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# Bean cultivar mixture allows reduced herbicide dose while maintaining high yield: A step towards more eco-friendly weed management

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## ABSTRACT

Integrated weed control methods are increasingly demanded to maintain high yield while alleviating negative environmental side effects of control measures. Five field experiments were conducted during 2015-2017 at three locations in Iran to determine the advantage of exploiting enhanced competitive ability of bean (Phaseolus vulgaris L.) via cultivar mixture to reduce herbicide dose. For all experiments, the experimental design was splitplot in randomized complete block with four replications. Main plots were assigned to the factor "herbicide dose" with 5 levels between 0 and 100 % of recommended dose and subplots comprised monoculture of a stand growth type cultivar (STAND), monoculture of a climbing growth type cultivar (CLIMB) and the substitutive (50 % STAND/50 % CLIMB) cultivar mixture (MIXCR). Without herbicide application, the yield of STAND, CLIMB and MIXCR was estimated at 588, 1026, and 1637 kg ha<sup>-1</sup>, and the corresponding weed biomass ( $W_0$ ) at 388, 215, and 239 g m<sup>-2</sup>, respectively. The herbicide dose to decrease  $W_0$  by 50 % (ed<sub>50</sub>) was estimated as 69 % of the recommended dose for STAND, while it was 53 and 45 % for CLIMB and MIXCR, respectively. Herbicide application at full rate increased the average yield of STAND by 2610 kg ha<sup>-1</sup>, but 1115 and 1532 kg ha<sup>-1</sup> for CLIMB and MIXCR, respectively. Potential yield of CLIMB at full herbicide rate application was 2141 kg ha<sup>-1</sup>, while STAND and MIXCR showed statistically the same potential of producing 3198 and 3169 kg  $h^{-1}$  bean yield. However, MIXCR could achieve its potential yield at half of the recommended dose. Relative yield total (RYT) was lower than or equal to one at 75 % and 100 % of the recommended dose, but with decreasing herbicide dose to 50 or 25 % RYT raised to 1.2. Careful selection of cultivars for growing in mixture that combines strong competitor with high yield cultivars is crucial to achieve potential yield with less dependence on herbicides. As we could show for bean, this will help to overcome conflicting management objectives to reach both environmental and economic goals in weed control and crop production.

## 1. Introduction

Weed control on conventional farms in Iran is mainly accomplished through herbicide application. Herbicides are highly effective in reducing crop yield loss and stabilizing weed infestations below the damage levels. Herbicides allowed simplification of cropping systems, expansion of monocultures and the adoption of reduced tillage systems (Johnson et al., 2009). However, the use of herbicides as the only weed management tool is increasingly being questioned because of herbicide costs and technical problems related to resistance of weed populations to one or several herbicides (Gherekhloo et al., 2016; Peterson et al., 2018). Nowadays, the trend is for reduced herbicide application rates to decrease both excessive costs and negative side effects on environment. On the other hand, some risks may be associated with reducing herbicide rates resulting in reduced crop yields, increased weed seed production and increased risk of herbicide resistance (Neve and Powles, 2005). Enhanced crop competitiveness via cultural methods integrated with herbicide application is considered as a solution for improving the efficiency of herbicide at reduced rates (Blackshaw et al., 2006).

Mix cropping, the practice of cultivating two or more crops

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#### Table 1

Characteristics of study locations with information on geographical, climatic and soil properties.

			Characteristics									
Study location	Latitude	Longitude	Altitude (m)	Soil texture	pН	Organic matter (%)	Annual temperature (° C)		Annual precipitation (mm)			
							2015	2016	2017	2015	2016	2017
Karaj Shiraz Behshahr	35°34' N 29°45' N 36° 41' N	50° 57' E 52° 28' E 53° 52' E	1361 1484 15	Sandy loam Sandy loam Loam	7.50 7.30 6.40	0.67 0.84 2.10	15.20 16.58 17.26	16.70 - -	16.60 - -	211 300 522	170  -	353 - -

simultaneously in the field, is an effective approach to make crops more competitive against weeds (Swanton et al., 2015). Weed suppression has often been found to be greater in mix crops compared to monoculture (Thole, 2012, but see Elsalahy et al., 2019) and, if rightly chosen, produce higher yields than either of the component crops (Liu et al., 2017). Therefore, it can be an option for at least partial weed control (Ganavel, 2015), but some potential disadvantages associated with mix cropping have limited its application in agricultural systems. The disadvantages are related mainly to lack of agricultural machinery especially when the component crops have different requirements for patterns of planting and harvesting, and for fertilizer and herbicide applications (Biabani et al., 2012).

