

Testing the Assumption of Constant Relative Yield Total in Replacement Series Experiments

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ABSTRACT

*The Relative Crowding Coefficient (RCC) is a common measure of interspecific competition in a replacement series experiment. However, the interpretation of RCC depends upon the validity of an assumed functional relationship in total species yield. We develop inference procedures based upon the Relative Yield Total (RYT), a function of species yields in mixture and monoculture plantings, that may be utilized to assess the plausibility of the species yield functional form under which RCC is a reasonable metric of interspecific competition. The performance of the proposed RYT inference procedures is evaluated on the basis of a simulation study. Using the proposed methodology, we conduct RYT inference in a replacement series experiment of bahiagrass (*Paspalum notatum*) and smutgrass (*Sporobolus indicus*).*

Keywords: Bootstrap, Competition index, Relative crowding coefficient, Simulation, Species competition.

1. Introduction

Replacement series experiments, first proposed by de Wit (1960), have been used extensively in ecology to assess the relative effects of intra- and inter-competition between two species (McGilchrist and Trenbath (1971); Harper (1977); Berendse (1979); Firbank and Watkinson (1985); for a review of competition indices, see Weigelt and Jolliffe (2003)). Competition among insect species (Novak, *et al.* (1993)) and tree species within a forest (Vanclay (2006)) have been explored using replacement series experiments. The work here is motivated by the study of competition between two plant species in an agricultural setting. Rana (2012) explored the competitive effects of bahiagrass

(*Paspalum notatum*) and each of two varieties of smutgrass: small smutgrass (*Sporobolus indicus*) and giant smutgrass, which is also known as West Indian dropseed (*Sporobolus indicus* var. *pyramidalis*) (Sellers, *et al.* (2011)). Bahiagrass is commonly used in Florida pastures because it produces well in low fertility and low input management conditions (Newman, *et al.* (2011)). Smutgrass, a bunch-grass, is a serious weed that negatively impacts the quality of grass in pastures. Rana (2012) conducted several replacement series experiments to assess the conditions under which bahiagrass was most competitive with smutgrass.

For the bahiagrass/smutgrass study, and for replacement series experiments in general, a fixed total number of individuals (plants), referred to as the *planting density* p , are in each experimental unit (planting container). Rana (2012) considered planting densities of $p = 2, 4$, and 8 . The proportions of each of two species, A and B, are varied. Expressed as a ratio of the number of individuals of species A to species B, at a fixed planting density, the smutgrass study was conducted using *planting ratios* 1:0, 1:1, and 0:1 for $p = 2$ and 1:0, 3:1, 1:1, 1:3, and 0:1 for $p = 4$ and 8 . The ratios 1:0 and 0:1 represent, respectively, species A and B being grown in monoculture. Note that, to obtain the specified planting ratios, the planting density must be even when using the ratios 1:0, 1:1, and 0:1 and a multiple of four when considering the ratios 1:0, 3:1, 1:1, 1:3, and 0:1. The number of replicates is often small; Rana (2012) had $n = 3$ replicate planting containers for each of the planting ratios. For each experimental unit k , $k = 1, 2, \dots, n$, the dry total biomass of each species in monoculture as well as in each mixture, is recorded.

Let $X_{i:j,k}$ denote the mean biomass *per individual* of species A in planting container k at a planting ratio of $i:j$, and similarly let $Y_{i:j,k}$ denote the mean biomass *per individual* of species of B in planting container k at a planting ratio of $i:j$.

The Relative Crowding Coefficient (*RCC*) and the Relative Yield Total (*RYT*) are common indices of interspecies competition in replacement series experiments (see. *e.g.*, Harper (1977) and Oberg, *et al.* (1996)). For all i, j , suppose $X_{i:j,k}$ are independent and identically distributed (iid) with $E(X_{i:j,1}) = \mu_{i:j} < \infty$ for all k , and $Y_{i:j,k}$ are iid with $E(Y_{i:j,1}) = \nu_{i:j} < \infty$ for all k . Given the population means $\mu_{i:j}$ and $\nu_{i:j}$ of the dry yield per plant biomass for species A and B, respectively, in planting ratio $i : j$, the RYT is

$$RYT_{i:j} = \frac{\mu_{i:j}}{\mu_{1:0}} + \frac{\nu_{i:j}}{\nu_{0:1}}$$

Let $\bar{X}_{i:j}$ and $\bar{Y}_{i:j}$ denote the sample mean dry yield per plant of species A and species B at planting ratio $i:j$, respectively. Then the sample $RYT_{i:j}$ is

$$\widehat{RYT}_{i:j} = \bar{X}_{i:j}/\bar{X}_{1:0} + \bar{Y}_{i:j}/\bar{Y}_{0:1}.$$

Note that if the design considers only ratios 1:0, 1:1, and 0:1, there is only one RYT, $RYT_{1:1}$, whereas three RYT ($RYT_{3:1}$, $RYT_{1:1}$, and $RYT_{1:3}$) may be of interest when the planting ratios 1:0, 3:1, 1:1, 1:3, and 0:1 are used. Although not common in the literature, here we use a “hat” symbol to emphasize when the quantities presented are statistics.

Several variants of the *RCC* appear in the literature. One common definition (see, e.g. Oberg, *et al.* (1996)), considered here, is

$$RCC_1 = \frac{\mu_{1:1}/\nu_{1:1}}{\mu_{1:0}/\nu_{0:1}}.$$

Noting that, when the five planting ratios (1:0, 3:1, 1:1, 1:3, and 0:1) are included in the design, RCC_1 does not use all available information, Novak, *et al.* (1993) proposed an expanded form of the RCC for these designs:

$$RCC_2 = \frac{[(\mu_{3:1}/\nu_{3:1})/3 + (\mu_{1:1}/\nu_{1:1}) + 3(\mu_{1:3}/\nu_{1:3})]/3}{\mu_{1:0}/\nu_{0:1}}.$$

Both RCC_1 and RCC_2 are estimated by substituting sample estimates for population parameters; that is,

$$\widehat{RCC}_1 = \frac{\bar{X}_{1:1}/\bar{Y}_{1:1}}{\bar{X}_{1:0}/\bar{Y}_{0:1}}$$

and

$$\widehat{RCC}_2 = \frac{[(\bar{X}_{3:1}/\bar{Y}_{3:1})/3 + (\bar{X}_{1:1}/\bar{Y}_{1:1}) + 3(\bar{X}_{1:3}/\bar{Y}_{1:3})]/3}{\bar{X}_{1:0}/\bar{Y}_{0:1}},$$

respectively.

