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Quantifying seed and establishment limitation to seedling recruitment of arable weeds: an example of barnyardgrass (*Echinochloa crus-galli*)

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Abstract

The establishment of plants in an ecosystem is limited by the availability of seeds and the availability of suitable sites for establishment. Describing plant population dynamics through the relative strength of seed and establishment limitation is an important concept in the study of natural ecosystems. To date, it is unclear whether this concept can be applied to describe populations of annual weeds in agricultural fields. Using a recruitment function, we show that limitation parameters prove valuable in describing seedling recruitment in weed populations. We conducted a seed addition experiment in three cornfields (*Zea mays* L.) and recorded seedling recruitment in populations of the economically important weed barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.]. Seed predation, competition with other weeds, and seed burial were prevented. We estimated the strength of seed and establishment limitation in the population with two parameters: *n*, which is the number of microsites, and *b*, which is the suitability of those sites to support a seedling. We further estimated the relative proportions of density-dependent and density-independent establishment limitation in the seedling population. Recruitment rates of *E. crus-galli* ranged from 31% to 36% across all evaluated seed densities and fields, which is high compared with results from other seed addition studies. Two of the three monitored populations were predominantly establishment limited at the highest evaluated seed density of 2,400 added seeds m^{-2}. Further knowledge about the relative strength of limitations in other weed populations will provide important information on how effective different weed management strategies can be.

Introduction

Plant population dynamics depend on a plant successfully passing through different stages of its life cycle (Fettig and Hufbauer 2017; Osipitan et al. 2019). Seedlings represent one of these stages. The recruitment of seedlings depends on two preconditions: the availability of seeds and the availability of sites for their establishment. Both can be limited.

Describing plant population dynamics by the relative strength of seed and establishment limitation is an important concept in the study of natural ecosystems (Clark et al. 2007; Eriksson and Ehrlén 1992; Miller et al. 2014; Poulsen et al. 2007; Spotswood et al. 2017). Previous research has studied parameters describing the type and strength of limitations in order to examine potential community assembly and competition (Aicher et al. 2011; Clark et al. 2013; Spotswood et al. 2017), the resistance of habitats to invasion (Miller et al. 2014), and the invasiveness of species (Spotswood et al. 2017). Despite the utility of the concept of relative strength to describe limitations of plant populations and their ability to thrive in an environment, to the best of our knowledge, only Boyd and van Acker (2004) conducted a field-based experiment in populations of weeds on agricultural sites. Based on the experimental results, they qualitatively demonstrated that both seed and establishment limitation were in effect.

In particular, populations of annual arable weeds depend on the availability of seeds to reestablish a population of plants as soon as the environmental conditions are favorable. To increase the chance of survival, many species that are considered arable weeds produce a lot of seeds (van Acker 2009). Another strategy is to produce seeds with an extended longevity (Davis et al. 2016). Both traits allow for the continuation of populations as soon as a seed reaches a site suitable for seedling emergence, also referred to as a microsite (Crawley 1990) or safe site (Harper 1977), at the right season. During the postdispersal seed stage, from seed shed until seedling emergence, factors such as dormancy, seed burial, and various factors of seed mortality (e.g., decay and predation) can alter the number of available seeds necessary for seedling recruitment. To be recruited into the seedling stage, each seed needs a microsite suitable for emergence (Crawley 1990; Eriksson and Ehrlén 1992). The number of suitable sites can be reduced by the lack of water, oxygen, nutrition, and light necessary for establishment. Certainly, before and after emergence, germinated seeds and growing seedlings can die due to various reasons, for example, predation, microbial attacks, competition, and/or drought. Therefore, seed addition experiments creating different seed densities at the same site are necessary to investigate the limitation concept based on recruited seedlings.

A recruitment function relates the number of seedlings of a species in 1 yr to the number of seeds that had arrived previously on a site. The parameters of a recruitment function can be estimated from experiments in which a sufficient number of different seed densities are added to the plots and the resulting seedlings are counted (Poulsen et al. 2007). The derived parameter values estimate the relative strength of seed and establishment limitation for a species on a site at the time of the observations. If the number of all viable seeds of the species is known, limitation can be estimated. All viable seeds include the number of newly added seeds and the seedbank on the site (Poulsen et al. 2007). If there is no seedbank and no seeds are added, the population will be totally seed limited. With every added seed, the system will become less seed limited but more limited by constraints for establishment. Factors that reduce the number of suitable sites and, consequently, limit establishment can act dependently on or independently of seed density (Poulsen et al. 2007). If recruitment rates are density independent because the number of suitable sites is much higher than the number of added seeds, every added seed has the same probability to recruit to seedling stage. However, from a biological perspective, it is obvious that at some point in any seed augmentation study, seedling emergence rates will decrease as seed density increases (Aicher et al. 2011; Duncan et al. 2009). Thus, density-dependent limitation factors, such as competition for resources, can affect successful establishment in early plant development stages. As individual plants grow, they claim more resources, and density-dependent effects become even stronger. Therefore it is important to clearly define the developmental stage of the populations that are observed, as the relative strength of seed and establishment limitation will change with plant life stages (Duncan et al. 2009).

