

Research Article

Cite this article: Leon RG, Oreja FH, Mirsky SB, Reberg-Horton C (2023) Addressing biases in replacement series: the importance of reference density selection for interpretation of competition outcomes. *Weed Sci.* **71**: 606–614. doi: [10.1017/wsc.2023.53](https://doi.org/10.1017/wsc.2023.53)

Received: 7 June 2023
Revised: 22 September 2023
Accepted: 25 September 2023
First published online: 5 October 2023

Associate Editor:
William Vencill, University of Georgia

Keywords:
Competition; crop; interference; weed

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Addressing biases in replacement series: the importance of reference density selection for interpretation of competition outcomes

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Abstract

Replacement series are used by researchers to understand how competition-related variables influence dynamics from the individual to the population and community levels, but this approach has been criticized because of inherent biases associated with plant size differences and density-dependent responses. The use of functional densities instead of demographic densities was proposed to minimize those biases. This work explored three models to determine reference densities for replacement series experiments based on (1) maximum biomass, (2) biomass at onset of diminishing returns (i.e., inflection point), and (3) nitrogen (N)-uptake equivalency. Replacement series experiments were conducted using redroot pigweed (*Amaranthus hybridus* L.):maize (*Zea mays* L.) and giant foxtail (*Setaria faberi* Herrm.):maize proportions of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1. The monoculture density for each species was established according to the three models. Density selection criteria resulted in major differences in competitive interactions between species. The use of functional densities at which the biomass accumulation inflection point for the smaller species allowed both species to exhibit either increases or decreases in biomass production depending on competitive interactions for all interspecific mixtures. Conversely, the maximum biomass model favored the larger species, almost completely inhibiting the growth of the smaller species, which resulted in a poor characterization of competitive responses of the smaller species. The N uptake equivalency model resulted in interactions closer to the predicted neutral competition. The model based on the biomass accumulation inflection point was the most sensitive and informative across all interspecific mixtures for both species. We propose that to reduce bias associated with species size differences when determining reference densities for replacement series experiments, at least two criteria must be met: (1) the experiment sensitivity allows measuring and quantifying the competitive responses for both species in all mixtures, and (2) the balance between density and carrying capacity of the system minimizes intraspecific competition.

Introduction

Weeds coexist in space and time with crops, sharing resources in limited supply such as light, nutrients, and water. Depending on their ability to capture resources, competing species can affect each other's growth and plant size distribution within the population, and even cause density-dependent mortality (Park et al. 2003). There are several ways to evaluate interspecific competition between crops and weeds, including pairwise, additive, replacement, or response surface experimental designs (Connolly et al. 2001; Cousens 1991; Swanton et al. 2015). Among these, the method most widely used is replacement series, which consists of comparing the growth of two species under monoculture and in different combinations while maintaining the overall plant density constant (Cousens 1991). This design has been used to obtain several indices that, based on growth or reproductive output, give an idea of species competitive ability. This information can then be employed to interpret processes such as plant community structure and stability, secondary ecological succession, crop–crop interactions in intercropping systems, crop–weed interactions, weediness, and invasiveness (Baumann et al. 2001; Cousens 1991; Doudová et al. 2017; Hoffman and Buhler 2002; Swanton et al. 2015; Vila and Weiner 2004).

Replacement series experiments are frequently utilized to determine which species is more likely to succeed in a situation of interspecific competition, and how the relative abundance of one species in relation to the other affects the outcome of the competition (Connolly et al. 2001). The main problem with this approach is that intrinsic differences in size between species can

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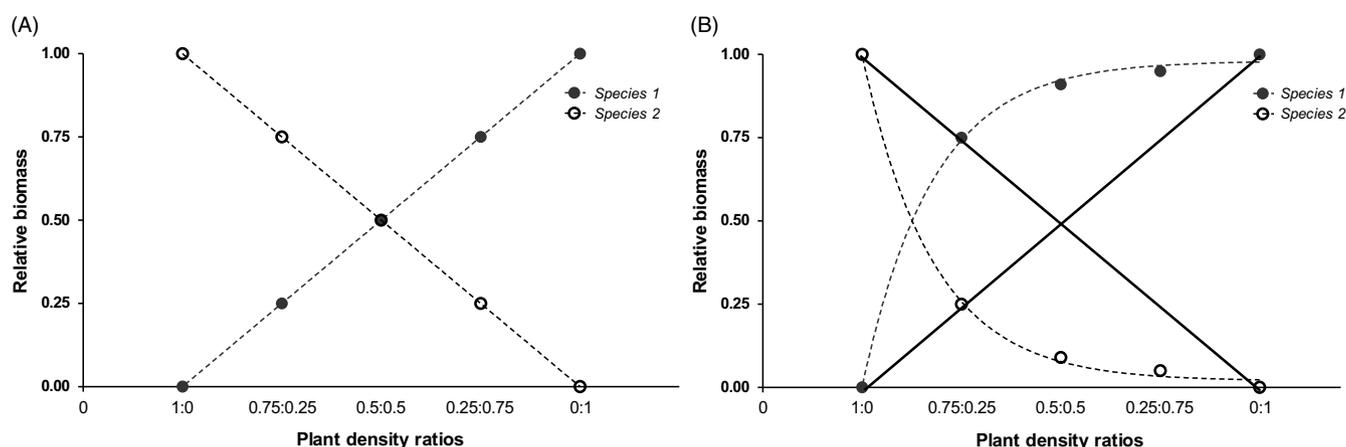