Harper (1977) describes four distinct relationships between the relative yields of species A and B (see Figure 1.1). For each species, per-individual yields are relative to the expected yield per individual in monoculture; that is, species A

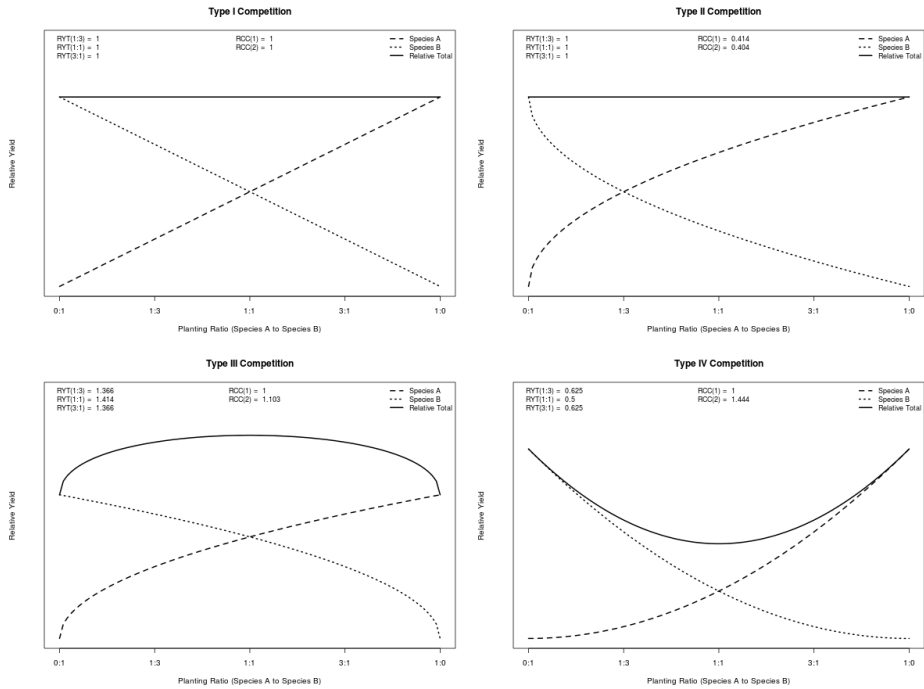


Figure 1.1: Four models of interspecific relative yield. Adapted from Harper (1977). For clarity, RCC_i is denoted $RCC(i)$, $i = 1, 2$. Furthermore, RYT is calculated at each planting ratio, with $RYT(i : j)$ denoting RYT at planting ratio $i:j$.

yield is scaled by $\mu_{1:0}$ and species B yield is scaled by $\nu_{0:1}$. Because RCC_1 and $RYT_{1:1}$ are functions of mean yields at only planting ratios 1:0, 1:1, and 0:1, we restrict attention to quadratic yield functions. When designs incorporate 3:1 and 1:3 planting ratios, quartic yield functions are theoretically possible but practically implausible and thus will not be considered.

In types I and II competition, $RYT_{1:1} = 1$. Thus, at a planting ratio of 1:1, the relative yield of A is the reflection of the relative yield of B about the line $y = 1/2$. If $RYT_{1:1} = 1$, then $RCC_1 = 1$ only if the relative yield is linear for both species. This may be interpreted as identical inter- and intra-species competitive effects. When $RYT_{1:1} = 1$ and $RCC_1 > 1$, however, species A has concave relative yield while species B has convex relative yield. The researcher infers that the competitive effect of species A on species B is stronger than the competitive effect of species A on itself. If $RYT_{1:1} = 1$ and $RCC_1 < 1$, species A has convex relative yield while species B has concave relative yield. Thus, the competitive effect of species B on species A is stronger than

the competitive effect of species A on itself.

Types III and IV indicate mutualistic or antagonistic interspecific relationships, respectively. In such cases the relative crowding coefficient is not a reasonable measure of interspecific competition. To see why, consider the examples of such competition illustrated in the bottom left and bottom right plots of Figure 1.1. In both cases the relative yield functions produce $RCC_1 \approx 1$, indicative of a lack of interspecific interaction, while in both cases there is a strong interspecific effect. This phenomenon may be observed whenever there exists $c \in [(0, 1/2) \cup (1/2, \infty)]$ such that the relative yields at 1:1 mixture $\bar{X}_{1:1}/\bar{X}_{1:0} \approx c$ and $\bar{Y}_{1:1}/\bar{Y}_{0:1} \approx c$. In this case,

$$\widehat{RCC}_1 = \frac{\bar{X}_{1:1}/\bar{X}_{1:0}}{\bar{Y}_{1:1}/\bar{Y}_{0:1}} \approx c/c = 1.$$

Hence, on the basis of RCC_1 alone the researcher concludes that neither species is out-competing the other. Type III competition is characterized by a mutualistic relationship between the two species; thus, the interpretation is that intraspecific competitive effects dominate interspecific competitive effects. Type IV competition is characterized by an antagonistic relationship between the two species with strong interspecific competitive effects for both species. Noteworthy is that $RYT_{1:1} \neq 1$ for competition types III and IV.

Upon inspection of Figure 1.1, it is clear that the use of RCC_2 as a measure of interspecific competition is also problematic in competition types III and IV. For competition types I and II, in which $RYT_{i:j} = 1$ for all i, j , RCC_2 may be interpreted in the same manner as RCC_1 . Therefore, whether the planting ratios are 1:0, 1:1, and 0:1 or 1:0, 3:1, 1:1, 1:3, and 0:1, the RCC is meaningful only if $RYT_{i:j} = 1$. Oberg, *et al.* (1996) developed inference procedures for testing $RCC_1 = 1$, assuming $RYT_{1:1} = 1$. However, no methods assessing the validity of this assumption have been considered.

The purpose of this paper is to develop methods for testing $RYT_{i:j} = 1$. The theory is developed in Section 2. The behavior of the proposed tests is explored via a simulation study in Section 3. The methods are used to draw inference for the replacement series experiments from Rana (2012) in Section 4. In the final sections, conclusions are summarized.

2. Tests of Constant Relative Yield

Plant weights tend to be normally distributed. For a replacement series experiment, the observed mean of plant weights of each species, which is the response

of interest, should be independent from one planting container to another. However, due to competitive effects, the means of species A and B within the same container should be correlated. Under this set of assumptions, a Wald test is developed to test the hypothesis of $RYT = 1$. First, a replacement series experiment with the planting ratios 1:0, 3:1, 1:1, 1:3, and 0:1 is considered. Here the hypothesis of interest is $RYT_{3:1} = RYT_{1:1} = RYT_{1:3} = 1$. The test can then be extended for replacement series studies with planting ratios of 1:0, 1:1, and 0:1 to test the hypothesis $RYT_{1:1}$. Because the Wald test is an asymptotic one and the number of replications tends to be small, bootstrap tests based on the derived test statistic are also considered. We begin by establishing the notation needed.

