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Crop Diversification to Control Powdery Mildew in Pea

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Abstract: Pea is a temperate grain legume cultivated worldwide that can be severely constrained by powdery mildew infection. Control by fungicides and the use of resistant cultivars is possible, but there is a growing interest in alternative control methods such as crop diversification, particularly in low input agriculture. The aim of this work was to assess the potential of intercropping pea with other crops and of pea cultivar mixtures for powdery mildew management on pea crop. Results show a reduction of powdery mildew on pea when intercropped by replacement at a 50:50 ratio with barley or with faba bean, but not when intercropped with wheat. A barrier effect seems to explain a major part of this decrease, especially in the pea/barley intercrop. This hypothesis was further supported by inoculated seedlings under controlled conditions, where powdery mildew infection on pea decreased with the distance to the inoculation point, this decrease being larger in the intercrop with barley than in the intercrop with wheat and in the pea monocrop. Powdery mildew was also reduced in the mixture of resistant and susceptible cultivars, with infection decreasing with the increasing proportions of the resistant one. Overall, this work shows that crop diversification may be a good strategy to reduce powdery mildew in pea.

Keywords: *Erysiphe pisi*; *Pisum sativum*; intercropping; cultivar mixtures

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1. Introduction

Pea (*Pisum sativum* L.) is a major temperate grain legume widely cultivated worldwide [1]. Its high protein content makes pea highly suitable for animal feed and a good alternative to meat as a source of proteins for human diets [2]. Like other legumes, pea fixes atmospheric nitrogen, enriching soils and contributing towards sustainability. However, as with any crop, pea production can be constrained by a number of pests and diseases [3]. Powdery mildew is an air-borne fungal disease caused by the biotrophic pathogen *Erysiphe pisi* DC. Infection affects all aerial parts of the plant, with losses that may reach 50% of yield [4]. Although methods such as planting early in the season or using early-maturing cultivars can be implemented for promoting escape from the disease, effective control is really achieved by using chemical fungicides or resistant cultivars. The problem with chemicals is that their use entails damage to the ecosystems and increases the economic costs of the farmers. As for resistant cultivars, only a limited number of them are available, and with a resistance that relies on a narrow genetic basis. Only three genes for resistance have been described, namely *er1*, *er2* and *Er3*, with only the first one being widely used in breeding programs all over the world [5]. The risk of the pathogen overcoming these resistances is high, especially when they occur in large areas of genetically homogeneous plants [5]. Even more, in addition to *E. pisi,* other species such as *E. trifolii* Grev. or *E. baeumleri* U. Braun and S. Takam. Magnus might also affect pea under certain conditions and are reported to overcome *er1* resistance [6–8].

Crop diversification, either by intercropping, i.e., mixing two or more crops, or by mixtures of genotypes of the same crop, has proven useful to reduce disease pressure in several crops [9,10]. The objective is to modify the traditional monocrop environment in such a way that it hampers the process of infection and the extension of the pathogen, or that it provides additional strengths to the host crop to fight the infection. This effect on diseases adds to other known advantages of diversification, such as an increase of yield and reduction of fertilizers, or the positive effects on beneficial insects and pollination [11–13].

Legumes are popular components in diversification strategies, given the ecological services they provide to other crops and to the environment as a whole. In particular, cereal–legume intercrops are of great interest because of the synergies they deliver and have been the subject of several studies, including their effects on disease reduction [14–16]. On the contrary, mixtures of legume cultivars have been less studied, with only some studies on common bean available so far.

Intercropping pea with cereals can reduce pea diseases such as ascochyta blight (*Peyronellaea pinodes* (Berk. and A. Bloxam) Avesk., Gruy. and Verkl.) [17–19] and broomrape (*Orobanche crenata* Forsk.) [20]. As for powdery mildew, only one experiment has been reported, which presented unclear effects of pea/oat mixtures on disease reduction [21].

With the objective to assess benefits of diversification in the control of powdery mildew in pea, we established a series of experiments, first to identify the most efficient intercrop, and second to determine the optimal proportion of resistant and susceptible pea cultivars in a mixture.

2. Materials and Methods

2.1. Field Trials

Six field trials were carried out in two different locations in the South of Spain (Córdoba and Almodóvar del Río) from 2015 to 2019 (Table 1) to study the effect of various intercrops on pea powdery mildew infection.

Table 1. Field trials carried out for the study of the effect of intercropping on the pathosystem pea/powdery mildew.

