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Article

Development of a Geo-Referenced Database for Weed Mapping and Analysis of Agronomic Factors Affecting Herbicide Resistance in *Apera spica-venti* L. Beauv. (Silky Windgrass)

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Abstract: In this work, we evaluate the role of agronomic factors in the selection for herbicide resistance in *Apera spica-venti* L. Beauv. (silky windgrass). During a period of three years, populations were collected in more than 250 conventional fields across Europe and tested for resistance in the greenhouse. After recording the field history of locations, a geo-referenced database has been developed to map the distribution of herbicide-resistant *A. spica-venti* populations in Europe. A Logistic Regression Model was used to assess whether and to what extent agricultural and biological factors (crop rotation, soil tillage, sowing date, soil texture and weed density) affect the probability of resistance selection apart from the selection pressure due to herbicide application. Our results revealed that rotation management and soil tillage are the factors that have the greatest influence on the model. In addition, first order interactions between these two variables were highly significant. Under conventional tillage, a percentage of winter crops in the rotation exceeding 75% resulted in a 1280-times higher risk of resistance selection compared to rotations with less than 50% of winter crops. Under conservation tillage, the adoption of >75% of winter crops increased the risk of resistance 13-times compared to rotations with

less than 50% of winter crops. Finally, early sowing and high weed density significantly increased the risk of resistance compared to the reference categories (later sowing and low weed density, respectively). Soil texture had no significant influence. The developed model can find application in management programs aimed at preventing the evolution and spread of herbicide resistance in weed populations.

Keywords: farm management; geographic information system; logistic regression model (LRM)

Nomenclature: *Apera spica-venti* L. Beauv.; Silky windgrass; APESV

1. Introduction

Chemical weed control through the use of herbicides has gained an increasing popularity among farmers over the last six decades. Since their appearance on the market, herbicides revolutionized the traditional concept of plant protection by removing weeds selectively, while avoiding labor-intensive and time-consuming soil cultivation practices, which ultimately resulted in cost-effective, safe and profitable food production [1].

For these reasons, herbicides represent the most extensively used weed control measure, accounting for up to 50% of the global plant protection market [2]. The high adaptability potential of weeds together with herbicide over-reliance have resulted in the selection for herbicide-resistant weed populations on a large scale [3]. At present, 210 weed species (~58% dicotyles and ~42% monocotyles) worldwide have evolved resistance to different herbicide modes of action [4]. Recommendations for delaying the evolution of herbicide resistance have been mainly focused on herbicide rotation and herbicide mixtures [5,6]. However, other factors are known to affect the evolution of herbicide resistance in weed populations.

The recent trend of increase in proportion of autumn-sown crops in the agricultural systems of Central and Eastern Europe has promoted the incidence of a specialized weed flora [1,7]. This ultimately led to significant income losses deriving from the increased costs for weed control [1], whose effectiveness and profitability can be jeopardized by herbicide resistance.

Soil tillage is another management measure that plays an important role in the spread of weed populations, potentially including resistant ones. In fact, although reduced (e.g., tine cultivation prior to drilling) and no-till methods are likely to have several benefits (e.g., conservation of soil moisture and structure, reduction of water and wind erosion, time optimization and lower work inputs), they can also promote the germination of certain weed species as a consequence of the permanence of their seeds in the upper soil layers [8]. This eventually results in higher rates of seedling emergence and population density, faster spread throughout the fields and higher competition ability, which may increase the likelihood of resistance emerging.

Finally, sowing date and weed density have a relevant impact on weed seed production, selection and survival [8]. In particular, early sowing and high weed density may enhance crop-weed

competition, which eventually results in the short-term spread of weeds [9] and, therefore, in increased probability of resistance development.

Among the numerous weed species spread worldwide, *Apera spica-venti* L. Beauv. (silky windgrass) is one of the most abundant in the agricultural fields of Central and Eastern Europe. It is an annual grass weed that can be mostly found in winter crops, especially in winter cereals and winter oilseed rape (*Brassica napus* L.) [7,10]. The seeds exhibit short primary dormancy [11,12] and germinate in late summer and fall, mostly on light-textured, humid soils [13,14]. Nevertheless, during mild winters, the seeds may continue to germinate, eventually causing infestations also in spring-sown crops [7,15]. Despite the presence of *A. spica-venti* also in North America and Canada, its relevance is higher in the Central and Eastern part of Europe (Germany, Switzerland, Poland, the Czech Republic, Slovakia and Hungary) and more recently in Northern Europe (Denmark and Sweden) [7]. The economic importance of this weed has been quantified in yield losses of up to 30% [16], and the economic threshold has been estimated at ~10–30 plants m^{-2} [17]. As the weed overgrows the crop during the last phenological development stages (July), *A. spica-venti* strongly competes for light [16]. During the last years, reports on herbicide-resistant *A. spica-venti* populations have accumulated [4].

