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The effect of mixed cultivars plantings on pest abundance and grain yields in rice

Pengaruh penanaman campuran kultivar padi sawah terhadap kelimpahan hama dan hasil produksi tanaman

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ABSTRACT

A field study was carried out to assess the effects of mixed cultivar plantings on grain yields and on the abundance of pests in rice. Increasing plantation species diversity through cultivar mixtures is often claimed to decrease pest problems while stabilizing or even increasing yield, but the effects on pest abundance of planting rice cultivar mixtures in Indonesia have not been extensively studied. We tested for changes in pest abundance in experimental plots planted with five genetically distinct rice cultivars, combined in two different mixture arrangements (seed mix and row mix). These mixes were cultivated in lowland paddy areas, in replicated randomized block designs, during two growing seasons. Pest abundance was measured weekly in all plots, and rice yields were measured at harvest time. The results showed that the average abundance of pests was reduced in plots planted with cultivar mixes, compared to those planted with monocrops comprised of each of the component cultivars. Plots planted with the seed mix showed consistently reduced brown plant-hopper (*Nilaparvata lugens* (Stål)) abundance compared to monocrops in each growing season, with a relative reduction in pest abundance of 29.83% at the end of season 1 and 6.61% at the end of season 2, respectively. Plots planted with the row mix consistently showed decreased stem borer abundance compared to monocrops in each growing season, with a relative reduction in pest abundance of 100% at the end of season 1 and 1.4% at the end of season 2, respectively. In terms of yield, plant height proved to be a consistent yield component character, correlating positively with plant yield for both seed mix and row mix in both growing seasons. Our results showed higher average yields –and plant heights--for the mixed genotype plots compared to pure genotype stands in 2013. We found a greater relative increase in the yield of seed mix plots than row mix, measuring 7.26% and 4.63%, respectively in 2013. Among the two types of mixtures, seed mix showed higher overall grain yield. Our findings suggest that rice farmers can both increase yield and decrease pest abundance by planting cultivar mixes.

Key words: brown plant-hoppers, cultivars mixture, rice, row mix, seed mix, stems borers

ABSTRAK

Penelitian lapang telah dilakukan untuk mengkaji pengaruh percampuran kultivar padi sawah terhadap kelimpahan hama dan hasil gabah. Peningkatan keragaman spesies tanaman melalui percampuran

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kultivar sering diklaim berdampak positif untuk mengurangi masalah hama sekaligus menstabilkan atau bahkan meningkatkan hasil, tetapi efek percampuran kultivar padi terhadap kejadian hama di Indonesia belum banyak dipelajari. Kami menguji perubahan kelimpahan hama dalam plot percobaan yang ditanami lima kultivar padi yang berbeda secara genetik, dikombinasikan dalam dua pola pengaturan campuran yang berbeda (*seed mix* dan *row mix*). Campuran ini ditanam di area sawah dalam bentuk rancangan acak lengkap berulang selama dua musim tanam. Kelimpahan hama di semua plot pengujian diukur setiap minggu dan hasil gabah pada saat panen. Hasilnya menunjukkan bahwa rerata kelimpahan hama menurun pada plot yang ditanami oleh campuran kultivar dibandingkan dengan plot yang ditanami oleh tanaman tunggal dari masing-masing komponen kultivar. Plot dengan penanaman *seed mix* konsisten terhadap penurunan hama wereng coklat dikedua musim tanam, dengan penurunan relatif kelimpahan hama adalah masing-masing 29,83% pada musim pertama dan 6,61% pada musim kedua dibandingkan dengan monokultur. Plot dengan penanaman *row mix* konsisten terhadap penurunan relatif penggerek batang dalam campuran, yaitu masing-masing 100% pada musim pertama dan 1,4% pada musim kedua dibandingkan dengan monokultur. Dalam hal hasil, tinggi tanaman adalah karakter komponen hasil yang konsisten menunjukkan efek positif pada kedua tipe campuran di dua musim tanam. Dari penelitian juga menunjukkan hasil rata-rata lebih tinggi untuk plot genotipe campuran dibandingkan dengan genotipe murni pada musim tanam 2013. Peningkatan relatif hasil gabah dalam pengujian *seed mix* lebih baik dibandingkan dengan *row mix* berturut-turut, yaitu 7,26% pada *seed mix* dan 4,63% pada *row mix*, pada musim tanam 2013. Diantara kedua tipe campuran, *seed mix* menunjukkan hasil yang lebih baik. Hasil penelitian ini menyarankan pada petani untuk meningkatkan hasil dan menurunkan kelimpahan hama melalui percampuran kultivar.

Kata kunci: padi, penggerek batang, percampuran kultivar, *row mix*, *seed mix*, wereng coklat

INTRODUCTION

Rice (*Oryza sativa* L.) remains one of the primary staple foods in Indonesia. Therefore, rice represents an important source of food security and self-sufficiency in that country, such that its supply must be kept secure and sustainable. However, invertebrate pests and plant diseases are limiting factors for crop production in Indonesia, possibly triggered by farmers' cultivation practices. Generally, rice farming at all scales--from individual farmers to large scale commercial producers--is still dominated by monoculture cultivation. In a monoculture system, every plant in a field is nearly identical genetically to its neighbor, so that all of the plants share the same phenotypic vulnerabilities to pests, and there are no plants with different advantageous traits to resist predation. This allows insect pests and pathogens to move easily from plant to plant and decimate crop fields. Since the post-WWII "Green Revolution", monoculture cropping has become dominant, not only in rice producing countries, but in agriculture globally, for both field and plantation crops. The advantages of monoculture include ease, efficiency, and lowered costs--for planting, harvesting and other operations--all of which can be mechanized and produce consistent yield and crop quality. However, the limited genetic variation of monoculture crops constitutes a liability that leaves plants vulnerable to outbreaks of pests and diseases. If all the plants in a field are susceptible to the same pest species, pest populations will spread rapidly from one plant to another once a field is invaded (Stukenbrock & McDonald 2008; Tooker & Frank 2012). As a result, monocropping has led inevitably to the extensive use of pesticides. Maximum crop yield is achieved via repeated applications of insecticide, which has negative effects on non-target organisms, and for human and environmental health (Pimentel et al. 1992). Alternatives to single species/genotype planting are needed to reduce the intensity of pesticide use and pest damage both (Tooker & Frank 2012).