Trial	Cord1-15(i)	Cord1-16(i)	Cord1-17(i)	Alm1-19(i)	Cord1-19(i)	Cord2-19(i)
Location	Córdoba	Córdoba	Córdoba	Almodóvar	Córdoba	Córdoba
Max. T (°C)	35.3	31.7	32.2	35.2	35.7	35.7
Min. T (°C)	−3.3	−2.3	−3.4	−0.8	−2.8	−2.8
Mean T (°C)	12.3	12.6	14.3	14.2	12.5	12.5
Rain (mm)	150	336	143.8	107.2	110.4	110

Each trial consisted of monocrops of pea, wheat, barley and faba bean (cvs. Messire, Califa, Henley and Brocal, respectively), intercrops of pea with each one of the other crops; and a monocrop of pea at 50% density (doubling the distance between rows). Each plot consisted of eight 3-m long rows (except for the plots with pea at 50% density, with only four rows), with 35 cm between rows. Sowing densities were 30 seeds/row for legumes, and 200 seeds/row for cereals. The intercropping system was alternate with replacement at 50%. This means that a row of each crop was alternatively sown, thus ending up with a rate of 50/50 in the plot (Figure 1). A randomized complete block design with four replications was used.

Disease severity (DS) was visually estimated as the percentage of whole plant canopy covered by powdery mildew. A single severity value was assigned to each plot as the overall DS on the two middle rows (discarding those plants at the extremes of the rows). Evaluations were made one week after first symptoms started and repeated every 7–10 days till plant senescence. The area under disease progress curve (AUDPC) was subsequently calculated with the following formula:

$$\mathrm{AUDPC} = \sum_{i=1} \frac{1}{2} \{ (y_{i+1} + y_i)(x_{i+1} - x_i) \} \quad (1)$$

where y_i = value of evaluated parameter at day 1, x_i = time (days) and n = total number of observations

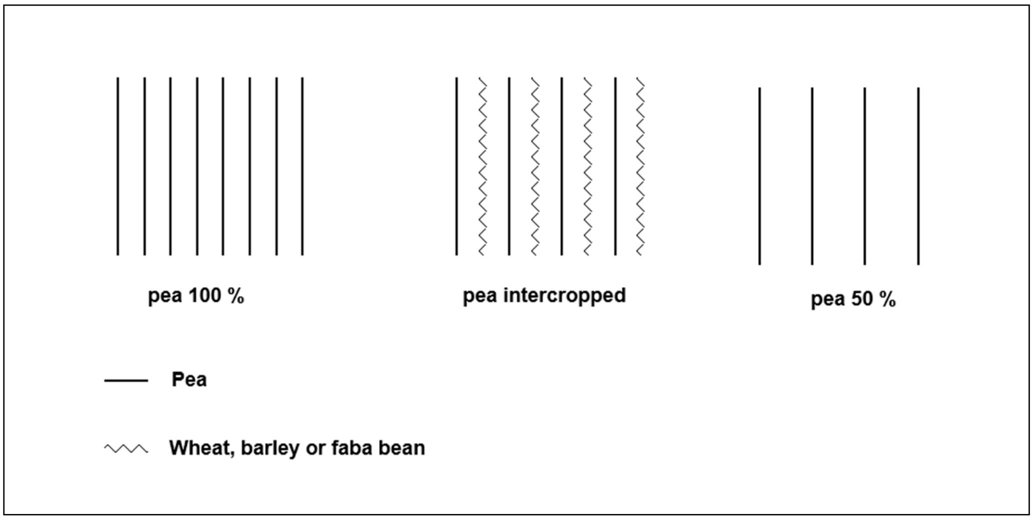


Figure 1. Experimental design for the intercropping experiments.

Crop height was assessed at full maturity of crops in trials Cord1-18(i), Alm1-19(i) and Cord2-19(i). Five plants of each crop per plot were measured from the ground to the top of plants, not stretching them, with the help of a ruler. Differences between the height of the companion crops and of pea in the intercropped plots were then calculated. The two central rows of each crop in each plot were harvested. Plants were dried in an oven at 60 °C for 3 days, and then plant biomass was assessed by weighing dry plants from each row. After this, plants were threshed and seeds were weighed. Biomass data were available for trials Alm1-19(i), Cord1-19(i) and Cord2-19(i), and grain yield data were available for trials Alm1-19(i) and Cord2-19(i).

The land equivalent ratios (LERs) of grain and biomass yield values were calculated as follows [22]:

$$LER_{px} = Y_{ip}/Y_{mp} + Y_{ix}/Y_{mx}$$

where LERpx represents the LER value (either for grain yield or biomass yield) of a given combination of pea and other crop "x" (faba bean, wheat or barley). Y_{ip} and Y_{mp} are the yields of pea intercropped and in monocrop, respectively; Y_{ix} and Y_{mx} are the yields of the other crops in intercrop or monocrop, respectively.