Because of the similarity of the requirements, crop cultivars can combine benefits of mix cropping and alleviate its disadvantages (Tooker and Frank, 2012). Yield advantage of cultivar mixtures has been shown for various crops (Reiss and Drinkwater, 2018) including barley (Hordeum vulgare L.) (Creissen et al., 2016), mustard (Brassica napus L.) (Ahmad et al., 2012), rice (Oryzae sativa L.) (Jareen et al., 2019), wheat (Triticum aestivum L.) (Gigot et al., 2013), soybean (Glycine max L.) (Crusciol et al., 2012) and bean (Phaseolus vulgaris L.) (Ssekandi et al., 2016). The superiority of cultivar mixtures over pure stands has been attributed generally to the significant variations of morphological characteristics including root system, plant height, and leaf orientation for weed suppression, but also of pathogen susceptibility allowing a reduction in fungicide applications (McDonald and Stukenbrock, 2016). These variations would result in efficient exploitation of environmental resources, specifically light interception, increased lodging resistance, improved disease resistance (Damicone et al., 2007), and better weed control (Bond and Grundy, 2001). Cultivars mixture of sub-species with different growth forms of either above or underground parts is expected to better use available resources and thus to limit their access for weeds, as compared to monocultures (Bilalis et al., 2010).

Red bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) is an important crop in Iran with around 110,000 ha area of cultivation and 2.5 t ha<sup>-1</sup> yield (http://www.amar.maj.ir). Red bean cultivars have various growth habits, ranging from determinate, short and erect to fully prostrated or climbing types. The natural genetic and phenotypic diversity that exist in legumes represent an excellent opportunity for mix cultivar systems in limiting empty space for weed establishment (Hall et al., 2003). Amini et al. (2014) showed that a mixture of short and tall cultivars of bean was associated with less weed biomass compared to the monoculture of them. Enhanced competition of crop cultivars via mix cropping allows reducing herbicide dose that offers less environmental risk. By this, it ideally links the demands for more sustainable farming principles with efficient weed control. Unfortunately, there are still only few studies on interactions of cultivar mixture with reduced herbicide dose (Kaczmarek and Matysiak, 2017).

Here, we set out to develop a more eco-friendly method for weed control in bean by determining the interactions between herbicide dose and two bean cultivars grown in monoculture and mixture under field condition in Iran. For this, we used cultivars differing in competitive ability and yield, and in seed size for later separation after harvest. Specifically, we asked the following questions: 1) at what minimal level of herbicide dose can the full yield, achieved under full herbicide dose, still be reached in mixture (MIXCR), 2) under what herbicide dose is the advantage of MIXCR over monocultures (relative yield total: RYT) highest, and 3) what is the effect of the cultivars grown in monocultures vs. MIXCR on weed cover and crop yield under five levels of herbicide applications (from control to recommended field dose, i.e. 0–100 %).

These questions are addressed in five replicated field experiments at three sites over three years and that varied in weed cover and composition, as well as in climatic and soil properties.

