing down the build-up of less sensitive *Panicum* populations. The latter may further be reduced by adding persistent soil-acting grass herbicides (such as dimethenamid-P) to POST tank mixes, thus avoiding survival of late emerging *Panicum* seedlings which might be missed by abovementioned foliar-applied herbicides.

The relative contribution from soil activity to weed control resulting from post-emergence applications was important for isoxaflutole and to a varying degree also for sulcotrione, mesotrione and tembotrione but not for topramezone and the ALS-inhibitors nicosulfuron and foramsulfuron (Table 4). Compared to seedlings growing in charcoal-free pots, doses of isoxaflutole required to obtain a 90% reduction in *Panicum* biomass, were ten-fold higher for seedlings growing in charcoal-topped pots. In our small pot experiments with regular overhead irrigation, considerable soil activity would be expected. Presumably, contribution from soil activity in the field will largely depend on soil moisture content. For example, in moist soils *P. capillare* seedlings at the three leaves stage may be controlled by two- and six-fold lower doses of tembotrione and sulcotrione,

Table 4: ED_{90} responses with standard errors of *P. dichotomiflorum*, *P. capillare* and *P. schinzii* to post-emergence HPPD- and ALS-inhibitors. Herbicides were applied at the three to four leaves stage of plants growing in charcoal-topped pots(+charcoal) and in charcoal-free pots (–charcoal) (Experiment 1)

Herbicide	*P. dichotomiflorum*		*P. capillare*		*P. schinzii*	
	–charcoal	+charcoal	–charcoal	+charcoal	–charcoal	+charcoal
	g ai ha^{-1}					
HPPD-inhibitors						
Isoxaflutole	–	–	231 ± 70.7a	1,256 ± 660.2b	1,043 ± 356.9a	94,996 ± 175,705.7b
Mesotrione	49 ± 17.1a	155 ± 44.8b	382 ± 188.9a	407 ± 101.3a	459 ± 156.9a	478 ± 155.7a
Sulcotrione	–	–	278 ± 115.4a	1,434 ± 449.6b	884 ± 236.0a	448 ± 85.7a
Tembotrione	67 ± 20.0a	46 ± 3.3a	139 ± 30.5a	247 ± 39.3b	26 ± 2.8a	40 ± 4.5b
Topramezone	34 ± 6.7a	27 ± 5.0a	8 ± 1.2a	8 ± 1.2a	6 ± 2.2a	9 ± 2.0a
ALS-inhibitors						
Foramsulfuron	–	–	4 ± 1.5a	5 ± 1.1a	8 ± 1.4a	10 ± 1.3a
Nicosulfuron	7 ± 2.8a	10 ± 1.5a	2 ± 0.4a	3 ± 0.2a	14 ± 3.6a	18 ± 4.3a

No significant differences (based on computed selectivity indices and corresponding *p*-values) between figures with the same letter, comparison within herbicide/species combination only (Experiment 1).

respectively, than in dry soils. This can be indirectly deduced from pot experiment 1 assuming soil activity in dry soils to be comparable with soil activity in charcoal-topped substrate and soil activity in moist soils to be comparable with soil activity in charcoal-free substrate. In general, contribution from soil activity of foliar applied β-triketones (mesotrione, sulcotrione and tembotrione) and isoxaflutole was more important for *P. capillare* than for *P. dichotomiflorum* and *P. schinzii*. This differential response may be attributed to interspecific differences in root pattern. Gross et al. (1992) found that most roots of 12-day-old *P. capillare* seedlings were close to the soil surface. This root pattern may favour root uptake of foliar-applied herbicides, thus increasing relative contribution from soil activity.

Experiment 2

Different locally naturalized *P. dichotomiflorum* populations showed a high variation in degree of sensitivity (based on ED_{90} doses) to HPPD-inhibiting herbicides mesotrione, sulcotrione and tembotrione but not to the highly effective herbicides topramezone and nicosulfuron (Table 5). ED_{90} doses of mesotrione, sulcotrione and tembotrione varied by a factor of up to 3. This diverse response may reflect genetic variability among *P. dichotomiflorum* populations. Interspecific hybrids are not known. Indeed, *P. dichotomiflorum* being a hexaploid and *P. capillare* and *P. schinzii* being diploids, hybridization is not expected to occur spontaneously. The high degree of intraspecific variability in herbicide sensitivity may be rather surprising for a species that only recently (beginning of the 1990 s) became a naturalized species in Flemish corn fields. Most likely, new populations with a different genetic background are continuously introduced in Flemish corn fields as casual grain aliens, thus increasing genetic variability among populations. In Belgium, seeds of *P. dichotomiflorum* are found in grain elevators in port areas as contaminant seeds of imported grain seeds (Verloove 2001). Nowadays, many *P. dichotomiflorum* populations are found as ephemeral populations in port areas, near grain stores, by roads or railway tracks, on dumps *etc*.. From these habitats they can gradually spread and naturalize in corn fields (Verloove 2001). According to Freckmann & Lelong (2003) *P. dichotomiflorum* exhibits substantial phenotypic variation in North-America. Spikelet length and form can range from less than 3 mm to nearly 4 mm and from shortly acuminate to subobtuse. In habit prostrate, ascending as well as erect plants can be observed.