In 1994, the first case of resistance to photosystem II (PSII)-inhibiting herbicides was reported in Switzerland [18]. Since then, several cases have been documented. In 1997, the first case of PSII-resistance was reported in Germany [19]; in 2005, three cases of resistance to acetolactate synthase (ALS)-inhibiting herbicides were documented in Poland, the Czech Republic and Germany [20–22]; in 2006, the first case of resistance to ALS-inhibitors was confirmed in Switzerland [23]; and in 2009, the first case of multiple resistance to PSII-, ALS- and acetyl-CoA-carboxylase (ACCase)-inhibiting herbicides was reported in Germany [4].

The objectives of this study were: (1) to develop a GIS (geographic information system) database for mapping the spread of herbicide-resistant *A. spica-venti* populations across Europe; (2) to assess and quantify the relative influence of crop rotation, soil tillage, sowing date, soil texture and weed density on the likelihood of resistance emergence in *A. spica-venti*; and (3) to provide farmers and consultants with rational and practicable solutions at the agronomic level aimed at preventing the evolution and spread of herbicide resistance in European cereal production systems.

2. Results and Discussion

2.1. Logistic Regression Analysis

The results of the greenhouse screening revealed that 80% of populations (210) were resistant to herbicides (mainly ALS-inhibitors), whereas resistance could not be confirmed in 53 cases (~20%). A great part of the investigated fields (72%) was characterized by a high percentage of winter crops in the rotations (>75%), whereas the rest of the cases (50%–75% and <50%) had a smaller proportion. Early sowing was less represented than middle and late sowing, accounting for 30% of total observations. Finally, conventional/conservation soil tillage and low/high weed density had a similar proportion in their categories (Table 1).

Table 1. Frequency distribution of cases (in %) within each category of analyzed factors (resistance, crop rotation, soil tillage, sowing date, soil texture and weed density). Frequency distribution of herbicide resistance is shown in each factor category.

Factors	%	Herbicide Resistance	
		Yes (%)	No (%)
Resistant			
Yes (verified)	79.8		
No (not verified)	20.2		
Total	100		
Crop Rotation *			
<50%	11.4	16.7	83.3
50%–75%	17.1	55.6	44.4
>75%–100%	71.5	95.7	4.3
Total	100		
Tillage			
Plow	57.0	74.0	26.0
Reduced/No-Till	43.0	87.6	12.4
Total	100		
Sowing Date **			
Middle/Late	70.0	76.1	23.9
Early	30.0	88.6	11.4
Total	100		
Soil Texture ***			
Middle/Heavy	88.6	82.4	17.6
Light	11.4	60.0	40.0
Total	100		
Weed Density ****			
Low/Middle	43.3	73.7	26.3
High	56.7	84.6	15.4
Total	100		

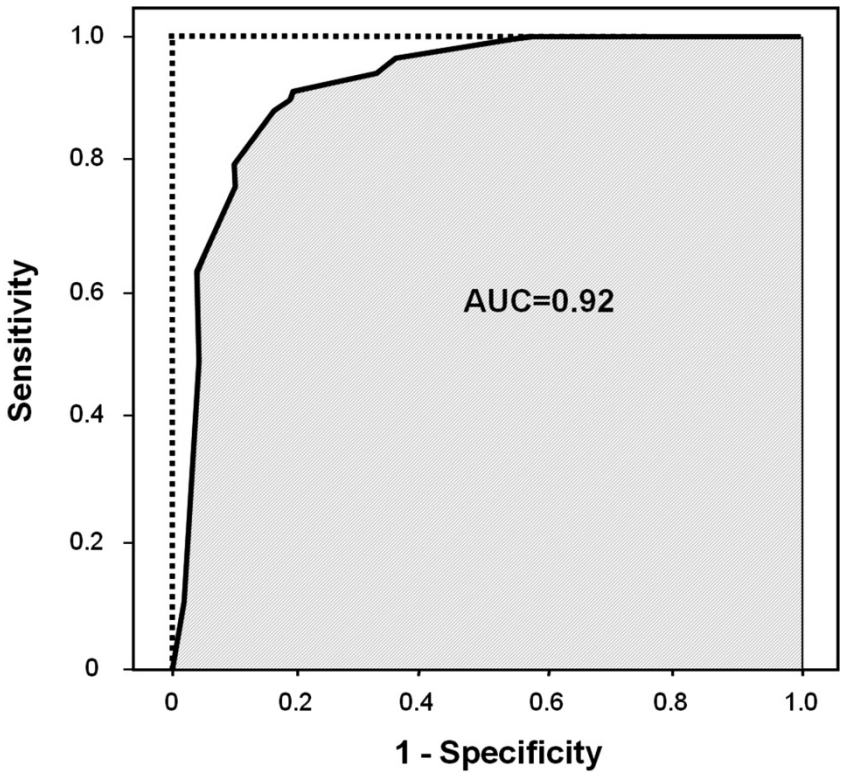
* % of winter crops in the rotation; ** (winter crops) Early: until October 05; Middle: until October 15; Late: after October 15; *** Light: sandy; Middle: sand-clay/silt; Heavy: clay/silt; **** Plants m^{-2} (Low: <10; Middle: 10–40; High: >40).

