Effects of crop mixtures on rust development on faba bean grown in Mediterranean climates

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Abstract

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

Introduction

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

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

Intercropping, consisting of growing different crops together simultaneously in the same piece of land at different mixing rates was a common practice in the past, being currently reevaluated for its potential to increase crop resilience (Finckh et al., 2000; Hauggard-Nielsen et al., 2006; Mikić et al., 2015). Intercropping is believed to have beneficial effects providing a degree of weeds, pests and disease reduction. This protection use however to be incomplete and
to be influenced by environmental factors (Boudreau, 2007; Malézieux et al., 2009), therefore needing monitoring and case by case adjustments.

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

**Materials and methods**

**Sites and soil**

The experiments were conducted in two countries with long tradition of faba bean cultivation in which rust is a major limiting factor, but in which cultivation practice are very contrasting. These were Palestine where rain fed faba beans are grown and Egypt where they are grown under flood irrigation. Locations were selected based on their known history of high and uniform infestation of *U. viciae-fabae*. These were Tulkarm at Palestine (32.31519° N, 35.02033° E, 75 m altitude), with light clay chromic luvisol with a pH of 7.0-7.5, in Kafr El-
Sheik, Egypt (30°47’ N, 30°59’ E, 0 m altitude) with loamy calcaric entisol with a pH of 7.5-8.0 during 2006-2007 and 2007-2008 seasons. In all these locations, due to their mild winter and hot spring, faba beans are autumn sown.

**Validation of optimal crop mixtures**

Four intercrop mixtures were used in this study: faba bean (cultivars Nobaria 1 at Egypt, and Baladi at Palestine) intercropped either with barley, with durum wheat, with oat or with triticale (cultivars Cory, Meridiano, Aspen and Peñarroya, respectively). The experimental unit consisted of 1.8 x 10 m plots, with 6 rows 10 m long, separated 0.3 m (Figure 1). Plots were laid out in a randomized complete block design with 4 replicates. Faba bean monocrop with a plant density of 100 faba bean plants m$^{-2}$ was used as a control. Species were mixed intercropped within rows. All species were sown by hand at the 1st of November in all environments. No chemical fertilizers or pesticides were applied across the experiment. Weeds were removed by hand weeding when needed. No irrigation was applied at Tulkarm whereas surface irrigation took place four times at Kafr El-Sheikh (400 m$^3$ ha$^{-1}$ were applied each time). The first irrigation was done after sowing, the second one during December the third during March, and the fourth during April.

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

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

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

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

Where $DS_i$ is the rust severity at evaluation date $i$, $ti$ is the number of days after the first observation on evaluation date $i$ and $k$ is the number of consecutive evaluations. The epidemic growth rate ($r$) was obtained by linear regression of the transformed DS against time: $\text{trans}(DS) = \text{logit}(DS) + 10 = \ln(DS/100 - DS) + 10$ (10 was added to obtain a positive number). Time of the first pustule’s appearance ($t0$) was calculated from the straight line slope equation $y = mx + b$, and was measured as number of days from sowing to first pustule’s appearance on the leaves (Barilli et al., 2009).

Plant stature and biomass assessments

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

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

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

Results

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

**Table 1**: Average temperature (°C) at each growing season and site of experimentation.

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<tbody>
<tr>
<td>Tulkarm, Palestine*</td>
<td>2006-2007</td>
<td>21.6</td>
<td>17.9</td>
<td>13</td>
<td>15.1</td>
<td>21.7</td>
<td>24.6</td>
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<td></td>
<td>2007-2008</td>
<td>23.7</td>
<td>18</td>
<td>14.9</td>
<td>15.2</td>
<td>15.5</td>
<td>19.2</td>
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<tr>
<td>Kafr El-Sheikh, Egypt**</td>
<td>2006-2007</td>
<td>23.2</td>
<td>20.1</td>
<td>19.3</td>
<td>20.2</td>
<td>22.8</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>2007-2008</td>
<td>25.9</td>
<td>21.7</td>
<td>18.1</td>
<td>19.2</td>
<td>25.4</td>
<td>27.8</td>
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**Table 2**: Total rain fall (mm) at each growing season and site of experimentation.

