

1 Effects of crop mixtures on rust development on faba bean grown in

2 Mediterranean climates

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15 Abstract

16 Faba bean (*Vicia faba*) is a temperate grain legume of major importance in Mediterranean
17 agriculture. Faba bean production can be severely constrained in the area by rust incited by
18 *Uromyces viciae-fabae*. Control with fungicides is possible, but there is a growing interest on
19 alternative control methods, compatible with organic agriculture. Intercropping faba bean with
20 cereals has been suggested as an alternative to maximize land use and increase crop resilience,
21 with potential beneficial effects reducing weeds and diseases. In the present work, we studied
22 the effect of intercropping faba bean with barley, triticale or wheat on faba bean rust
23 development. Field studies performed over two consecutive seasons in two different countries
24 showed that faba bean rust is significantly reduced when faba bean is intercropped with cereals
25 confirming the value of faba bean-cereal intercropping for the management of faba bean rust.
26 These results confirm that, under our conditions, intercropping faba bean with cereals (oat,
27 barley, wheat or triticale) could usefully contribute to the management of faba bean rust.

29 **Keywords:** Intercropping, Organic farming, Disease management, *Uromyces viciae-fabae*,
30 *Vicia faba*

31

32 **Introduction**

33 Faba bean (*Vicia faba* L.) is a temperate annual legume crop cultivated worldwide. It is an
34 important source of protein in developing countries and is used as a human food and feed
35 (Karkanis et al., 2018). It is also grown for green manure and can significantly improve yields
36 of cereals and other crops. Faba bean needs a cool season for better growth and the optimum
37 temperatures for production range from 18 to 27°C. It is well adapted to Mediterranean-type
38 climates, where it is sown in autumn, and to cold-temperature regions where it is sown in spring
39 (Mínguez and Rubiales, 2020).

40 Faba bean rust (*Uromyces viciae-fabae* Pers.) is one of the main diseases of faba bean
41 in nearly every area in the world where faba bean is grown and can cause yield loss up to 70%
42 in early infection (Stoddard et al., 2010). Control is possible by the use of fungicides (Emeran
43 et al., 2012) and resistant cultivars (Ijaz et al., 2017; Sillero et al., 2017). However, there is an
44 increasing demand for unconventional management practices as fungicides are expensive,
45 unfriendly for the environment and people, and can lose effectivity against pathogens. At the
46 same time, resistant cultivars are limited and resistance available so far is only incomplete
47 (Sillero et al., 2000).

48 Intercropping, consisting of growing different crops together simultaneously in the same
49 piece of land at different mixing rates was a common practice in the past, being currently
50 reevaluated for its potential to increase crop resilience (Finckh et al., 2000; Hauggard-Nielson
51 et al., 2006; Mikić et al., 2015). Intercropping is believed to have beneficial effects providing a
52 degree of weeds, pests and disease reduction. This protection use however to be incomplete and

53 to be influenced by environmental factors (Boudreau, 2007; Malézieux et al., 2009), therefore
54 needing monitoring and case by case adjustments.

55 Cereal-legume combinations are among the most common intercropping systems
56 practiced worldwide (Willey, 1979) providing advantages in yield particularly in low-input
57 systems (Bedoussac et al., 2015). The increased N use efficiency in cereal/ legume intercrops
58 can reduce the need of fossil-based fertilizer N by about 26% on a global scale (Jensen et al.,
59 2020). Intercropping with cereals has already been reported to improve alternaria leaf spot,
60 chocolate spot, fusarium wilt and broomrape control in faba bean (Sharaiha et al., 1989;
61 Fernández-Aparicio et al., 2007, 2011; Sahile et al., 2008a; Yang et al., 2014; Zhang et al.,
62 2019) as well as on diseases of the cereals (Luo et al., 2021). However, negative (Zhang et al.,
63 2019) or insufficiently quantified reports are available on the potential of intercropping for faba
64 bean rust management (Terefe et al., 2016; Ijaz et al., 2017; Villegas-Fernández et al., 2017).
65 However, even for the diseases in which significant reduction was reported, this was incomplete
66 and variable among environments. The objective of the current study was to quantify the effect
67 of intercropping faba bean with cereal species on the development of *U. viciae-fabae* in faba
68 bean in Mediterranean environments.