The developed LRM showed a good fitness, calibration and discrimination (Table 2). According to the Nagelkerke R^2, the model could explain the 64% of total variability in selection for herbicide resistance. The discrimination capacity of the fitted model resulted in 90% of correctly classified cases, with greater sensitivity (it correctly classified 97% of resistant cases) than specificity (it correctly classified 64% of non-resistant cases). ROC analysis showed a good discrimination, with an AUC of 0.92 (95% CI: 0.88–0.97) (Figure 1).

Table 2. Fitness, calibration and discrimination of the logistic regression model (LRM). The goodness of fit of the developed model was tested by Log Likelihood, Cox-Snell R^2 and Nagelkerke R^2. Calibration was tested by Hosmer-Lemeshow statistic (Chi^2, degrees of freedom and *p*-value) and discrimination by the percentage of correctly classified cases.

Statistical method	Value	Degrees of freedom	*p*-value
Goodness of fit			
Log Likelihood (LL)	−64.10		
Cox-Snell R^2	0.40		
Nagelkerke R^2	0.64		
Calibration			
Hosmer-Lemeshow Chi^2	4.02	6	0.674
Discrimination Percentage of correct classification	90.1		
Valid cases	263		

Figure 1. "Sensitivity" *vs.* "1-Specificity" plot. Solid line represents the calculated Receiver Operating Characteristic (ROC) curve, which indicates the discrimination capability of the LRM. Dotted line represents the perfect ROC curve (Area under the curve [AUC] = 1). Non-resistant cases wrongly classified by the model (false positives) are represented in the *x*-axis (1-Specificity). Resistant cases correctly classified by the model (true positives) are represented in the *y*-axis (Sensitivity).



LRM for the predictors resulted in two predictors with greater relative influence on the model. All factors, except for soil texture (p = 0.614), were statistically significant at 95% (crop rotation, soil tillage, sowing date and the interaction between crop rotation and soil tillage) and 90% confidence

level (weed density). Rotation management, soil tillage and their interactions were the most important predictor variables (Table 3).

According to the analyzed data, the highest risk of herbicide resistance occurred in the presence of a high proportion of winter crops in the rotations (>75%); in these cases, under conventional tillage, the probability of resistance selection showed to be 1274.82-times higher compared to <50% of winter crops. This probability decreased as the proportion of spring-sown crops in the rotation increased (50%–75% of winter crops under conventional tillage), reaching the limit of 21.13-times compared to <50% of winter crops. Finally, under conservation tillage, a high percentage of winter crops in the rotations (>75%) resulted in a 12.75-fold increase of resistance selection compared to <50% of winter crops.