As biomass measurements, each random variable $X_{i:j,k}$ and $Y_{i:j,k}$ is non-negative and finite with probability 1. Hence, $E[(X_{i:j,k})^p]$ and $E[(Y_{i:j,k})^p]$ are assumed to be finite for all $p \in [1, \infty)$. Recall that, for each species and planting ratio we suppose replicate per-plant yield measurements are i.i.d. for $k = 1, 2, \dots, n$, with $E(X_{i:j,1}) = \mu_{i:j}$ and $E(Y_{i:j,1}) = \nu_{i:j}$ denoting the mean per-plant yield of species A and species B at planting ratio $i:j$, respectively. Let $\text{Var}(X_{i:j,1}) = \sigma_{i:j}^2$, and $\text{Var}(Y_{i:j,1}) = \tau_{i:j}^2$ denote per-plant yield variance for species A and species B at planting ratio $i:j$, respectively. Denote $\text{Cov}(X_{i:j,1}, Y_{i:j,1}) = (\sigma\tau)_{i:j}$. We suppose $\mu_{i:j} > 0$ for $i:j \in \{1:0, 3:1, 1:1, 1:3\}$ and $\nu_{i:j} > 0$ for $i:j \in \{3:1, 1:1, 1:3, 0:1\}$, so that mean total biomass is positive whenever a given species is employed at a particular planting ratio. Lastly, to simplify notation we let $R_{i:j} \equiv RYT_{i:j}$ and $\hat{R}_{i:j} \equiv \widehat{RYT}_{i:j}$, where $i:j \in \{3:1, 1:1, 1:3\}$. We further take $\mathbf{R} \equiv (R_{3:1}, R_{1:1}, R_{1:3})'$ and $\hat{\mathbf{R}} \equiv (\hat{R}_{3:1}, \hat{R}_{1:1}, \hat{R}_{1:3})'$.

2.1. Designs with All Planting Ratios

We now let $\mathbf{Z}'_k = (Z_{k,1}, Z_{k,2}, \dots, Z_{k,8})$ with $Z_{k,1} = X_{1:0,k}$, $Z_{k,2} = X_{3:1,k}$, $Z_{k,3} = X_{1:1,k}$, $Z_{k,4} = X_{1:3,k}$, $Z_{k,5} = Y_{0:1,k}$, $Z_{k,6} = Y_{3:1,k}$, $Z_{k,7} = Y_{1:1,k}$, and $Z_{k,8} = Y_{1:3,k}$. Suppose \mathbf{Z}_k are iid for $k = 1, 2, \dots, n$ where $Z_{k,i}$ have finite second moments for all k, i . Let

$$E(\mathbf{Z}'_1) \equiv \boldsymbol{\mu}' = (\mu_{1:0}, \mu_{3:1}, \mu_{1:1}, \mu_{1:3}, \nu_{0:1}, \nu_{3:1}, \nu_{1:1}, \nu_{1:3})$$

and let $\text{Var}(\mathbf{Z}_1) = \boldsymbol{\Sigma}$. Under the assumption that \mathbf{Z}_k are iid, and with $\bar{\mathbf{Z}} = n^{-1} \sum_{k=1}^n \mathbf{Z}_k$, by the multivariate central limit theorem (see, e.g., Lehmann and Casella (1998)), $\sqrt{n}(\bar{\mathbf{Z}} - \boldsymbol{\mu}) \rightsquigarrow N(\mathbf{0}, \boldsymbol{\Sigma})$, where \rightsquigarrow denotes convergence

in distribution. In order to construct the asymptotic distribution of $\hat{\mathbf{R}}$ under the above assumptions, we employ the multivariate delta method (Lehmann and Casella, 1998). To do so necessitates assumptions about the structure of $\boldsymbol{\mu}$ as well as the existence of a function $f : \mathbb{R}^8 \mapsto \mathbb{R}^3$ such that $f(\bar{\mathbf{Z}}) = \hat{\mathbf{R}}$ and $f(\boldsymbol{\mu}) = \mathbf{R}$. The function $f(\cdot)$ must satisfy several properties. Using the multivariate delta method, we establish

$$\sqrt{n}(f(\bar{\mathbf{Z}}) - f(\boldsymbol{\mu})) = \sqrt{n}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow N(\mathbf{0}, \boldsymbol{\Sigma}_R),$$

where $\boldsymbol{\Sigma}_R \equiv (\nabla f_{\boldsymbol{\mu}})\boldsymbol{\Sigma}(\nabla f_{\boldsymbol{\mu}})'$. Here $\nabla f_{\boldsymbol{\mu}} \equiv \{\frac{\partial f_i}{\partial t_j}|_{\mathbf{t}=\boldsymbol{\mu}}\}$, $i = 1, 2, \dots, 3$ and $j = 1, 2, \dots, 8$, the 3×8 matrix of partial derivatives of f evaluated at $\boldsymbol{\mu}$.

Define $f : \mathbb{R}^8 \mapsto \mathbb{R}^3$ such that $f(\mathbf{t}) = (f_1(\mathbf{t}), f_2(\mathbf{t}), f_3(\mathbf{t}))'$ and $f_i(\mathbf{t}) = \frac{t_{i+1}}{t_1} + \frac{t_{i+5}}{t_5}$. Then $f(\bar{\mathbf{Z}}) = (\hat{R}_{3:1}, \hat{R}_{1:1}, \hat{R}_{1:3})'$, and $f(\boldsymbol{\mu}) = (R_{3:1}, R_{1:1}, R_{1:3})'$, so that the elements of $f(\bar{\mathbf{Z}})$ and $f(\boldsymbol{\mu})$ are the sample and population analogues of Relative Yield Total at planting ratios 3:1, 1:1, and 1:3, respectively. Under the assumption that there exists $\epsilon > 0$ such that $\min_{i,j}(\mu_{i:j}, \nu_{i:j}) > 2\epsilon$, and by noting that all partial derivatives $\frac{\partial f_i}{\partial t_j}$ exist for $\|\boldsymbol{\mu} - \mathbf{t}\|_{\infty} < \epsilon$ and are furthermore continuous at $\boldsymbol{\mu}$, the function f is totally differentiable in an ϵ -neighborhood of $\boldsymbol{\mu}$. Thus, with $\nabla f_{\boldsymbol{\mu}}$ given by

