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## About the interplay of sensitive and resistant biotypes in weed populations - simulation exercises for *Echinochloa crus-galli* in maize crops

Über das Zusammenspiel von sensitiven und resistenten Biotypen in Unkrautpopulationen – eine Übung anhand von *Echinochloa crus-galli* in Mais

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### Abstract

Weed species easily establish in crops with a similar life cycle. Especially mono-cropping provides best conditions for such weeds. In maize this is true for *Echinochloa crus-galli*, which is a C4 plant and one of the worst weeds worldwide. In Germany *E. crus-galli* is one of the few typical weed species in maize. When recurrent herbicide treatments are applied, development of herbicide resistance is likely.

Since maize is typically only treated once with herbicides, later germinating weeds can escape and produce seeds. These escaping weeds are not selected for herbicide resistance. Hence, they still have a more sensitive gene pool. *E. crus-galli* emerges with the highest density in spring and continues to germinate over the whole vegetative period of maize. In that way the early germinating biotypes are selected for herbicide resistance while the later germinating biotypes are not.

To avoid the reproduction of *E. crus-galli* effort is made to suppress these later germinating weeds. Using undersown cover crops is one way to do that. We hypothesize that suppressing the later germinating biotypes enhances the development of herbicide resistance.

We simulated the development of herbicide resistance in populations of *E. crus-galli*, composed of sensitive and resistant biotypes, in a continuous maize cropping system. We used the model PROSPER for our simulations. We assumed three levels of suppression of the later germinating weeds (0%, 30%, and 100%).

The results show a faster development of herbicide resistance, when the later germinating individuals are suppressed. Nevertheless, the suppressive effect of undersown crops is able to lower the weed density even with high resistance level in the population.

**Keywords:** Cover crop, *Echinochloa crus-galli*, population dynamic, simulation model, suppression

### Zusammenfassung

Unkräuter können sich besonders leicht auf solchen Flächen etablieren, auf denen ihnen ähnliche Feldfrüchte stehen. Dabei wird ihre Etablierung durch den kontinuierlichen Anbau dieser Kulturpflanze gefördert. Reguliert werden die Unkrautdichten meist mit wiederholtem Einsatz immer derselben Herbizide. Der Grund dafür ist die geringe Verfügbarkeit von unterschiedlichen Wirkstoffen, mit der Folge einer möglichen Resistenzentwicklung. Im Maisanbau zeigt *Echinochloa crus-galli* so eine Entwicklung. Diese C4-Pflanze ist in Deutschland eines der wichtigsten Unkräuter in Mais.

In Deutschland wird Mais üblicherweise nur einmal mit Herbizid behandelt. Da *E. crus-galli* mit abnehmender Rate über die gesamte Vegetationsperiode von Mais keimt, werden von dieser einen Herbizidbehandlung nur die früh auflaufenden Biotypen unterdrückt. Später auflaufende Unkräuter können ihre sensitive Genetik weiter vermehren. Um die Reproduktion der später auflaufenden Biotypen zu verhindern, werden verschiedene Maßnahmen angewendet. Eine davon ist der Einsatz der Untersaat. Wir behaupten, dass die Unterdrückung der später auflaufenden Biotypen die Entwicklung der Herbizidresistenz verstärkt.

Wir betrachten die Entwicklung der Herbizidresistenz von *E. crus-galli* im kontinuierlichen Maisanbau in drei Varianten: 0%, 30% und 100% Unterdrückung der spät auflaufenden Biotypen. Dazu nutzen wir das populationsdynamische Modell PROSPER.

Bei Unterdrückung der spät auflaufenden Biotypen zeigen die Ergebnisse eine schnellere Entwicklung der Herbizidresistenz. Die Dichte von *E. crus-galli* wird von der Untersaat aber trotz hohem Resistenzniveau gesenkt.

**Stichwörter:** *Echinochloa crus-galli*, Populationsdynamik, Simulationsmodell, Unterdrückung, Untersaat

## Introduction

*Echinochloa crus-galli* (L.) P. B. is one of the widest spread summer annual weed species on land under arable use worldwide (HOLM et al., 1977). In Germany it has become one of the most important weed in maize cropping (MEHRTENS et al., 2006). The summer annual weed species can produce more than 300.000 seeds/m<sup>2</sup> with only 10 plants/m<sup>2</sup> plants (NORRIS, 1992). Most of these seeds feed seed predators undergo decay or lose their viability within one year (BAGAVATHIANNAN and NORSWORTHY, 2013). However, other seeds enter the seed bank and germinate over a long period of time, starting in spring and ends in late summer (OGG and DAWSEN, 1984; NORRIS, 1996).