69

70 **Materials and methods**

71 **Sites and soil**

72 The experiments were conducted in two countries with long tradition of faba bean cultivation
73 in which rust is a major limiting factor, but in which cultivation practice are very contrasting.

74 These were Palestine where rain fed faba beans are grown and Egypt where they are grown
75 under flood irrigation. Locations were selected based on their known history of high and
76 uniform infestation of *U. viciae-fabae*. These were Tulkarm at Palestine (32.31519° N,
77 35.02033° E, 75 m altitude), with light clay chromic luvisol with a pH of 7.0-7.5, in Kafr El-

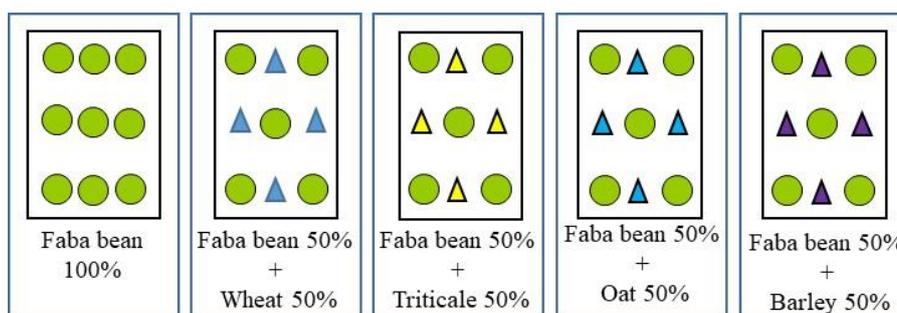
78 Sheik, Egypt (30°47' N, 30°59' E, 0 m altitude) with loamy calcareous entisol with a pH of 7.5-
79 8.0 during 2006-2007 and 2007-2008 seasons. In all these locations, due to their mild winter
80 and hot spring, faba beans are autumn sown.

81

82 **Validation of optimal crop mixtures**

83 Four intercrop mixtures were used in this study: faba bean (cultivars Nobarria 1 at Egypt, and
84 Baladi at Palestine) intercropped either with barley, with durum wheat, with oat or with triticale
85 (cultivars Cory, Meridiano, Aspen and Peñarroya, respectively). The experimental unit
86 consisted of 1.8 x 10 m plots, with 6 rows 10 m long, separated 0.3 m (Figure 1). Plots were
87 laid out in a randomized complete block design with 4 replicates. Faba bean monocrop with a
88 plant density of 100 faba bean plants m⁻² was used as a control. Species were mixed
89 intercropped within rows. All species were sown by hand at the 1st of November in all
90 environments. No chemical fertilizers or pesticides were applied across the experiment. Weeds
91 were removed by hand weeding when needed. No irrigation was applied at Tulkarm whereas
92 surface irrigation took place four times at Kafr El-Sheikh (400 m³ ha⁻¹ were applied each time).
93 The first irrigation was done after sowing, the second one during December the third during
94 March, and the fourth during April.

95 The rust susceptible faba bean cv. Brocal was grown in a single, long row perpendicular to the
96 rows of the tested plots to act as rust spreader. No artificial inoculation was performed as faba
97 bean rust was known to occur every year naturally on both sites.