$$\nabla f_{\boldsymbol{\mu}} = \begin{bmatrix} \frac{-\mu_{3:1}}{\mu_{1:0}^2} & \frac{1}{\mu_{1:0}} & 0 & 0 & \frac{-\nu_{3:1}}{\nu_{0:1}^2} & \frac{1}{\nu_{0:1}} & 0 & 0 \\ \frac{-\mu_{1:1}}{\mu_{1:0}^2} & 0 & \frac{1}{\mu_{1:0}} & 0 & \frac{-\nu_{1:1}}{\nu_{0:1}^2} & 0 & \frac{1}{\nu_{0:1}} & 0 \\ \frac{-\mu_{1:3}}{\mu_{1:0}^2} & 0 & 0 & \frac{1}{\mu_{1:0}} & \frac{-\nu_{1:3}}{\nu_{0:1}^2} & 0 & 0 & \frac{1}{\nu_{0:1}} \end{bmatrix},$$

by the multivariate delta method we have

$$\sqrt{n}(f(\bar{\mathbf{Z}}) - f(\boldsymbol{\mu})) = \sqrt{n}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow N(\mathbf{0}, \boldsymbol{\Sigma}_R),$$

where $\boldsymbol{\Sigma}_R \equiv (\nabla f_{\boldsymbol{\mu}})\boldsymbol{\Sigma}(\nabla f_{\boldsymbol{\mu}})'$. Some algebra shows that the s^{th} element along the diagonal of $\boldsymbol{\Sigma}_R$ is

$$\left(\frac{\mu_s \sigma_{1:0}}{\mu_{1:0}^2}\right)^2 + \frac{\sigma_s^2}{\mu_{1:0}^2} + \frac{2(\sigma\tau)_s}{\mu_{1:0}\nu_{0:1}} + \frac{\tau_s^2}{\nu_{0:1}^2} + \left(\frac{\nu_s \tau_{0:1}}{\nu_{0:1}^2}\right)^2$$

and that the $(s, t)^{th}$ element of $\boldsymbol{\Sigma}_R$ is

$$\frac{\mu_s \mu_t \sigma_{1:0}^2}{\mu_{1:0}^4} + \frac{\nu_s \nu_t \tau_{0:1}^2}{\nu_{0:1}^4},$$

where $s \in \{1, 2, 3\}$ corresponds to planting ratio $(3-s+1):s$ and it is understood that 2:2 is equivalent to 1:1. Symbolic evaluation of the product $(\nabla f_{\boldsymbol{\mu}})\boldsymbol{\Sigma}(\nabla f_{\boldsymbol{\mu}})'$ was performed in MATLAB.

Consider now the problem of making family-wise inference about $\{R_{3:1}, R_{1:1}, R_{1:3}\}$ (e.g. the elements of \mathbf{R}). This is the problem we must address in order to make inferences about interspecific competition between species A and B with RCC_2 . We may use RCC_2 only if $RYT_{i:j} = 1$ across all planting ratios. Thus, in order to use RCC_2 we must fail to reject the null hypothesis that $\mathbf{R} = \mathbf{1}$. That is, we may use RCC_2 only if we fail to reject the null hypothesis $H_0 : \mathbf{R} = \mathbf{1}$. To this end, if $|\Sigma_R| > 0$ and $\Sigma_R^{\frac{1}{2}}$ is the ‘‘square root’’ matrix of Σ_R such that $\Sigma_R^{\frac{1}{2}}\Sigma_R^{\frac{1}{2}} = \Sigma_R$, then $\sqrt{n}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow N(\mathbf{0}, \Sigma_R)$ implies that $\sqrt{n}\Sigma_R^{-\frac{1}{2}}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow N(\mathbf{0}, \mathbf{I}_3)$. Therefore, $n(\hat{\mathbf{R}} - \mathbf{R})'\Sigma_R^{-1}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow \chi_3^2$, and for any consistent estimator $\hat{\Sigma}_R$ of Σ_R ,

$$n(\hat{\mathbf{R}} - \mathbf{R})'\hat{\Sigma}_R^{-1}(\hat{\mathbf{R}} - \mathbf{R}) \rightsquigarrow \chi_3^2. \quad (2.1)$$

From (2.1), it follows that under $H_0 : \mathbf{R} = (R_{3:1}, R_{1:1}, R_{1:3})' = \mathbf{1}$,

$$X^2 = n(\hat{\mathbf{R}} - \mathbf{1})'\hat{\Sigma}_R^{-1}(\hat{\mathbf{R}} - \mathbf{1}) \stackrel{H_0}{\rightsquigarrow} \chi_3^2. \quad (2.2)$$

An asymptotic level- α Wald test of H_0 may be conducted by rejecting $H_0 : \mathbf{R} = (R_{3:1}, R_{1:1}, R_{1:3})' = \mathbf{1}$ whenever $n(\hat{\mathbf{R}} - \mathbf{1})'\hat{\Sigma}_R^{-1}(\hat{\mathbf{R}} - \mathbf{1}) > \chi_{3,1-\alpha}^2$, where $\Pr(\chi_{3,1-\alpha}^2 > \chi_3^2) = 1 - \alpha$.

2.2. Designs with 1:0, 1:1, and 0:1 Planting Ratios

When the experimental design incorporates only monocultures and a 1:1 planting ratio, we may construct the large-sample distribution of $\hat{R}_{1:1}$ by borrowing from the calculations of the previous section. In this case, several diagonal elements of Σ are zero, and hence the distribution of \mathbf{Z}_1 is now degenerate. However, in what follows we shall see that the borrowed calculations are independent of all degenerate random variables in \mathbf{Z}_1 . We will thus arrive at the same solution had we redefined \mathbf{Z}_k with degenerate random variables omitted. Moving forward, suppose now $f : \mathbb{R}^8 \rightarrow \mathbb{R}$ is the function f_2 defined earlier. The gradient of $f_2(\mathbf{t})$ evaluated at $\mathbf{t} = \boldsymbol{\mu}$ is then the second row of $\nabla f_{\boldsymbol{\mu}}$. Hence, by the univariate delta method (see, e.g., Lehmann and Casella (1998)),

$$\sqrt{n}(f_2(\bar{\mathbf{Z}}) - f_2(\boldsymbol{\mu})) = \sqrt{n}(\hat{R}_{1:1} - R_{1:1}) \rightsquigarrow N(0, \sigma_R^2),$$

where $\sigma_R^2 \equiv \left(\frac{\mu_{1:1}\sigma_{1:0}}{\mu_{1:0}^2}\right)^2 + \frac{\sigma_{1:1}^2}{\mu_{1:0}^2} + \frac{2(\sigma\tau)_{1:1}}{\mu_{1:0}\nu_{0:1}} + \frac{\tau_{1:1}^2}{\nu_{0:1}^2} + \left(\frac{\nu_{1:1}\tau_{0:1}}{\nu_{0:1}^2}\right)^2$. With $\hat{\sigma}_R^2$ a consistent estimator of σ_R^2 , an asymptotic two-sided level- α test of

$H_0 : R_{1:1} = 1$ is conducted by rejecting H_0 whenever

$$\left| \frac{\sqrt{n}(\hat{R}_{1:1} - 1)}{\hat{\sigma}_R} \right| > t_{n-1, 1-\alpha/2},$$

where $t_{n-1, 1-\alpha/2}$ is the $1 - \frac{\alpha}{2}$ quantile of a t distribution with $df = n - 1$.

