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INTRODUCTION OF A NON DESTRUCTIVE METHOD FOR THE INVESTIGATION OF HERBICIDE EFFICACY IN GREENHOUSE BIOASSAYS BASED ON IMAGE ANALYSIS

Ortrud Jaeck[1], Alexander Menegat[1], Martin Weis[1], Hanwen Ni[2] and Roland Gerhards[1]

ABSTRACT

The North China Plain (NCP) is the main winter wheat growing region in China. In winter wheat, Bromus japonicus is an important annual monocotyledonous weed, which is difficult to control and causes high yield losses as a consequence for lack of effective herbicides. For this reason dose-response studies were conducted in a greenhouse to test the efficacy of several herbicides on B. japonicus. Two ACCase inhibitors, five ALS inhibitors and one PS(II) inhibitor were tested at six different application rates. Efficacy on B. japonicus was measured by dry weight assessment as well as non-destructively by leaf coverage measurements with a bi-spectral camera. The efficacy of the tested ACCase and PS(II) inhibitors was not satisfactory for weed management, even at high dosages. However the tested ALS inhibitors showed high efficacies. Coverage measurements with the bi-spectral camera correlated very well with the dry matter weight of B. japonicus. Therefore it can serve as an easy, rapid and non-destructive method to determine herbicide efficacy over time and also under field conditions. It is necessary to introduce new herbicides to the NCP for efficient weed control, to ensure the security of the national food supply. Therefore comprehensive dose-response studies are necessary to evaluate herbicides of interest with different modes of action on all important weeds of the NCP.

Key words: North China Plain, Japanese brome, *Bromus japonicus,* herbicide, bi-spectral camera, coverage

INTRODUCTION

The North China Plain is the most important winter wheat growing region of China. With about 50 % of the national yield, production of wheat in the NCP makes a substantial contribution to the national food security (Wu *et al*. 2006, Zhang *et al*. 1999). Besides other limiting factors such as water supply, weed infestation can also strongly reduce winter wheat yield. During two surveys performed in

[1]University of Hohenheim, Institute of Phytomedicin, Department of Weed Science, Stuttgart, Germany.
[2]China Agricultural University, College for Agronomy and Biotechnology, Beijing, China.
Corresponding author's email: ortrud.jaeck@uni-hohenheim.de

2009 and 2010, we noted that *B. japonicus* was an abundant and widespread grass weed in winter wheat, and was difficult to control with the available herbicides. Infestation can result in large yield losses. Furthermore, we noticed that herbicide application rates required to achieve control were often excessive, which results in increased selection pressure and potential for resistance development to progress more rapidly.

For these reasons, dose-response studies were conducted in a glasshouse with the aims (1) to evaluate the efficacy of different herbicides, which are not available in the NCP for control of *B. japonicus*, (2) to screen different herbicides with respect to resistance of *B. japonicus* and in addition (3) to test coverage measurement with a bi-spectral camera for assessment of herbicide activity.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of the Chinese biotype of *B. japonicus* were derived from agricultural sites from the Hebei province in the NCP. The sensitive biotype was derived from Herbiseed, UK. Experiments were conducted in a greenhouse with day/night length of 12 h and temperature regime of 15 °C/5 °C. Seedlings were transplanted in the 1-leaf-stage (BBCH 11).

Herbicides

Eight herbicides were selected for dose-response studies; these included fenoxaprop-p-ethyl, pinoxaden + clodinafop, iodosulfuron + mesosulfuron, flucarbazone and isoproturon which are common in the NCP, and pyroxsulam + florasulam, propoxycarbazone and sulfosulfuron which are not currently available in the NCP. Each herbicide was applied at six application rates, with the highest rate consisting of the recommended field rate (Table-1).

Application

Herbicides were applied to *B. japonicus* in the 3-leaf stage (BBCH 13) with a laboratory sprayer (Aro, Langenthal, Switzerland) equipped with a broadcast nozzle (8004 EVS, TeeJet Spraying Systems Co., Wheaton, IL, USA) using a velocity of 800 mm/s, at a height over plants of 500 mm, a water volume of 400 l/ha and a pressure of 300 kPa.

Assessment of Herbicide Activity

Leaf-coverage of *B.japonicus* was measured three weeks after herbicide application with a bi-spectral camera. Herbicide activity on *B.japonicus* was visually rated and afterwards aboveground biomass was harvested and dried for 48 h at 80 °C before dry weight was obtained.