For annual weed species, arable fields provide plenty of suitable sites for emergence. As such, on a recently tilled field, seedling recruitment is expected to be limited predominantly through the number of available seeds (i.e., seed limited). However, weed control reduces the available microsites and, therefore, limits the establishment of seedlings (i.e., establishment limitation). If suitable microsites are numerous, density-independent establishment limitation will present the main constraint on seedling establishment. Density-dependent limitation, on the other hand, increases with seed numbers (Aicher et al. 2011). To estimate the strength of the limitations of a population, the parameters of a recruitment function can be utilized to derive the number of suitable microsites, independent of the number of available seeds (Miller et al. 2014). The higher the number of suitable microsites, the higher the susceptibility of a habitat to the invasion by a plant species. Various types of recruitment functions have been proposed (Aicher et al. 2011; Duncan et al. 2009; Poulsen et al. 2007; Spotswood et al. 2017), of which the Skellam function (Skellam 1951) seems to be best suited to describe the recruitment of annual plant species (Aicher et al. 2011).

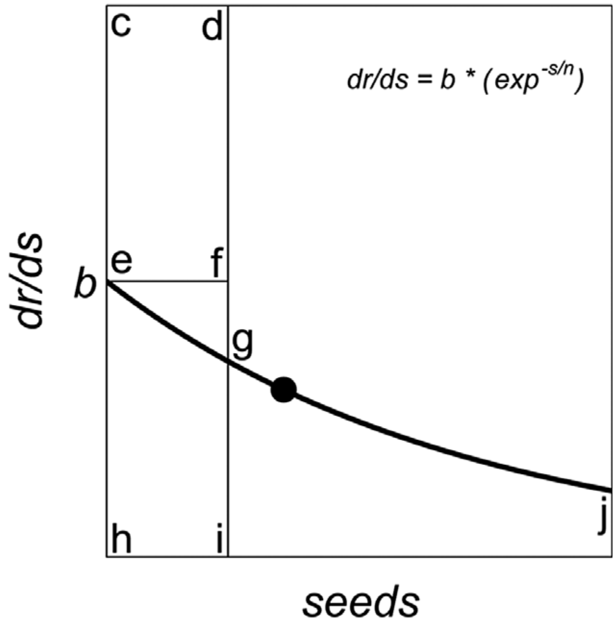

Figure 1. The ratio of additional recruits per added seed (*dr/ds*), dependent on the number of seeds (*s*) in a seed addition experiment ($dr/ds = b * (\exp^{-s/n})$). Parameter n is the number of microsites, and b is the proportion of those sites suitable for a seedling to establish. The magnitude of limitations is presented as areas above and beneath the curve *dr/ds.* The selected density of seeds is indicated by the vertical line in the plot. Lowercase letters define the areas by marking their corner points. The total number of seedlings, r, is represented as area (h i g e). The dot on the curve gives the equilibrium where $L_S = L_E$. If more seeds are added, seedling recruitment becomes increasingly establishment limited. The number of seeds that did not germinate because of density-independent establishment limitation $DI\ L_E$ is represented as the area (e f d c). If no seeds are added, parameter b is equal the ratio of density-independent establishment limitation. The number of ungerminated seeds due to density-dependent establishment limitation $DD\ L_E$ is represented as the area (g f e).

Figure 1 depicts the first derivation of the Skellam function, which is the change in number of seedlings per added seed for a given population of seeds. All quantities, which are number of emerged seedlings, seed limitation, density-dependent establishment limitation, and density-independent establishment limitation, are represented by areas. The respective area can be directly translated into the number of seedlings that either did or did not emerge at the chosen seed density. If the number of added seeds converges toward infinity, the number of seedlings approaches bn, the maximum number of recruits. At a certain number of added seeds, the magnitude of seed limitation becomes equal to the magnitude of establishment limitation ($L_S = L_E$; black dot in Figure 1).

With the Skellam function, all unobserved seed losses can be attributed to a decrease in available microsites, thus an increase in establishment limitation. This includes all mortality factors that can occur in the postdispersal seed stage, such as seed predation and seed decay. Various studies have shown that seed predation can be both density dependent and density independent (Daedlow et al. 2014; Westerman et al. 2008). It is challenging in the field to experimentally obtain an unambiguous estimate of seeds lost due to these factors. Thus, effects of seed predation, seed decay, and dormancy remain unknown in many seed addition experiments in the field. Nevertheless, high rates of non-emerged seedlings in field experiments are typically attributed to these processes (Bagavathiannan and Norsworthy 2013; Clark et al. 2007). Excluding seed predators from seed addition experiments allows their influence on emergence dynamics to be avoided

(Blubaugh and Kaplan 2016; White et al. 2007). Seed decay, germination without establishing a seedling (i.e., fatal germination), and the disposition of seeds into the seedbank and emergence in later years are difficult to distinguish, particularly in field experiments. These sources of seed loss hide the fate of the seed from the observer in a field experiment. Therefore, it is reasonable to avoid any soil disturbance after dispersal of the weed seeds. In addition frequent counts of emerged seedlings are required in order not to miss short-lived seedlings.