Additional field trials were performed over four seasons (Table 2) in which the powdery mildew susceptible pea cv. Messire and its resistant isoline Eritreo [8] were mixed at different ratios (100/0, 75/25, 50/50, 25/75 and 0/100). Mixtures were made by alternating different rows of each cultivar (Figure 2). Disease severity (DS) in cv. Messire was evaluated as described above for the intercropping trials.

Table 2. Field trials carried out for the study of the effect of cultivar mixtures on the pathosystem pea/powdery mildew.

Trial	Cord1-15(c)	Cord1-16(c)	Alm1-19(c)	Cord1-19(c)	Cord2-19(c)
Location	Córdoba	Córdoba	Almodóvar	Córdoba	Córdoba
Max. T (°C)	35.3	31.7	35.2	31.2	31.2
Min. T (°C)	−2.2	−2.3	−0.8	−2.8	−0.7
Mean T (°C)	13.8	12.9	13.5	12.0	12.9
Rain (mm)	121.8	281.8	107.8	110	109.4

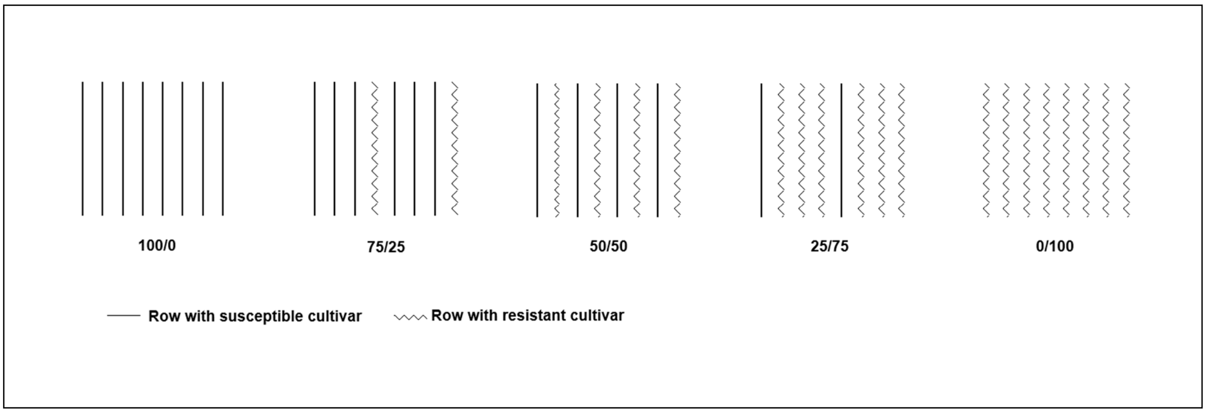
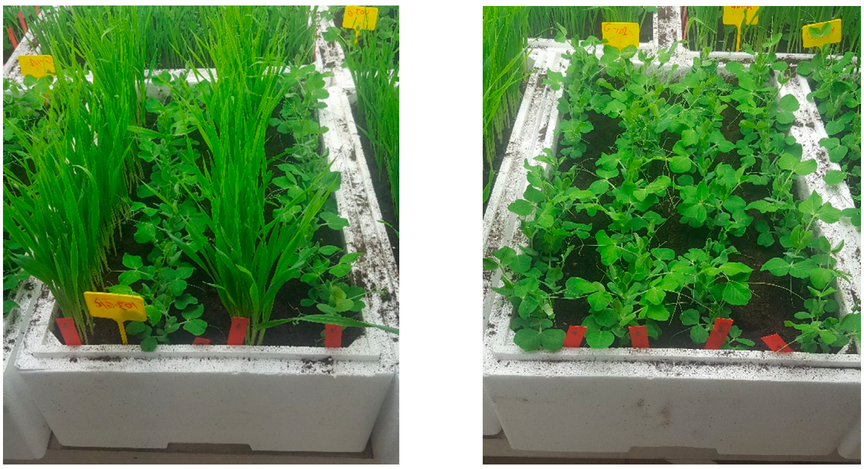


Figure 2. Experimental design for cultivar mixture experiments. Proportions are for Messire/Eritreo cultivars.