2.3. Bootstrap Tests of Significance

In most replacement series experiments, the number of replicates is small, often less than five. Further, when working with plants, such as the grass in the study of interest, or insects, the distribution of biomass tends to be skewed right because no weight can be negative. As a consequence, the Wald test is unlikely to have desirable properties in areas of the parameter space of greatest interest from a biological perspective. Thus, we consider bootstrap tests of significance using the Wald test statistic X^2 given by (2.2).

For each observed sample, $B = 1000$ bootstrap samples are drawn, indexed here by b . It is important for the bootstrap sample to be drawn in accordance with the design of the study. For the bahiagrass/smutgrass study, the design was a completely randomized design. Thus, within each planting ratio, observations of biomass associated with a planting container were drawn at random and with replacement. By drawing the observation for a planting container (experimental unit), any correlation among plants within a container is preserved.

For each bootstrap sample, the test statistic, X_b^2 is calculated. Let $\hat{\mathbf{R}}$ and $\hat{\Sigma}_R$ denote sample estimates of \mathbf{R} and Σ_R . Further, let $\hat{\mathbf{R}}_b$ and $\hat{\Sigma}_{R,b}$ denote estimates of \mathbf{R} and Σ_R in the b^{th} bootstrap replicate drawn from the observed data. Following the second guideline of Hall and Wilson (1991), the bootstrap test statistic X_b^2 is

$$X_b^2 = n(\hat{\mathbf{R}}_b - \hat{\mathbf{R}})' \hat{\Sigma}_{R,b}^{-1} (\hat{\mathbf{R}}_b - \hat{\mathbf{R}}). \quad (2.3)$$

The p -value associated with the test statistic is $p = 10^{-3} \sum_{b=1}^{1000} I(X_b^2 > X^2)$.

3. Simulation Study

A simulation study was conducted to assess the size and power of the asymptotic Wald testing procedure of $H_0 : \mathbf{R} = \mathbf{1}$ using the test statistic given by (2.2). The study design was a $3 \times 4 \times 6$ factorial arrangement of three factors: the number of replicates n per planting ratio, $n \in \{3, 4, 6, 8, 10, 20, 100\}$; a scaling parameter s for the mean vector such that the means increase with increasing s , $s \in \{2, 5, 10\}$; and the type of interspecies competition (I, II, III, or

IV). For competition types I and II the null hypothesis $H_0 : \mathbf{R} = \mathbf{1}$ is true, and for competition types III and IV the null hypothesis $H_0 : \mathbf{R} = \mathbf{1}$ is false. Thus, there were 72 settings of the simulation parameters, 36 for which the null hypothesis was true and 36 for which the null hypothesis was false. One thousand samples were generated for each combination of the simulation parameters.

For each simulated sample, per-plant yields were generated. For the monocultures (1:0 and 0:1), independent gamma random variates with mean a and variance ab^2 were generated. For each of the mixtures (3:1, 1:1, and 1:3), random variates were drawn from a two-dimensional Gumbel($\alpha = 2$) copula with gamma marginal distributions using the `copula` package in R (Yan, 2007). The marginal gamma distributions were parameterized by a and b , with mean ab and variance ab^2 . In all cases, the b parameter was fixed at one so that each marginal mean was equal to the a parameter. We let $\boldsymbol{\mu}$ denote the vector of marginal means.

Competition type defined the vector of marginal gamma means, $\boldsymbol{\mu}$. The four functional forms of competition type considered in the simulation experiment are plotted in Figure 1.1. Let t correspond to the proportion of species Y plants in a container, e.g., $t = 0$ for planting ratio 1:0 and $t = 0.25$ for planting ratio 3:1. To assess Wald test size under Type I competition, the functions $f_X(t) = (1 - t)$ and $g_Y(t) = t$ were used to construct the simulation means of species X and Y , respectively, so that the vector of marginal gamma means is

$$\boldsymbol{\mu} = s[f_X(0), f_X(0.25), f_X(0.5), f_X(0.75), \\ g_Y(0.25), g_Y(0.5), g_Y(0.75), g_Y(1)]'.$$

Note that the means are increased by the factor s , which is why we refer to it as a scaling parameter. Under Type II competition, the functions $f_X(t) = 1 - \sqrt{t}$ and $g_Y(t) = \sqrt{t}$ were used to calculate the simulation means of species X and Y , respectively. To assess the power of the Wald test, under Type III competition, the functions $f_X(t) = \sqrt{1-t}$ and $g_Y(t) = \sqrt{t}$ were used to calculate the simulation means of species X and Y , respectively. Lastly, the functions $f_X(t) = (1-t)^2$ and $g_Y(t) = t^2$ were used to calculate the simulation means of species X and Y , respectively, under Type IV competition.

The use of the copula provided correlated positive observations for mixtures of plants within a planting container. However, the independence among planting containers was maintained, and a desired marginal mean vector $\boldsymbol{\mu}$ obtained. The gamma marginal distributions with $b = 1$ are asymmetric and right skewed.

The empirical size and power of the asymptotic Wald test and the bootstrap test based on the Wald test statistic were assessed at the 5% significance level. The empirical size under Types I and II competition (“Wald Size” and “Bootstrap Size” in Table 3.1) and the empirical power under Types III and IV competition (“Wald Power” and “Bootstrap Power” in Table 3.2) were determined based on 1000 simulated samples. The mean of the bootstrap p -values, $\bar{p}_B = 10^{-3} \sum_{i=1}^{1000} p_i$, is reported in Tables 3.1 and 3.2, along with the median of the bootstrap p -values, $m(p_B)$.