Figure 1. Theoretical relationship between relative biomass and plant density ratios of (A) two species of the same size and (B) two species with species 1 being larger than species 2. Dashed lines represent the regression lines for both species, and solid lines represent theoretical line of parity (1:1) between species.

introduce a major bias in the outcome of replacement series experiments. Assuming all species, regardless of their size, can compete equally is problematic, and many researchers have relied on this assumption (intentionally or not) to conduct experiments in which one individual of species A is counted as one individual of species B to determine the replacement ratios. Plant size is one of the most important ecological traits that can either influence or reflect the ability of plants to capture resources (Goldberg and Landa 1991), among others such as seed size, seedling size, and emergence time (Freckleton and Watkinson 2001). If the sizes of the two plant species are similar, it is more likely that their potential to acquire resources is also similar (Goldberg and Landa 1991); therefore, the relative yields (based on their respective monocultures) would be similar and close to 0.5 for both species in the same proportion (Taylor and Aarssen 1989; Figure 1A). For this reason, replacement series experiments are particularly useful to compare competitive ability among genotypes of the same species (Ethridge et al. 2023a, 2023b; Sibony and Rubin 2003). Conversely, comparing species that are dramatically different in size poses the question of how one can determine the densities that would represent a 1:1 equivalency. Those densities must permit both species to exhibit their potential competitive ability; in other words, they must create conditions for a “fair fight.” It is critical to keep in mind that size differences increase the risk of setting the outcome of the study a priori, because the species with larger individuals (species 1) has a higher probability to use the resources of the system than the smaller one (species 2) (Weiner 1985). This inevitably causes deviations from the parity line 1:1 with a concave curve shape (Figure 1B) and vice versa (Akey et al. 1991; Babić and Mahmoodi 2013; Lodge et al. 2009; Szymura et al. 2018; Wang et al. 2006). Therefore, our capacity to properly quantify and characterize the traits involved in competitive ability for both species can be masked by the innate differences in plant size (Freckleton and Watkinson 2001).

Although the bias associated with differences in plant size has been recognized for a long time, no consistent criteria are used to decide upon or justify the selection of plant density per species in replacement experiments. Even de Wit (1960), who first published a detailed description of the use, analysis, and interpretation of replacement series, did not provide a clear system to determine reference densities. This researcher mentioned the importance of considering a “maximum or equilibrium density” for the experimental system, but how to allocate the number of individuals

to each species was left unclear. Other researchers have also focused on finding mixture densities that maximize yield for both species (Taylor and Aarssen 1989). Connolly et al. (2001) proposed the use of functional densities based on equivalent sizes (e.g., biomass or leaf area) or resource use (e.g., shoot nitrogen [N] content) instead of demographic densities. Nevertheless, the approaches used by researchers to determine those functional densities vary and in many cases are arbitrary and lack a robust rationale.

Because total biomass per area is a better indicator of resource use than the number of individuals (Goldberg and Werner 1983), the former should be an equitable variable to guide density selection. However, what criteria must be considered to use biomass accumulation is still an open question. Most systems that use maximum biomass accumulation in pure stands have done so for both species (Harper 1977; Law and Watkinson 1987; Taylor and Aarssen 1989). However, we propose two alternative ways for using biomass to avoid biases associated with individual size and demographic density. One is considering the maximum shoot biomass per area produced by the smaller species according to a density-response curve in monoculture, which then is used to determine the density of both species in a competition experiment (Figures 2 and 3). Thus, both species have the potential to reach the same overall biomass, but their competitive ability will determine which one will do it first. The rationale for using the maximum biomass of the smaller species is based on the improbability of the smaller species competing with the larger one if the latter is growing at a density that allows the accumulation of biomass at levels unachievable by the former. Furthermore, if the selected monoculture reference densities are too high, intraspecific competition might inadvertently contribute to the overestimation of interspecific competition (Szymura et al. 2018). Thus, another advantage of the proposed approach is that it decreases both intraspecific competition and the risk of density-dependent mortality during the experiment (Weiner 1985; Weiner and Freckleton 2010). This same approach can be used to determine densities based on the equivalency of a resource that is the focus of the competition study. For example, one can use the densities at which both species exhibit the same uptake potential for a nutrient of interest (Poffenbarger et al. 2015).

An alternative way to determine the reference densities is to consider using the density at which the smaller species reaches the onset of diminishing returns in biomass accumulation (i.e., inflection point; Figures 2 and 3). This inflection point is when intraspecific competition initiates for the smaller species. In this