As a C4 plant *E. crus-galli* possesses best prerequisites to become a serious weed in maize (MURPHY and LEMERLE, 2006). It has the ability to reduce the yield of maize significantly (BOSNIC and SWANTON, 1997), thus it is a serious threat to maize production. Farmers meet this risk by a consequent weed control, including recurred applications of herbicides. However, their usage has a great disadvantage since herbicides induce strong selection pressure with growing probability to develop herbicide resistance (POWLES and YU, 2010). For *E. crus-galli*, target-site resistance against ALS-inhibitors is the main discovered herbicide resistance (HEAP, 2015) but others are likely, including multigene non-target site resistance (CLAERHOUT et al., 2015).

It is difficult, maybe even impossible, to conduct field trials on the development on herbicide resistance. Hence, computer based simulation models are important tools helping to predict and explain this effect on in-field weed populations (RENTON et al., 2014). In this study we use the simulation model PROSPER developed at the University of Rostock (REDWITZ et al., in prep.) provided as an R package to analyse the population development of *E. crus-galli*.

Integrated weed management combines chemical control with non-chemical control methods, including cultural control. A method to increase weed competition of the poorly competing maize crop is to introduce undersown crops, like legumes or grass/legumes mixtures. Acting as cover crops, these crops are undersown in the crop stand of maize four to five weeks after maize and after an initial weed control, which is, at least in conventional agriculture, done with herbicides (JUNG et al., 2012).

We investigated the effects of such undersown cover crops on the population of *E. crus-galli* under continuous maize cropping on the development of herbicide resistance in simulation exercises. We assumed three levels of weed suppression by the undersown cover crop on late germinating seedlings. We hypothesized an effect of the additional weed suppression on both, reduction of the population growth and an enhanced development of herbicide resistance within the population of *E. crus-galli* by additional weed suppression. Hence, our simulation studies reveal, how sensitive and resistant biotypes of a population of *E. crus-galli* interplay with each other and how this interplay is modified by cultural control measures.

## Materials and Methods

### The simulation model PROSPER

The population dynamic model PROSPER (REDWITZ et al., in prep.) is used to model the effect of a suppressed weed cohort (without selection pressure) on the development of herbicide resistance in *E. crus-galli* population. This model simulates weed population dynamics with specific genetics under selection pressure. It uses the individual genetics to calculate the stochastic reaction of the population. The genetic part is employed according to the published model PERTH (RENTON et al., 2011). PROSPER provides an adaptable standard combination of functions to describe the population dynamic in R (R CORE TEAM, 2015).

We use PROSPER to simulate the development of target-site herbicide resistance of a self-fertile annual weed species in the annual cropping system continuous maize (Fig. 1). At the end of the vegetation period, a certain amount of seeds exists in the autumn seed bank (seed bank autumn). During winter, only a proportion of those seeds survive (seeds\_suv\_winter) whereas the remaining

seeds are death (seed death). The extant viable seeds form the soil seed storage of the weed population in spring (seed bank spring). In spring the seedlings germinate in two cohorts (seedling 1 and seedling 2). The first cohort is affected by post-emergence herbicides. Assuming resistance is not affecting germination resistant plants in the first cohort has a higher chance to survive. In contrast, the second cohort is not affected by herbicide but is suppressed without any selection pressure. The two cohorts are then affected by a natural dying rate regardless of the resistance status and the applied treatment. The remaining plants produce and shed seeds on the ground (seeds). The number of seeds is reduced by the prevalent post-dispersal seed predators before they enter the seed bank (seed bank autumn). Hence in autumn, the seed bank consists of these new seeds and viable, non-germinated seeds from the summer seed bank (seed bank summer). These seeds together have the chance to survive until the next spring, when the described life-cycle starts again.