Table-1. Herbicides and their application rates used in the experiment.

HRAC class	Commercial name	Active ingredient	Application rates used [g a.i./ha]					
			1	1/2	1/4	1/8	1/16	1/32
A	Ralon Super®	fenoxaprop-P-ethyl	63.60	31.80	15.90	7.95	3.98	1.99
A	Axial + Topik 100®	pinoxaden + clopdinafop	56.73	28.37	14.18	7.09	3.55	1.77
B	Broadway®	pyroxsulam + florasulam	20.04	10.02	5.01	2.51	1.25	0.63
B	Atlantis WG®	iodosulfuron + mesosulfuron	18.00	9.00	4.50	2.25	1.13	0.56
B	Attribut®	propoxycarbazone	66.34	33.17	16.59	8.29	4.15	2.07
B	Monitor®	sulfosulfuron	20.00	10.00	5.00	2.50	1.25	0.63
B	Everest 70 WG®	flucarbazone	29.40	14.70	7.35	3.68	1.84	0.92
C2	Arelon flüssig®	isoproturon	1500	750	375	187.5	93.75	46.88

Bi-spectral Camera

The bi-spectral camera takes pictures simultaneously in the infrared (> 700 nm) and red (620-660 nm) wave lengths. Both images were overlaid and normalized to a similar grey level intensity before creating a differential image (infrared-red).The differential image enhances the contrast between green leaf matter and background. This facilitates the calculation of a binary image by setting a grey level threshold (Fig. 1). From this binary image, plant coverage was calculated by counting number of white pixels and dividing by the total number of pixels.

Experimental Design and Statistical Analysis

Experimental layout consisted of a completely randomized design with 4 replications for each treatment. One replication consisted of four *B.japonicus* plants per pot. Statistical analysis was performed using the statistical software *R (*R Development Core Team 2011). Dose-response analyses were performed with the *R* extension package drc (Ritz and Streibig, 2005). For comparison of biotypes, data were normalized according to Streibig (1995), the Streibig-function was fitted using the following equation $y = C + ((D - C) / (1 + \exp(b \ln(x / ED50))))$ (Streibig, 1988) and ED50-values were tested on mean differences (F-test, $\alpha=0.05$). For comparison of coverage measurements and dry weight measurements, Pearson’s product-moment correlations were done with the procedure cor.

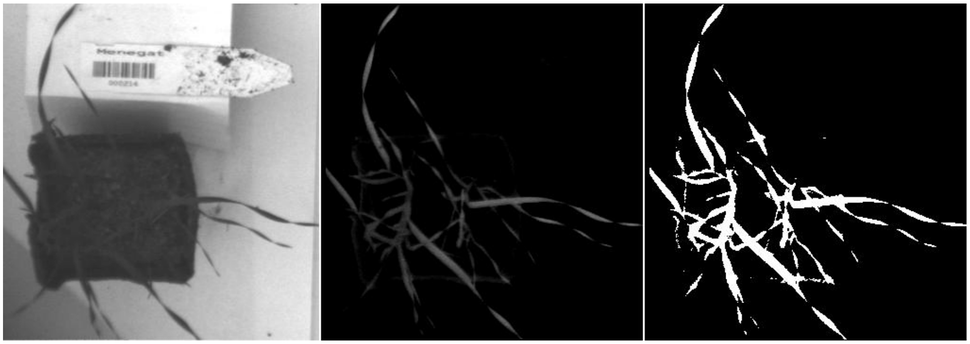

Figure 1. Infrared (left), differential (middle) and binary (right) image of *B. japonicus.*

RESULTS

Herbicide Efficacy and Resistance

The ACCase-inhibitors fenoxaprop-p and pinoxaden + clodinafop did not control *B. japonicus,* even at the highest dosage. The PS(II)-inhibitor isoproturon as well as the ALS-inhibitor flucarbazone, with an activity of 63.75 % and 66.25 %, respectively, did not show satisfactory inhibitory activity as well (Table 2). The tested ALS-inhibitors pyroxsulam + florasulam, iodosulfuron + mesosulfuron, propoxycarbazone and sulfosulfuron all showed high efficacies, however none of these herbicides was able to completely control *B.japonicus.* The herbicides sulfosulfuron and pyroxsulam + florasulam with 83.75 % and 82.5 % control, respectively, showed the highest efficacies of the herbicides under evaluation. Comparison of ED50-values of the NCP-population with the "sensitive" standard showed no indication of evolved resistance of *B. japonicus* against the herbicides commonly used in the NCP, as observed in Figure 2 and Figure 3 for the herbicides flucarbazone and iodosulfuron + mesosulfuron.