In this study, we quantified seedling recruitment and the relative strength of limitations for the economically important weed barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.]. The seed addition experiment was set up with four seed densities of *E. crus-galli* and controls in three agricultural cornfields (*Zea mays* L.). *Echinochloa crus-galli* was chosen as the model weed species based on practical and theoretical considerations. The species is a weed that 1) is common in cornfields, 2) is a strong competitor to corn and can cause significant economic losses (Bajwa et al. 2015a; Bosnic and Swanton 1997; de Mol et al. 2015), and 3) was not abundant in the local soil seedbank. Cornfields are especially suitable habitats for *E. crus-galli*, as these species share common environmental requirements. Both are monocot summer annuals with a C_4 metabolism. Furthermore, *E. crus-galli* sheds its seeds before corn harvest, which ensures maintenance of the population. We controlled for two factors—predation and seed burial—by enclosing experimental plots with plastic frames, ensuring there was no disturbance of the soil surface, and carrying out frequent seedling counts throughout the vegetation period.

Questions addressed are: What are the model parameters for seedling recruitment to estimate limitations in an *E. crus-galli* population in an agricultural field? Is the chosen method suitable to estimate the relative strength of seed and establishment limitation in a weed population in agricultural fields? Is the method suitable to determine the relative proportion of density-dependent and density-independent establishment limitation in the seedling population?

Materials and Methods

Sites

The experiments were conducted in northeastern Germany (state of Mecklenburg-West Pomerania). The 30-yr average precipitation (1981 to 2010) is 640 mm yr^{-1}, and the 30-yr mean annual air temperature is 9.0 C (DWD 2017).

The seed addition experiments started in August 2014 with dispersal of *E. crus-galli* in three cornfields. Samples taken before the dispersal in two soil depths of 0 to 5 and 5 to 20 cm were processed with an elutriator and a stereo microscope was used to examine the cleaned sample for seeds (Pannwitt et al. 2021). The samples showed that no *E. crus-galli* seeds were present at the experimental sites. All fields had a history of at least 3 yr of continuous corn. Corn is a common crop in the region; as silage, the biomass goes into livestock feeding or biogas production. The three fields differed in soil properties: field A (11.8 ha, 53.55010°N, 11.14040°E) was a sandy soil with a minimum of 48% sand, up to 40% silt, and 5% to 12% clay; field B (10.1 ha, 53.97543°N, 11.98061°E) was mostly a loamy sand with a minimum of 42% sand, 25% to 40% silt, and 14% to 18% clay; and field C (7.0 ha, 54.03830°N, 12.04487°E) was a sandy loam with at least 21% sand, up to 50% silt, and 24% to 29% clay. Field A was approximately 80 km away from fields B and C, which were about 8 km apart from each other. Before the seed addition experiment, the management of the three fields followed common practices for the region. Manure, mineral fertilizer, and herbicides were applied, and shallow non-inversion tillage was practiced with on-farm equipment.

Weed Seeds

Seeds of *E. crus-galli* were purchased from Appels Wilde Samen (Darmstadt, Germany). They originated from a batch harvested in summer 2014 immediately preceding the start of the experiment. These seeds are not likely to germinate in autumn of the year they are planted because of innate dormancy (Maun and Barrett 1986).

Experimental Design

To estimate recruitment functions, we dispersed *E. crus-galli* in plots, randomly assigned to five different densities of either 0, 300, 600, 1,200, or 2,400 viable seeds m^{-2}. Seed predation was inhibited by using 0.75-m-high plastic frames (colorless Polyethylene 300, Buck & Sohn Kunststoffe, Hamburg, Germany) around plots to exclude predators. The upper 0.15 m of the frames was bent downward and outward at an angle of 45° to prevent animals from climbing over and feeding on the seeds. Additionally, to avoid the effect of interspecific competition on seedling recruitment, weeds (but not the crop) other than *E. crus-galli* were removed in the plots after seeding of *E. crus-galli*. Crop density was similar in all treatments. There were two nonseeded control plots (0 seeds m^{-2}) per block. A randomized complete block design with a total of 18 plots (1.5 by 1.5 m) in three blocks (13.5 by 10.5 m) per field was established. Each block contained six plots spaced at least 1.5 m apart. The blocks were placed at least 40 m away from the field margin.