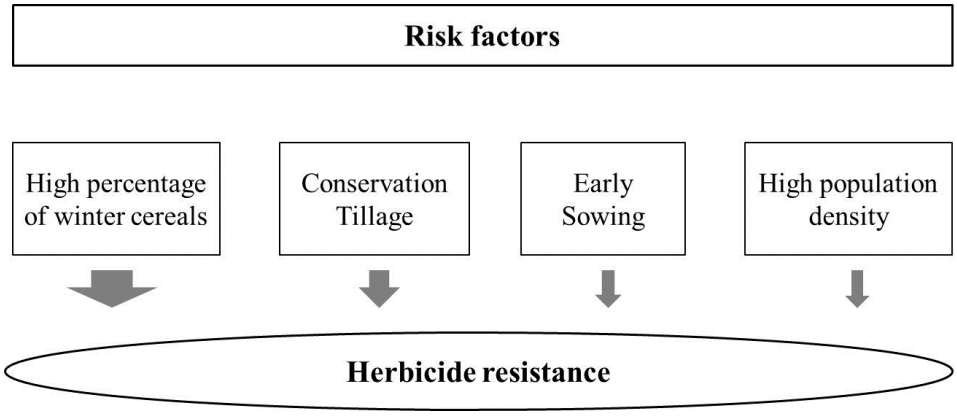
Table 3. Predictor variables of herbicide resistance for the LRM. Probability of resistance selection with respect to the reference category is shown for each statistically significant factor and is given by the crude (univariate model) and adjusted odds ratio (multivariate model with all factors considered as independent variables in the regression). The significance of the predictor variables is shown by Wald statistic and 95% confidence interval (CI) of the adjusted OR in the multivariate model. Percentage distribution of the factors influencing herbicide resistance is given in terms of relative contribution of the predictor variables.

Factors [a]	OR[b]	OR[c]	Wald	95% CI	% [d]
Crop Rotation (ref. <50%)			36.69		53.38
50%–75%	6.25	21.13 **	7.21	2.28–195.83	
>75%–100%	112.50	1274.82 **	31.38	104.46–15557.23	
Tillage (ref. Plow)	2.49	87.67 **	10.05	5.52–1393.08	14.62
Sowing Date (ref. Middle/Late)	2.44	4.65 **	6.91	1.48–14.61	10.05
Weed Density (ref. Low/Middle)	1.96	2.11 *	2.31	0.81–5.52	3.36
Rotation * Tillage (ref. <50% & Plow)			12.77		18.58
(50%–75%) & (Reduced/ No–Till)		0.04 **	4.61	0.00–0.75	
(>75%–100%) & (Reduced/ No–Till)		0.01 **	12.13	0.00–0.09	
Constant		0.02	13.17		

* Factor significant at 90% confidence level (p-value ≤ 0.100); ** Factor significant at 95% confidence level (p-value ≤ 0.050); [a] The displayed factors are maintained in the final model after the modeling process; [b] Crude Odds Ratio (OR); [c] Adjusted Odds Ratio (OR); [d] Relative contribution of predictors.

The rest of the agronomic factors also resulted in a potential risk of resistance emergence. Early sowing and high density of *A. spica-venti* (>40 plants m^{-2}) increased the probability of resistance selection 4.65- and 2.11-times compared to later sowing and lower densities, respectively (Table 3). In conclusion, the predominant presence of winter crops (>75%) in the rotation systems, together with conservation tillage, early sowing and high population density lead to a higher chance of herbicide resistance in *A. spica-venti* (Figure 2). Also, the effect of soil tillage under crop rotation practices significantly affects the selection of resistance.

Figure 2. Flow chart showing the risk factors involved in the development of herbicide resistance. All factors, with the exception of soil texture, significantly increased the probability of resistance occurrence in *A. spica-venti*. The size of the arrows is proportional to the significance of each factor.



Our results underline the role of farm management in the development of herbicide resistance. In fact, most interviewed farmers had reported reduced herbicide performance in the last 1–3 years despite adoption of rational chemical weed control strategies (single herbicide applications at the recommended field rate, tank mixtures and/or rotation of active ingredients). Statistical analysis of the collected data revealed that each factor, with the exception of soil texture, significantly increased the likelihood of resistance development.

Rotation management proved the most significant factor influencing the probability of resistance selection in *A. spica-venti*. In particular, a proportion of winter crops in the rotations exceeding 75% greatly increases the risk of herbicide resistance compared to situations in which winter and summer crops are more evenly distributed over the years. This is in accordance with most existing literature and can be attributed to two main reasons: (1) continuous cropping tends to favor and select the species within the weed flora that have phenological and physiological similarities to the crop (e.g., grass weeds in cereals) [24,25]. This eventually results in the establishment of an adapted, resilient weed flora with more competition ability, as it would be in a more diverse cropping system [25,26]; and (2) in consequence of repeated use of the same cultural practices over several years, the tolerance of weeds to the direct control methods (e.g., chemical weed control) is likely to develop and spread more quickly [25].

Also, conservation tillage practices resulted in an increased risk of resistance development in *A. spica-venti* compared to conventional tillage situations (e.g., moldboard plowing). It has been widely documented that low soil disturbance systems can cause a shift of the weed flora towards a troublesome composition [25]. In the case of winter cereal cropping systems, for example, minimum tillage practices can promote the dominance of grasses with low-dormant seeds (e.g., *Alopecurus myosuroides*, *A. spica-venti*, *Bromus* spp.) within only a few years [25]. Consequently, annual grass weeds, especially those whose seed anatomy enables easy wind dispersion, are becoming more prevalent since the adoption of conservation tillage practices [26,27].