## 2. Materials and methods

#### 2.1. Field experiments

Five field experiments were conducted under sprinkler irrigation at three locations and over several years, in Behshahr (2015), Shiraz (2015) and Karaj (2015, 2016 and 2017) (see Table 1 for the characteristics of the study locations). A moldboard plough followed by a disk was used to prepare the seedbed in May. Based on soil analyses, fertilizers were applied prior to planting or as topdressing. The experimental design was a randomized complete block in a split plot arrangement of treatments with four replications. Main plots were the application rates of 0, 25, 50, 75 and 100 % of the recommended dose of the herbicide Imazethapyr (Pursuit®, 100 SL, 100 g a.i. L<sup>-1</sup>, BASF plc). Subplots comprised monoculture of Akhtar with standing growth type (STAND), monoculture of Goli with climbing growth type (CLIMB) and a mixture (MIXCR) of the two cultivars. Sub-plots measured 3 by 6 m including six rows of bean with a row spacing of 50 cm and 10 cm within the rows. Bean seeds were sown in mid-June at the recommended density of 40 plants m<sup>-2</sup>. For MIXCR the planting rows were alternately assigned to each cultivar. Herbicide doses were applied on the natural weed infestation at the 2-4 leaf growth stage of bean using an electric knapsack sprayer (MATABI) fitted with flooding fan spray nozzle (Goizeper S. Cooperative Company, Guipuzcoa, Spain) and operated at a pressure of 240 k Pa and a volume rate of 260 L h<sup>-1</sup>.

Prior to herbicide application (two leaf stage of bean), weed species density was measured in 100 cm long to 50 cm wide rectangles per subplot and then converted to plants  $m^{-2}$ . Above ground biomass were also measured at bean growth stage 30 (BBCH scale following Meier (2001)) that coincides with 12 full leaves and more than 12 side shoots visible in three randomly chosen areas of 50 cm  $\times$  50 cm within four middle planting rows. In addition, a quadrat of 50 cm  $\times$  50 cm was fixed between two middle planting rows of each sub-plot to monitor ground coverage by plants. The quadrats were gridded into one hundred sub-squares of 25 cm<sup>2</sup> (5 cm  $\times$  5 cm) and the percent bean ground cover at each square was visually scored and recorded. Each sub-square with plant cover was assumed one percent coverage. Bean yield was harvested by hand from one m<sup>2</sup> area per sub-plot in mid-September and expressed as kilograms/hectare (kg ha<sup>-1</sup>) at 13 % seed moisture.

## 2.2. Statistical analyses

A mixed model was used to analyze the main effects of herbicide dose (main-plots) and cropping system (sub-plots) and their interaction. Herbicide dose and cropping system were considered as fixed effects in the model, whereas locations and years were considered as random effects. Data analysis was performed in R-studio version 1.1.453 (https

#### Table 2

Weed species population per square meter of the experimental fields in the year of the study. Number of individuals with standard error value in parenthesis. "Other weeds" includes the species with average abundance less than 2 plants  $m^{-2}.$ 

	Weed density (plants $m^{-2}$ )							
Weed species	Karaj		Shiraz	Behshahr				
	2015 2016 2017			2015	2015			
Amaranthus retroflexus L.	33 (4.0)	46 (8.0)	41 (7.3)	19 (3.2)	47 (8.0)			
Chenopodium album L.	21 (2.7)	38 (11.0)	27 (4.3)	8 (6.0)	-			
Xanthium strumarium L.	16 (3.1)	12 (4.1)	9 (3.2)	15 (4.0)	-			
Heliotropium europaeum L.	24 (5.0)	15 (3.9)	17 (6.4)	-	12 (4.0)			
Solanum nigrum L.	11 (2.0)	21 (5.5)	25 (4.1)	7 (3.9)	19 (5.0)			
Echinochloa crus-galli L.	19 (4.7)	26 (8.5)	21 (5.0)	19 (3.9)	32 (7.8)			
Other weeds	6 (1.7)	11 (3.0)	8 (3.0)	5 (1.4)	12 (3.3)			

://rstudio.com) using package '*lme4*' (Bates et al., 2018). As there was no significant deviation from normality, no data transformation was required. Where the interaction between herbicide dose and cropping system was significant, the effect of herbicide dose on the response variables (either bean yield or weed biomass) was described using nonlinear regression. Non-linear regression provides comparison of response to herbicide rates via comparing estimated parameters. These comparisons of the rates of change would not be possible using ANOVA (Ritz et al., 2015).