Rice cultivar breeding activities continue to produce new varieties, often with a focus on using crop varieties that are more tolerant or resistant to pests and diseases, in order to provide options for maintaining sustainable insect pest management programs in agricultural systems. Generally, the benefits of new varieties do not last long. Pathogen and pest resistance of new cultivars usually is overcome by new virulent pathogen strains or pests within a few years. Although first-generation cultivars have continually been replaced by new pest-resistant ones, this "arms race" between plant and pathogen/pest is a challenge for the sustainable management of new rice varieties, and for efforts to reduce use of fertilizers and pesticides (Sester et al. 2008). One potential strategy being investigated is the use of cultivar mixtures. Genotypically diverse mixtures can foster plant–plant interactions that may lead to improved resistance to pests and pathogens (Tooker & Frank 2012). Theories suggest that a complex plant species habitat promotes diversity of insect prey and predator populations (Root 1973; Bach 1980; Russell 1989), specifically predicting a reduced abundance of herbivore pests and increased species richness and abundance of their natural enemies (Root 1973).

There has been only limited applied research that explores the influence of intraspecific crop/plant diversity on insect pests and/or arthropods in agricultural systems. Nevertheless, cultivar mixtures have been shown to improve pest and disease suppression in some crop studies (Power 1991). The study of Jie et al. (2003) reports suppression of the white-backed plant-hopper (*Sogatella furcifera* (Horváth)) in plots cultivated with a mixture of resistant and susceptible rice varieties, indicating that this approach could efficiently suppress the development of *S. furcifera* and maintain rice yield without increased losses.

The most important advantage of growing cereal mixtures is the introduction of biodiversity which, because of the distinctive features of the introduced plants, makes better use of the environment's resources without disrupting its biological balance (Michalski et al. 2004; Szemplinski & Budzynski 2011). Indeed, in contrast to cultivar monocultures, a variety of biological (genetic and epidemiological) pest reducing mechanisms are active in genetically diverse sowing mixtures. Biological diversity within mixtures enables component plants to make better use of the habitat and agro-technical conditions; this is reflected in higher and more stable yields compared to those of cultivars sown separately (Gacek & Nadziak 2000; Tratwal & Walczak 2010; Walczak et al. 2011). Trenbath (1974) concluded that elements of such a mixture may complement each other, potentially resulting in more complete and efficient utilization of nutrients or water, and boosting average yields for mixture plots above those of single-component pure stands. Meanwhile Zhu et al. (2000) has demonstrated that genetic heterogeneity provides disease suppression and increased land productivity.

The use of cultivar mixtures in Indonesia may be implemented using elite rice varieties and diverse local rice varieties. Rice plant breeding activities there have resulted in more than 260 high yield rice varieties with varied levels of pest resistance. These have been approved for release as nationally-developed elite varieties but only a few are widely used by rice farmers. Generally, Indonesian farmers tend to plant the same preferred varieties every plant season, thus increasing the chances of genetic vulnerability to pest infestation. Promoting mixed cultivar agriculture as a method of pest control may be particularly beneficial for local farmers. Although the use of mixed rice varieties costs more, these costs will be offset by reductions in the use of expensive pesticides and fungicides, and in associated health and environmental risks. In their research, Zhu and colleagues showed that, by growing a simple mixture of rice (*Oryzae sativa*) varieties across thousands of farms in China, the spread of rice blast disease was restricted to acceptable levels that required no treatment with fungicide (Wolfe 2000).

In the present study, two mixtures (seed mix and row rix) were prepared, each containing a different combination of the same 5 genotypes of varying susceptibility and resistance to primary rice pests, particularly the brown plant-hopper. These two mixtures were cultivated, each in their own stand, to evaluate performance resisting insect pests. Performance of the mixed-variety plots was compared to that of plots cultivated with each of the 5 component rice varieties in pure-genotype stands. Crops were grown over two cropping seasons. The objective of this study was to assess whether use of rice genotype mixtures affects pest occurrence, as well as yield component measures (tiller and plant height), and grain yields.

MATERIALS AND METHOD

Experimental sites

The study was conducted over the same 10-week period during the dry growing seasons of

2012 and 2013, at the Center for Forecasting of Plant Pest Organisms, Jatisari, Cikampek, and West Java. The experimental location is a relatively flat area at an altitude of 10–15 meters a.s.l. The average air temperature is 27 ^{o}C, the average air pressure 0.001 millibars, solar radiation averages +70%, and relative humidity averages 85%. The range of annual rainfall is 1200–3300 mm/year. The experiment site is generally characterized by alluvial soil.