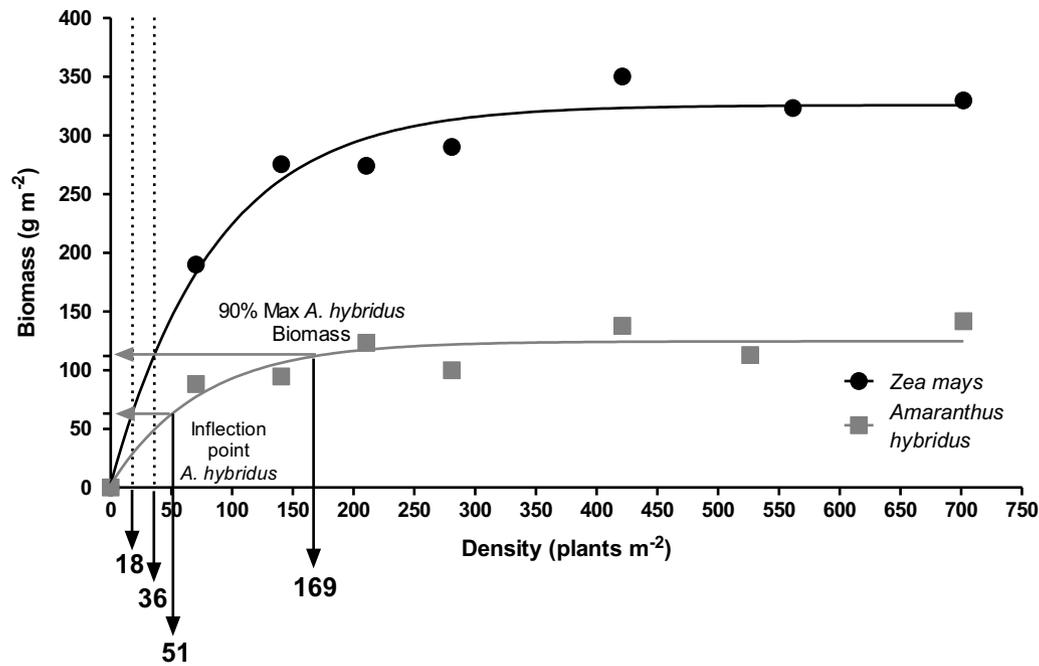


Figure 2. Aboveground biomass (g m^{-2}) across densities (plants m^{-2}) for maize and *Amaranthus hybridus*. Vertical arrows indicate the maize (black numbers) and *A. hybridus* (gray numbers) densities at which *A. hybridus* reaches inflection point and 90% of maximum biomass. Horizontal arrows indicate *A. hybridus* biomass at the inflection point and 90% of maximum biomass.

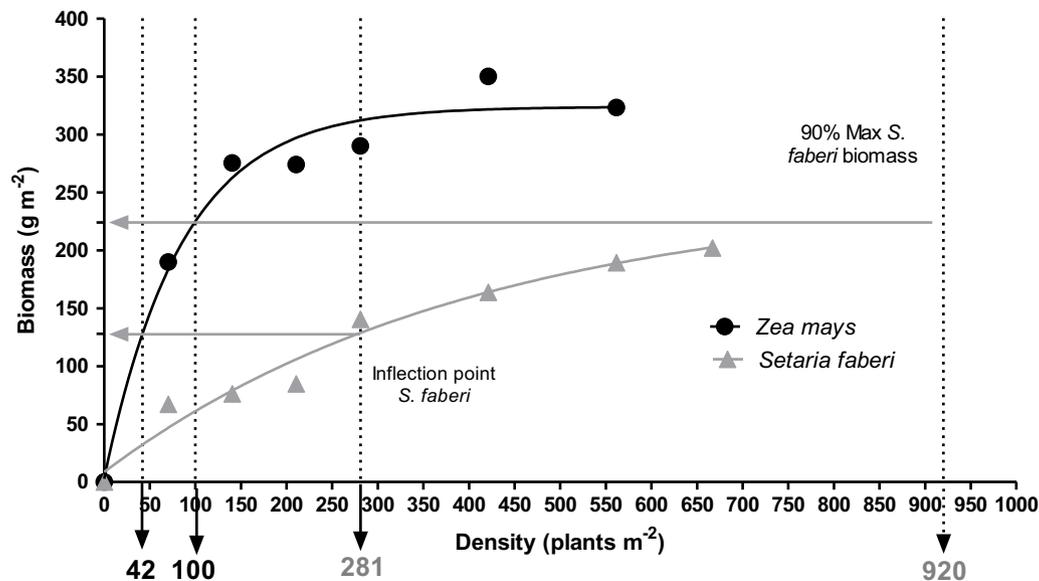


Figure 3. Aboveground biomass (g m^{-2}) across densities (plants m^{-2}) for maize and *Setaria faberi*. Vertical arrows indicate the maize (black numbers) and *S. faberi* (gray numbers) densities at which *S. faberi* reaches inflection point and 90% of maximum biomass. Horizontal arrows indicate *S. faberi* biomass at inflection point and 90% of maximum biomass.

context, both species will have densities that can potentially produce similar biomass per area, but none of them has reached intraspecific competition levels that can undermine their own growth favoring the other species. In other words, this is a situation in which there is space for both species to increase their biomass depending on their interspecific competitive ability.

The objective of the present research was to characterize how the proposed models to determine reference densities can shape the outcome and interpretation of replacement series experiments

to study plant competition (interference). Ultimately, this study was intended to provide a framework and criteria for functional density selection of replacement series studies.

Materials and Methods

Two replacement series experiments were conducted under greenhouse conditions at North Carolina State University in Raleigh, NC, using a completely randomized design with three

Table 1. Monoculture densities and ratios for maize in competition with either *Amaranthus hybridus* or *Setaria faberi* based on different models to determine reference densities for replacement series experiments.