Table 3.1: The empirical size of the asymptotic Wald and the bootstrap tests using a 5% significance level. For each number of replicates per planting ratio, n , and for each scale parameter, s , the mean of the bootstrap p -value across all 1000 simulated samples is \bar{p}_B and the median bootstrap p -values is $m(p_B)$. Note that ‘Type’ refers to competition type.

n	s	Type	\bar{p}_W	\bar{p}_B	$m(p_W)$	$m(p_B)$	Wald Size	Bootstrap Size
3	2	I	0.348	0.553	0.281	0.58	0.342	0.003
3	2	II	0.328	0.542	0.237	0.57	0.373	0.007
3	5	I	0.358	0.565	0.282	0.585	0.304	0.003
3	5	II	0.335	0.557	0.249	0.567	0.315	0.002
3	10	I	0.368	0.579	0.315	0.606	0.273	0.002
3	10	II	0.358	0.581	0.28	0.596	0.265	0.001
4	2	I	0.345	0.491	0.281	0.494	0.315	0.041
4	2	II	0.365	0.512	0.3	0.521	0.292	0.035
4	5	I	0.379	0.514	0.343	0.528	0.247	0.025
4	5	II	0.392	0.531	0.342	0.533	0.23	0.013
4	10	I	0.391	0.523	0.358	0.53	0.243	0.019
4	10	II	0.405	0.54	0.346	0.543	0.214	0.017
6	2	I	0.393	0.484	0.362	0.484	0.247	0.058
6	2	II	0.409	0.503	0.393	0.512	0.225	0.056
6	5	I	0.442	0.522	0.421	0.512	0.183	0.041
6	5	II	0.425	0.508	0.412	0.517	0.188	0.042
6	10	I	0.432	0.509	0.417	0.506	0.155	0.041
6	10	II	0.434	0.514	0.416	0.524	0.166	0.035
8	2	I	0.398	0.467	0.374	0.462	0.246	0.08
8	2	II	0.415	0.488	0.397	0.494	0.213	0.056
8	5	I	0.451	0.509	0.453	0.519	0.158	0.05
8	5	II	0.454	0.514	0.432	0.513	0.128	0.036
8	10	I	0.448	0.504	0.425	0.494	0.118	0.036
8	10	II	0.449	0.506	0.445	0.518	0.136	0.044

Continued Table 3.1: The empirical size of the asymptotic Wald and the bootstrap tests using a 5% significance level. For each number of replicates per planting ratio, n , and for each scale parameter, s , the mean of the bootstrap p -value across all 1000 simulated samples is \bar{p}_B and the median bootstrap p -values is $m(p_B)$. Note that ‘Type’ refers to competition type.

n	s	Type	\bar{p}_W	\bar{p}_B	$m(p_W)$	$m(p_B)$	Wald Size	Bootstrap Size
10	2	I	0.434	0.493	0.433	0.502	0.166	0.054
10	2	II	0.421	0.482	0.412	0.484	0.195	0.069
10	5	I	0.457	0.504	0.452	0.502	0.139	0.063
10	5	II	0.451	0.502	0.43	0.501	0.137	0.049
10	10	I	0.462	0.506	0.442	0.501	0.105	0.043
10	10	II	0.443	0.49	0.425	0.48	0.122	0.049
20	2	I	0.446	0.483	0.451	0.49	0.135	0.059
20	2	II	0.472	0.507	0.467	0.503	0.114	0.057
20	5	I	0.467	0.493	0.463	0.49	0.101	0.055
20	5	II	0.483	0.508	0.489	0.518	0.1	0.067
20	10	I	0.489	0.512	0.507	0.532	0.087	0.054
20	10	II	0.457	0.481	0.446	0.474	0.106	0.067
100	2	I	0.487	0.497	0.49	0.504	0.07	0.052
100	2	II	0.489	0.498	0.491	0.504	0.064	0.048
100	5	I	0.498	0.504	0.507	0.513	0.061	0.055
100	5	II	0.493	0.499	0.494	0.5	0.054	0.048
100	10	I	0.501	0.506	0.517	0.524	0.055	0.044
100	10	II	0.499	0.504	0.514	0.514	0.061	0.052

Table 3.2: The empirical power of the bootstrap testing procedure under across all simulation studies. For each number of replicates per planting ratio, n , and for each scale parameter, s , the mean of the bootstrap p -values across all 1000 simulated samples is \bar{p}_B and the median bootstrap p -value is $m(p_B)$. Note that ‘Type’ refers to competition type.

n	s	Type	\bar{p}_W	\bar{p}_B	$m(p_W)$	$m(p_B)$	Wald Power	Bootstrap Power
3	2	III	0.115	0.35	0.00	0.318	0.737	0.046
3	2	IV	0.386	0.595	0.365	0.614	0.204	0.002
3	5	III	0.086	0.323	0.00	0.282	0.778	0.042
3	5	IV	0.335	0.571	0.269	0.576	0.226	0.00
3	10	III	0.032	0.254	0.00	0.199	0.893	0.042
3	10	IV	0.252	0.517	0.164	0.52	0.309	0.00
4	2	III	0.129	0.296	0.00	0.224	0.711	0.174
4	2	IV	0.418	0.557	0.401	0.569	0.126	0.004
4	5	III	0.061	0.202	0.00	0.134	0.818	0.25
4	5	IV	0.337	0.497	0.279	0.482	0.187	0.004
4	10	III	0.023	0.128	0.00	0.077	0.921	0.377
4	10	IV	0.203	0.39	0.118	0.367	0.336	0.007
6	2	III	0.105	0.219	0.00	0.126	0.748	0.312
6	2	IV	0.413	0.507	0.382	0.493	0.079	0.007
6	5	III	0.039	0.119	0.00	0.057	0.86	0.485
6	5	IV	0.282	0.393	0.217	0.356	0.18	0.013
6	10	III	0.007	0.047	0.00	0.015	0.968	0.743
6	10	IV	0.15	0.258	0.067	0.203	0.44	0.056
8	2	III	0.088	0.181	0.00	0.096	0.775	0.375
8	2	IV	0.399	0.477	0.379	0.465	0.063	0.01
8	5	III	0.026	0.075	0.00	0.023	0.908	0.664
8	5	IV	0.24	0.324	0.169	0.282	0.224	0.029
8	10	III	0.004	0.022	0.00	0.004	0.985	0.892
8	10	IV	0.105	0.179	0.036	0.13	0.556	0.182

Continued Table 3.2: The empirical power of the bootstrap testing procedure under across all simulation studies. For each number of replicates per planting ratio, n , and for each scale parameter, s , the mean of the bootstrap p -values across all 1000 simulated samples is \bar{p}_B and the median bootstrap p -value is $m(p_B)$. Note that ‘Type’ refers to competition type.