A four-parameter sigmoidal function was used to describe the relationship between crop yield (y) and herbicide dose (*dose*):

$$y = Y_0 + \frac{a}{1 + \exp(-B(dose - rd_{50}))}$$
(1)

where  $Y_0$  is the crop yield (kg ha<sup>-1</sup>) when no herbicide is applied, *a* is the increase in crop yield that occurs with herbicide application at the recommended dose,  $rd_{50}$  is the dose that increases yield by  $(a-Y_0)/2$  and *B* determines the slope of the curve.

A three-parameter logistic was used to parametrize herbicide dose (*dose*) effect on weed biomass (*W*) as follows:

$$v = \frac{W_0}{1 + \left(\frac{dose}{eds_0}\right)^b} \tag{2}$$

where  $W_0$  is weed biomass (g m<sup>-2</sup>) at no herbicide treatment,  $ed_{50}$  is the dose that decreases  $W_0$  by half and *b* denotes the slope of the curve. R-package of *drc* was used for non-linear regression analysis and model fit (Ritz et al., 2016).

The RYT index was calculated to evaluate the advantage of mix cropping over the monocultures at each herbicide dose (Willey, 1985) as:

$$RYT = \frac{Y_{CS}}{Y_{CC}} + \frac{Y_{SC}}{Y_{SS}}$$
(3)

where  $Y_{CS}$  is the yield of climbing cultivar in MIXCR,  $Y_{SC}$  represents the yield of stand cultivar in MIXCR and  $Y_{CC}$  and  $Y_{SS}$  are respectively the yields in CLIMB and STAND. A RYT value greater than one indicates the advantage of mix cropping over monoculture.

Semivariance was used to express the degree of relationship between squares in the gridded plots as follows (e.g. Robertson, 2008):

$$\gamma(h) = \left[\frac{1}{2N(h)}\right] \sum \left(Z_i + Z_{i+h}\right)^2 \tag{4}$$

where  $\gamma$  (*h*) is semivariance for interval distance class *h*,  $Z_i$  and  $Z_{i+h}$  are measured sample values at points *i* and *i* + *h*, respectively, and *N* (*h*) is the total number of sample couples for the separation distance *h*. Kriging interpolation method (Somerville et al., 2020) was used to map the percent ground cover of bean in each planting system.

Model fit was assessed using the root mean square of error (RMSE), adjusted  $R^2$  and the standard error of parameter estimates.

## 3. Results

v

The mixed model analysis indicates that location /year effect, which were considered as random effects, were not significant neither on crop yield nor on weed biomass, but for the main fixed effects and the interactions, both crop yield and weed biomass were significantly affected. Five location/year data series were used for a global model fit, and parameters were estimated by fitting models to the entire data set.

Table 2 shows the average density of the main weed species in the experimental fields at each location and year. The study farms at Karaj and Shiraz were subjected to weed competition studies over the past 5 years, with annual summer weeds including redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.)



Fig. 1. Relationship between bean yield and herbicide dose. Fitted lines are sigmoidal model (Eq. 1) for STAND, CLIMB and MIXCR.

#### Table 3

Parameter estimates of fitting model (1) to crop yield with herbicide dose for STAND, CLIMB and MIXCR. Standard errors of estimates are shown in parenthesis.

Cronning		Paramete	er estimates			
System	a (kg ha <sup>-1</sup> )	Y <sub>0</sub>	rd <sub>50</sub>	В	Adjusted R <sup>2</sup>	RMSE
STAND	2610 (215.0)	588 (62.0)	54 (2.7)	-3.70 (0.68)	0.93	262
CLIMB	1115 (395.0)	1026 (73.0)	40 (19.0)	-1.77 (0.60)	0.84	285
MIXCR	1532 (165.0)	1637 (47.0)	39 (5.0)	-2.10 (0.40)	0.89	181

STAND, monoculture of Akhtar; CLIMB, monoculture of Goli; MIXCR, cultivar mixture; *a*, the increase in crop yield that occurs with herbicide application at recommended dose;  $Y_0$ , the crop yield when no herbicide is applied;  $rd_{50}$ , the dose that increases yield by  $(a-Y_0)/2$ ; *B*, the slope of curve at linear part; RMSE, root mean square of error.