99 **Figure 1:** Diagram of planting patterns in the field experiments

100 **Disease assessment**

101 Disease severity (DS) was evaluated by visual estimation of the percentage of leaf area covered
102 with rust pustules. DS evaluation was estimated using 10 randomly chosen plants per plot. First
103 DS evaluations were made four weeks after onset of the first symptoms on the spreader rows
104 of faba bean cv. Brocal. This was followed by two additional DS evaluations, at two weeks
105 interval. The third DS evaluation was used as final disease severity (DSf). DS data were used
106 to calculate the area under the disease progress curve (AUDPC) using the formula:

$$107 \quad \text{AUDPC} = \sum_{i=1}^k 1/2[(DS_i + DS_{i+1})(t_{i+1} + t_i)]$$

108

109 Where DS_i is the rust severity at evaluation date i , t_i is the number of days after the first
110 observation on evaluation date i and k is the number of consecutive evaluations. The epidemic
111 growth rate (r) was obtained by linear regression of the transformed DS against time: $\text{trans}(DS)$
112 $= \text{logit}(DS) + 10 = \ln(DS/100 - DS) + 10$ (10 was added to obtain a positive number). Time of
113 the first pustule's appearance (t_0) was calculated from the straight line slope equation $y = mx$
114 $+ b$, and was measured as number of days from sowing to first pustule's appearance on the
115 leaves (Barilli et al., 2009).

116

117 **Plant stature and biomass assessments**

118 To study the effect of plant height on rust development, the height of 10 randomly chosen plants
119 per plot, was measured for faba bean and the associated crop. Measurements were recorded at
120 faba bean pod filling stage. The averages of the 10 plants were used for statistical analysis.

121 To study the effect of plant biomass on rust development, all plants within 0.5 m² of each plots
122 were collected by cutting the plants at soil level when the faba bean was at pod filling stage.
123 Then, faba bean plants were separated from the associated crop and then fresh biomass of each
124 crop was weighted separately.

125 **Statistical analysis**

126 Analysis of variance (Two-way ANOVA) was conducted using PROC MIXED of SAS/STAT
127 software (version 9.0 for Windows) (SAS institute 2002). The analysis was carried out for each
128 season and each location separately. The terms fitted in the analysis model included the effects
129 of block and intercrop mixture. The residuals (errors) were obtained and investigated for any
130 departure from normality using Shapiro-Wilk test. The results of this test showed no significant
131 deviations from normality for all studied variables ($P > 0.05$) indicating that ANOVA is
132 appropriate for the analysis of the data. Multiple comparisons among pairs of intercrop mixtures
133 were made by the Tukey-test.

134

135 **Results**

136 Uniform rust infection was observed in both seasons at both locations. Infection started earlier
137 in Egypt than in Palestine in both seasons (Fig. 2) which could be explained by cooler winters
138 in Palestine (Table 1 and 2). Onset of symptoms (t_0) occurred around 107-109 days after sowing
139 in Egypt and 115-116 days in Palestine in both seasons. No year x treatment interaction effect
140 was observed.

141 **Table 1:** Average temperature ($^{\circ}\text{C}$) at each growing season and site of experimentation.

Location	Season	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Tulkarm, Palestine*	2006-2007	21.6	17.9	13	15.1	21.7	24.6
	2007-2008	23.7	18	14.9	15.2	15.5	19.2
Kafr El-Sheikh, Egypt**	2006-2007	23.2	20.1	19.3	20.2	22.8	25.3
	2007-2008	25.9	21.7	18.1	19.2	25.4	27.8

142

143 **Table 2:** Total rain fall (mm) at each growing season and site of experimentation.

Location	Season	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
Tulkarm, Palestine*	2006-2007	148.3	82	77.8	104	10.5	0.0	422.6
	2007-2008	32.7	151	22.7	151.5	105.9	6.2	470
Kafr El-Sheikh, Egypt**	2006-2007	3.2	10	17.5	44.1	9	11.5	95.3
	2007-2008	28	12.5	37.8	38	0.0	0.0	116.3

144 *rain fed, **irrigated

145 There was a general trend for slight retardation on the onset of infection (longer t_0) in the studied
146 intercrops, although these were seldom significant (Table 3). Apparent growth rate (r) in
147 monocrops was 0.167 and 0.114 respectively for Palestine and Egypt in 2006-2007 and 0.166
148 and 0.236 in 2007-2008. A general non-significant trend of reduction was observed in the
149 intercrops ranging in Palestine from 0.161 to 0.164 in 2006-2007 and 0.154 to 0.158 during
150 2007-2008 and in Egypt from 0.088 to 0.114 during 2006-2007 and 0.223 to 0.228 during 2007-
151 2008.