n	s	Type	\bar{p}_W	\bar{p}_B	$m(p_W)$	$m(p_B)$	Wald Power	Bootstrap Power
10	2	III	0.069	0.145	0.00	0.062	0.801	0.462
10	2	IV	0.365	0.435	0.314	0.398	0.077	0.01
10	5	III	0.019	0.051	0.00	0.011	0.934	0.75
10	5	IV	0.194	0.264	0.116	0.208	0.295	0.063
10	10	III	0.001	0.009	0.00	0.001	0.997	0.961
10	10	IV	0.064	0.118	0.019	0.077	0.676	0.353
20	2	III	0.026	0.061	0.00	0.015	0.912	0.714
20	2	IV	0.257	0.302	0.185	0.24	0.183	0.042
20	5	III	0.001	0.006	0.00	0.00	0.997	0.971
20	5	IV	0.072	0.102	0.02	0.054	0.674	0.472
20	10	III	0.00	0.00	0.00	0.00	1.00	1.00
20	10	IV	0.006	0.015	0.00	0.006	0.967	0.932
100	2	III	0.00	0.00	0.00	0.00	1.00	1.00
100	2	IV	0.007	0.011	0.001	0.003	0.974	0.958
100	5	III	0.00	0.00	0.00	0.00	1.00	1.00
100	5	IV	0.00	0.00	0.00	0.00	1.00	1.00
100	10	III	0.00	0.00	0.00	0.00	1.00	1.00
100	10	IV	0.00	0.00	0.00	0.00	1.00	1.00

Based on Table 3.1, it is evident that the asymptotic Wald test has size well above the nominal level for small means and small numbers of replications. As the mean increases, the gamma becomes less skewed, allowing the asymptotic properties of the test to be observed for smaller numbers of replication. However, the observed size was at least 0.087 with 20 replications and the means considered here. Few replacement series experiments, especially those involving plants, have 20 or more replications. Therefore, the utility of the asymptotic Wald test for these studies is questionable at best. For three replications,

the size of the bootstrap test was well below the nominal level. When at least four replications are conducted, the bootstrap test based on the Wald statistic had empirical size ranging from 0.035 to 0.069 for the parameters considered in this study, which is reasonably close to the 0.05 nominal level.

Because the asymptotic Wald test had size well above the nominal level, its power is artificially inflated. For a sample of size 100, where its size began to approach the nominal level, its power was comparable to the bootstrap test. One hundred replications is rare in a replacement series experiment so the Wald test cannot be recommended. The bootstrap test had less power under type IV competition than under type III competition. For both type III and type IV competition, the power increased with the mean. When $s = 2$, the simulation means ranged from 0.5 to 2 under the null hypothesis, from 1 to 2 under Type III competition, and from 0.125 to 2 under type IV competition. The low power observed for type IV competition is likely explained, at least in part, by the presence of small means for some of the ratios. The power of the bootstrap test is low until $n = 20$ replications are present for type III competition, but more are required for type IV competition. However, if $s = 10$ and the means vary from 2.5 to 10, the bootstrap test has good power under type Iii competition with 6 replications.

4. Application

Smutgrass (*Sporobolus indicus*) is an invasive weed species in Florida pastures where bahiagrass (*Paspalum notatum*) is frequently used for cattle forage (Mislevy *et al.* (1999); Ezenwa *et al.* (2006)). Although controlling smutgrass in a small plot may be a manageable task, for cattle ranchers, the task is an economic challenge; any chemical or mechanical treatment must be applied to a vast area. Newman, *et al.* (2011) estimated that bahiagrass is planted on over two million acres in the state of Florida. Because large-scale alteration of soil pH is an economically viable treatment option, Rana (2012) conducted a set of replacement series experiments to assess the effect of pH on interspecific competition between bahiagrass and smutgrass. The two factors, pH with three levels (4.5, 5.5, and 6.5) and planting density with two levels $p \in \{4, 8\}$, were crossed, resulting in six treatment combinations. A replacement series experiment with three replications was conducted for each treatment. Replicate containers were planted at ratios 1:0, 3:1, 1:1, 1:3, and 0:1). Within the greenhouse, planting containers were placed at random. After a fixed period of

growth, all individuals were sacrificed. Above-ground biomass was separated from root matter and dried. Total dry weight per species, per planting container was recorded. For a fixed treatment, let the random variable $X_{i:j,k}$ and $Y_{i:j,k}$ denote the mean dry biomass of bahiagrass and smutgrass, respectively, from the k^{th} planting container at planting ratio $i : j$, $k = 1, 2, 3$. Sample means as well as unbiased estimates of variances and covariances are listed in Table 4.1. The study's goal was to assess the effects of planting density and pH on the relative competitiveness of the two species. Because RCC was to be used as the measure of competitiveness, the hypothesis of $RYT = 1$ for all planting ratios needed to be tested first. Both the asymptotic Wald test and the bootstrap test of significance based on the Wald test statistic were conducted (see Table 4.2). Because the Wald test has an observed size well above the nominal level, it is not surprising that its use led to rejection of H_0 in three of the six $\text{pH} \times p$ treatment groups. Further, because the bootstrap test was extremely conservative when only three replications were considered, the failure to reject the null hypothesis $H_0 : \mathbf{R} = \mathbf{1}$ for any of the six treatment groups is also to be expected. Thus, we would tend to proceed with the use of the RCC to assess inter-specific competition. However, we should recognize that the potential of a type II error is quite high.

Table 4.1: Per-plant means (grams), variances, and covariances from the greenhouse experiment of Rana (2012). $\hat{\mu}_{i:j}$ and $\hat{\sigma}_{i:J}^2$ are the sample mean and variance of per-plant mean dry biomass of bahiagrass. $\hat{\nu}_{i:j}$ and $\hat{\tau}_{i:J}^2$ are the sample mean and variance of per-plant mean dry biomass of smutgrass. $(\widehat{\sigma\tau})_{i:j}$ is the sample covariance of bahiagrass and smutgrass per-plant mean dry biomass measurements at planting ratio $i:j$.

Study	p	pH	Planting					
			Ratio	$\hat{\mu}_{i:j}$	$\hat{\nu}_{i:j}$	$\hat{\sigma}_{i:j}^2$	$\hat{\tau}_{i:j}^2$	$(\widehat{\sigma\tau})_{i:j}$
1	4	4.5	1:0	3.125	.	1.32	.	.
1	4	4.5	0:1	.	0.592	.	0.163	.
1	4	4.5	3:1	1.911	0.5	2.787	0.7	0.408
1	4	4.5	1:3	3.233	0.468	6.463	0.128	0.883
1	4	4.5	1:1	3.233	0.212	1.981	0.15	0.138
2	8	4.5	1:0	1.38	.	0.474	.	.
2	8	4.5	0:1	.	0.479	.	0.82	.
2	8	4.5	3:1	1.594	0.351	0.846	0.13	0.25
2	8	4.5	1:3	2.5	0.411	0.93	0.34	0.78
2	8	4.5	1:1	1.867	0.418	2.66	0.162	0.572
3	4	5.5	1:0	6.325	.	1.118	.	.
3	4	5.5	0:1	.	4.45	.	1.183	.
3	4	5.5	3:1	5.833	5.67	3.314	4.493	-2.937
3	4	5.5	1:3	4.3	3.789	3.36	0.507	0.833
3	4	5.5	1:1	7.767	2.867	15.801	0.723	1.778

Continued Table 4.1: Per-plant means (grams), variances, and covariances from the greenhouse experiment of Rana (2012). $\hat{\mu}_{i:j}$ and $\hat{\sigma}_{i:j}^2$ are the sample mean and variance of per-plant mean dry biomass of bahiagrass. $\hat{\nu}_{i:j}$ and $\hat{\tau}_{i:j}^2$ are the sample mean and variance of per-plant mean dry biomass of smutgrass. $(\widehat{\sigma\tau})_{i:j}$ is the sample covariance of bahiagrass and smutgrass per-plant mean dry biomass measurements at planting ratio $i:j$.