Nevertheless, the significant interactions between rotation management and soil tillage evidenced the importance of combining appropriate rotation systems (*i.e.*, a higher proportion of summer crops)

with conventional tillage to reduce the risk of resistance development in *A. spica-venti*. In fact, even where conventional tillage is used, agricultural systems tending to monoculture are still at risk of resistance. On the other hand, if a percentage of winter crops exceeding 75% is foreseen in the rotation, the adoption of conservation tillage will increase the risk of resistance compared to a situation in which less than 50% of winter crops are foreseen in the rotation.

Sowing date confirmed as well its influence on the likelihood of resistance selection in *A. spica-venti* populations. Results revealed that early sowing of the winter crop increased the risk of herbicide resistance when compared to later sowing dates. Studies have shown that weed species characterized by early germination are favored by early sowing. Hartzler (2000) [28], for example, documented the correlation between the increase in the selection of *Ambrosia trifida* L. (giant ragweed) in the cropping regions of the U.S.A. and the move to earlier sowing dates of maize and soybean; this was consequent to weed seedling emergence after planting, which results in reduced effectiveness of control measures in both pre- and post-emergence. As *A. spica-venti* is a weed notoriously characterized by a short dormancy period and early emergence, the anticipation of winter crop sowing dates can play a decisive role in promoting recurrence of infestations and, therefore, increasing the risk for selection of herbicide-resistant populations. This problem can be particularly relevant in years with favorable weather conditions, which promote high seed germination rates, high population densities and, ultimately, higher competition ability.

Finally, high *A. spica-venti* densities proved to increase the likelihood of resistance selection. In accordance with several sources, weed density can have a relevant influence on the rate of herbicide resistance development (e.g., higher weed competition ability, higher seed production and faster spread and reduced penetration of herbicides into the plant foliage) [8]. Overall, this factor resulted in *A. spica-venti* being twice as prone to evolve resistance in high density patches as in areas with low weed density.

The role of soil texture in the probability of resistance selection in *A. spica-venti* could not be statistically confirmed. Although the preference of this weed for light-textured soils (*i.e.*, sandy soils) has been widely documented, most infested fields were characterized by a middle soil texture. Light soils (*i.e.*, sandy soils) and heavy soils (*i.e.*, clay soils) were under-represented.

3. Experimental Section

3.1. Sampling Strategy

Harvesting of *A. spica-venti* seeds took place over a period of three years (from 2007 till 2010) at 263 conventional fields across Europe (Germany, Poland and the Czech Republic). The provenance of the populations was homogenously distributed across the three countries (40% from Germany, 30% from Poland and 30% from the Czech Republic). The winter cropping system mainly consisted of wheat, barley and oilseed rape. Most of the interviewed farmers had observed reduced herbicide performance (especially ALS-inhibitors) over a 1 to 3 year period. Weed control consisted mostly of single herbicide applications in the spring (March-April). Seed collection was performed randomly throughout the fields, in different patches across each field and from different plants (>60 panicles) shortly before winter crop harvest. Samples were taken at a distance of more than 4 m from the field

borders. Finally, the seeds collected from each field were pooled together (*i.e.*, mixed populations) and stored accordingly at 4 °C until ready for greenhouse screening.

3.2. Screening for Herbicide Resistance

3.2.1. Greenhouse Screening: Seed Germination Tests

Seed germination tests were performed after breaking of primary dormancy. One hundred ripe seeds per population (in six replicates) were sown in 6 by 6 cm paper pots (Jiffy® Pots, Jiffy Products Ltd., Winchester, UK). Each pot was filled with sterilized sandy loam soil (5.1% organic carbon content; pH 6.9) and kept well-watered. Growing conditions consisted of 20/15 °C (with a 12 h photoperiod) and a light intensity of 122 μmol m^{-2} s^{-1}. After 7–10 days, emerged seedlings per pot were counted.

3.2.2. Greenhouse Screening: Whole-Plant Pot Assays

A variable amount of seeds for each collected population (depending on the germinative ability) was sown in 8 by 8 cm paper pots (Jiffy® Pots, Jiffy Products Ltd., Winchester, UK) (in three replicates, according to standard experimental design) to give at least 20 plants per pot. Growing conditions were the same as above. Plants were kept well-watered and fertilized twice a week until harvest (8-8-6 N-P-K) (Wuxal® Super, Horticentre Ltd., Drury, New Zealand).