and black nightshade (*Solanum nigrum* L.), cocklebur (*Xanthium strumarium* L.), common heliotrope (*Heliotropium europaeum* L.) and the annual grass barnyardgrass (*Echinochloa crus-galli* L.) repeatedly grown. This resulted in a relatively homogeneous distribution of these weed populations within the experimental plots. At Behshahr site, infestations of *C. album* and *X. strumarium* were low, while *A. retroflexus, S. nigrum*, *H. europaeum* and *E. crus-galli* were consistently dominant in the experimental field. Some species such as *E. colonum* L. Link, yellow foxtail (*Setaria glauca* L.), jimsonweed (*Datura strumarium* L.) and bathurst burr (*X. spinosum* L.) were also present in the plots, however with average densities lower than 2 plants m<sup>-2</sup> (cf. "other weeds" in Table 2).

### 3.1. Crop yield

Parameter  $Y_0$  represents the potential of maintaining crop yield when no weed control is performed. Therefore, higher  $Y_0$  means more bean yield with no attempt for weed control.

In STAND,  $Y_0$  was estimated at 588 (SE = 62) kg ha<sup>-1</sup> (Fig. 1, Table 3). For CLIMB and MIXCR,  $Y_0$  was estimated 1026 (SE = 73) and 1637 (SE = 47) kg ha<sup>-1</sup>, respectively. Therefore, if cropping system has to suppress weeds by itself with no additional weed control measures, MIXCR has a significantly much higher potential to produce more yield, i.e. respectively 1.59 and 2.78 times higher than CLIMB and STAND.

Parameter a computes the amount of increase in bean yield with herbicide application at full rate, thus it shows how yield is saved with

herbicide application. In STAND, *a* was estimated 2610 (SE = 215) kg ha<sup>-1</sup>, while for CLIMB and MIXCR was 1115 (SE = 395) and 1532 (SE = 165) kg ha<sup>-1</sup>, respectively.

The potential yield i.e.  $(Y_{0+}a)$  for STAND, MIXED, and CLIMB was estimated at 3198, 3169, and 2141 kg ha<sup>-1</sup>, respectively. Therefore, with herbicide application, STAND and MIXCR statistically produced the same yield.

Parameter  $rd_{50}$  indicates herbicide dose efficiency to save yield. The estimated value for STAND was 54 % (SE = 2.7 %) of the recommended dose indicating that when 54 % of the recommended dose is applied, a 1305 kg ha<sup>-1</sup> higher yield is obtained (half of parameter *a*).

The  $rd_{50}$  value in CLIMB was similarly estimated 40 % (SE = 19 %) of the recommended dose that could increase bean yield by 557 kg ha<sup>-1</sup>. In MIXCR, the  $rd_{50}$  value was estimated at 39 % (SE = 5 %) of the recommended dose, which could increase the bean yield by 766 kg ha<sup>-1</sup>.

#### 3.2. Weed control effect

Eq. 1 generally gave a good description of weed biomass as affected by herbicide dose (Fig. 2, Table 4). As described above, parameter  $W_0$ represents weed biomass at cropping systems that received no herbicide dose, therefore, a larger  $W_0$  can be interpreted as a lower potential of the cropping system to suppress weeds. Consistently over all experimental sites and years,  $W_0$  was significantly smaller in CLIMB and MIXCR than in STAND. The  $W_0$  values for STAND, MIXCR, and CLIMB were estimated at 388 (SE = 24.1), 239 (11), and 215 (SE = 12.3) g m<sup>-2</sup>. Therefore, without herbicide application, CLIMB and MIXCR had respectively 45 and 38 % less weed biomass than STAND.

Exploring the relationship between parameters  $Y_0$  and  $W_0$  (Fig. 3) shows how bean yield is affected by weed competition if herbicide is not

## Table 4

Parameter estimates of the standard dose-response model (Eq. 2) fitted to total weed biomass with herbicide dose. Standard errors of estimates are shown in parenthesis.