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153 **Table 3.** The effect of intercropping faba bean with cereals on rust development: time of the first pustule's appearance
 154 (t_0), apparent growth rate (r), final disease severity (DS_f) and AUDPC

Intercrops ¹	Tulkarm, Palestine								Kafr El-Sheikh, Egypt							
	2006-2007 ²				2007-2008 ²				2006-2007 ²				2007-2008 ²			
	$t_0^{3,4}$	r	DS_f	AUDPC	t_0	r	DS_f	AUDPC	t_0	r	DS_f	AUDPC	t_0	r	DS_f	AUDPC
FB 100%	115.1 ^b	0.167 ^a	35.8 ^a	765.4 ^a	119.3 ^a	0.166 ^a	39.8 ^a	724.4 ^a	109.4 ^a	0.114 ^a	47.5 ^a	782.3 ^a	107.2 ^b	0.236 ^a	54.6 ^a	1012.8 ^a
FB50% + B50%	117.8 ^b	0.163 ^a	23.9 ^b	522.9 ^b	117.2 ^a	0.154 ^a	20.0 ^b	505.1 ^b	111.5 ^a	0.114 ^a	34.0 ^b	472.0 ^b	109.7 ^a	0.223 ^a	38.0 ^b	573.5 ^b
FB50% + O50%	117.3 ^b	0.163 ^a	23.1 ^b	539.0 ^b	116.5 ^b	0.154 ^a	19.3 ^b	533.0 ^b	119.5 ^a	0.088 ^a	34.3 ^b	500.7 ^b	107.7 ^b	0.225 ^a	42.8 ^{ab}	760.4 ^{ab}
FB50% + T50%	115.3 ^b	0.161 ^a	26.1 ^b	629.4 ^b	117.0 ^a	0.158 ^a	23.5 ^b	614.4 ^{ab}	111.1 ^a	0.114 ^a	29.9 ^b	434.9 ^b	108.2 ^b	0.228 ^a	45.1 ^{ab}	779.1 ^{ab}
FB50% + W50%	118.7 ^a	0.164 ^a	24.9 ^b	586.2 ^b	117.9 ^a	0.157 ^a	24.1 ^b	526.4 ^b	110.2 ^a	0.132 ^a	33.3 ^b	541.3 ^b	108.5 ^b	0.226 ^a	42.4 ^{ab}	714.7 ^b

155 ¹ FB100%: faba bean monocrop, FB+B: faba bean 50% intercropped with barley 50%, FB+O: faba bean 50% intercropped with oat 50%, FB+T: faba bean 50% intercropped with triticale
 156 50%, FB+W: faba bean 50% intercropped with wheat 50%.

157 ² Per each year, column with the same superscripts are not statistically different (Tukey test, $p < 0.05$).

158 ³ t_0 was calculated from the straight line slope equation $y = mx + b$.

159 ⁴ r : the epidemic growth rate, t_0 : time of the first pustule's appearance, AUDPC: area under the disease progress curve, DS: the percentage of leaf area covered with rust pustules. DS_f : the final
 160 disease severity.