Herbicide treatments were carried out at the 2–3 leaf stage (BBCH 12-13) using a precision spray chamber (Aro, Langenthal, Switzerland). The tested herbicides consisted of five ALS-inhibitors [Lexus® (flupyrsulfuron-methyl 50% w/w), DuPont de Nemours; Glean® (chlorsulfuron 75% w/w), DuPont de Nemours; Monitor® (sulfosulfuron 80% w/w), Monsanto; Atlantis® OD (mesosulfuron-iodosulfuron 1.24% w/v), Bayer CropScience; Oust® XP (sulfometuron-methyl 75% w/w), DuPont de Nemours], two ACCase-inhibitors [Ralon® Super (fenoxaprop-P-ethyl 6.9% w/v), DuPont de Nemours; Select® 240 EC (clethodim 24.1% w/v), Stähler] and one PSII-inhibitor [Arelon® flüssig (isoproturon 50% w/v), Stähler] (Table 4). The appropriate surfactants [Trend® 90 (isodecyl alcohol ethoxylate 90% w/v), DuPont de Nemours; MonFast™ (ethoxylated fatty alcohol 60% w/v), Monsanto; Parasommer® (paraffin oil 65.4% w/v), Stähler] were added when necessary according to suppliers' recommendations. The delivery volume (8004 EVS, Teejet® spraying systems Co., Illinois, USA) was calibrated to spray 400 L ha^{-1}. The velocity of the nozzle was set at 800 mm s^{-1}, the distance from the sprayed surface at 500 mm and the spraying pressure at 300 kPa. All herbicides were tested at the recommended field rate (according to manufacturer`s instruction for the control of *A. spica-venti*) and the fourfold of it. The purpose of the fourfold application rate was to clearly discriminate target site resistant populations for further laboratory investigations (data not shown). Two reference standards (herbicide-susceptible and herbicide-resistant) were included in each assay. Both standards were chosen following previous greenhouse investigations (data not shown). Herbicide performance was evaluated 15 and 30 days after treatment (DAT) according to OEPP/EPPO (European and Mediterranean Plant Protection Organization) guidelines [29]. Finally, resistance was classified into two main categories: resistant and non-resistant (susceptible).

Table 4. Herbicides used for the greenhouse resistance screening tests.

Trade name Surfactant (%)	Active ingredient (a.i.)	Amount of active ingredient (g a.i./Kg-L)	Applied doses (g a.i./ha)
Lexus® Trend 90® * (0.1%)	flupyrsulfuron-methyl	500	15, 60
Glean® Trend 90® (0.1%)	chlorsulfuron	750	15, 60
Monitor® MonFast™ ** (0.1%)	sulfosulfuron	800	10, 40
Atlantis® OD	mesosulfuron iodosulfuron	10.44 2	7.4, 29.6
Oust® XP Trend 90® (0.1%)	sulfometuron-methyl	750	37.5, 150
Ralon® Super Trend 90® (0.1%)	fenoxaprop-*P*-ethyl	69	82.8, 331.2
Select® 240 EC Parasommer® *** (0.5%)	clethodim	241.9	241.9, 967.6
Arelon® flüssig	isoproturon	500	1500, 6000

* active ingredient: isodecyl alcohol ethoxylate (DuPont de Nemours); ** active ingredient: fatty alcohol ethoxylate (Monsanto); *** active ingredient: paraffin oil (Stähler).

3.3. Field History

The field history of the samples was provided by the farmers, who were requested to complete questionnaires reporting the following information: (1) field location (GPS coordinates or address); (2) crop rotation of the last 5–7 years, (3) soil tillage, (4) soil texture, (5) sowing date of the winter crop and (6) weed density. Categories within each factor are shown in Table 1. The factors 2–6 functioned as explanatory variables for the developed model. The results of greenhouse assays for verification of actual resistance after herbicide treatment functioned as categorical response for the model, using "yes" (resistance confirmed) and "no" (resistance not confirmed).