Genetic materials

Five rice genotypes expressing a range of resistance levels to pests—particularly the brown plant-hopper--were used to study the effect of mixed cultivar plantation on pest prevalence. Two types of mixes were created (seed mix and row mix), each comprised of a different combination of the cultivars (Table 1). All mixed component genotypes were chosen with no prior knowledge of their performance in mixtures. We sought genotypes that produced similar yields with a similar time to maturity, to control for natural yield variation between varieties and so that all plants would grow at similar rates. In addition, we selected genotypes that would introduce variety in plant architecture. The plant architecture of two genotypes (IPB 4S and IPB 117-4-1-1) is distinct from the other genotypes, with larger stems, longer and wider leaves, longer panicles, and fewer tillers.

Experimental design and field experiments

The experimental field area was laid out in a randomized complete blocks design with four replications. There were seven treatments, consisting of pure stands of each of the five rice genotypes, and a stand of each of the 2 genotype mixtures (Figure 1). The size of a single plot was 41.4 m^2 (length 4.60 m, width 9.0 m). Each plot consisted of 23 rows with an inter-row distance of 0.20 m and row length of 9.0 m. Each plot was separated by Mekongga rice variety on all sides, serving as a border to limit interplot interactions. The Mekongga border measured 2.0 m in between plots and 5.0 m between each block. Mekongga is moderately resistant to brown plant-hopper (biotype 2 and 3) and bacterial leaf blight disease. Seeds of the experimental genotypes were sown on a wet-bed for seedling production. Seedlings of each individual genotype were grown for use in the control/monocrop plots and the alternating-row plots. The rest of the seedlings were grown from

Table 1. Lowland rice genotypes used in this study and their reactions to different biotypes of brown plant-hopper

Genotypes	Reaction to BPH (biotype)			Source	Maturity (days)
	1	2	3		
IPB 117-F-4-1-1	MR	MR	MR	Bogor Agricultural University	110
IPB 4S	MR	MR	MR	Bogor Agricultural University	112
IR-64	R	R	MR	Rice research center	115
Inpari 11	S	S	S	Rice research center	108
Inpari 13	R	R	R	Rice research center	103

S: susceptible; MR: moderate resistant; and R: resistant.

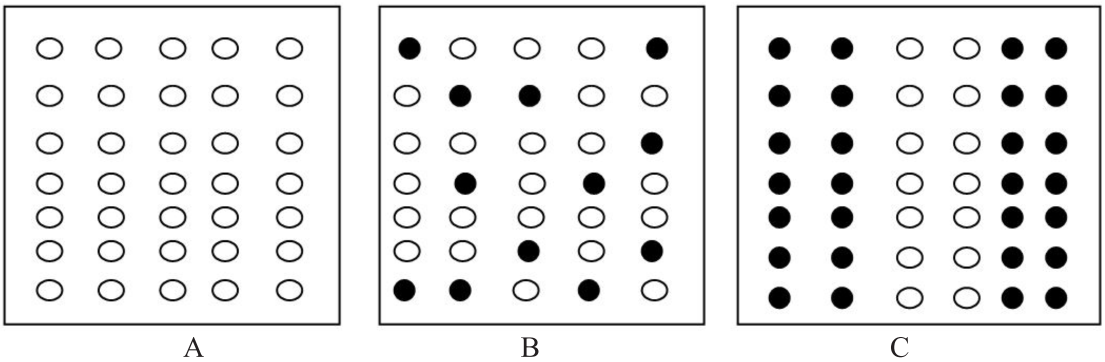


Figure 1. Planting patterns used in experimental plots containing different rice cultivars. A: pure stand of component cultivars (single genotype per stand); B: seed mixtures containing equal proportions of five rice genotypes (1:1:1:1:1); C: row mixtures (alternate strip planting) of five rice genotypes (alternating every nine rows of five planted genotypes). ○: single genotype; ●: mix genotype.

seed mixtures, for use in the random/mix rows. The seed mixtures were prepared by mixing equal proportions of five different genotypes before sowing, thereby preventing the identification of individual genotypes in the mixture by the observer. In between the monocrop plots, in order to keep them separate and avoid cross-contamination, we planted buffer areas using an "alternate strip layout", wherein seedlings for each pure genotype were planted in successive rows (genotype 1 in row 1, genotype 2 in row 2, through genotype 5). A section of alternate strip layout was sown after every ninth row of the single genotype plots, and using the same inter and intra-row distances as the respective single crops. Two seedlings aged 18-days after sowing (DAS) were planted per hole in the experimental fields. The in-row distance between holes was 0.20 m. Plant maintenance was carried out in accordance with conditions and crop requirements in the field. Normal fertilizer rate was 250 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5, 100 kg ha^{-1} K_2O. No pesticide was applied.

Treatments for the 2013 season replicated those of 2012, except that the mixture populations were derived from combine-harvested seeds from the previous year. Three types of field plots were established: five plots cultivated with component genotypes grown as pure stands, plots planted with the seed mixture, and plots planted with the row mixture.

Data collection

Measurements of tiller number, plant height, and pest populations were conducted during the vegetative phase until one week before harvest. Data was collected in all plots, for two important rice pests: brown plant-hoppers (*Nilaparvata lugens* (Stål)), and stem borers (*Scirpophaga incertulas* Walker). We measured the occurrence of pests in the experimental plots by visual identification in the field. During the growing season we observed five randomly selected plants in each of 4 experimental plots per treatment, for a total of 20 plants per treatment, per year. The number of individual pests on the selected plants in each plot was counted two weeks after planting, and subsequently every week thereafter, for 10 weeks. Tiller number and plant height were also measured at the same time. Pests were counted in situ and not harmed or removed for observation. After the initial random selection of sample plants, we examined the same plants during each weekly observation. Yields were calculated for all plots by measuring the mass of grain harvested for each replication, after cleaning and drying the samples. The population density and infestation intensity of pests was calculated from the population counts.