Table-2. Efficacy of herbicides on Chinese biotype, *B. japonicus* at recommended field rates.

Herbicide	**Mean herbicide efficacy on *B. japonicus* [% activity]**
Fenoxaprop-p-ethyl	7.5
Pinoxaden + Clodinafop	0
Pyroxsulam + Florasulam	82.5
Iodosulfuron + Mesosulfuron	71.25
Propoxycarbazone	78.75
Sulfosulfuron	83.75
Flucarbazone	66.25
Isoproturon	63.75

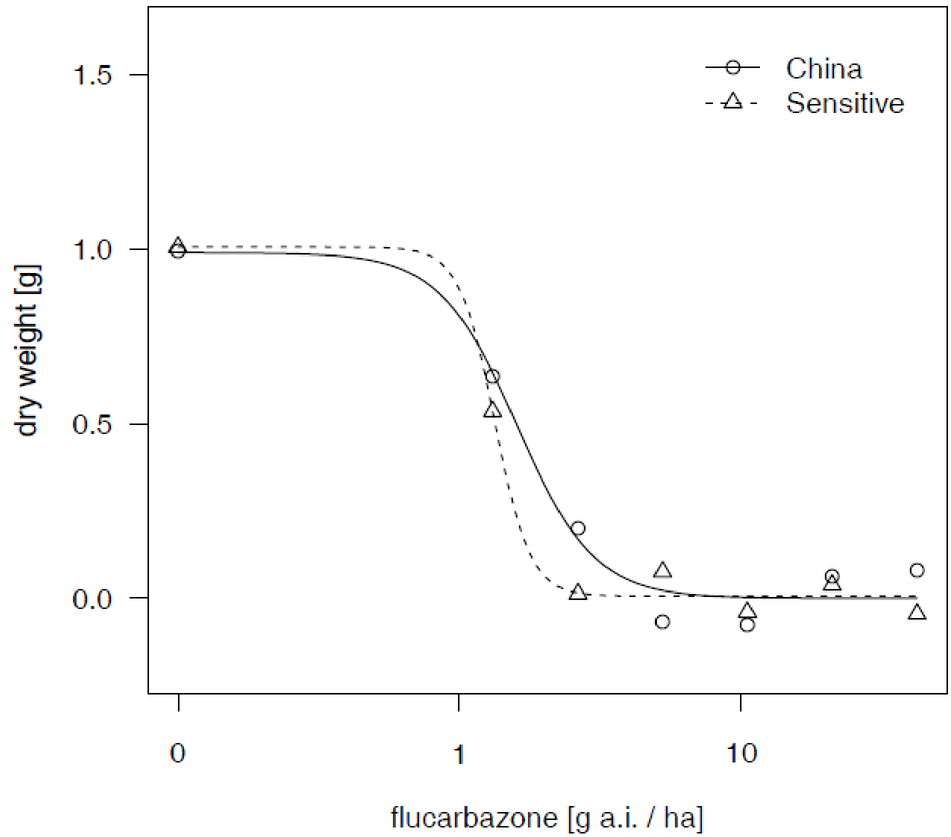

Figure 2. Dose-response relationship for the herbicide flucarbazone upon dry weight [g] of the Chinese and "sensitive" biotypes of *B. japonicus*.

Comparison of Methods

Correlation analyses showed that coverage measurements with the bi-spectral camera correlated very well with dry weight data. Each correlation was significant and correlation coefficients ranged between 0.62 and 0.93 (Table 3). Furthermore it was possible to fit the Streibig-function to the coverage data (Figure 4) so that identification of herbicide resistance would be potentially possible using the method of coverage measurement with bispectral camera as well as for method using dry weight measurement.