Model	A. <i>hybridus</i> : maize		Ratio	S. <i>faberi</i> : maize		Ratio
	plants m ⁻²			plants m ⁻²		
Inflection point	87	39	2:1	470	70	7:1
Maximum biomass	127	27	5:1	1,533	167	9:1
N uptake	117	13	9:1	117	13	9:1

replications, from July to October 2001. A commercial potting mixture (Fafard® 4P Mix, Sun Gro® Horticulture, Agawam, MA, USA) was used to fill 3-L pots to grow maize (*Zea mays* L.), redroot pigweed (*Amaranthus hybridus* L.), and giant foxtail (*Setaria faberi* Herrm.). Based on this equivalency, a replacement series was designed with 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1 of weed:maize ratios based on the three proposed models for density selection (Table 1).

Shoot biomass (g m⁻²) response to increasing plant density was determined using monocultures of maize, *A. hybridus*, and *S. faberi* grown for 35 d (Poffenbarger et al. 2015; Figures 2 and 3). The goal was to identify densities that minimize intraspecific density-dependent mortality (Marshall and Jain 1969). This relationship was used to determine 90% of maximum biomass of the smaller species in the replacement series experiment and the density at which this biomass was obtained. Then, we determined the density in monoculture at which the larger species (i.e., maize) reached the same biomass as the smaller one (Figures 2 and 3; Table 1). Similarly, monoculture density-response curves were used to obtain the density at which the smaller species initiated diminishing returns in biomass accumulation (i.e., inflection point), and this biomass was also used to determine the equivalent density of maize in monoculture (Figures 2 and 3). Density selection based on N uptake was done using a complete aggregated data set provided from Poffenbarger et al. (2015), who studied *S. faberi* and *A. hybridus* in competition with maize and determined in a greenhouse study that 36 weed plants pot⁻¹ (948 plants m⁻²) exhibited equivalent N uptake as 4 maize plants pot⁻¹ (105 plants m⁻²) over a period of 35 d. Once these densities were estimated, replacement series using the aforementioned ratios were established (Table 1). Both maize and weed seeds were sown at a higher density than required and thinned to the designated densities, watered every 2 d in the first 20 d and every day in the last 15 d to maintain field capacity, and fertilized (20-10-20) every 7 d with 0.5 g pot⁻¹ (125 mg N kg⁻¹ soil). The greenhouse was maintained at 28 ± 5 C and a 14-h photoperiod. The experiment was terminated 35 d after emergence, shoots were harvested and dried at 70 C, and dry biomass was recorded.

Shoot biomass per pot from monocultures was subjected to ANOVA using a general linear model procedure in R (R Development Core Team 2021). Shoot biomass was square root-transformed to ensure homogeneity of variance. Tukey's honestly significant difference was used for means separation. Relative biomass (RB) and relative yield total (RYT) were calculated as described by Harper (1977):

$$RB_{sp1} = \frac{\text{Biomass}_{Sp1\text{mixture}}}{\text{Biomass}_{Sp1\text{monoculture}}} \quad [1]$$

where RB_{sp1} is the relative biomass produced by species 1, $\text{Biomass}_{Sp1\text{mixture}}$ is the biomass produced by species 1 growing in

the mixture with species 2, and $\text{Biomass}_{Sp1\text{monoculture}}$ is the biomass produced by species 1 growing in monoculture. The impact of the competition on overall productivity was estimated with RYT (Harper 1977):

$$RYT = RB_{sp1} + RB_{sp2} \quad [2]$$

Furthermore, RB_{sp1} and RB_{sp2} were subjected to polynomial (quadratic) regression procedures using the GraphPad Prism 6 (GraphPad Software, Boston, MA, USA) program.

Results and Discussion

Competition Outcomes of Reference Density Selection Models

As proposed by Firbank and Watkinson (1985), we first grew pure stands of each species, so we could identify the thresholds at which intraspecific competition would start. Having density-response curves for monocultures is not only useful to determine the reference densities for the mixture treatments but also for comparing and reinterpreting other studies (Jolliffe et al. 1984). In general, the maximum biomass and the N-uptake models had the greater weed:maize ratios (5:1 to 9:1), while the inflection point model had the lowest (2:1 to 7:1) (Table 1). The differences in plant density were evident across models, especially for weeds. Also, the maximum biomass model had the highest densities. For example, in the case of *A. hybridus*, this model had a density 45% higher than the inflection point model, but the opposite was observed for maize density, with the former model being 30% lower than the latter (Table 1). *Setaria faberi* required much higher densities to meet the goals of the different models for competing with maize. Thus, in the case of the maximum biomass model, *S. faberi* density was 1,533 plants m⁻². It is important to remember that this study was harvested 35 d after planting. Therefore, densities this high do not necessarily cause density-dependent mortality during this time frame, which was one of the goals of all models. The maximum biomass model resulted in densities that were 3- and 13-fold higher than the inflection point and N-uptake models for both *S. faberi* and maize. These results illustrate how target growth goals will influence the densities that must be used for each species as well as the ratios between them, but these two variables will not be proportional across models. For instance, it is possible to have the same ratios and dramatically different densities, as was the case for *S. faberi*:maize for the maximum biomass and N-uptake equivalency models.