Data analysis

This study compared the yield and pest resistance of plantations comprised of pure stands of cultivars of a single genotype vs. stands of mixed-genotype cultivars. For each configuration, we measured physical traits including tiller number and plant height, and also measured pest incidence. All collected data were analyzed with statistical software (SPSS 16.0). In the case of pests with higher baseline abundance, the percentage reduction in abundance for plots planted with genotype mixtures was calculated relative to reductions observed in pure single-genotype stands (Finckh & Mundt 1992). Pest count data of pests were analyzed using two-way analysis (weeks, genotypes/mixtures) of variance (ANOVA) for each season separately using the general linear model (GLM) procedure. The general model equation was the following:

$$y_{ij} = \mu + \tau_i + \beta_{ij} + (\beta\tau)_{ij}$$

Where μ is an intercept, τ_i is the weekly invertebrate (pest) count, β_{ij} is the effect of genotype (pure stand vs. mixtures), and $(\beta\tau)_{ij}$ are interaction effects. ANOVA was applied to test the null hypothesis that the timing of observations/measurements; and the planting configuration of genotypes/mixtures, as well as the interaction between these two interventions, has no influence on the number of pests and PNESs found, nor on tiller number and plant height. Mean values and standard deviations were estimated. Duncan's analyses were applied to determine the difference between results found for each planting configuration (genotypes), and for different observation times.

The decrease in the relative efficacy of pest predation was calculated by comparing the number of pests in the plots planted with mixed-genotype cultivars, vs. the number of pests found in pure single-genotype stands, according to the methods of Finckh & Mundt (1992):

The relative efficacy (%) = [(A1-A2)/A1] × 100%

Where A1 is the number of pests counted in the single genotype plots, and A2 is the number of pests counted in plots planted with mixed-genotypes.

RESULTS

The occurrence of pests in the field

The ANOVA results calculated for the incidence of stem borers and brown plant-hoppers for both growing seasons are presented in Table 2. The effects of observation date, planting configuration by genotype (single vs. mixed) and the interaction of the two, were all highly significant (at $P < 0.001$) for two of the five genotypes. Such highly significant results indicated a differential response in the infestation intensity of stem borers, and in the population of brown plant-hoppers, between the plots planted with cultivars of mixed genotypes, compared to those planted with a single-genotype of either IPB 4S and IR64, during the ten-week experimental period.

The mean infestation intensity (%) of stem borers and mean population of brown plant-hoppers, in relation to planting configuration of rice genotypes in the two growing seasons, are presented in Table 3. In the 2012 growing season, Duncan analysis indicated that the results from plots planted with the seed mix vs. row mix were not greatly different from each other: 0.065% and 0.00% respectively ($P > 0.05$). When pure-genotype plots planted with genotypes IPB 4S, IPB 117-F-4-1-1, and Inpari 13, were compared with plots planted with the seed mix or the row mix, we found only a small difference in infestation intensity of stem borers for the pure vs. mixed-genotype paired plot comparisons and this difference was not statistically significant ($P \leq 0.05$). The highest infestation of stem borers was observed in single-genome stands planted with genotype IR64, and Inpari 11, measured at 0.291% and 0.153%.

Average brown plant-hopper populations were low in all of our experimental plots in 2012, and we found no significant difference ($P \leq 0.05$) in this measure when comparing the seed mix plots with the row mix plots, nor between the results for either of the mixed genotype plots compared to the single genotype plots planted with cultivars IPB 117-F-4-1-1, Inpari 11, and Inpari 13. There was, however, a significant difference between the mixed-genotype plots and plots planted with pure stands of IPB 4S and IR64. The plots planted with pure stands of IPB 4S and IR64 had the highest population of brown plant-hoppers compared to the mixed genotype plots and to the other single genotype plots. Inpari 13 proved to be a more resistant genotype compared to the other component genotypes, with low measures of infestation intensity (%) for stem borers, and of plant-hopper population, in plots planted with this genotype. By contrast, IR64 is the most susceptible genotype of the 5 tested, with the highest infestation intensity of stem borers and population of brown plant-hoppers.

The 2013 growing season saw an increase in the incidence of pests across all plots, compared to that measured in 2012. Both types of mixture showed significantly different results ($P \leq 0.05$) for the intensity of stem borer attacks, where row mixes were mixed types with a lower value of stem borer attack intensity of 1.013%. Among the pure stands, the results of the intensity of stem borer

Table 2. ANOVA results for average performance of the genotypes (pure stand vs. mixtures) based on weekly observation of traits measured during the 2012 & 2013 growing season

Source	F/P-statistic (2012 experiment)			F/P-statistic (2013 experiment)		
	Weeks	Stand type (pure or mixed)	Week x Stand type	Weeks	Stand type (pure or mixed)	Week x Stand type
Pests occurrence:						
Stem borers	3.446^{***}/0.001	3.125^{***}/0.006	1.960^{***}/0.00	27.541^{***}/0.00	8.408^{***}/0.00	2.80^{***}/0.00
Brown plant-hoppers	14.443^{***}/0.00	5.459^{***}/0.00	2.340^{***}/0.00	20.078^{***}/0.00	3.810^{***}/0.001	1.14^{*}/0.1

Significance level ***P-value 0.001–0.01; **P-value 0.01–0.05; *P-value 0.05–0.1, NS, non-significant.

attacks on the IPB 4S and IR64 genotypes were not significantly different from row mix types. While the single genotypes of Inpari 11 and Inpari 13 have lower intensity of stem borer attacks, which are 0.613% and 0.545% respectively. In contrast, the genotype IPB 117-F-4-1-1 has the highest intensity of stem borer attack, which is 2.336% compared to other single genotypes and mixed types. With regard to brown plant-hopper counts, pure plots of genotype IPB 117-F-4-1-1 had the lowest population of the pest, compared to other single-genotype plots, and compared to the mixed genotypes. There was no significant difference found between the population of brown plant-hoppers measured in plots planted with either of the mixed genotypes, and that measured in the single-genotype plots ($P \leq 0.05$).