2.2. Controlled Conditions Experiment

An experiment was performed on seedlings under controlled conditions to investigate possible barrier effects of cereals on powdery mildew dispersal. Seeds were planted in 4 rows separated 7 cm in polystyrene boxes (34 × 55 × 16 cm, width:length:height) filled with a mixture of sand and peat at a 1:3 ratio (v:v) following three treatments: pea monocrop, pea/wheat intercrop and pea/barley intercrop. The numbers of seeds per row were 18 for pea and 150 for the cereals. Treatments were replicated five times. In the intercrops, the first row was always a cereal, with pea in the second and fourth rows (Figure 3).The fifteen boxes were placed in a growth chamber with a photoperiod of 12 h of visible light (150 µmol m^{-2} s^{-1} photon flux density) at 25 °C, and 12 h of darkness at 20 °C. Seventeen days after sowing, seedlings were inoculated with *Erysiphe pisi* isolate CO-07. The isolate originated from infected pea crop in Córdoba and was maintained on living pea plants at IAS-CSIC. *E. pisi* spores from infected leaves were blown with the help of an air compressor in a perpendicular direction towards the first row of each box, distributing them homogenously along the line of the row. Numbers of leaves of cereal plants at the time of inoculation were counted. Ten days after inoculation, pea plants on the second and fourth row of each box were evaluated for severity of powdery mildew. Then, dry biomass of cereal plants was measured as described above for the field trials.



(**A**) (**B**)

Figure 3. Pea monocrop (**A**) and pea intercropped with barley (**B**) in the experiment under controlled conditions.

2.3. Statistical Analysis

For the intercropping trials, areas under the disease progress curves (AUDPCs) were calculated using data of powdery mildew severity by trapezoidal integration. Data of

AUDPC were standardized (SAUDPC), considering the length of the evaluation period [23]. The effect of the treatment (pea monocrops and the different combinations) on the dependent variable (area under disease progress curve) was examined for each environment (i.e., year × location) using Dunn's test, while Friedman's test was used to compare the effect of the treatment for the set of environments. Both tests were used because the data did not satisfy the requirements of parametric tests regarding normality, homogeneity of variance or sphericity. The O'Brien test was used to study the homogeneity of the variances, while the Shapiro–Wilk Test was used to examine whether data conformed to a normal distribution. The means were compared using Dunn's test with a Bonferroni adjustment at $p = 0.05$ [24].

The effects of treatment and environment on LER values (grain and biomass yields) were subject to two-way analysis of variance (ANOVA) because these data satisfied the normality and homogeneity of variance requirements of ANOVA. To test whether the LER value of each treatment differed from the hypothesized value ($\mu \neq 1$), the confidential interval (C.I.) for the mean of each treatment was calculated, and one-sample t-test was performed. Factorial ANOVAs were also carried out for biomass of the companion crops and height differences.

The effect of the percentage of sowed resistant cultivar (cv. Eritreo) with respect to the susceptible one (cv. Messire) was studied employing the exponential equation of Kiyosawa and Shiyomi [25]:

$$\partial y/(\partial r) = -by \tag{2}$$

where y represents the severity of symptoms on the susceptible cultivar (SAUDPC), r represents the ratio of the resistant cultivar and b is the rate of decrease in disease per unit increase in the resistant cultivar (i.e., slope). Overall, with higher disease severity, there is a higher effect due to the resistant cultivar. The previous equation was linearized as $\text{Lny} = a - b \times r$, in which a is a constant. For each ambient, the linearized equation fit the data well based on the coefficient of determination ($R^2 > 0.620$; $p < 0.001$) and the pattern of residuals [26]. Subsequently, we linearized the selected model and compared the regression lines for each ambient based on their homogeneity of variances, slopes and intercepts.

In the experiment conducted under controlled conditions, the effect of the treatment on the disease severity on pea leaves was subject to ANOVA. Previously, the data were arcsin-transformed to satisfy the normality and homogeneity of variance. Because we were more interested to compare the treatments, the sowing rows were used as blocks. Treatment and environment means were compared using Tukey's HSD test at $p = 0.05$. Biomass and number of leaves of barley and wheat were compared by ANOVA. All the data were analyzed using the software Statistix 10 (Tallahassee, FL, USA).

3. Results

3.1. Intercropping in the Pathosystem Pea/Powdery Mildew

3.1.1. Field Trials

Powdery mildew disease was present in pea in all trials, with a wide range of incidence across them (Table 3).

Table 3. Final powdery mildew disease severity (DS) for each treatment of the different intercropping trials carried out (SE: standard error).