Despite the major differences in plant density and ratios (Table 1), total biomass accumulation for the reference densities in monoculture did not differ among models for any of the species ($P > 0.05$; Table 2). Moreover, in most cases, biomass ratios favored maize over both weeds (i.e., weed:maize ranged from 1:1 to 1:19), and with the exception of *A. hybridus* in experiment 2, weed:maize biomass ratios were similar for inflection point and N-uptake models, always favoring maize (1:3 to 1:7). Conversely, the maximum biomass model maintained more equitable ratios between weed:maize (1:1 to 1:5). The fact that all models yielded similar biomass accumulation for weeds and maize suggests that plants adjusted growth to the differences in density and met the carrying capacity of the system. It must be highlighted that in almost all cases, the biomass accumulation of the maize monoculture in the replacement experiments (Table 2) was higher than biomass obtained in the calibration experiments (Figures 2 and 3), surpassing the expected equivalency with the weeds. Thus,

Table 2. Maize, *Amaranthus hybridus*, and *Setaria faberi* biomass in monocultures in experiments 1 and 2.^a

Model	Experiment 1						Experiment 2					
	<i>A. hybridus</i> :maize (g pot ⁻¹)		Ratio	<i>S. faberi</i> :maize (g pot ⁻¹)		Ratio	<i>A. hybridus</i> :maize (g pot ⁻¹)		Ratio	<i>S. faberi</i> :maize (g pot ⁻¹)		Ratio
Inflection point	11 (3)	60 (24)	1:6	26 (14)	75 (17)	1:3	20 (14)	192 (55)	1:10	33 (3)	237 (83)	1:7
Max biomass	15 (3)	42 (17)	1:3	37 (8)	42 (11)	1:1	15 (5)	155 (32)	1:10	39 (8)	204 (98)	1:5
N uptake	8 (4)	48 (17)	1:6	17 (9)	48 (10)	1:3	10 (6)	193 (42)	1:19	24 (7)	195 (18)	1:8

^aValues in parentheses are standard errors of the mean.

the prediction that, at the selected reference densities, weeds as well as maize would produce similar biomass was not met, and maize demonstrated a greater ability to exploit the carrying capacity of the system than the weeds.

Maize and weed relative biomass exhibited concave and convex patterns, respectively, indicating a competitive advantage of the former over the latter under almost all models (Figures 4 and 5). Models based on the inflection point of biomass accumulation allowed a more progressive change in relative biomass accumulation across mixtures than the model based on the maximum weed biomass, which exhibited almost full weed suppression by maize at the 0.5:0.5 ratio. This was particularly evident for *A. hybridus* (Figure 4). Thus, the maximum biomass model saturated growth responses for the smaller species, making it not possible to quantify changes in growth when the larger species represented more than 25% of the mixture. Conversely, the inflection point model allowed growth of both species at all mixtures. Although it can be concluded from the maximum biomass model that maize was more competitive than the weeds, this is not a very informative result. The objective of a replacement series experiment should not be to obtain a binary answer. Instead, one should aim for generating a proper characterization of changes in biomass accumulation across multiple competitive ratios. This allows a robust regression analysis to be conducted to properly quantify responses to competition pressure (Cousens 1991).

The use of the equivalency based on N-uptake potential did not result in competition patterns similar to the maximum biomass model. In fact, the N-uptake equivalency model was the only instance in which one or both species exhibited an RB that did not deviate from the expected values under neutral competition (Figures 4E and F and 5E and F). Similar to the inflection point model, the N-uptake equivalency model allowed characterization of growth responses across all mixtures. However, it seems that ensuring N availability to meet the requirements of both species reduced competition, at least during the duration of the present study, making it more difficult to identify competitive interactions. The diametrically opposed results of the maximum biomass and N-uptake equivalency models raise the question of whether the former created a system allowing the full expression of the competitive ability of both species and/or meeting the N demands of both species, thus “masking” or minimizing the intensity of the competition. Although it is difficult to answer this question with certainty, considering RYT can help interpret those results.

Model selection did not have a clear impact on RYT. None of the models resulted in RYT below 1. This indicated that the carrying capacity of the system was not limiting for any of the models and density ratios. In fact, there were cases in which mixtures of *A. hybridus* and maize increased RYT above the levels obtained with monocultures (Figure 4C and 4E). This was also

observed in a few cases with mixtures of *S. faberi*:maize (Figure 5A and 5E). In all those cases, the increase in RYT was driven by maize, indicating that some of the weed:maize ratios created interspecific competitive interactions that were less limiting for maize growth than intraspecific ones.

Competitive Responses and Plant Density

The competitive interactions generated by the evaluated models were clearly different. This is not surprising, considering the major differences in number of individuals per species and the consequent differences in density ratios and biomass production ratios among models (Jolliffe 2000; Tables 1 and 2). These results could be interpreted as contradictory to the idea that competitive interactions in a replacement experiment do not change much with variation in the total density (Cousens 1991; Cousens and O'Neill 1993). Our models had different total densities but also different proportions between species, which makes it difficult to evaluate the role of density in competitive interactions. However, in the case of *S. faberi* versus maize, ratios were similar in the different models (Table 1), particularly for the maximum biomass and the N equivalency models (9:1 for both), while plant densities were dramatically different (13-fold difference). Competitive interactions had a more pronounced reduction in *S. faberi* biomass production in the maximum biomass model and no deviation from the predicted null competition response in the N equivalency model (Figure 5). Therefore, physiological processes that respond to plant density must be considered for the design of the experiment, because they might indeed modify competitive responses at different densities.