The pattern of pest incidence during the ten weeks of observation fluctuated over time in both the mixed-genotype and the single-genotype plots (Figure 2). During the 2012 growing season, in all plots, it appears that the infestation intensity of stem borers rose, peaked at six weeks, and then declined through the tenth week. A similar pattern was seen in the population of brown plant-hoppers. Plant-hopper counts also peaked at weak 6, and plateaued from week 6 to 8, then declined subsequently until the tenth week. This shows that the peak incidence of stem borer and brown plant-hoppers occurs at the same time. We noted that in 2012 plots planted with only genotype IR64 showed the highest pest incidence compared with the other single-genotype plots and with the mixed genotype plots. Results for the 2013 season were somewhat different. The occurrence of pests in the 2013 growing season was generally higher across all plots than the previous season, with a peak infestation intensity of stem borers occurring in the ninth week, and the peak of brown plant-hopper population occurring in the fourth and sixth weeks during the growing phase. This pattern held true for all plots. Thus, the peak incidence of the two species did not occur at the same time, unlike the prior year. Overall, pest incidence for mixed-genotype plots was lower than for some of the single-genotype plots. This indicates that planting genotype mixtures can reduce the incidence of pests, compared to plots only planted with single component genotypes.

A relative decline in the effectiveness of plant mixtures in reducing the incidence of pests in mixed-genotype plots is shown in Figure 3. Higher relative pest reductions were observed in 2012 (3A) than in 2013. In 2012, relative pest reduction in plots planted with row mix was 100% for stem borers and 47.37% for brown plant-hoppers, while plots planted with seed mix saw a relative decrease for stem borers and brown plant-hoppers of 46.55% and 29.83%, respectively.

During the growing season in 2013 both positive and negative effects, with regard to relative reduction of pests, were found in mixed-genotype plots (3B). Infestation intensity of stem borers in plots planted with the seed mix was higher than the average intensity measured in plots planted with its component genotypes. This is indicated by a negative -30.52% relative decline of these pests. By contrast, plots planted with the row mix shows a positive effect with the value of relative decline at 1.4% compared to its component genotypes. There were similarly inconsistent results for measures of brown plant-hopper populations. The average

Table 3. Average occurrence of pests found on plants of five rice genotypes grown as pure stand and genotype mixtures during the 2012 & 2013 growing season

Genotypes/ mixtures	Means of infestation intensity (%)		Means of number population	
	Stem borers (2012)	Stem borers (2013)	brown plant-hopper (2012)	brown plant-hopper (2013)
IPB 4S	0.079 a	0.707 ab	0.090 a	0.590 a
IPB-117-F-4-1-1	0.000 a	2.336 c	0.035 b	0.290 b
IR 64	0.291 b	0.936 ab	0.115 a	0.800 a
Inpari 11	0.153 ab	0.613 a	0.025 b	0.670 a
Inpari 13	0.085 a	0.545 a	0.020 b	0.675 a
Seed mix	0.065 a	1.341 b	0.040 b	0.565 a
Row mix (Strip)	0.000 a	1.013 ab	0.030 b	0.765 a

Different combinations of letters indicate statistically significant at $P \leq 0.05$ (DNMRT).