Echinochloa crus-galli seeds were dispersed on the soil surface on August 20 and 21, 2014, in fully grown corn stands. Late summer is the natural period for seed shed of *E. crus-galli* in the region. Seed quantities were based on seed weight. The initial 1,000-seed weight was 1.38 g, assuming that most of the seeds were viable. Seeds were sown uniformly on the plots by hand. A subsequent viability test (tetrazolium chloride test, $n = 591$) indicated that viability of the seed batch was only 63%. These values were corrected by a second seeding on August 29. In the second batch, randomly taken from the original batch, chaff, which contained empty seeds, was removed, and seeds were cleaned. Subsequent tests showed that 1,000-seed weight increased to 1.54 g and viability to 89% ($n = 353$) (Pannwitt et al. 2017). Seeds from the second batch were added to reach the intended densities of viable seeds.

Management of Experimental Blocks

After seeding of *E. crus-galli,* the soil inside the plots was left undisturbed, except for the passage of harvest machinery (autumn 2014, occasional wheel tracks) and corn sowing (spring 2015). Before any emergence of *E. crus-galli* and before corn sowing in early spring 2015, the blocks were treated with a nonselective herbicide (glyphosate, 450 g ai L^{-1}, Glyphos Supreme, FMC, Germany). A professional field trial service provider (Hetterich Fieldwork GbR, Schwarzach am Main, Germany) seeded corn in rows at a row distance of 0.75 m in spring 2015. The seeding blades disturbed just the soil surface and only in the corn rows. In the experimental area, mineral fertilizer was applied manually at a similar rate as in the surrounding field (field A: 20 kg ha^{-1} N and 40 kg ha^{-1} P; field B: 140 kg ha^{-1} N and 60 kg ha^{-1} K; field C: 70 kg ha^{-1} N and 70 kg ha^{-1} P). The agronomic measures in the experimental area resembled those in the surrounding field to enable uniform crop development.

To achieve a competition-free starting point for *E. crus-galli*, an herbicide ineffective on *E. crus-galli* (Arrat®, WG BASF, dose: 50 g ai ha^{-1} tritosulfuron and 100 g ae ha^{-1} dicamba) was applied in all blocks at the beginning of June. Three to four weeks later, another herbicide, also ineffective on *E. crus-galli* (Bromotril 225 EC, ADAMA Deutschland GmbH, dose: 225 g ai L^{-1} bromoxynil) was applied at the 6-leaf stage of the crop in the blocks in fields A and C. There was no second herbicide application in field B because of extremely low weed abundance. From the middle of July onward, plots were occasionally hand weeded by clipping dicot and monocot weeds at ground level.

Assessing Weed Emergence

From the first appearance of seedlings on May 6 until July 31, newly emerged *E. crus-galli* seedlings were counted in a 0.5 by 0.5 m quadrat in the center of each plot and marked with colored toothpicks. Seedlings were counted every 2 wk. By the end of July, emergence had slowed, and seedlings were counted once per month (end of August and shortly before harvest between September 21 and 30).To avoid double counts, the colors of the toothpicks changed periodically, resulting in five discriminable periods of emergence.

Statistical Analysis

We tested different functions that model seedling recruitment dependent on the number of seeds. To avoid confusion with seeds (s) in the formulas, the term "recruits" is used as the synonym for seedlings. Preliminary model analysis by comparing deviance information criterion (Spiegelhalter et al. 2002) showed that both the Beverton-Holt recruitment function proposed by Poulsen et al. (2007) and the general recruitment function used by Duncan et al. (2009) were unsuitable to describe the data. The comparison showed that another model adapted from Brännström and Sumpter (2005) to model recruitment, r (recruits m^{-2}), as a function of added seeds, s (seeds m^{-2}) (Eq. 1) was suitable to answer our questions:

$$r = n * (1 - \exp^{-s/n}) \qquad [1]$$

The underlying assumption is that a number of seeds arrive on a site with a number of microsites, n. Added seeds are distributed uniformly. Each microsite is capable of supporting only a single recruit, and each seed has the same probability to land on any microsite. If the number of added seeds is much higher than the number of microsites, the number of recruits reaches a maximum at the number of microsites ($r \rightarrow n$). To relax the assumption that every microsite is suitable to support a seedling, Skellam (1951) extended the function by the parameter b:

$$r = bn * (1 - \exp^{-s/n}) \qquad [2]$$

Here, b represents the proportion of microsites that are available (i.e., suitable) for a seedling. This parameter is constant, that is, independent of the density of added seeds (Aicher et al. 2011). Hence, the microsite encompasses all factors that prevent seedling emergence, such as seed mortality, dormancy, and seed predation. The number of recruits approaches a linear function with the slope equal to b if low numbers of seeds are added compared with the number of microsites ($s << n$):

$$r = bs \qquad [3]$$

When large amounts of seeds are added ($s >> n$), the number of recruits reaches a maximum at the number of suitable microsites (bn). This is the maximum number of recruits that can emerge at a site.