	Cord1-15(i)	Cord1-16(i)	Cord1-17(i)	Alm1-19(i)	Cord1-19(i)	Cord2-19(i)
Pea 100%	29.3	65.7	64.9	30.0	69.9	69.5
Pea/barley	13.8	47.7	53.3	5.1	59.4	43.8
Pea/faba bean	15.0	33.7	66.6	15.0	63.3	51.0
Pea/wheat	17.3	59.2	59.2	10.4	64.1	57.1
Pea 50%	12.0	53.6	48.8	30.0	69.2	49.4
SE	5.5	6.0	6.0	4.2	11.9	5.8

The global analysis revealed significant differences ($p < 0.05$) among treatments for SAUDPC (Figure 4). Powdery mildew infection on pea was reduced when pea was intercropped with barley or with faba bean (a decrease of 44% and 32% in SAUDPC, respectively). Powdery mildew was not significantly reduced on pea intercropped with wheat or when grown in monocrop at 50% density.

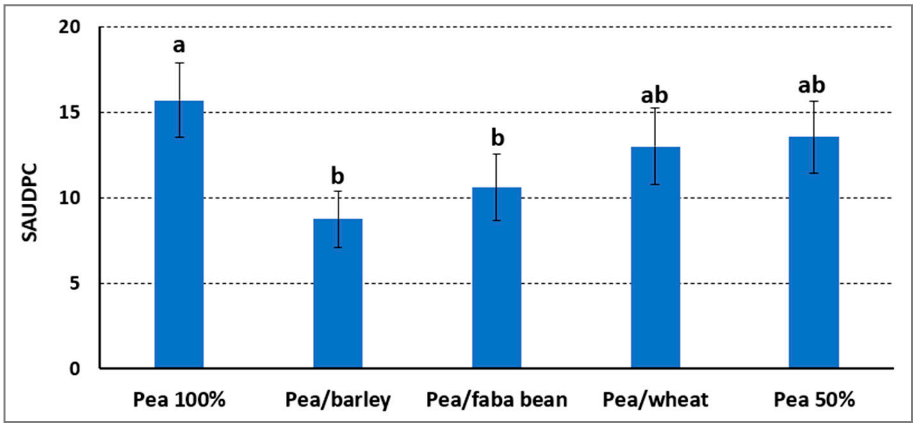


Figure 4. Standardized area under disease progress curve (SAUDPC) for severity of powdery mildew in pea in the different treatments evaluated across the six intercropping field trials. Different letters mean significant differences (Dunn's test corrected by Bonferroni, $p < 0.05$). Bars for standard errors are shown.

Pea/barley intercrops presented LER values slightly higher than those of pea/wheat, but differences were not significant. Likewise, no LER values significantly deviated from 1 (Table 4).

Table 4. Land equivalent ratio (LER) values for grain yield (trials Alm1-19(i) and Cord2-19(i)) and biomass (trials Alm1-19(i), Cord1-19(i) and Cord2-19(i)). No significant differences between any of them in each case were detected, and they did not significantly deviate from 1.

	LER Grain Yield	LER Biomass
Pea/barley	1.06	1.07
Pea/faba bean	1.03	0.92
Pea/wheat	0.85	0.96

The factorial analysis for biomass of the companion crops, with crop and cultivation system (intercrop or monocrop) as fixed factors, revealed a significant interaction between factors ($p < 0.05$); barley biomass was higher than that of the other crops, but even higher when intercropped with pea, which did not occur with the others (Figure 5). ANOVA for plant height differences also detected significant differences between crops ($p < 0.05$), with barley showing a higher height difference with pea than wheat or faba bean (Table 5).

Table 5. Height differences between the companion crops and pea in each intercropped plot in trials Cord1-18(i), Alm1-19(i) and Cord2-19(i). Different letters on the same crop mean significant differences (LSD test, $p < 0.05$).