	Parameter es	stimates			
Cropping System	Wo	ed <sub>50</sub>	В	Adjusted R <sup>2</sup>	RMSE
STAND CLIMB MIXCR	388 (24.1) 215 (12.3) 239 (11.4)	69 (7.14) 53 (5.0) 45 (3.8)	2.10 (0.5) 2.30 (0.4) 2.10 (0.3)	0.87 0.81 0.87	59.00 49.20 44.65

STAND, monoculture of Akhtar; CLIMB, monoculture of Goli; MIXCR, cultivar mixture;  $W_0$ , weed biomass (g m<sup>-2</sup>) at no herbicide treatment;  $ed_{50}$ , the dose that decreases  $W_0$  by half; b, the slope of the curve at linear decrease part; RMSE, root mean square of error.



Fig. 2. Relationship between total weed biomass and herbicide dose. Fitted lines are standard dose-response model (Eq. 2) for STAND, CLIMB and MIXCR.



Fig. 3. Scatter plot of estimated values of weed biomass W<sub>0</sub> vs. crop yield Y<sub>0</sub> for Karaj (2015, 2016, 2017), Behshar (2015) and Shiraz (2015).



**Fig. 4.** Relative yield total (RYT) calculated for cultivar mixture of the climbing and stand cultivars at each herbicide dose. The dashed line is a refrence line of RYT = 1. Error bars show the standard error of means.

applied. The position of points along the bisector line suggests that in STAND, the mainly large values of  $W_0$  relate to lower values of  $Y_0$ , thus, a large weed biomass grew in the absence of herbicide application that led to a significant yield loss.

In contrast, the MIXCR points were all distributed above the line indicating less weed biomass presence that corresponded to the larger  $Y_0$  values. CLIMB points were placed around the bisector line. In CLIMB lower values of weed biomass were observed compared to MIXCR, while bean yield was also lower. Therefore, weeds were controlled more in CLIMB but resulting bean yield remained low, while in MIXCR, weed

biomass was between CLIMB and STAND, but bean yield was significantly higher.

Parameter  $ed_{50}$  for STAND, CLIMB and MIXCR was estimated at 69 % (SE = 7.14), 53 % (SE = 5), and 45 % (SE = 3.8) of the recommended dose, respectively. Therefore, CLIMB and MIXCR were estimated to have, respectively, 23 and 35 % lower  $ed_{50}$  than STAND.

## 3.3. Relative yield total (RYT)

To evaluate the advantage of MIXCR over STAND or CLIMB, RYT was



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Fig. 5. 3-D maps for percent ground cover of bean in (A) STAND, (B) CLIMB, and (C) MIXCR at the 12-leaf stage of the beans. Semivariance (Eq. 3) was fitted to data of ground cover obtained from 5 cm to 5 cm squares. An average over five data sets (Karaj-2015, 2016, 2017; Shiraz-2015; Behshahr-2015) was used to produce ground cover map.

calculated for each herbicide dose (Fig. 4). RYT was lower than or equal to 1 at high herbicide doses (75 and 100 % of the recommended dose) but became more than one at lower herbicide doses indicating the advantage of MIXCR. Therefore, herbicide use at higher rates alleviates the advantage of MIXCR in weed suppression.

#### 3.4. Bean ground cover map

Fig. 5 shows the 3-D maps of percent ground cover of bean at the 12 leaves stage, providing a quantitative outlook of bean ground cover status between planting rows in STAND, CLIMB and MIXCR plots. In STAND, percent ground cover rapidly decreased with increasing distance from planting rows. At a distance of 20 cm from the planting rows, percent ground cover was lower than 20 %. In contrast, in CLIMB or MIXCR the percent ground cover was more than 70 % in between rows space indicating that MIXCR and CLIMB provided less space for weed growth and were more efficient in light interception by crop.