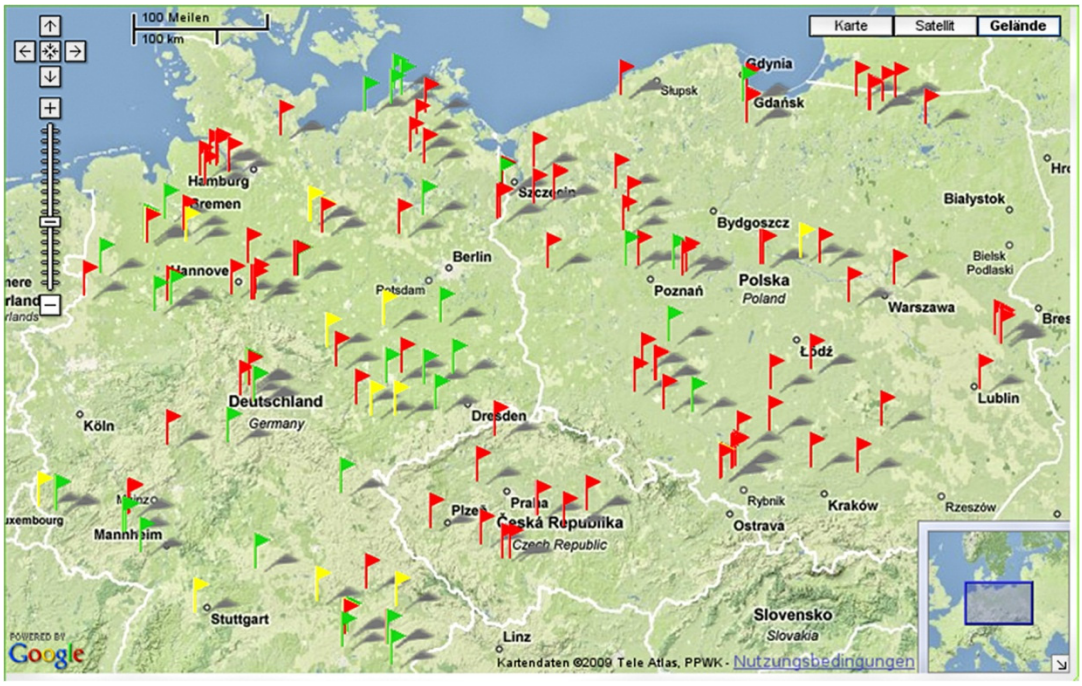
3.4. Technical Features of the GIS Database "Weedscout"

The information obtained from the questionnaires (field history) and the results of greenhouse assays (resistant/non-resistant) was used to develop a database in which the distribution of herbicide-resistant *A. spica-venti* populations is mapped. Weedscout 2.0, provided by Proplanta (Proplanta GmbH & Co. KG, Stuttgart, Germany), is a geo-referenced database and role-based web application for the storage, management and spatial representation of weed species and other location-related parameters. The multilingual administration interface of the software is based on the HTML PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN", which is CCS3-compliant and Crossbrowser compatible (IE 6.0+®, FF 2+®, Safari 3.0+®, Opera 9.0+®, Chrome®). The server-side scripting language is based on PHP® 5.2.10 and the web database on MySQL® 4.0.27-standard de-iso-8859-1.

The cartographic representation of the data (available in aerial and terrain view) is run by Google maps 2.0. Different zoom levels allow detailed view of target areas. An online form was created for the upload of raw data (questionnaires), with the geocoding (*i.e.*, the conversion of addresses into geographic coordinates) being automatically carried out by Google GClientGeocoder (Google maps-API). If no exact address can be given, the GPS coordinates (*i.e.*, longitude and latitude) of

locations are obtained through the marker function of Google Maps and automatically transferred into the form. After the greenhouse assays for verification of herbicide resistance, each population was identified by a colored flag, which is red in the case of verified resistance, green in the case of unverified resistance and yellow in the case of suspected populations that could not be tested due to seed scarcity or inability to germinate (Figure 3). The search results can be modulated by several filters (e.g., country, populations of interest and weed species), and specific geo-tools allow the calculation of distances and surfaces. Finally, an export function enables the backup and processing of metadata in XLS format.

Figure 3. Screenshot example (terrain view) of the geographic information system (GIS) database "Weedscout" [30] showing the distribution of *A. spica-venti* populations in Germany, Poland and the Czech Republic. Cases of verified resistance are identified by the red flags, cases of unverified resistance by the green flags and cases of suspected resistance by the yellow flags.



The advantages of developing a geo-referenced database of herbicide-resistant *A. spica-venti* populations across Europe are manifold: (1) easy and fast acquisition of data for different purposes (not limited to herbicide resistance); (2) easy and fast acquisition of spatiotemporal features (e.g., evolution and spread of herbicide-resistant populations); (3) easy access to other GIS sources, such as point locations, distances and further documentary data; and (4) the creation of a specific weed database with global coverage ability. Further benefits include: (1) online access, which enables users to work on their data safely and effectively (many users can work on their own data at the same time); (2) free scalability, which enables data analysis at different levels (global, national, regional and target-specific); and (3) multi-filtering features, which enable flexible access to the metadata through the selection of specific criteria (weed species, populations of interest, country of provenance, field history *etc.*).