The importance of density-dependent responses has been the main criticism of the use of replacement series experiments. However, as indicated by Cousens and O'Neill (1993), that should not be a limitation if the study is properly designed; hence the value of understanding the impact that the reference density might have on the ability to meet the objectives of the study. It is important to remember that the decision about model selection to determine reference densities is arbitrary. There is no way to know with complete certainty the equivalent reference density between two species, especially as the magnitude of the differences in size and morphology increase. We contend that because models are simplifications of complex processes, a key criterion for their selection is the sensitivity to competitive responses in both species studied. Using this criterion, the model based on maximum biomass is the least informative, because in most interspecific ratios, the smaller species' relative biomass was close to zero (Figures 4 and 5). When this happens, the experiment is not providing information about competitive responses for most of the ratios, at least for the smaller species. Conversely, the inflection point and the N equivalency models allowed characterization of

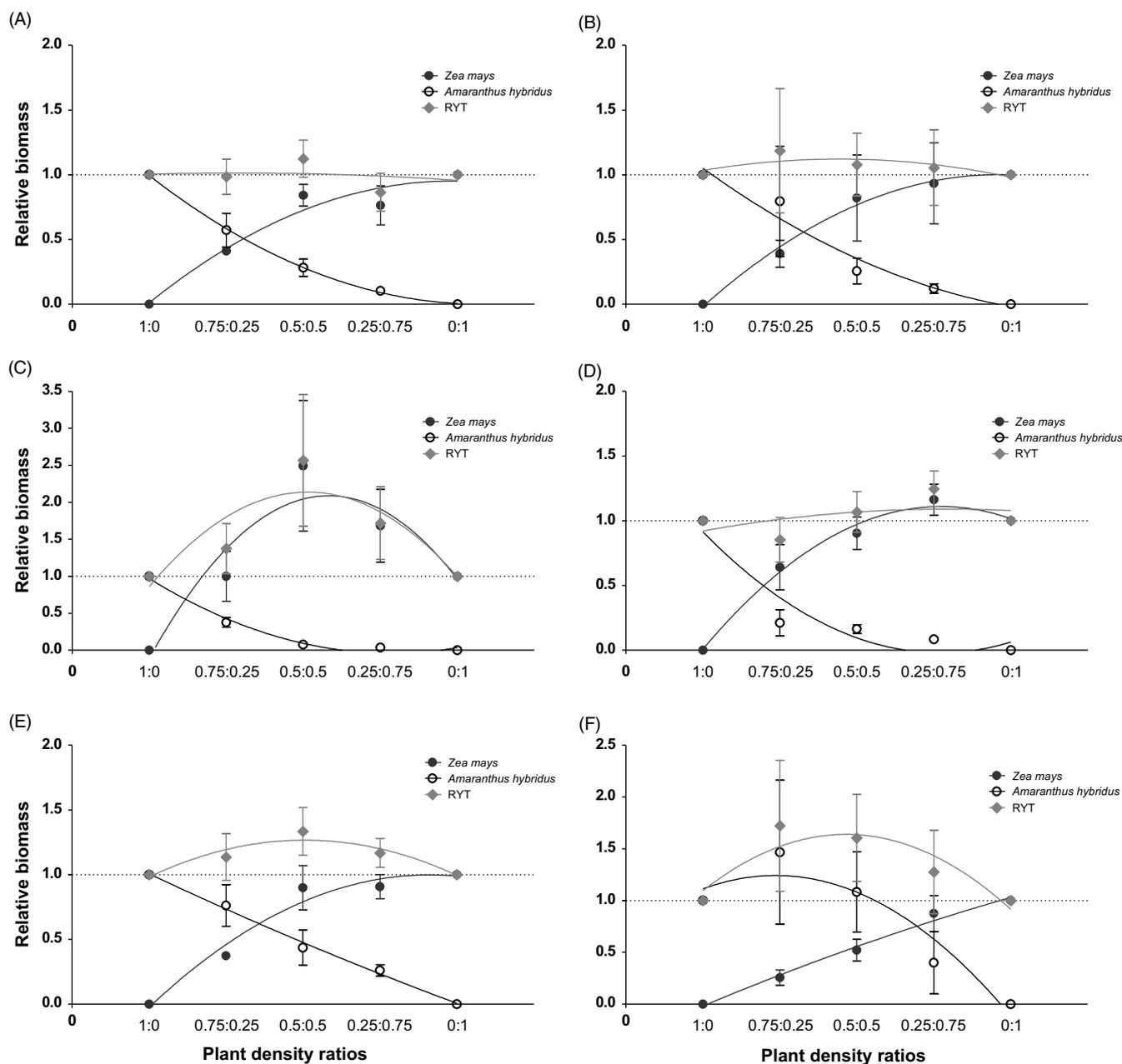


Figure 4. Relative biomass of *Amaranthus hybridus*:maize replacement series from experiment 1 (left) and experiment 2 (right) with densities based in inflection point (A and B), maximum biomass (C and D), and equal N uptake (E and F). Black circles represent maize relative biomass, white circles represent *A. hybridus* relative biomass, and gray diamonds represent relative yield total biomass (RYT). Dotted line represents a relative biomass of 1 for all the proportions. The points and error bars represent data means and standard errors.

changes in competitive responses by allowing growth of both species.