	Height Difference with Pea (cm)
Barley	20.0 a
Wheat	12.9 b
Faba bean	12.3 b

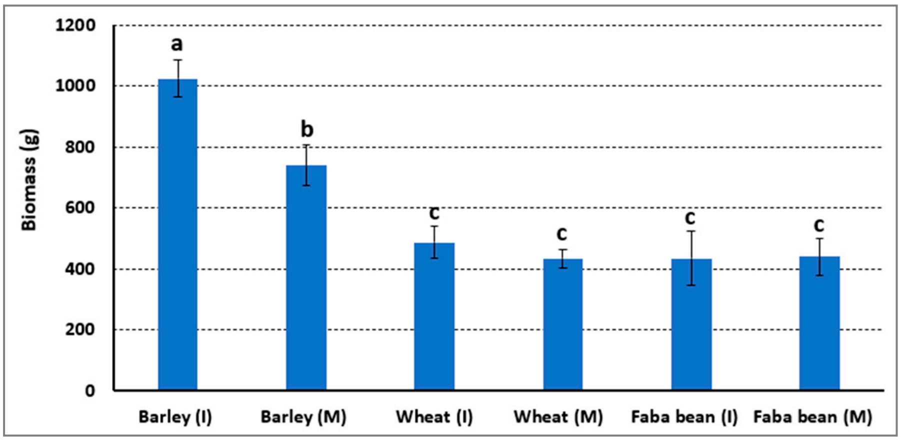


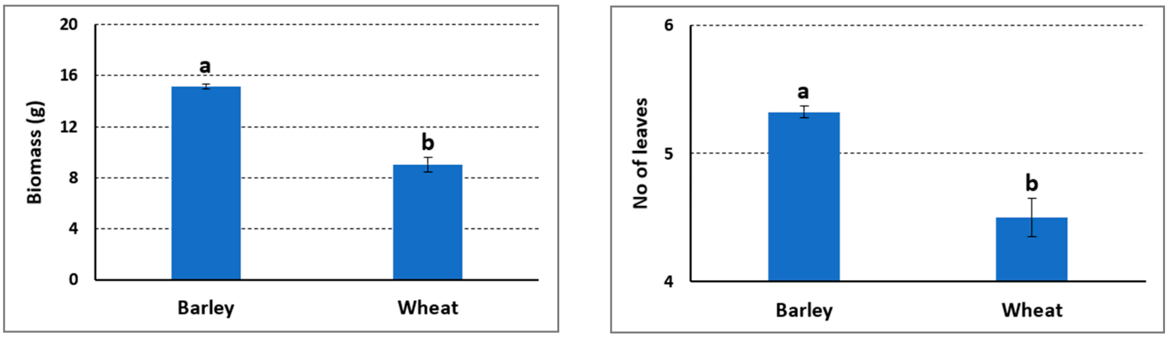
Figure 5. Biomass for the different companion crops (I: intercrop; M: monocrop) in trials Alm1-19(i), Cord1-19(i) and Cord2-19(i). Different letters mean significant differences (Tukey's test, $p < 0.05$). Bars for standard errors are shown.

3.1.2. Controlled Conditions Experiment

Factorial analysis showed significant differences ($p < 0.05$) for powdery mildew severity for factors treatment and row (distance to the focus of inoculum), with no interaction among them. Powdery mildew severity was highest in pea monocrop, followed by pea intercropped with wheat, and lowest in pea intercropped with barley (Table 6). In all treatments, powdery mildew severity decreased with distance to the inoculation point, being lower in the fourth than in the second pea row. Dried biomass and number of leaves of barley were significantly higher ($p < 0.05$) than those of wheat (Figure 6).

Table 6. Powdery mildew disease severity (%) on pea seedlings grown in rows 2 and 4 of the boxes. Different letters per treatment (pea monocrop, pea/wheat and pea/barley intercrops) mean significant differences (Tukey test, $p < 0.05$).

	Row 2	**Row 4**
Pea	33.5 a	24.1 b
Pea/wheat	11.9 c	10.5 d
Pea/barley	5.2 e	3.6 f



(**A**) (**B**)

Figure 6. Dry biomass (**A**) and number of leaves (**B**) of barley and wheat in the experiments under controlled conditions. Different letters mean significant differences (Tukey test, $p < 0.05$). Bars for standard errors are shown.

3.2. Cultivar Mixtures in the Pathosystem Pea/Powdery Mildew

A wide range of disease severity was found (Table 7). In all trials, powdery mildew decreased in susceptible cv. Messire as the proportion of the resistant cv. Eritreo increased in the mixture. No powdery mildew was recorded in the plots with 100% of Eritreo.

The results were adjusted to a non-linear regression curve, with a pseudo R^2 of 0.5829 (Figure 7).

Table 7. Final powdery mildew disease severity (%) in cultivar Messire for each treatment of the different cultivar mixtures trials carried out (S/R: proportions of susceptible cultivar Messire and resistant cultivar Eritreo; SE: standard error).