DISCUSSION

The dose-response study showed that none of the herbicides available in the NCP have sufficient inhibitory activity on *B. japonicus.* The additionally tested herbicides propoxycarbazone, pyroxsulam +

florasulam and sulfosulfuron, which are not currently available in the NCP, had the highest activities on *B. japonicus,* but did not result in complete control. These results reveal the necessity of introducing more effective herbicides to the NCP for control of *B. japonicus* in winter wheat together with additional methods for cultural weed control.

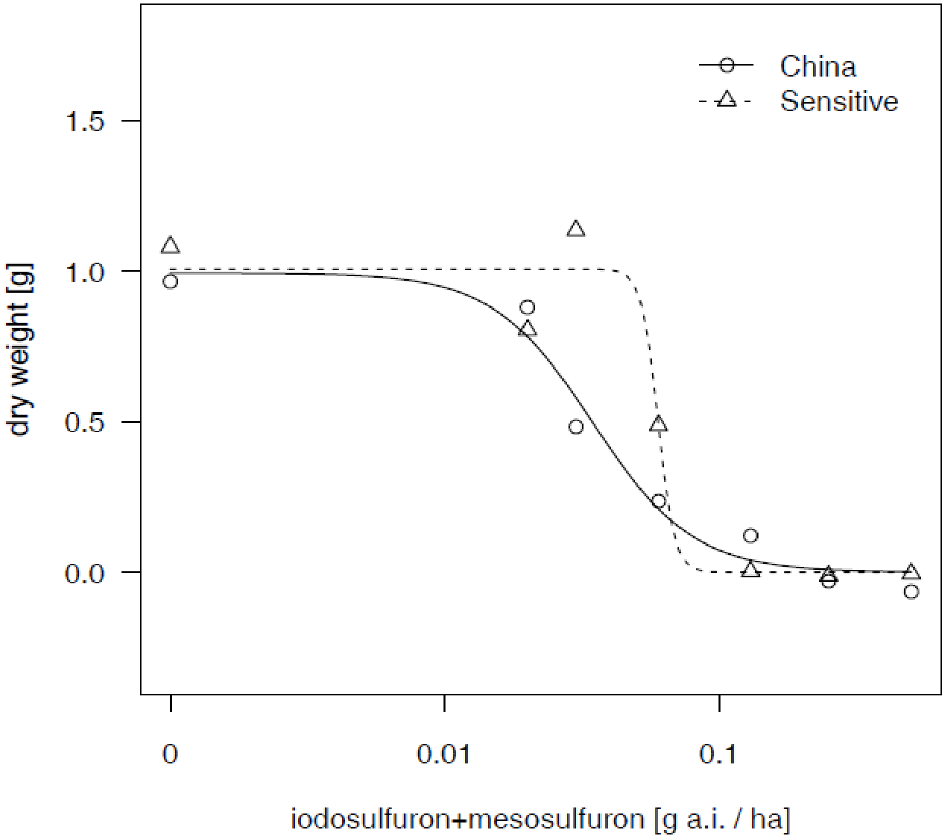

Figure 3. Dose-response relationship for the herbicide iodosulfuron + mesosulfuron upon dry weight [g] of the Chinese and "sensitive" biotypes of *B. japonicus*.

Herbicide resistance of *B. japonicus* against herbicides common in the NCP was not detected in this screening trial. Obviously, the herbicides commonly used in the NCP had a much lower efficacy than the ALS-inhibitors, which have not yet been introduced to the NCP. This could be an indication that there is an evolved tolerance of *B. japonicus* to these herbicides and that the "sensitive" standard used in this study was not really sensitive. This could be seen in tendencies in the dose-response studies, for example, of iodosulfuron + mesosulfuron where the sensitive standard was less susceptible to the herbicide than the biotype from the NCP. The only group of herbicides which showed satisfactory control was the group of ALS-inhibitors.

However, many weeds have evolved rapid resistance to ALS inhibitors; in the USA, resistant populations exist in Kansas to propoxycarbazone, pyroxsulam and sulfosulfuron (Peterson, 2007). Those herbicides showed the highest activity in the dose-response-studies, but are not currently available in the NCP. Due to issues associated with known resistance to herbicides, a resistance management program is indispensable when introducing new herbicides to the NCP in which other weed control strategies than herbicides are utilized for sustainable control of *B. japonicus.*

Table-3. Correlation coefficients of different treatments and biotypes.