The more seeds that are available in a system, the more seed limitation decreases. Once $r = bn$ seed limitation is zero, adding seeds will not result in more recruits. Therefore, the difference between the maximum number of recruits (bn) and the actual number of recruits (r) defines seed limitation, L_S:

$$L_S = b * n - r \qquad [4]$$

Establishment limitation, L_E, is caused by all factors that reduce recruitment. Therefore, establishment limitation can be expressed as the number of recruits that did not germinate from the seed pool:

$$L_E = s - r \qquad [5]$$

To determine whether a system is more seed or more establishment limited, the ratio of L_S/L_E is calculated. If $L_S/L_E > 1$, the system is more seed limited; if $L_S/L_E < 1$, it is more establishment limited. The equilibrium at which seed numbers are sufficient so that $L_S = L_E$ can be estimated as follows:

$$s = b * n \qquad [6]$$

We follow the approach of Spotswood et al. (2017) wherein total establishment limitation is the sum of density-dependent ($DD\ L_E$) and density-independent ($DI\ L_E$) establishment limitation. The magnitude of $DI\ L_E$ is defined as the number of seeds per unit area that do not germinate because of density-independent constraints: $DI\ L_E = s(1 - b)$. This implies that density-dependent and density-independent limitations are additive and vary with the number of seeds. Density-dependent L_E, which is the number of seeds that did not germinate because of density-dependent constraints, is calculated as:

$$DD\ L_E = L_E - DI\ L_E = sb - bn(1 - \exp^{-s/n}) \qquad [7]$$

A Bayesian framework was applied to fit the Skellam function to the data. A negative binomial (NB) distribution of the number of seedlings was assumed:

$$y_i \sim NB(p_i, k) \qquad [8]$$

In this equation, y_i is the number of observed seedlings in plot i, while p_i is the number of recruits as modeled by the Skellam function, and k is the error term. As priors for b and n, gamma-distributed parameters $X \sim \Gamma(\alpha, \beta)$ were used. The parameters α (shape) and β (rate) were derived from a Poisson fitted maximum-likelihood estimation of the data. A correction for the number of seeds in the seedbank was not necessary, as no seeds of *E. crus-galli* were found in the seedbank. To avoid zero values in the data, 1 was added to the number of seedlings and 10 to each

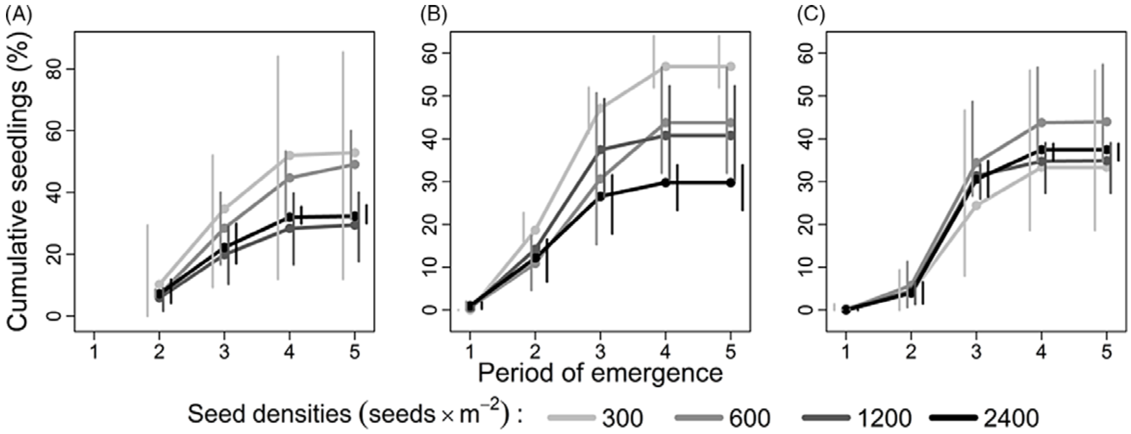

Figure 2. Percentage seedling recruitment after different seed densities in fields A, B, and C, cumulated over the course of five sampling periods from May until September 2015. Vertical bars give the minimum and maximum percentage seedling recruitment. Note that the *y* axis in plot A has a different range than in B and C. No seedlings emerged in field A in the first period. $n = 3$ plots for each period and density.

seed density. The error term k was given a gamma distributed non-informative prior.

We used JAGS v. 4.2.0 accessed from the RJAGS package v. 4-6 using R v. 3.3.3 (R Core Team 2017). The model was run with three chains and 300,000 iterations with no thinning and a burn-in of 10,000 iterations. Convergence of the chains and autocorrelation were monitored for each parameter. Effective sample size was well above 10,000 samples (Kass et al. 1998). The modes of the posterior distributions and the lower and the upper bounds of the 95% highest-density intervals (HDI) or highest posterior density characterize the parameter estimates (Kruschke 2010).

Results and Discussion

Seedling Recruitment

Figure 2 gives an overview of how the percentage of seedling recruitment differed among fields and seed densities. In field A, cumulated across all densities, 35% of all seedlings emerged. The percentage of seedling recruitment decreased with increasing seed densities, ranging from 29% to 52% between densities. The same inverse density-dependent effect was observed in field B, where 36% of all seeds became seedlings, with a range of 29% to 56%, depending on the density. As Boyd and van Acker (2004) showed, this qualitative assessment can provide information about the presence of establishment limitation. They found density dependence in percentage seedling emergence for canola (*Brassica napus* L.), green foxtail [*Setaria viridis* (L.) P. Beauv.], wild mustard [*Brassica kaber* (DC.) L.C. Wheeler], and wild oat (*Avena fatua* L.) in agricultural fields. The inverse density dependence we observed in *E. crus-galli* recruitment indicated that the weed population was not exclusively seed limited under the observed conditions.