#### Simulation exercise with an undersown cover crop

**Tab. 1** Parameters of the population dynamic model PROSPER for *E. crus-galli* with two cohorts of seedlings.

**Tab. 1** Parameter des populationsdynamischen Modells PROSPER von *E. crus-galli* mit zwei Kohorten von Keimlingen.

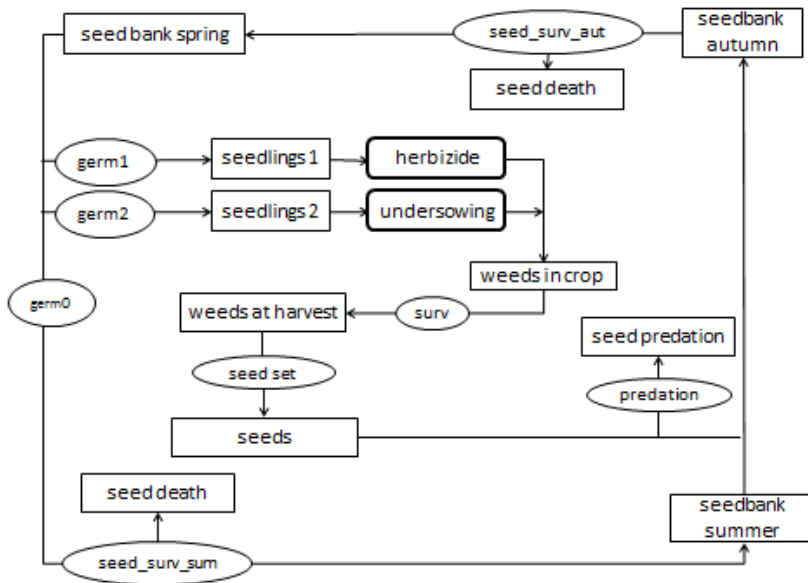
Parameter	Description	Unit	Value	Source
<b>duration</b>	time span of the simulation	years	20	
<b>start freq</b>	initial frequency of resistance alleles		0.001	
<b>dom</b>	dominance of resistance alleles		1	
<b>epistasis</b>	no epistasis used			
<b>repetitions</b>	repetitions of the simulation		15	
<b>suppression</b>	probability of dying due to the cover crop	proportion	1/0.3/0	
<b>seed production first cohort</b>	$200 * \text{density} / (e^{(0.5)} + \text{density} / 200000)$	pc.		BOSNIC and SWANTON, 1997
<b>seed production second cohort</b>	$100 * \text{density} / (e^{(0.5*2)} + \text{density} / 30000)$	pc.		BOSNIC and SWANTON, 1997
<b>surv</b>	natural surviving probability of seedlings	proportion	0.675 ± 0.02062	CLAY et al., 2005
<b>seed_surv_winter</b>	probability of surviving the winter	proportion	0.8	BAGAVATHIANNAN and NORTHWORTHY, 2013
<b>seed_surv_summer</b>	probability of surviving the summer	proportion	0.1	BAGAVATHIANNAN and NORTHWORTHY, 2013
<b>germ1</b>	germination rate	proportion	0.08	OGG and DAWSEN, 1984
<b>germ2</b>	half of germ1 is assumed	proportion	0.04	
<b>germ 0</b>	not germinated seeds	pc.		
<b>predation</b>	probability of predation	proportion	0.65	BAGAVATHIANNAN and NORTHWORTHY, 2013

We simulate a population of *E. crus-galli* in continuous maize with a cover crop as an example for suppression of late germinating weeds. The effect of suppression in the second cohort is simulated on three levels: 0% seedlings suppressed, 30% seedlings suppressed and 100% seedlings suppressed. We did not include a scenario in which the second cohort is again controlled with herbicides. The population was modelled over 20 years and repeated 15 times for

each level of suppression. For this study we implemented parameters for population dynamic from literature. Details of all parameters used are given in Table 1.

### Resistance genetics

Our simulation is an exercise to understand general patterns in population behaviour. We chose a simple target-site resistance with complete dominance (dom), no epistasis and a low starting frequency (start freq) to achieve that goal. Additionally, we did not account for the hexaploid genome of *E. crus-galli* (MAUN and BARRET, 1986). In our simulations the *E. crus-galli* has only one copy of the gene and is behaving similar to a diploid species.



**Fig. 1** Structure of the population dynamic model PROSPER adapted for cover crops and two cohorts of seedlings. Rectangles are model results, ovals are parameters. The herbicide effect is dependent on the genetics and a normal distributed random effect of success in spraying. Cover crop has no selection effect and is modeled as complete suppressing seedling 2, partly suppressing seedling 2 and no suppressing of seedling 2.