161 Faba bean rust developed during the months of March and April in Palestinian trials
 162 under warmer and drier conditions in 2007 than in 2008 and reached maximum values of
 163 diseased tissue (DSf) in monocropped faba bean plots (maximum DS of 35.8% in 2007 and
 164 39.8% in 2008). However, as disease started a few days later in 2008, AUDPC was slightly
 165 lower in 2008 in spite of a slightly higher final DS. All cereal in intercrop mixtures showed
 166 significantly less faba bean rust in comparison with monocropped faba beans. This cereal-
 167 mediated reduction being higher in 2007-2008 (average AUPDC reduction of 25.6%) than in
 168 2006-2007 (23.5%), with all cereals having similar effects except for barley (Table 3).

169 Higher rust severities were achieved in Egyptian trials, with 47.5% and 54.6% final DS
 170 in faba bean monocrops in 2007 and 2008, respectively. Rust started a few days earlier in 2008,
 171 and reached higher DS, resulting in a much larger ADPC in 2008 than is 2007. All intercrops
 172 reduced faba bean rust significantly in comparison with monocrops, with an overall reduction
 173 of 30.2% in 2007 and of 37.7% in 2008.

174 Faba bean plant stature varied with the environment, being taller in 2006-2007 in both
 175 locations (Table 5). There was no effect of the various intercrops on faba bean height in both
 176 locations except a significant reduction in faba bean height when it was mixed with barley at
 177 Kafr El-Sheikh during 2006-2007 season. The associated crop was higher in 2006-2007 in both
 178 locations.

179 **Table 5:** The effect of intercropping on plant height

Intercrops ¹	Tulkarm, Palestine		Kafr El-Sheikh, Egypt	
	2006-2007	2007-2008	2006-2007	2007-2008
FB100%	118.1 ^a	99.0 ^a	147.3 ^a	101.1 ^a
FB50% + B50%	116.3 ^a (94.6) [*]	104.0 ^a (85.6)	125.0 ^b (85.5)	100.8 ^a (63.1)
FB50% + O50%	115.3 ^a (135)	98.9 ^a (107.8)	141.7 ^{ab} (112.4)	101.8 ^a (62.5)
FB50% + T50%	114.1 ^a (106.5)	97.0 ^a (89.4)	144.9 ^a (128)	102.6 ^a (90.6)
FB50% + W50%	114.3 ^a (84.4)	98.9 ^a (94.1)	137.1 ^{ab} (92.5)	92.2 ^a (63.7)

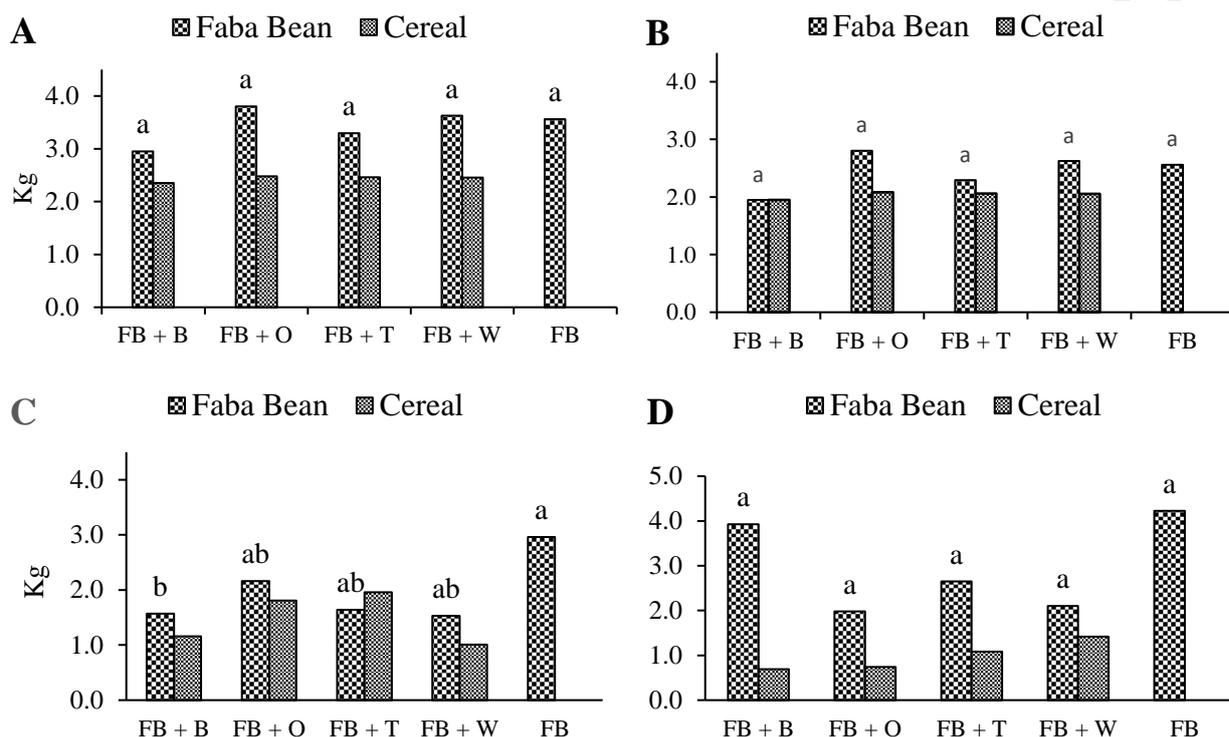
180 ¹ FB100%: faba bean monocrops, FB+B: faba bean 50% intercropped with barley 50%, FB+O: faba bean 50%
 181 intercropped with oat 50%, FB+T: faba bean 50% intercropped with triticale 50%, FB+W: faba bean 50%
 182 intercropped with wheat 50%.