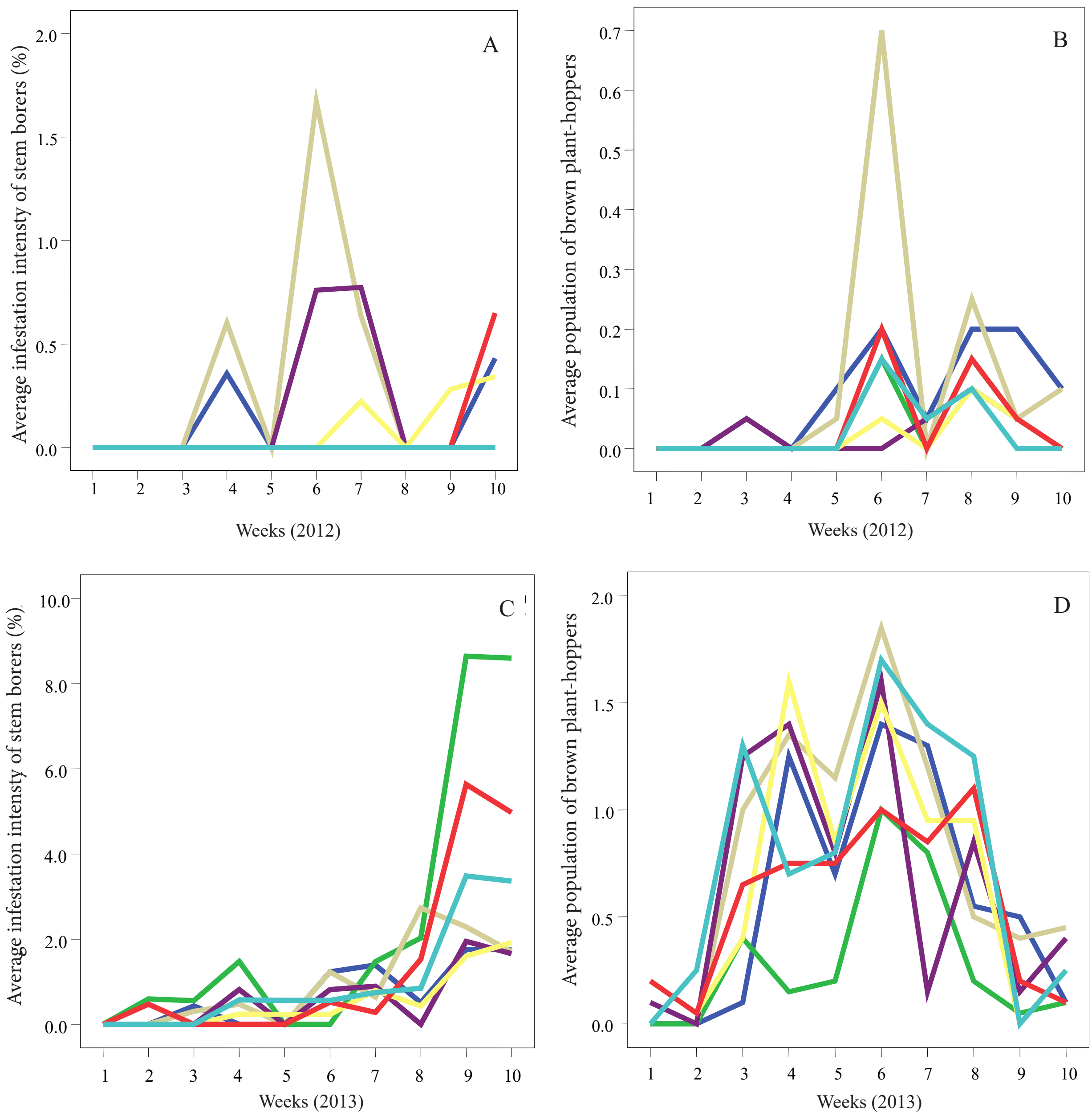


Figure 2. Average pest performance observed weekly in stand types (pure and mixed) during the 2012 & 2013 growing season. Panel A: average infestation intensity of stem borers (%) 2012; B: average population of brown plant-hoppers 2012; C: average infestation intensity of stem borers (%) 2013; D: average population of brown plant-hoppers 2013. ▬: IPB-4S; ▬: IPB-117-4-1-1; ▬: IR64; ▬: Inpari 11; ▬: Inpari 13; ▬: Seed mix; ▬: Row mix/Strip.

plant-hopper population in plots planted with the seed mix was less than the average population measured in plots planted with the component genotypes, while plots planted with the row mix experienced a higher average population of brown plant-hoppers compared with the plots planted with the component genotypes. The relative decline measured for the seed mix was 6.61%. Thus, it seems that in 2013 only the seed mix, and not the row mix, outperformed the plots planted with its component single-genotypes.

Effect of mixture arrangements on the tillers number, plant height and yield

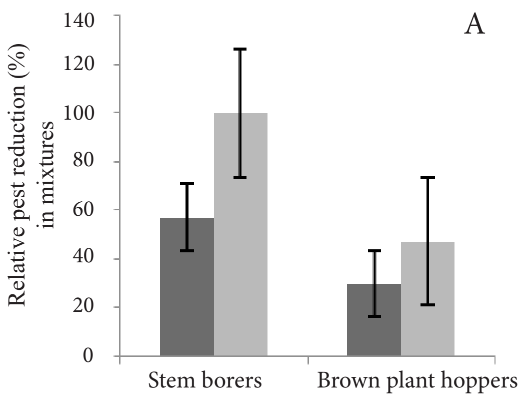
The effect of observation timing (week of observation or WO), genotype (pure vs. mixed) and the interaction of WO x genotype for plant height and effective tillers number were all highly significant (0.001< P < 0.01), during both growing seasons, except for the effect of WO x genotype on plant height in 2013.

There were significant differences between different genotype configurations on the number of effective tillers, in both growing seasons. The highest number of effective tillers was recorded in plots planted with pure stands of IR-64, while plots planted with either the single genotype IPB 117-F-4-1-1, or with a mixture containing that genotype, consistently produced the lowest number of effective tillers. When compared, results for plots planted with seed mix vs. row mix showed no significant difference in the number of effective tillers in either 2012 or 2013. Results for plant height were significantly higher in mixed-genotype plots vs. single-genotype plots ($P \leq 0.05$) in both seasons (Table 4). In addition, plant height in plots planted with the seed mix was highest of all, and higher than those measured in plots planted with the row mix.

Generally speaking, the mixed-genotype plots in 2013 appeared to perform better than in 2012 for grain yield advantage. The difference in mean grain yield in 2012 between seed mix plots and row mix plots was not significant ($P < 0,05$), totaling 26.4 kg plot^{-1} and 26.5 kg plot^{-1} respectively (Figure 4). However, during the 2013 growing season, the grain yield of plots planted with seed mix was significantly higher than the average yield of those planted with row mix. Furthermore, the average yields of both types of mixed-genotype plots were significantly higher than the average yields of plots planted with component genotype cultivars for this year. By calculating the average of the relative effect of mixed-genotype plantings on yield (compared to single-genotype plantings), we found an increased relative yield of 7.26% for the seed mix and 4.63% for the row mix (Figure 5).

DISCUSSION

In general, in locations with moderate baseline pest incidence (the only baseline levels we tested in this study), plots planted with seed mix and row mix seem to have the same capacity to suppress the infestation intensity of stem borers, and the populations of brown plant-hoppers, resulting in reduced occurrence of these pests in both types of the mixed-genotype test plots.