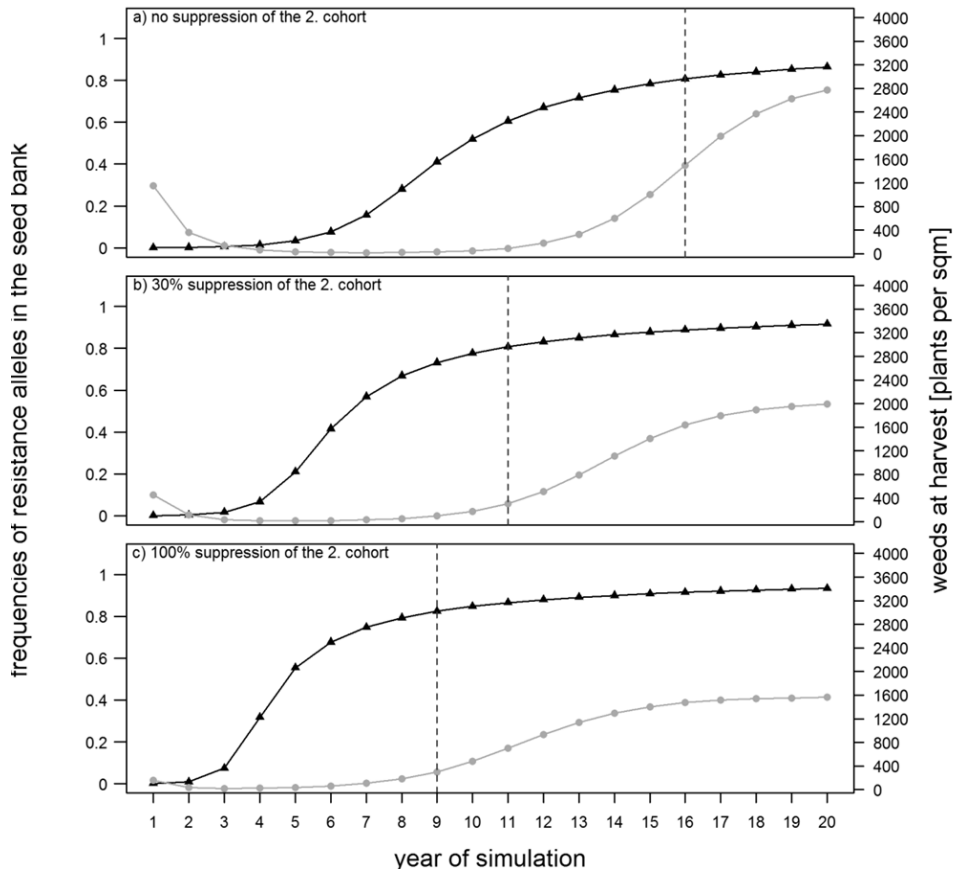
**Abb. 1** Struktur des populationsdynamischen Modell PROSPER angepasst mit zwei Kohorten von Keimlingen und Untersaat mit Rechtecken als Ergebnisse, Kreise als Parameter. Der Wirkungsgrad des Herbizids ist abhängig von der Genetik und Normalverteilung des randomisierten Einfluss des Erfolgs der Applikation. Modellierung der Untersaat (ohne Selektionsdruck): unterdrückt seedling 2 vollständig, teilweise, nicht.

### Results

The simulations of the pure maize stand without any other crop results in a high number of weeds at harvest in the first years, which is rapidly decreasing under the suppressive force of herbicides (Fig. 2a). These herbicides on the other hand select for the resistant alleles in the gene pool of the weed population and increase the frequency of resistant alleles reaching 80% after 16 years. The enhanced resistance level in the *E. crus-galli* population is followed by an increase of population size reaching 2800 weeds/m<sup>2</sup> after 20 years.

Simulating with the same starting population, but adding a 30% suppressive effect on the second cohort of the cover crop undersown in maize lower the weed density at harvest in the beginning (Fig. 2b). Compared to the pure continuous maize cropping the development of herbicide

resistance becomes faster, reaching 80% resistance alleles already after 11 years. The weed density at harvest amounts to 2000 weeds/m<sup>2</sup> after 16 years.



**Fig. 2** Results for three simulation runs of PROSPER with different amount of suppression of a second seedling cohort of *E. crus-galli*: (a) 0%, (b) 30% and (c) 100% suppression. Frequency of resistance alleles is black and number of weeds at harvest is grey. For better comparison between the simulations, we marked the point when the frequency of resistance alleles in the population reaches 0.8 with a dashed line.

**Abb. 2** Ergebnisse aus drei Simulationsläufen von PROSPER mit unterschiedlichen Anteilen an Unterdrückung der zweiten Kohorte von *E. crus-galli*: (a) 0 %, (b) 30 % und (c) 100 %. Frequenzen der Resistenzallele sind schwarz, Anzahl der Unkräuter vor der Ernte ist grau. Zur besseren Vergleichbarkeit wurde der Punkt, an dem die Frequenz der Resistenzallele 0.8 erreicht mit einer gestrichelten Linie markiert. Die gestrichelte Linie markiert die 0.8 Marke der Frequenz.