In field C, 37% of all seeds became seedlings. However, in contrast to the other two fields, percentage of seedling recruitment in field C showed a narrower range. The lowest percentage of seedling recruitment (33%) appeared for plots with the lowest seed density (300 seeds m^{-2}). Recruitment at the other three densities was higher (34% to 44%). This order suggests density-independent seedling recruitment in field C. The results indicate that in field C, seed limitation was more dominant than in fields A and B. It is not possible to make a quantifiable distinction between density-dependent and density-independent limitations with this qualitative method. However, this first analysis indicates that there are differences between the factors that alter the suitability of microsites and, as a result, limit seedling establishment in the experiments. These factors seem to have a restrictive effect on seedling recruitment in field C at the lower densities rather than a stimulating effect on seedling recruitment at higher seed densities. The observed recruitment rates were two to five times higher than found in other, seminatural environments, such as experimental (6%) and natural grassland communities (4% to 16.4%) (Aicher et al. 2011; Spotswood et al. 2017). Clark et al. (2007) reported an average percentage emergence of 15% in their meta-analysis of 159 species in three different habitat types (intertidal, grassland, forest). Frequent seedling monitoring, rather than counting seedlings only once, was a methodological advantage in our experiment. It allowed for a thorough census of all emerged seedlings, whether they survived the whole vegetative period or not. Boyd and van Acker (2004), who monitored seedling emergence once per week, found the percentage of seedling recruitment of four weed species in wheat (*Triticum aestivum* L. 'AC Barrie') ranged from 20% to 56%. The high average recruitment rates in our experiment confirm the assumption that cornfields are particularly suitable habitats for *E. crus-galli*. Nevertheless, we assert that frequent monitoring is necessary to get a thorough picture about seedling recruitment.

Seed and Establishment Limitation

Figure 3 shows the number of seeds per square meter that did not recruit to the seedling stage because of limitations, that is, seed and establishment limitation. The parameters of the recruitment function are given in Table 1. The curve illustrates the change in seedling numbers per seed and square meter (dr/ds) dependent on the number of added seeds (s). dr/ds is the first derivation of the recruitment-seed function, thus areas in Figure 3 represent values in units of seeds per square meter.

In fields A and B, the ratio of seedlings per added seed dr/ds decreases (solid black line) with increasing seed number, s. The number of seeds that did not recruit seedlings due to establishment limitation increases accordingly (area e g d c). With every added seed, the number of seeds that did not emerge due to seed limitation decreases. In both fields A and B, the equilibrium is reached within the maximum range of 2,400 added seeds m^{-2}. Knowledge about the equilibrium of seed and establishment limitations ($L_S = L_E$) is especially beneficial if the size and recruitment rate

Table 1. Mode and lower and upper highest-density interval (HDI) limits for the parameter estimates of *b* and *n* of the Skellam function fit to cumulative *Echinochloa crus-galli* seedling densities in 2015.

	Parameter *b*			Parameter *n*		
		HDI			HDI	
	Mode	Low	High	Mode	Low	High
A	0.42	0.32	0.57	2,948	1,319	6,148
B	0.50	0.41	0.60	2,505	1,346	4,572
C	0.36	0.29	0.43	2.4×10^8	8.4×10^7	5.4×10^8