Overall, the developed database represents a useful instrument for studies aimed at monitoring the evolution and spread of herbicide resistance in *A. spica-venti* populations over time and space, thus allowing precise identification of agricultural areas with a potential risk of resistance.

3.5. Presentation of the Model and Statistical Analyses of the Data

Statistical analyses of the collected data were performed with the software SPSS® 15.0 (SPSS Inc., Chicago, IL, USA). The relationships between variables were analyzed using Spearman correlations, multicollinearity detection and contingency tables. A Logistic Regression Model (LRM) was used to describe the relationships of the explanatory variables (crop rotation, soil tillage, soil texture, sowing date and weed density) and first order interactions with herbicide resistance.

Logistic regression is a statistical method that uses one or more explanatory variables to predict the probability of a categorical response [31–33]. The response variable in LRM is the log of the odds ratio (logit). The logit transforms a variable constrained between zero and one (p) into a continuous variable, which is linear with respect to the vector of the explanatory variables:

$$Logit(p) = \log\left[\frac{p}{1-p}\right] = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k \quad (1)$$

where p is the probability of occurrence of the event, β_0 is a scalar intercept parameter and $\beta_1 \ldots \beta_k$ the slope coefficients for the explanatory variables $X_1 \ldots X_k$. The maximum likelihood method was carried out to estimate the parameters of the model. The criteria for the inclusion/exclusion of variables were: probability of inclusion of 0.05 and probability of exclusion of 0.10. The Wald statistic indicated the significance of the explanatory variables in herbicide resistance. The interpretation and assessment of significance for the explanatory variables were performed by using the p-value and the 95% confidence interval (CI) of the exponents of the coefficients β (e^{β} = OR). Explanatory variables were accepted, with significance levels close to 10%. To assess the goodness of fit of the developed model, statistical measures were used (Log Likelihood, Cox-Snell R^2, Nagelkerke R^2), a calibration test (Hosmer-Lemeshow test) and a measure of discrimination (percentage of correctly classified cases, area enclosed under the curve ROC: AUC).

In the ROC (receiver operating characteristic) analysis, sensitivity (true positive rate) is plotted against 1-specificity (false positive rate) to show the relationship between true positives (*i.e.*, resistant cases correctly classified by the model) and false positives (*i.e.*, non-resistant cases wrongly classified by the model). The area enclosed under the curve (AUC) is a measure of the overall performance of the predictive model and is expressed as the average value of sensitivity for all possible values of specificity. The perfect classification corresponds to 100% sensitivity (no false negatives) and 100% specificity (no false positives), with AUC = 1. The differences between the log likelihood of the full model and the log likelihood of a model without each predictor variable were calculated. The relative contribution of a predictor variable was defined as the ratio of its log likelihood difference to the sum of the all log likelihood differences multiplied by 100 [34].

4. Conclusions

In conclusion, this study confirmed the significant role of rotation management, soil tillage, sowing date and weed density in the selection of herbicide resistance in *A. spica-venti*. According to these results, growers should focus more effort on preventing the evolution and spread of herbicide-resistant populations. The rotation of crops having different growing periods is a mandatory management measure for 'breaking' the regular life cycle of weeds and delaying the selection of resistant plants. This should be corroborated by the adoption of conventional tillage methods (*i.e.*, plowing) to suppress seedling emergence from deep soil layers. In fact, the inversion of soil layers after the light induction of the seeds (which is necessary for germination) will result in impaired emergence of the deeply buried seedlings ("fatal germination") and, therefore, in reduced weed density and more effective control [35]. Further measures should include increased crop competitiveness (e.g., higher seed density) for keeping weed population density as low as possible. Finally, early sowing of winter crops should be avoided when dealing with *A. spica-venti* infestations, as delaying the sowing date would facilitate weed suppression by seedbed preparation prior to planting. These measures, adequately combined together, will provide growers with useful tools for preventing and containing the spread of *A. spica-venti* in arable lands and will result in the decreased likelihood of herbicide resistance. Strategies relying solely on rotation of herbicide modes of action, although undoubtedly effective, will not ultimately result in successful long-term management of *A. spica-venti* in intensive cropping systems.

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