S/R in Mixture (%)	Cord1-15(c)	Cord1-16(c)	Alm1-19(c)	Cord1-19(c)	Cord2-19(c)
100/0	19.0	66.7	28.6	54.5	41.6
75/25	10.8	52.6	30.4	34.6	37.1
50/50	7.3	45.1	18.8	18.0	9.6
25/75	6.5	33.9	16.1	18.9	17.0
SE	2.8	1.7	4.7	2.7	5.3

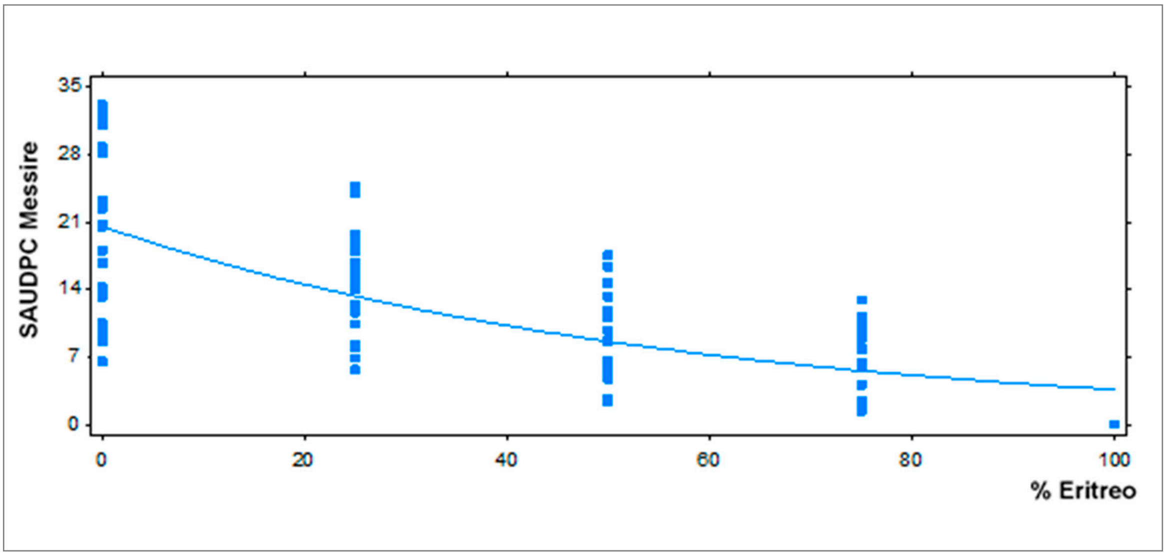


Figure 7. Nonlinear regression fitted curve for powdery mildew SAUDPC in susceptible cultivar Messire with varying proportions of resistant cultivar Eritreo across five different experiments.

4. Discussion

Crop diversification, consisting of growing different crops or cultivars simultaneously in the same piece of land, is considered to increase crop resilience [27]. Cereal–legume intercropping might be particularly interesting in low-input systems by reducing the requirements for fossil-based fertilizer N [14,28]. A number of reports has shown reduction of pests and diseases [10,29–32]. However, these effects are only quantitative and influenced by environmental factors, therefore requiring monitoring and case by case adjustments. In this work we quantified the reduction of powdery mildew on pea under different cropping systems, either intercropped with barley, wheat or faba bean, monocropped at reduced plant density, or in mixtures of resistant and susceptible pea cultivars. We opted for alternate-row, replacement intercropping at 50% proportion. An addition intercropping system (i.e., introducing rows of the second crop in between the rows of the first crop, so in practice halving the distance between rows) was not studied, but we speculate that doubling plant density would reduce aeration and increase relative humidity around leaves, thus favoring the infection and proliferation of the pathogen [10].

Our results show a significant reduction of powdery mildew on pea when intercropped with barley or with faba bean (44% and 32% SAUDPC reduction, respectively). This tendency was consistent across six field trials, in two different sites, within a time span of four years, under an ample range of disease pressures. It is the first time that such a wide study on the effect of intercropping on pea powdery mildew has been reported. Zivanov et al. carried out one field trial of pea intercropped with oat, with inconclusive effects on powdery mildew; they reported a 20–30% disease reduction in pea leaves, but no effect on global disease on pea plants [21]. Our results are in line with what has been found for other diseases in grain–legume intercropping systems, with disease reductions in the range of 20–50% [10]. It is also similar to the described reduction of Ascochyta blight on pea when intercropped with barley [19], although Fernández-Aparicio et al. reported higher reductions in mixtures with triticale and faba bean [18].