## 4. Discussion

#### 4.1. Yield advantage of mix cropping

Mix cropping is a common practice in marginal agroecological environments, which fulfills a variety of functions, including complementary use of growth factors, such as soil nutrients, light, and water; reduced pest and disease incidence, reduced soil erosion, more total biomass production and higher yield stability (Mao et al., 2012; Weltzien and Christinck, 2017). Mix cropping provides a high-density multispecies cropping system (Trenbath, 1999), which is not achieved in monoculture; because of a competition-density effect (decrease in mean size of surviving plants with increasing density); alteration in the size structure of the population (size hierarchy development); and density-dependent mortality (self-thinning) (Park et al., 2003). A cultivar mixture with appropriately chosen components reduces intra-specific competition as it provides resource uptake from different micro-zones (Li et al., 2008; Zhao et al., 2018). We selected two bean cultivars differing in canopy form for mixture. In beans, earlier studies showed differential uptake of underground resources due to different root types between standing and climbing varieties used in our study (Motesharezadeh and Savaghebi, 2012; Yadegar et al., 2015). Biabani et al. (2012) reported that mix cropping of short and tall soybean cultivars created a wavy type canopy when planting alternate rows of shorter and taller plants. This provided a greater potential for intercepting radiation and thus, increased dry matter production. MIXCR owns a high-density cropping, more ground cover, and a wavy expanded canopy in which two co-growing cultivars have their specified underground and above ground resources. These explain the RYT values greater than one; however, we obtain higher RYT when MIXCR was treated with low herbicide rates, and increasing herbicide dose led to lower RYT values indicting a diminished advantage of MIXCR. Based on this, we conclude that an important advantage of MIXCR is in its weed suppression ability.

## 4.2. Weeds find less unused resources in mix cropping

Because of more efficient light interception or underground uptake, resources remain less available to weeds (Rodríguez, 2006). Cultivar mixture can lead to an expanded canopy, which rapidly covers the ground surface between crop rows (Weerarathne et al., 2017).

In our study, we indeed found a higher percent ground cover of MIXCR (Fig. 5) as compared to the monocultures. Therefore, less vacant space was left for weeds in between crop rows and a smaller amount of light may have passed through the canopy to stimulate weed seed germination or competition. In STAND with the recommended herbicide dose, higher yield was obtained compared to plots with 75 % of the recommended dose. In contrast, in MIXCR or CLIMB, the yields obtained

with 50 % of the recommended dose were not different from 100 % of the recommended dose. We show that MIXCR provides an enhanced competitive advantage resulting in partial weed control. Applying herbicide at the recommended dose in MIXCR would be equivalent to herbicide over-usage. To integrate herbicide dose application with other weed control alternatives such as mix cropping, the dose can be optimized to avoid excessive use. Reducing pesticide doses below the recommended doses whenever possible is a straightforward approach to reduce the risk of adverse side effects. To adopt this approach decisionmaking has to be improved. The parameters to consider optimizing herbicide doses are: weed flora and growth stage, crop competitiveness, climatic conditions, application technique, formulation/adjuvant and combination with other pesticides (Kudsk, 2008). Although tillage and crop rotation are being used to reduce weed damage, herbicides remain pivotal for weed management in current conventional bean cultivation systems. We suggest mix cropping as a remediation that decreases the reliance on chemical control.

## 4.3. A solution for the harvesting issue

Limitations for the use of cultivar mixture of either crops or cultivars are potential differences in harvesting time combined with a lack of machinery for separately harvesting the crop/cultivars. Attention must therefore be paid to a careful selection of crop cultivars with identical maturity time, as simultaneously harvesting of non-synchronized mixed cropped cultivars with regard to timing of maturity may decrease the marketability of products. For beans, the size, color, and cookability of the seeds may differ in this case. To deal with this issue, we chose cultivars significantly differing in seed size, with seed weight (100 seeds) of STAND of about 45 g and for CLIMB of 25 g. This large difference in size of seeds provides efficient winnowing of seeds after harvest and the seeds of the two cultivars can be separately supplied to market.

The current study clearly shows the potential of mix cropping for decreasing weed cover and thus herbicide use while maintaining high yield. CLIMB is a better competitor due to its increased vegetative growth, but its twisted climbing stems produce less grain yield. In contrast, STAND produces higher grain yield, but with high reliance on herbicide application. MIXCR ideally combines the high competitor CLIMB with the high yield producer STAND to achieve high yield with less dependence on herbicides. Exploiting this complementarity through mixed cultivar cropping allows for a more efficient and eco-friendly weed management.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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