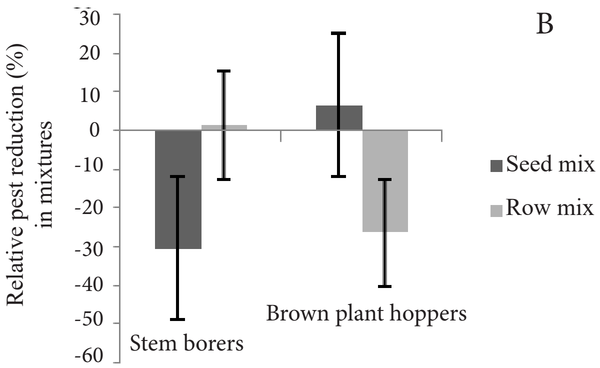


Figure 3. Relative pest reduction between plots sown with seed vs. row genotype mixtures. Panel A: 2012 growing season; B: 2013 growing season.

Table 4. Mean growth measured for plants of five rice genotypes grown in pure stands and mixed genotype stands in two growing seasons (2012 & 2013)

Genotypes/ mixtures	2012 growing season		2013 growing season	
	Number of efective Tillers	Plant height (cm)	Number of efective Tillers	Plant height (cm)
IPB 4S	10.625 a	90.293 a	11.875 a	90.144 a
IPB 117-F-4-1-1	8.250 b	87.585 b	8.665 b	90.373 a
IR-64	18.225 c	76.455 c	17.400 c	67.990 b
Inpari 11	16.450 d	75.055 d	14.910 d	74.221 c
Inpari 13	14.500 e	81.970 e	17.260 c	76.536 d
Seed mix	12.750 f	92.191 f	15.141 d	84.628 e
Row mix /Strip	13.125 f	88.733 g	15.720 d	81.995 f

Different letters indicate statistical differences between combinations at $P \leq 0.05$ (DNMRT).

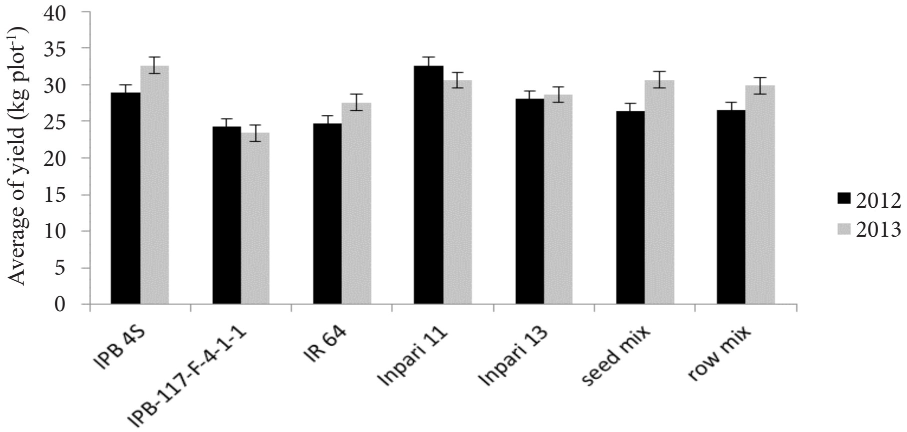


Figure 4. Average yields of rice genotypes sown in pure stands and in mixtures at for two growing seasons (2012 & 2013).

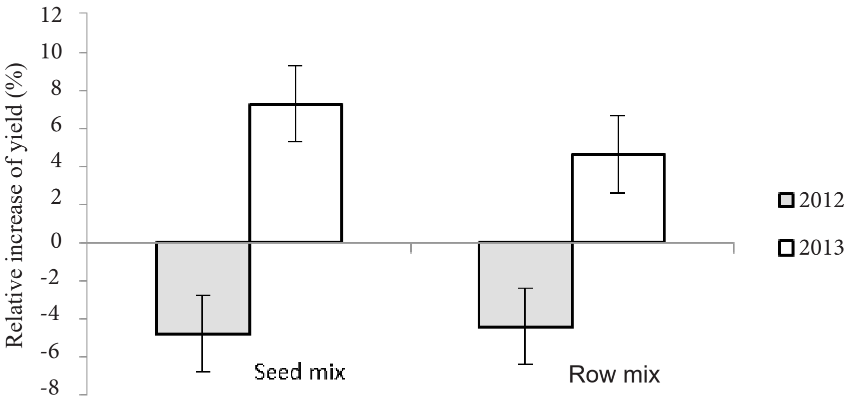


Figure 5. Relative increase (%) in yields 2012 vs. 2013 for two types of rice genotype mixtures.

Theoretically, according to several authors (Trenbath 1993; Bailey & Lazarovits 2003; Kaut et al. 2008) there are several mechanisms by which cereal mixtures can affect crop pests and diseases. Experimental results have shown (Trenbath 1993; Kaut et al. 2008; Walczak et al. 2011; Tratwal et al. 2014) that plots planted with cultivar mixtures are often less damaged by pests and disease organisms compared to pure, single-cultivar stands. Many authors (Trenbath 1993; Kaut et al. 2008) point out that the practice of planting a cultivar in the presence of associated plants in a mixed plot can lower the rate of population growth of attacking organisms. Firstly, the associated plants cause the plants of the target species to be less suitable hosts by altering the plant-pest ecosystem; secondly, associate plants interfere directly with the activities of the attacking pest, through biochemical inhibition (allelopathy) or acting as a physical obstacle to pest activities; and finally, they change the micro-environment within the intercrop such that natural enemies of the pest are favored (Trenbath 1993). Earlier literature suggests that use of genotype mixtures can reduce the impact of pests and diseases by diversifying various mechanisms including: crop physiological resistance, direct and indirect effects of plant architecture and physiology, and conservation of PNESs (Plant Natural Enemies Species) and facilitation of their ability to suppress aerial pests (Ratnadass et al. 2011).