183 * Faba bean plant height and in brackets the height of the associated cereal crop.

184 Means in the same column with similar superscripts are not statistically different (Tukey test, $p < 0.05$).

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When faba bean fresh biomass was assessed no significant reduction was observed in both seasons and both locations except intercropping with barley in Egypt in 2006-2007 season (Fig. 2). Intercropping faba bean with triticale gave the highest accumulative faba bean fresh biomass in Egypt during 2008.



191 **Figure 2:** Plant fresh biomass of monocropped faba bean at full plant densities and when intercropped with oat
 192 (O), barley (B), wheat (W) and triticale (T). Data correspond to the mean values in kg/m² made at Tulkarm,
 193 Palestine 2007 and 2008 (Fig. A and B.), and at Kafr El-Sheikh, Egypt 2007 and 2008 (Fig. C and D). Data are
 194 mean (n = 4). Treatments with the same letter are not statistically significant different (Tukey test, p < 0.05).
 195

196 Simple correlation coefficients between disease severity and plant height and plant fresh
 197 weight were computed and presented in Table (4). Significant positive correlation was found
 198 between DSf in each season in both countries. High negative significant correlation was found
 199 between DSf on faba bean and the height and fresh biomass of the associated crop in both
 200 seasons and both countries. Positive non-significant correlation was found between DSf and
 201 faba bean plant fresh weight.

202

203 **Table 4:** Correlation coefficients for final disease severity (DSf) and plant fresh weight and plant height.

Palestine					
	DSf 2008	Weight of faba bean 2007	Height of faba bean 2007	Weight of associated crop 2007	Height of associated crop 2007
DSf 2007	0.922 (0.000)	0.114 (0.631)	0.135 (0.570)	-0.908 (0.000)	-0.898 (0.000)
		Weight of faba bean 2008	Height of faba bean 2008	Weight of associated crop 2008	Height of associated crop 2008
DSf 2008		0.208 (0.379)	-0.146 (0.540)	-0.905 (0.000)	-0.906 (0.000)
Egypt					
	DSf 2008	Weight of faba bean 2007	Height of faba bean 2007	Weight of associated crop 2007	Height of associated crop 2007
DSf 2007	0.620 (0.000)	0.410 (0.073)	0.293 (0.210)	-0.529 (0.016)	-0.866 (0.000)
		Weight of faba bean 2008	Height of faba bean 2008	Weight of associated crop 2008	Height of associated crop 2008
DSf 2008		0.008 (0.975)	0.049 (0.836)	-0.385 (0.094)	-0.620 (0.004)