Study	p	pH	Planting					
			Ratio	$\hat{\mu}_{i:j}$	$\hat{\nu}_{i:j}$	$\hat{\sigma}_{i:j}^2$	$\hat{\tau}_{i:j}^2$	$(\widehat{\sigma\tau})_{i:j}$
4	8	5.5	1:0	3.913	.	0.187	.	.
4	8	5.5	0:1	.	3.42	.	0.181	.
4	8	5.5	3:1	4.661	2.1	1.31	2.373	1.207
4	8	5.5	1:3	6.25	1.972	12.902	1.987	-4.837
4	8	5.5	1:1	6.5	1.717	1.24	0.481	0.435
5	4	6.5	1:0	5.508	.	1.575	.	.
5	4	6.5	0:1	.	3.667	.	2.823	.
5	4	6.5	3:1	4.922	3.8	1.588	1.99	1.747
5	4	6.5	1:3	4.533	3.78	10.703	0.398	-1.981
5	4	6.5	1:1	3.5	3.467	0.902	1.376	0.907
6	8	6.5	1:0	3.233	.	0.4	.	.
6	8	6.5	0:1	.	3.83	.	0.49	.
6	8	6.5	3:1	3.722	2.233	0.289	3.406	0.62
6	8	6.5	1:3	4.983	2.383	2.173	0.69	0.242
6	8	6.5	1:1	3.45	2.675	0.306	1.579	0.338

Table 4.2: The asymptotic Wald and bootstrap test of $H_0 : \mathbf{R} = \mathbf{1}$. The Wald test statistic is X_W^2 , and the Wald test asymptotic p -value is $p_W = \Pr(\chi_3^2 > X_W^2)$.

pH	n	X_W^2	$m(X_B^2)$	p_W	p_B
4.5	4	2.06	3.698	0.56	0.66
5.5	4	3.401	3.921	0.334	0.535
6.5	4	18.329	4.595	< 0.001	0.214
4.5	8	47.355	5.45	< 0.001	0.159
5.5	8	5.534	4.739	0.137	0.448
6.5	8	35.052	6.604	< 0.001	0.347

5. Discussion

Consider a replacement series experiment that is conducted in a greenhouse with one hundred experimental units per planting ratio. Here the experimental unit is generally a planting container. If five ratios are considered (1:0, 3:1, 1:1, 1:3, and 0:1), 500 containers are needed. Often, as in the application considered here, multiple replacement series experiments are conducted simultaneously to evaluate the effects of other factors, such as pH and planting density, on the competitive effects of the two species. Rana (2012) had six replacement series experiments, which would require 3000 experimental units when using $n = 100$ replications. It is evident that conducting these studies with this many replications quickly becomes prohibitive when considering the space, material, and personnel needed. In Tables 3.1 and 3.2 the power of the Wald test does not approach the nominal 0.05 level with fewer than one hundred experimental units per planting ratio. Thus, the asymptotic Wald test cannot be recommended for replications in the range researchers commonly consider feasible. At the same time, it is evident that the bootstrap test has poor properties with less than four replications.

Although the bootstrap test does control the size for four or more replications, it has low power for the numbers of replications commonly considered in replacement series experiments. As a consequence, one can be led to use the *RCC* when the assumption of $RYT = 1$ is not satisfied. For example, on the basis of the bootstrap test of $H_0 : \mathbf{R} = \mathbf{1}$ in the replacement series experiment of Rana (2012), we fail to reject H_0 for all $\text{pH} \times p$ treatment groups.

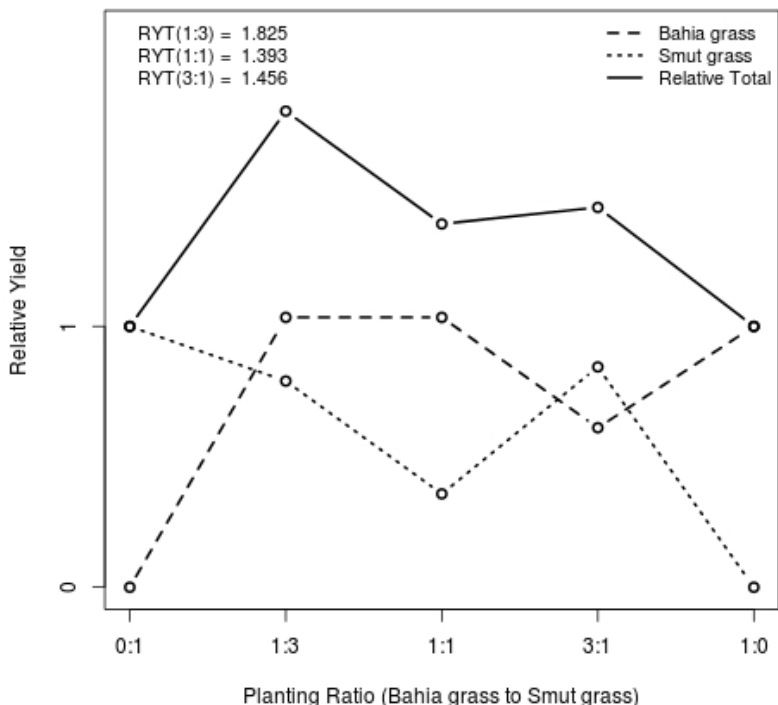


Figure 5.1: A relative yield plot for Bahia grass and Smut grass in Study 1 of Table 4.1.

The variability in biological studies tends to be high, making it difficult to detect significant effects. Consider the relative yield plot for Study 1 in Table 4.1 (Figure 5.1). The assumption of $RYT_{i,j} \neq 1$ appears questionable for at least some i, j , leading us to wonder whether natural variation in the data could be the cause or whether a type II error was made when the hypothesis $H_0 : \mathbf{R} = \mathbf{1}$ was not rejected.

Given the high variability associated with most biological studies, more replications are needed, in general, to obtain precise estimates. Although the focus is on RYT here, similar concerns can be raised about the precision with which RCC is estimated. This is often ignored in replacement series experiments.

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