The results of this study are in accord with previous reports on cereal crops, which show that mixing varieties can suppress the development of insect pests. Jie et al. (2003), reported that using two genotype varieties, in a mixture comprised of a 2 : 1 ratio of resistant to susceptible varieties, can efficiently suppress the population of an infestation of whitebacked plant-hoppers (*S. furcifera*), thus ensuring rice yield and reducing losses. Further, results of research by Walczak et al. (2009) show that cultivation of winter wheat in mixtures rather than pure stands resulted in reduced population of cereal leaf beetles (by up to 41.6% in comparison to pure stands) and increased yields (by up to 1.5 dt·ha-1 compared to pure stands). The results of field experiments (Tratwal et al. 2014) conducted in 2010 and 2011 with spring barley cultivars and their mixtures showed that numbers of cereal leaf beetle larvae, bird cherry-oat aphids, and grain aphids, were reduced in mixed cultivar stands in comparison with single-cultivar stands. Grain yields from mixtures increased in comparison with those from pure sowings by up to 6.48 dt ha-1 in 2010 and up to 3.99 dt ha-1 in 2011. Tratwal & Walczak (2010) also attribute an important decline in the incidence of pests, to the use of a combination of three species of cereals (wheat, barley and oats). They found pest populations in mixed-species plantations were 70–90% less than those in areas planted with a single-species genotype.

The results of this study indicate that differences in pest resistance and plant architecture found in different rice genotype varieties can affect the incidence of pests in fields planted with a mixture of the varieties, due to differences in the interactions between the component varieties in the mixture. Zhu and his group argued that the more genotypically diverse the component rice cultivars used in intercropping, the better rice blast is controlled (Ning et al. 2012).

In our study, grain yields for both genotype mixtures (seed mix and row mix) were higher in 2013 than in 2012. However, a relative increase in yield only occurred in 2013 for the seed mix and row mix. Moreover, even though pest occurrence was higher in 2013, the mixed-genotype plots did not show any decrease in grain yield, while some single-genotype plots (like IPB 117-F-4-1-1, IR 64 and Inpari 13) showed the opposite result (i.e. lower pest occurrence and lower yield). Because of interactions amongst the component genotypes such as competition and complementarity, which affect desired trait expression, mixtures provide a buffer against variation in environmental conditions, so that yield is stable across environments and over time. We obtained only plant height as a consistent yield component character showing a positive effect on seed mix and row mix for both growing seasons. Compensation was also observed in cultivar mixtures where the components differed in plant height (Khalifa & Qualset 1974).

Combining different crop varieties with complementary strengths is a way to reduce the yield fluctuations associated with any particular variety (Bowden et al. 2001). Grain yield in mixed-genotype plots is influenced by intraspecific competition between component pure genetic lines that begins during early development and continues through physiological maturity. It seems that complementary relationships between plants from different genetic lines allow each of the varieties to inhabit different ecological niches with regard to growth habit, shading, or other factors, thus decreasing interplant competition and increasing grain yield for fields planted with mixed-genotypes (Gallandt et al. 2001).

As in our study, grain yield advantages of about 1.5 to 3% for mixed-genotype plantings, compared to plots planted with only single component cultivars, have been previously reported for other small grain cereals such as oat, winter wheat, rice, and winter barley (Helland & Holland 2001; Gallandt et al. 2001; Cowger & Weisz 2008; Revilla-Molina et al. 2009; Creissen et al. 2016). Østergård et al. (2005) cultivated six mixtures containing three unique components of spring barley (*Hordeum vulgare* L.) in each, as well as planting single-variety plots of each component variety. The researchers found that plots planted with mixtures had more stable yields, on average, and higher yield ranking than plots of pure cultivars. Therefore, genotype mixtures offer the advantage of different components which complement one another in their adaptation to yield limiting factors and environmental variation, resulting in increased yield (Wolfe 2000; Biabani et al. 2008).

CONCLUSION

Cultivating plants in mixed-genotype stands provides a promising strategy to decrease the incidence of pests in the field, while maintaining or even increasing yield. Although baseline pest levels were only moderate during our test growing seasons, our results nonetheless showed that the occurrence of pests in mixed-genotype plantations was lower than in some of those planted with single-genotypes. Specifically, our data from 2012 shows that mixed-genotype plots experienced a relative percent decrease in stem borer infestation and brown plant-hopper population, respectively, of 46.55% and 29.83%. The plots planted with row mix show a higher percentage reduction in pest incidence than that achieved in those planted with the seed mix; 100% reduction for stem borers and 47.37% reduction for brown plant-hoppers. Results from plots planted with the seed mix showed a reduced population of brown plant hoppers as indicated by a relative decline of 6.61% in the 2013 season only, while results from the row mix plots showed no such decline. Row mix plots did, however, consistently show a decline in stem borer population, with a relative decrease of 100% in 2012 and 1.4% in 2013. In terms of yield, plant height proved to be a consistent yield component character, correlating positively with plant yield for both seed mix and row mix in both growing seasons. Our results showed higher average yields –and taller height--for the mixed genotype plots compared to pure genotype stands. In the second growing season, we also observed a higher yield for the seed mix than the row mix, with an increase relative yield of 7.26% for the seed mix and 4.63% for the row mix, respectively.

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