Carrying Capacity of the System

It is commonly assumed that reference densities should be high enough to create conditions that will secure strong interference between species (Cousens 1991). Choosing a model based on maximum biomass will certainly increase the likelihood that one or both species experience limitation in availability and access to resources, accentuating the growth reductions caused by competition. However, this model tends to favor high plant densities that could result in significant intraspecific competition, especially

as plants get larger, which could be misinterpreted as interspecific competition (Firbank and Watkinson 1985; Szymura et al. 2018) and even result in density-dependent mortality (Marshall and Jain 1969). The density to reach maximum biomass depends on the time that plants have been growing; while plants are smaller, the density to reach maximum biomass is higher (Weiner and Freckleton 2010). For this reason, competitive ability must be considered over a well-defined period. Otherwise, with longer periods, there is a higher risk of intraspecific competition and density-dependent mortality (Weiner and Freckleton 2010). Therefore, the calibration of the reference density must have the same duration as the intended replacement series experiment. Although experiment duration will depend on many factors (e.g.,

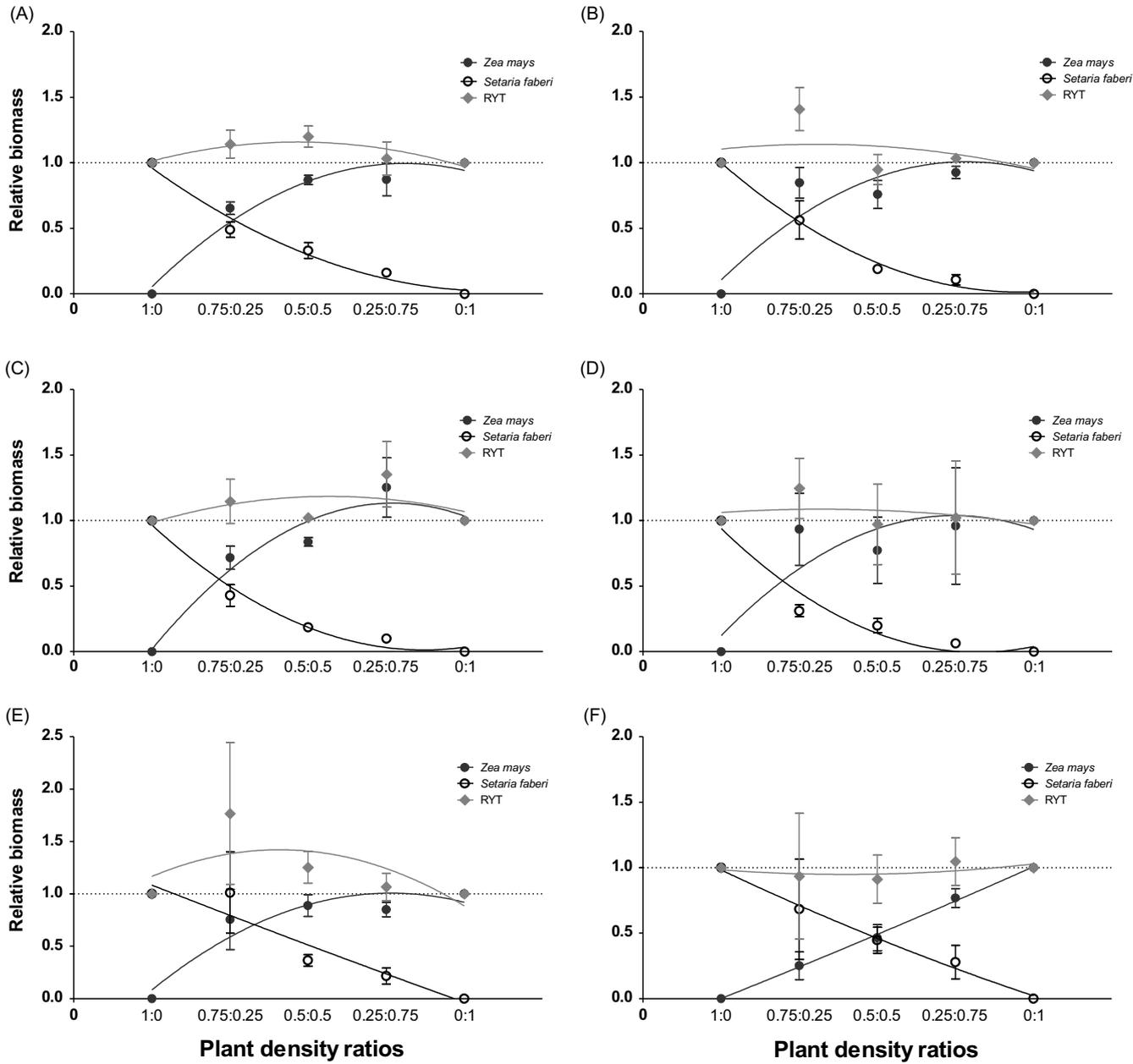


Figure 5. Relative biomass of *Setaria faberi*:maize replacement series from experiment 1 (left) and experiment 2 (right) with densities based in inflection point (A and B), maximum biomass (C and D), and equal N uptake (E and F). Black circles represent maize relative biomass, white circles represent *S. faberi* relative biomass, and gray diamonds represent relative yield total biomass (RYT). Dotted line represents a relative biomass of 1 for all the proportions. The points and error bars represent data means and standard errors.

space, materials availability), the duration should ideally be representative of the time and phenology in which the species would compete under normal conditions.