Herbicide	**biotype**	**Correlation coefficient r**
Fenoxaprop-p-ethyl	China	0.62
	Sensitive	0.81
Flucarbazone	China	0.77
	Sensitive	0.80
Iodosulfuron + mesosulfuron	China	0.85
	Sensitive	0.83
isoproturon	China	0.82
	Sensitive	0.82
pinoxaden + clodinafop	China	0.68
	Sensitive	0.78
propoxycarbazone	China	0.92
pyroxsulam + florasulam	China	0.92
	Sensitive	0.87
sulfosulfuron	China	0.93
	Sensitive	0.86

Comparison of dry weight and coverage methods for assessment of herbicide efficacy and resistance showed that evaluation of coverage is a suitable method for detecting herbicide efficacy as well as herbicide resistance. For assessment of herbicide activity it is was more useful than even dry weight assessment. Herbicide activity is better modeled in this system because an activity of 100 % equals green leaf surface coverage of 0 %, whereas with dry weight measurements a residual biomass will persist and it is not possible to accurately predict herbicide activity from this data. That is to say, use of a bispectral camera to assess coverage provides equivalent data to visual assessment of herbicide activity, with the difference that the coverage assessment method is more precise. It is an easy method to employ, especially with high throughput. This technique, in addition, is non-destructive so that assessment of the dynamics of herbicide activity is possible and the optimal timing of herbicide activity assessment can be met.

Future research should be conducted to develop comprehensive dose-response studies to evaluate more effective herbicides for the most abundant and serious weeds of the NCP.

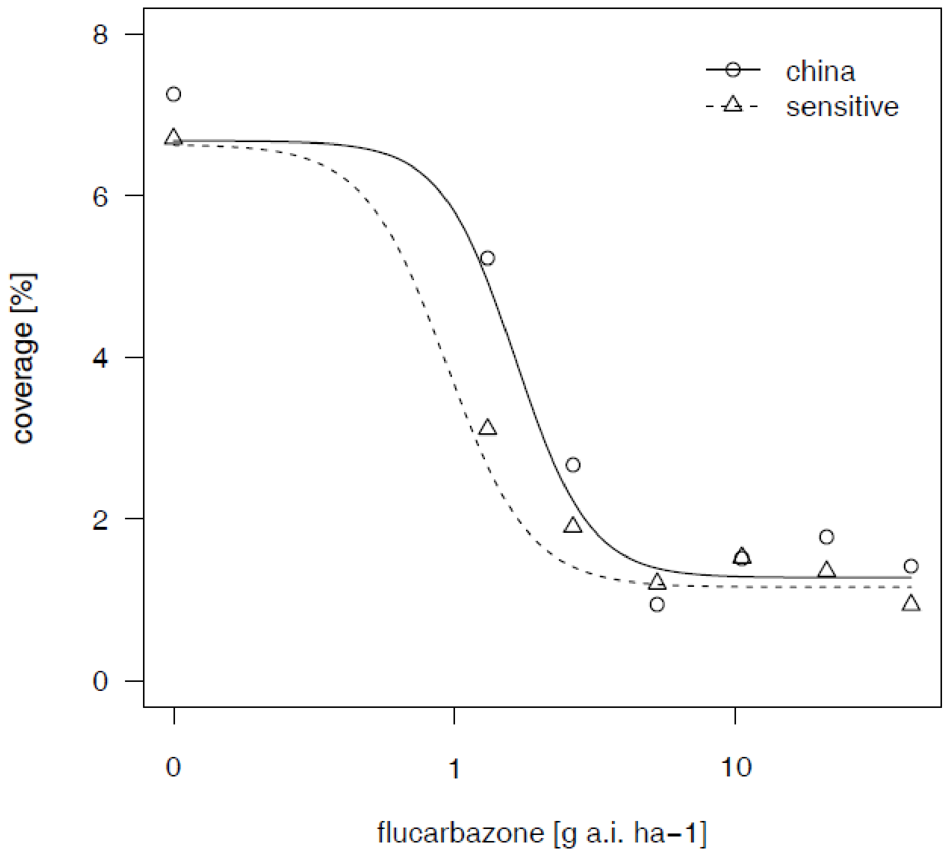

Figure 4. Dose-response relationship for the herbicide flucarbazone upon coverage [%] of the Chinese and "sensitive" biotypes of *B. japonicus*.

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