204 Actual P values are between brackets.

205

206 **Discussion**

207 Intercropping is useful for agriculture in terms of yield stability, increase in total yield, pest and
208 disease management, weed management, erosion control, and soil fertility amongst others
209 (Hauggard-Nielson et al., 2006). The results of this study, obtained over two growing seasons
210 in two countries, confirm that intercropping faba bean with cereals is an effective strategy for
211 controlling faba bean rust. The experiment was designed based on replacing half of the host
212 plant species (faba bean) with the associated cereal crop. Reducing host plant density and
213 increasing the distance between faba bean plants resulted in reduction of the amount of
214 susceptible plant tissue per field area unit leading to reduction in the likelihood of spore
215 deposition (Fernández-Aparicio et al., 2011).

216 The reduction in rust severity due to intercropping with cereals observed in this study is
217 in agreement with a number of occasional reports of reductions on faba bean rust in Denmark
218 (Fernández-Aparicio et al., 2006), highland Ethiopia (Terefe et al., 2016; Eshesu et al., 2018)
219 and Spain (Villegas-Fernández et al., 2017). On the contrary Zhang et al. (2019) reported a
220 reduction in other faba bean diseases, but not significant for rust. Reduction has been reported
221 for other aerial diseases of faba bean such as chocolate spot and alternaria spot (Sharaiha et al.,
222 1989; Sahile et al., 2008b; Fernández-Aparicio et al., 2011). The cereals in the intercropping
223 system might work as a natural barrier that intercept rust urediospores carried by wind from
224 infecting faba bean plants resulting in reducing the amount of available effective inoculums to
225 infect other faba bean plants. This hypothesis of barrier effect of the accompanying crops is
226 supported by the significant association of the reduction of rust severity on faba bean with the
227 plant stature and fresh biomass of the accompanying crop. Nutritive status of the plant is known
228 to affect disease severity, with increased application of nitrogen fertilizer favouring rust and
229 other diseases. Luo et al. (2021) found that the relative control effect of intercropping on
230 diseases of wheat intercropped with faba bean increased as application of N increased,
231 suggesting that rational application of nitrogen fertilizer in intercrops contributes to disease
232 control. As long as we focus on low input agriculture, we did not study fertilization effects, but
233 following the studies of Luo et al. (2021) we hypothesise that faba bean rust reduction effects
234 of intercrops would increase with nitrogen fertilization.

235 The stratified dispersal of faba bean rust was modelled by Sapoukhina et al. (2010) to
236 identify the spatial patterns of cultivar mixtures that could slow epidemic spread, showing that
237 mixtures creates a natural barrier to the progression of a smooth epidemic wave. Besides
238 reduction in dispersal phase of rust, additional effects on rust life cycle other than dispersal
239 could be derived from alterations in faba bean nutritive status and changes in microclimate
240 inside canopy promoted by the cereal component (Boudreau and Mundt, 1992). Similar effects

241 have been reported for pea ascochyta blight spores in pea intercropped with cereal (Schoeny et
242 al., 2010), chocolate spot reduction in faba bean-maize and faba bean-barley intercrops (Sahile
243 et al., 2008 a,b; Guo et al., 2020) and powdery mildew reduction in pea-cereals intercrops
244 (Villegas-Fernández et al., 2021).

245 In this study, faba bean rust development was significantly affected by intercropping of
246 faba bean with cereals. These results confirm that, under our conditions, intercropping faba
247 bean with cereals (oat, barley, wheat or triticale) at 50:50% seeding ratio could usefully
248 contribute to the management of faba bean rust. In this study, the effect of intercropping on
249 yield and yield losses due to leaf rust was not evaluated

250

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256

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