The model using reference densities based on equivalency for N uptake was the only one that had curves closer to neutral competition. This could have two major interpretations (although others are surely possible). First, ensuring N availability to both species will accentuate competition for other factors such as light interference, which in the present study should not be too intense, because there was ample space between pots. Second, ensuring the necessary availability of a critical resource increases the possibility of both species exhibiting their full competitive potential, possibly because intraspecific competition is minimized (Jolliffe 2000).

The model based on the inflection point of the smaller species had reference densities sufficiently high to favor interspecific competition, while minimizing intraspecific competition, which is reflected in an RYT close to the full capacity of the system (Connolly et al. 2001).

Caveats

In the present study, two species with very different sizes were compared, and although this comparison may be construed as extreme, its type is not uncommon in the literature. We believe that the models and criteria to determine reference densities proposed here are helpful in cases where differences in plant size and

architecture are important, but we recognize that as those differences diminish, the need to take them into consideration is also reduced. Certainly, in cases in which the comparisons are between two genotypes or populations of the same species or parental versus progeny lines or even species within the same genus (Ethridge et al. 2023a, 2023b; Ziska 2017), it might be acceptable to assume a 1:1 density ratio as a reference. A 1:1 density ratio experiment was not included here as a reference due to limited greenhouse space. However, the fact that all studied density ratios favored the weeds (Table 1) and that maize always produced more biomass despite this (Table 2) suggests that a 1:1 density ratio would have favored maize even more.

The fact that RYT did not decline is an indication that the reference densities for both species were not too high for the resources available within the system (i.e., carrying capacity). When the overall productivity of the system is reduced, especially when both species suffer reductions in their relative growth, it is not clear whether the reduction is due solely to interspecific competition or to the lack of resources that created a stress in both species. This may seem like semantics, but ensuring adequate conditions for growth for both species is important to reduce biases. An analogy would be comparing the performances of two racehorses that are seriously malnourished. Is that race really representative of the racing potential of the horses if both were in optimum health? One must not confuse competitive ability with stress tolerance or resilience. In the present experiment, most models resulted in RYT close to stable productivity (i.e., RYT ~ 1; Supplementary Tables 1 and 2), and a few cases with higher productivity under inter- than intraspecific conditions (Figures 4 and 5). Hence, the relation of the carrying capacity of the experiment and the reference densities of all three models was such that interspecific competition was likely the driving mechanism by which relative biomass changed, and the importance of overall scarcity of resources was diminished.

Although the present study provided evidence of the limitations of using the maximum biomass model, it is not clear which model between the inflection point and the N equivalency is the most informative or desirable. The challenge for making this decision relies on the fact that both meet our selection criteria. Thus, both models present sensitivity across the different ratios, both have a nonlimiting carrying capacity, and both species are growing in densities below intraspecific competition thresholds. This conundrum can be solved by proper identification of the main objective of the study (Cousens 1991; Cousens and O'Neill 1993). Thus, if the study is intended to characterize, in general terms, the competitive ability and interactions between two species, the inflection point model seems to be the least biased, because it does not explicitly favor any given resource. Conversely, if the study seeks to determine which species is more efficient in the use of a specific resource (e.g., N) and describe how efficiency differences can result in competitive displacement of growth, the resource equivalency model seems to be more informative. When the levels of the resource of interest satisfy the normal requirements of both species, it is more likely that the differences in growth will be due to competitive ability and not stress tolerance. One could argue that stress tolerance is part of competitive ability, but experimentally this could be a confounding effect. Furthermore, stress tolerance can be studied at the individual level, while competitive ability must be studied under multi-individual conditions.

Focusing only on biomass production is a limitation in the present study. Assessing other life-history traits can provide a more comprehensive view of how competitive dynamics between two

species affect their survival, coexistence, and population increase (Jolliffe et al. 1984). It is likely that reference densities will vary depending on the trait(s) of interest (Marshall and Jain 1969). This is another reason why establishing a clear goal and identifying the physiological or developmental process of interest when designing the study is critical to ensure the replacement series experiment yields interpretable and useful data.

As a corollary of the present research, it can be stated that replacement series are an experimental tool with very specific applications and that their greatest value is to describe the physiological mechanisms that drive interspecific competitive responses. The idea that a replacement series experiment can be used to determine which species is more competitive regardless of the ecological context, albeit widely applied, is simply incorrect. Therefore, researchers should approach the use of replacement series from a physiological perspective rather than a demographic one. In this regard, ensuring that the reference densities allow elucidating the responses of not just one but both species must be at the core of the experimental design. Otherwise, the experiment will be biased and likely result in what can be described as an “unfair fight” from which little can be learned.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/wsc.2023.53>

Acknowledgments. This research was supported by the USDA-NRCS Conservation Innovation grant no. NR213A750013G031; USDA-NIFA Agriculture and Food Research Initiative grant no. 2019-68012-29818; USDA-NIFA Integrated Research, Education, and Extension Competitive Grants Program grant no. 2021-51300-34899; USDA-ARS project no. 8042-22000-167-033-S; and Hatch project no. NC02906. The authors declare no conflict of interest.

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