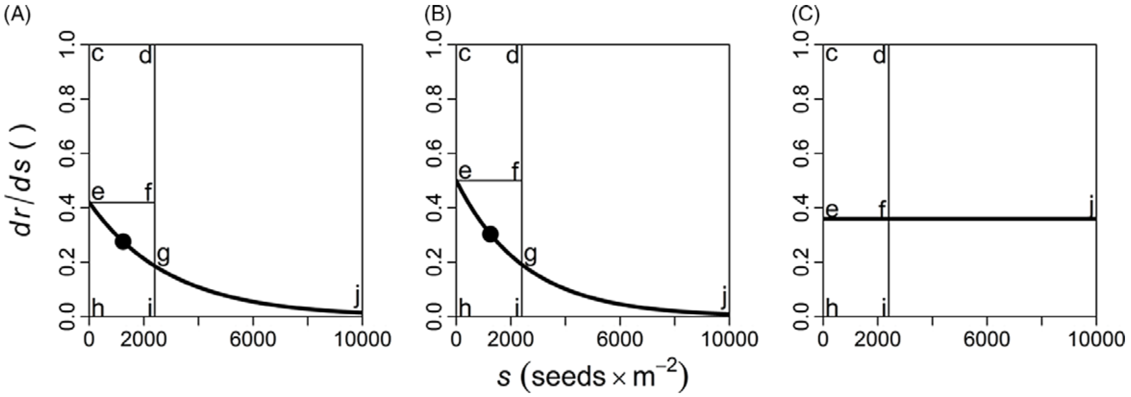

Figure 3. The ratio of additional seedlings per added seed and square meter (*dr/ds*), dependent on the number of seeds per square meter (*s*) in fields A, B, and C ($dr/ds = b * \exp^{-s/n}$). Parameter *n* is the number of microsites, and *b* is the proportion of those sites suitable for a seedling to establish. Areas above and beneath the curve *dr/ds* represent the magnitude of limitations. Lowercase letters define the areas by marking their corner points. The total number of seedlings at a seed density of 2,400 seeds m^{-2} (vertical line) is represented as area (h i g e). The dot on the graph gives the equilibrium where $L_S = L_E$. The number of seeds that did not emerge because of density-independent establishment limitation (*DI* L_E) is represented as the area (e f d c). The number of non-emerged seedlings due to density-dependent establishment limitation (*DD* L_E) is represented as the area (g f e). No corner point (g) is visible in field C because of the extremely low *DD* L_E. The area that represents seed limitation (i j g) is clipped at 10,000 seeds m^{-2}.

of a seedbank is known. If the number of available seeds from an ambient seedbank is below the equilibrium, that is, the population is seed limited, intercepting additional seeds is the most efficient means to reduce seedling recruitment. The results of the recruitment function demonstrate that at 2,400 added seeds m^{-2} of *E. crus-galli* in fields A and B, the populations were predominantly establishment limited. Whereas the presence of establishment limitation was already implied by the qualitative visual analysis (Figure 1), the recruitment function quantifies the degree of seed and establishment limitation at any given seed density. In our experiment, the lack of an ambient seedbank allowed for the mapping of seedling recruitment of a single annual seed input. Consequently, it was possible to separate the added seeds into those that emerged and those that failed to emerge. If an ambient seedbank exists, the size and the emergence rate from the seedbank must be estimated in control plots.

In contrast to fields A and B, seed limitation was important in field C across all seeding densities (Figure 3). Calculating the equilibrium resulted in an estimate of 86,400,000 added seeds m^{-2}. We consider this result to be of only limited informative value, as this would imply a plant density of 26,120,765 plants m^{-2}, which is biologically impossible. However, the result indicates more microsites (*n*) in field C than in fields A and B. Technically this result implies that in field C, the added number of seeds was not sufficient to saturate microsites and to induce density-dependent limitation (Figure 3). We assume that a density-dependent effect similar to that observed for fields A and B would have been detected in field C if the experimentally added seed numbers had been higher. The ambiguous results in field C demonstrate the importance of using adequately high numbers of different seed densities if recruitment functions are to be determined. Therefore, it is very important to take into account the potential number of produced seeds of the species in question, as well as emergence data from other studies and environments. The Skellam function confirmed the results of the qualitative analysis (Figure 1) in that if seed limitation is dominant and density-dependent establishment limitation is negligible, parameter *b* represents the actual recruitment rate.

With the parameters of the Skellam function, it is possible to estimate the number of suitable microsites, that is, the maximum number of seedlings that can emerge at a site (*bn*). The estimated number of recruits was similar in fields A and B, namely 1,238 and 1,252 seedlings m^{-2}, respectively. The number of suitable microsites, *bn*, can be utilized as a measure of a habitat's invasion susceptibility to a given plant species (Miller et al. 2014). The number of suitable microsites in our experiment was up to 110 times higher, depending on the habitat type, than in studies of subalpine habitats (Miller et al. 2014) and up to 8 times higher, depending on the species, in pastures (Spotswood et al. 2017). Although these experiments encompassed other species and habitats, our results are in line with an ecological perspective, as we presented the contrasting environment of an annually cultivated agricultural field and a highly adapted weed species. In fields A and B, half and two-thirds, respectively, of all suitable microsites were occupied, even though we added only 2,400 seeds m^{-2}. Therefore, we estimate that both fields are highly susceptible to the establishment of *E. crus-galli.* This estimation seems even more likely regarding the achievable numbers of produced seeds. Pannwitt et al. (2019) estimated that the seed production of *E. crus-galli* on the same sites ranged, field-wise, from 58,157 ± 8,064 seeds m^{-2} (field A) to

Table 2. The calculated number of recruits (*r*) at the end of the season, the number of seeds that did not germinate because of seed limitation (L_S), establishment limitation (L_E), and density-independent ($DI\ L_E$) and density-dependent ($DD\ L_E$) establishment limitation as the number of seedlings per square meter at a density of 2,400 added seeds m^{-2}.[a]

	At 2,400 seeds m^{-2}				
	r	L_S	L_E	$DI\ L_E$	$DD\ L_E$
A	691	550	1,709.00	1,390	319
B	768	478	1,632.00	1,206	426
C	854	86,664,968	1,546.25	1,546	0.004

[a] Values are calculated from the cumulative seedling numbers in the year 2015 and the corresponding parameter estimates (Table 1).