Furthermore, ranking of populations according to the degree of sensitivity (based on ED_{90} doses) depended on HPPD-inhibitor applied (Table 5). Populations 'Adegem' and 'Damme' were significantly less sensitive to mesotrione than population 'Ursel'. Compared to other *P. dichotomiflorum* populations tested, population 'Bellem' required a two- to three-fold lower dose of tembotrione to achieve 90% reduction in biomass. Population 'Bellem' was also significantly more sensitive to sulcotrione than 'Adegem'. Contrary to mesotrion, sulcotrione and tembotrione, no significant intraspecific differences in ED_{90} response were found for topramezone and nicosulfuron. Except for tembotrione, all *P. dichotomiflorum* populations were controlled by doses lower than authorized in the field. The *P. dichotomiflorum* populations 'Adegem' and 'Lembeke' required the maximum authorized field dose to achieve 90% reduction in biomass.

Despite being rather small compared to interspecific variation in herbicide sensitivity, intraspecific variation in herbicide sensitivity may further complicate the appropriate choice of HPPD-inhibiting herbicides and doses as well. Low dose applications may increase the risk of unsatisfactory control of some *P. dichotomiflorum* populations. This is particularly true for mesotrione, sulcotrione and tembotrione for which ED_{90} doses vary substantially among populations. Similar to abovementioned interspecific differences in sensitivity, these intraspecific differential responses may be attributed to differences in foliar uptake, differential herbi-

Table 5: ED_{90} responses with standard errors of geographically distinct *P. dichotomiflorum* populations to foliar-applied HPPD-inhibitors (mesotrione, sulcotrione, tembotrione and topramezone) and nicosulfuron (Experiment 2) applied at the three true leaves stage

Herbicide	*P. dichotomiflorum* population				
	Adegem	Bellem	Damme	Lembeke	Ursel
	g ai ha^{-1}				
HPPD-inhibitors					
Mesotrione	68.9 ± 13.10a	57.3 ± 10.44ab	79.0 ± 18.23a	55.8 ± 9.54ab	36.8 ± 9.03b
Sulcotrione	186.4 ± 31.83a	105.7 ± 15.74b	177.6 ± 43.20ab	133.1 ± 23.92ab	
Tembotrione	101.4 ± 24.88a	38.6 ± 7.29b	75.1 ± 19.10a	105.9 ± 28.23a	57.7 ± 21.53ab
Topramezone	10.0 ± 1.24a	10.0 ± 2.19a	3.6 ± 2.97a	9.2 ± 2.76a	11.3 ± 3.26a
ALS-inhibitor					
Nicosulfuron	7.6 ± 1.50a	5.2 ± 1.26a	6.6 ± 1.35a	9.2 ± 2.75a	

No significant differences (based on computed selectivity indices and corresponding *p*-values) between figures with the same letter, comparison within herbicide only (Experiment 2).

cide metabolism and/or differential sensitivity or activity of the HPPD enzyme.

Experiment 3

The ED_{90} response (expressed absolute in Table 6 or relative to ED_{90} dose for *P capillare* plants treated at the one leaf stage in Fig. 1 and 2) of *P. capillare, P. dichotomiflorum* and *P. schinzii* to foliar-applied topramezone and nicosulfuron was largely dependent on growth stage. Topramezone sensitivity of *P. schinzii and P. dichotomiflorum*, and of *P. capillare* respectively decreased exponentially and linearly with seedling age (Fig. 1). Topramezone sensitivity dropped drastically beyond the two true leaves stage for *P. schinzii* and the three true leaves stage for *P. dichotomiflorum*. At the four true leaves stage, ED_{90} doses were two- to eleven-fold higher than at the one leaf and two true leaves stage, irrespective of *Panicum* species (Table 6). Amongst *Panicum* species, *P. schinzii* was least sensitive to topramezone, irrespective of leaf stage. At the two and three leaves stages, *P. dichotomiflorum* required lower ED_{90} doses than *P. capillare* seedlings.