Numerous mechanisms have been suggested to explain the effect on intercropping on plant diseases [10,33]: morphological and physiological changes in host, reduced density of the host crop (dilution effect), a barrier effect to spore dispersion, alteration of the microclimate or inhibition of the pathogen by allelochemicals. One or more of these mechanisms may be present in a particular intercropping system. From our field experiments, it is difficult to elucidate the mechanisms behind the powdery mildew reduction. We might speculate on the dilution effect, as host plants are reduced to 50% in the mixture, so the production of secondary inoculum would be reduced. Additionally, pea rows are at twice the distance from one another, so it is more difficult for the fungal spores to travel to produce new infections. However, the fact that there were no significant differences in pea monocrops at 100% and 50% density does not support this dilution effect. Moreover, the better aeration in pea monocrop at 50% density did not result in a reduction of powdery mildew.

The barrier effect by the non-host crop, then, may play an important role in hampering the spread of the disease, especially with this alternate-row design. The non-host crop acts as a physical barrier to spore movement from row to row, hindering the development of successive cycles of infection. In this case, barley and faba bean were more effective than wheat in reducing powdery mildew on pea. Barley produced more biomass than wheat and faba bean, making it a denser barrier; it also produced more biomass in intercrops with pea than as a monocrop, which may indicate that barley benefits from the synergy with pea but also benefits from the lower sowing density, with less barley plants competing with each other from resources in the same space. As for plant height, the difference between barley and pea is greater than that of wheat and faba bean. This suggests a strong barrier effect by barley in the decrease of powdery mildew. The role of faba bean, on the other hand, seems more complex to clarify, but it is also likely that the barrier effect plays an important role, as has been described for the reduction of diseases in wheat intercropped with faba bean [16]. The barrier effect of barley was further supported by the results of the experiment under controlled conditions. This effect could be assessed independently by controlling the direction of the flow of spores through the rows of the cereal before reaching the pea plants. Evaluated symptoms were those originated from the primary infection, avoiding the complexities of second cycles of infection that would be accumulative. Results of biomass and number of leaves in this experiment confirm faster and greater development of barley over that of wheat, even at the seedling stage, which may account for its higher efficiency as a barrier despite the similarities between both crops.

It has been reported in many cases that the combination of pea with cereals confers benefits in terms of LER (grain and forage), although it is not always so [12,34]. Combining pea and faba bean is less common, but again, positive and negative effects on yield have been described [35,36]. In our experiments, no grain or biomass yield advantage or disadvantage was found. This neutral effect of intercropping on yield facilitates its use in the control of powdery mildew in pea.

The use of varietal mixtures offers a different approach to biodiversity when it is not desired to grow different crops in the same field. The employment of resistant cultivars is an efficient and sustainable strategy to control diseases, but they pose some drawbacks if they are not properly managed. One of the main problems is the overcoming of resistance by the pathogen. The chance of this happening is higher with the multiplication in space and time of the resistant variety; the resistant genes are repeatedly exposed to the pathogen, which by competitive selection may finally find a way to surmount the resistance [37]. The rationale behind the utilization of cultivar mixtures is to have in the field a sufficient "amount" of resistance genes to prevent the disease from causing important damage, but not so many as to exert too high a selection pressure on the pathogen that might finally lead to the overcoming of the resistance. For these mixtures to be effective, it is important that there exist contrasting resistance levels to the disease [38]. Cultivar Eritreo is a near-isogenic line of cultivar Messire carrying gene *Er3*, which confers hypersensitive resistance to powdery mildew [8]. Given that it is a monogenic resistance, there exists a high risk of being overcome by the pathogen, so the mixture with another variety appears as a good strategy to safeguard the resistance [9]. The results show that the SAUDPC values adjust to a non-linear regression with the percentage of resistant cultivar in the mixture, so disease symptoms in the susceptible cultivar decreases as the proportion of the resistant one increases. It is possible to get a remarkable reduction of disease even with a small percentage of the resistant variety (25%), as previously described for *Septoria tritici* blotch in wheat [39,40]. This significant disease decrease with the introduction of just one row of the resistant variety may also point to the importance of the barrier effect, which has been observed in other cultivar mixtures in which the resistant cultivar hampers the movement of spores to other rows of susceptible cultivars [41]. Determining the final optimal proportions of the components of the mixtures may be a complex task that takes into account different factors [34,42], although the expected levels of disease in the area may condition the proportion of the resistant variety required.

In conclusion, in this work it has been established for the first time that diversification is a good tool for the control of powdery mildew in pea, whether it is by mixing pea with another crop or by mixing two cultivars of pea. This adds up to the known advantages of diversification for agriculture, which is of great importance in the context of sustainable agriculture and especially when it comes to organic farming, where the use of fungicides is not accepted. Future work should focus on other diseases of pea, such as rust, and on identifying the best options to simultaneously face different biological stresses, including weeds.

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