203,643 ± 37,739 seeds m^{-2} (field C). Maun and Barrett (1986) reported seed production for *E. crus-galli* ranging from 2,000 to 40,000 seeds per plant. Field C seems even more susceptible to the establishment of seedlings due to its higher number of microsites, which leads to a higher maximum number of seedlings.

Consequently, an agronomic strategy requires preventing the establishment of *E. crus-galli* in the fields. While reducing seed numbers of annual species may be reasonable (Davis 2006), we found in our experiment that, at least in fields A and B, manipulating the microsite could have a stronger effect on seedling recruitment, because the number of seeds is not a constraint for this species at these sites.

Seedlings are an important target to manage weeds on arable sites. However, to estimate whether a plant population will expand, remain stable, or decrease, it is important to include additional plant life stages, especially reproducing individuals, in the studies. In general, knowledge about seed and establishment limitations can improve decision-making processes for weed management strategies. Generalized for (climatic) regions or cultivation techniques, such information will assist in developing more targeted management strategies. Moreover, the vulnerability of sites to the establishment of new weeds can be better foreseen.

Determination of Density-Dependent and Density-Independent Establishment Limitation

Figure 3 gives the number of seeds that did not emerge due to density-independent establishment limitation as the product of $1 - b$ times the number of added seeds. Both density-independent (area e f d c) and density-dependent (area g f e) establishment limitations increased with higher seed numbers in fields A and B. Parameter b of the recruitment function, the proportion of microsites available to seedlings, varied from 0.36 to 0.50 between the fields. This parameter indicates differences in density-independent mortality processes. It is lowest in field C, where it accounts for the whole reduction of seedling recruitment of the seeded densities, because only minimal density-dependent limitation was active. As already explained, the estimation of seed and establishment limitations would have diverged much less in field C if seed numbers had been sufficient to saturate microsites as they did in fields A and B.

Table 2 shows the magnitude of density-dependent and density-independent limitations for an added density of 2,400 seeds m^{-2}. More seedlings emerged in field B than in field A. While total establishment limitation was higher in field A, the density-dependent component had the strongest effect in field B. This reflects the lower number of microsites, n, in field B. However, a microsite for seedling establishment is not solely the physical site, but a continuum of conditions that support or prevent seedling recruitment (Bullied et al. 2012; Harper 1977). Factors responsible for the remaining density-independent establishment limitation are currently unknown. Two limitation factors with high variability in time and space, that is, seed predation and seed burial, were prevented (Davis et al. 2013). Nevertheless, rain or wind may have led to a dislocation of seeds into deeper soil layers, where some may have died. Some seeds may have been (or become) dormant. These effects resulted in seeds not emerging as seedlings during the observation period and thus going uncounted. High recruitment rates in our experiments show that we were successful in controlling for the majority of establishment-limiting factors, while those factors we were not able to control appeared to have little or no effect.

In agricultural fields, agronomic activities such as soil cultivation and herbicide application effectively limit weeds independent of their densities (Ball 1992). By excluding seed burial, herbicides, interspecific competition, and seed predation from the experiment, we excluded as much density-independent limitation as possible. The values of density-dependent establishment limitation reflect the effects of density-dependent factors, tested for by adding different seed densities. We tested for the complete density-dependent limitation without further differentiation of factors. Intraspecific competition is one factor of density-dependent establishment limitation. It can be induced by affecting the availability of water and light for a single *E. crus-galli* seed (Long et al. 2015).

It was possible to estimate the magnitude of seed and establishment limitation with the parameter values gained from the Skellam function. Knowledge about the relative strength of limitations for a population together with the possible magnitude of seedfall or seed introduction provides important information on the potential effectiveness of different weed management strategies. For example, reducing the number of available seeds through seed predation or harvesting techniques that destroy weed seeds (Shergill et al. 2019) will have minor effects on seedling numbers as long as seed numbers are not reduced below the equilibrium. Only if the population is already seed limited will restricting additional seed input significantly reduce the number of seedlings. This reduction can be achieved either via depletion of an existing seedbank or via the prevention of weed seed dispersal. In an establishment-limited population, the number of seedlings will only decrease if the suitability of the sites for emergence is reduced. Both population modeling and practical weed management can benefit from understanding this difference. For population modeling, we expect more realistic results when arable weed populations are simulated. The decision-making processes in practical weed management can become more sophisticated. As reviewed by Bajwa et al. (2015b), our target species, *E. crus-galli*, can be managed by a broad range of strategies combining cultural, chemical, and biological control techniques. With knowledge about the limitation of either seeds or microsites for establishment, the control techniques can be selected that most effectively target the present limitation. *Echinochloa crus-galli* is a species that is extremely well equipped to survive, grow, and spread in arable cornfields (Bajwa et al. 2015a). We strongly recommend further investigations of seed and establishment limitations to test the usability of the concept in other species and species–crop combinations and to incorporate other plant life stages in these studies.

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