Nicosulfuron sensitivity of *P. schinzii* and *P. capillare* linearly decreased with increasing number of true leaves (Fig. 2). Contrary to *P. capillare* and *P schinzii*, *P. dichotomiflorum* showed highest sensitivity at the two true leaves stage (Table 6). However, beyond the two leaves stage, sensitivity was lowered again. *Panicum capillare* plants were

Table 6: ED_{50} and ED_{90} responses with standard errors of *P. dichotomiflorum, P. capillare* and *P. schinzii* to foliar-applied topramezone and nicosulfuron as influenced by growth stage (Experiment 3)

Species	Growth stage†	Topramezone		Nicosulfuron	
		ED_{50}	ED_{90}	ED_{50}	ED_{90}
		g ai ha^{-1}			
P. dichotomiflorum	BBCH 11	0.8 ± 1.17a	3.3 ± 4.26a	0.3 ± 3.39a	11.7 ± 81.64ab
	BBCH 12	1.5 ± 0.43a	4.6 ± 2.80a	3.3 ± 0.86b	5.3 ± 2.55a
	BBCH 13	2.9 ± 0.34a	7.2 ± 1.63a	5.3 ± 0.83bc	11.4 ± 3.52b
	BBCH 14	11.5 ± 2.01b	37.5 ± 15.45b	7.5 ± 1.39c	22.6 ± 11.66b
P. capillare	BBCH 11	1.1 ± 0.51a	2.6 ± 1.42a	0.3 ± 0.26a	1.0 ± 0.47a
	BBCH 12	1.3 ± 0.36a	7.8 ± 3.56b	0.6 ± 0.11b	1.5 ± 0.51a
	BBCH 13	2.1 ± 0.48a	16.0 ± 8.08bc	0.9 ± 0.09c	1.7 ± 0.37a
	BBCH 14	5.0 ± 0.60b	20.6 ± 5.38c	1.3 ± 0.10d	4.8 ± 0.73b
P. schinzii	BBCH 11	1.7 ± 0.40a	5.9 ± 2.80a	1.8 ± 0.33a	6.2 ± 2.09a
	BBCH 12	7.7 ± 0.71ab	11.5 ± 2.27a	4.5 ± 0.73b	11.4 ± 3.77ab
	BBCH 13	9.7 ± 1.44b	35.0 ± 10.68b	6.2 ± 0.71b	13.0 ± 3.52ab
	BBCH 14	13.0 ± 3.44b	61.0 ± 85.40b	6.5 ± 1.07b	24.9 ± 10.55b

† BBCH 11, one true leaf; BBCH 12, two true leaves, BBCH 13, three true leaves; BBCH 14, four true leaves.
No significant differences (based on computed selectivity indices and corresponding *p*-values) between figures with the same letter, comparison within herbicide and effective dose only (Experiment 3).

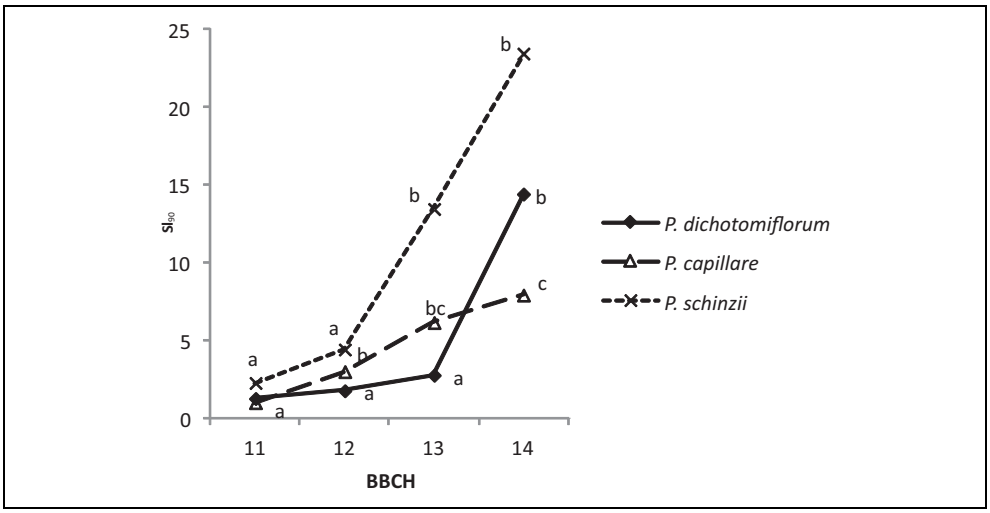

Fig. 1: The performance of topramezone for *P. capillare, P. dichotomiflorum* and *P. schinzii* as influenced by growth stage (Experiment 3). The performance is expressed as a SI_{90} index. i.e. ED_{90} dose relative to ED_{90} response for first leaf stage plants of *P. capillare* (i.e. 2.6 g ai ha^{-1} topramezone). No significant differences (based on computed selectivity indices and corresponding *p*-values) between data points with the same letter; comparison within species only.