The last simulation represents a complete suppression (100%) of the second cohort, while all other parameters stay the same (Fig. 2c). Such a suppressive cover crop in maize would restrict the number of weeds at harvest to 200 weeds/m<sup>2</sup> in the starting period. However, the development of herbicide resistance is accelerated reaching 80% frequency of resistant alleles after 11 years. Weed densities at harvest after 20 years are 1600 weeds/m<sup>2</sup>.

## Discussion

*E. crus-galli* is a highly dynamic weed species: the seed production is large (BOSNIC and SWANTON, 1997) and so is their dying rate over the seasons (BAGAVATHIANNAN and NORTHWORTHY, 2013),

resulting in a fast exchange of the seed bank. This flexibility explains our results on the population development. All three simulations result in a similar population development: in the first years the number of weeds at harvest decreases until the frequency of resistant alleles is high and the herbicide loses effectiveness. Then the weed density increase until it reaches a plateau. RENTON et al. (2011) describe a similar development of the population of *Lolium rigidum* in wheat crop under the recurrent selection pressure of herbicides. In their simulation study all applied herbicide rates (75%, 100%, 150%) resulting in a drop down and a later increase of the population after treatment.

The development of herbicide resistance in this simulation (Fig. 2a) shows a common structure: slow start, fast development and slow ending. It has already been shown that a target-site resistance with high dominance can spread within few years (i.e. JASIENIUK and MAXWELL, 1994; RENTON et al., 2011). Because of the specific population dynamic *E. crus-galli* has the ability to evolve herbicide resistance even faster: most of the seed bank lasts only one year while *E. crus-galli* produces thousands of seeds with only few individuals (NORRIS, 1992). Thus, the seed bank provides only a relatively small buffer for selection processes to the newly produced seeds with high herbicide resistant allele. Besides the small buffer in the soil, the extended germination period of *E. crus-galli* acts like a second buffer within the germinated population itself.

Later germinating individuals – the second cohort in our simulations – do not undergo selection for herbicide resistance. Even though the second cohort has a much lower potential for seed production than the first (BOSNIC and SWANTON, 1997; TRAVLOS et al., 2011), the small amount of produced seed still have the ability to slow down the development of herbicide resistance for years by providing sensitive seeds. When these later germinating seeds of *E. crus-galli* are suppressed, the development is nearly twice as fast (Fig. 2a compared to 2c). It is evident, that suppression of the second cohort by an additional chemical control instead of cultural control would increase both the weeds at harvest and the share of resistance weeds in the population much more strongly, given that the herbicide is struck by non-target-site resistance in the *E. crus-galli* population.

So far, the model PROSPER and the parameters used have some serious draw backs. One is the missing hexaploid genetic: since most genes in a hexaploid species are three times expressed, selection pressure is diluted and slows down. Another is the high self-pollination rate of *E. crus-galli* (MAUN and BARRET, 1986), which lowers the probability for outcrossing of resistant genes. In the current model we do not account for these effects, hence, the fast development might be slowed down. These are functions that will be implemented in further versions of PROSPER. Research towards a fully parameterized population dynamic of *E. crus-galli* for Northern Germany is in progress (PANNWITT et al., 2015).

Undersown cover crops are a management tool to suppress late emerging seedlings. They are part of the diversified weed control measurements, which is promoted to decelerate the development of herbicide resistances (BECKIE, 2006; HEAP, 2014; NORSWORTHY et al., 2012).

We learned from our simulation exercises that the interplay between resistant and sensitive biotypes is strongly modified by the effects of e.g. undersown cover crops on the population of *E. crus-galli*. While we are not yet able to consider all details of the genetics of *E. crus-galli* in the current model, it is worthwhile to account for this interplay in resistant management strategies. In our scenarios about the effects of undersown cover crop as a cultural control method the population growth of *E. crus-galli* is more capped in the long perspective. However, the share of herbicide resistant biotypes within the population increased.

We do not advertise our simulation studies in order to weaken the role of undersown cover crops as integrated weed management tool. Our results definitely support an adapted weed management based on the biology of the weed species. However, in case of proven herbicide resistance it appeared to be worthwhile in the simulations studies to let those still sensitive parts of the population grow. That later emerging individuals of *E. crus-galli* reduce the yield of maize

only to a very small extent (BOSNIC and SWANTON, 1997), offers good perspectives allowing late emerging sensitive plants to reproduce and thereby diluting the resistance within the seed bank.

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