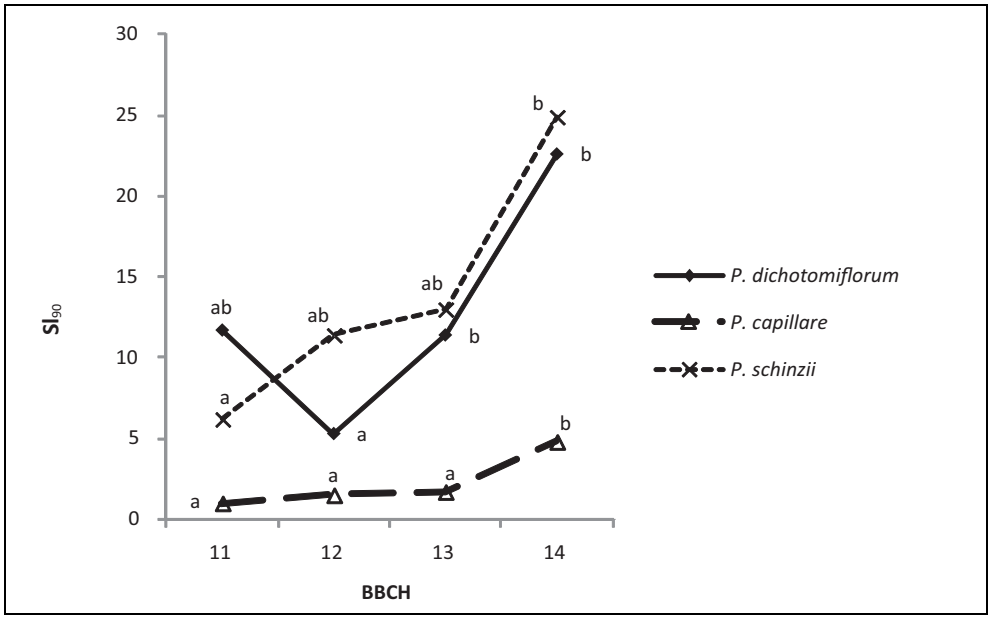

Fig. 2: The performance of nicosulfuron for *P. capillare*, *P. dichotomiflorum* and *P. schinzii* as influenced by growth stage (Experiment 3). The performance is expressed as a SI_{90} index. i.e. ED_{90} dose relative to ED_{90} response for first leaf stage plants of *P. capillare* (i.e. 1.0 g ai ha^{-1} nicosulfuron). No significant differences (based on computed selectivity indices and corresponding p-values) between data points with the same letter; comparison within species only.

significantly less sensitive to nicosulfuron when treated at the four true leaves stage than at all other leaf stages. Four leaves stage plants of *P. schinzii* were four-fold less sensitive than plants at the one leaf stage. *Panicum dichotomiflorum* sensitivity was four-fold lower at the four true leaves stage than at the two true leaves stage. Amongst *Panicum* species, nicosulfuron sensitivity was lowest for *P. schinzii* (except for one leaf stage seedlings) and highest for *P. capillare*, irrespective of leaf stage.

The reduced sensitivity with seedling age can be explained by a lower penetration, since herbicide penetration is hampered as plants age owing to the development of a thicker cuticle or altered cuticle composition (Aldrich & Kremer 1997). Moreover, herbicide metabolism may be higher as plants age as shown by Singh & Singh (2004) for glyphosate and trifloxysulfuron.

Overall, results of these experiments indicate that difficulties may arise in the successful chemical control of *Panicum* grasses due to interspecific differences in herbicide sensitivity in particular for HPPD-inhibiting herbicides. Compared to *P. dichotomiflorum*, satisfactory POST control of *P. schinzii* has required seven-to eight-fold higher doses of topramezone and mesotrione in the conducted pot experiments. Hence, correct identification of *Panicum* species before treatment or foreknowledge about the composition of *Panicum* species present is a prerequisite to avoid insufficient *Panicum* control. Foreknowledge can be obtained by studying flower parts of surviving *Panicum* plants in the fall which enables correct identification. In addition, successful control of *P. capillare*, *P. dichotomiflorum* and *P. schinzii* largely depended on growth stage at the time of herbicide application. Sensitivity of *Panicum* seedlings to topramezone and nicosulfuron linearly or exponentially decreased with increasing number of true leaves at the time of herbicide application: at the four leaves stage, sensitivity was two- to eleven-fold lower than at the one leaf stage. Poor control by topramezone and nicosulfuron in the field can be expected in circumstances where *P. dichotomiflorum* and *P. schinzii* seedlings in particular are developed beyond the three true leaves stage.

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