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<tbody>
<tr>
<td>Tulkarm, Palestine*</td>
<td>2006-2007</td>
<td>148.3</td>
<td>82</td>
<td>77.8</td>
<td>104</td>
<td>10.5</td>
<td>0.0</td>
<td>422.6</td>
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<td></td>
<td>2007-2008</td>
<td>32.7</td>
<td>151</td>
<td>22.7</td>
<td>151.5</td>
<td>105.9</td>
<td>6.2</td>
<td>470</td>
</tr>
<tr>
<td>Kafr El-Sheikh, Egypt**</td>
<td>2006-2007</td>
<td>3.2</td>
<td>10</td>
<td>17.5</td>
<td>44.1</td>
<td>9</td>
<td>11.5</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>2007-2008</td>
<td>28</td>
<td>12.5</td>
<td>37.8</td>
<td>38</td>
<td>0.0</td>
<td>0.0</td>
<td>116.3</td>
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*rain fed, **irrigated
There was a general trend for slight retardation on the onset of infection (longer $t_0$) in the studied intercrops, although these were seldom significant (Table 3). Apparent growth rate ($r$) in monocrops was 0.167 and 0.114 respectively for Palestine and Egypt in 2006-2007 and 0.166 and 0.236 in 2007-2008. A general non-significant trend of reduction was observed in the intercrops ranging in Palestine from 0.161 to 0.164 in 2006-2007 and 0.154 to 0.158 during 2007-2008 and in Egypt from 0.088 to 0.114 during 2006-2007 and 0.223 to 0.228 during 2007-2008.
### Table 3. The effect of intercropping faba bean with cereals on rust development: time of the first pustule’s appearance ($t_0$), apparent growth rate ($r$), final disease severity ($DSf$) and AUDPC

<table>
<thead>
<tr>
<th>Intercrops</th>
<th>2006-2007</th>
<th>2007-2008</th>
<th>Kafr El-Sheikh, Egypt</th>
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<tbody>
<tr>
<td></td>
<td>$t_0$</td>
<td>$r$</td>
<td>DSf</td>
</tr>
<tr>
<td>FB 100%</td>
<td>115.1</td>
<td>0.167</td>
<td>35.8</td>
</tr>
<tr>
<td>FB50% + B50%</td>
<td>117.8</td>
<td>0.163</td>
<td>23.9</td>
</tr>
<tr>
<td>FB50% + O50%</td>
<td>117.3</td>
<td>0.163</td>
<td>23.1</td>
</tr>
<tr>
<td>FB50% + T50%</td>
<td>115.3</td>
<td>0.161</td>
<td>26.1</td>
</tr>
<tr>
<td>FB50% + W50%</td>
<td>118.7</td>
<td>0.164</td>
<td>24.9</td>
</tr>
</tbody>
</table>

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 50%, FB+W: faba bean 50% intercropped with wheat 50%.

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

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

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

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

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

<table>
<thead>
<tr>
<th>Intercrops¹</th>
<th>Tulkarm, Palestine</th>
<th>Kafr El-Sheikh, Egypt</th>
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<tbody>
<tr>
<td>FB100%</td>
<td>118.1⁵</td>
<td>147.3⁶</td>
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<tr>
<td>FB50% + B50%</td>
<td>116.3⁵ (94.6)*</td>
<td>104.0⁵ (85.6)</td>
</tr>
<tr>
<td>FB50% + O50%</td>
<td>115.3⁵ (135)</td>
<td>98.9⁵ (107.8)</td>
</tr>
<tr>
<td>FB50% + T50%</td>
<td>114.1⁵ (106.5)</td>
<td>97.0⁵ (89.4)</td>
</tr>
<tr>
<td>FB50% + W50%</td>
<td>114.3⁵ (84.4)</td>
<td>98.9⁵ (94.1)</td>
</tr>
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</table>

¹FB100%: faba bean monocrops, 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 50%, FB+W: faba bean 50% intercropped with wheat 50%.
²Faba bean plant height and in brackets the height of the associated cereal crop.
³Means in the same column with similar superscripts are not statistically different (Tukey test, p < 0.05).
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.

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

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

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<tr>
<td>DSf 2007</td>
<td>0.922</td>
<td>0.114 (0.631)</td>
<td>0.135 (0.570)</td>
<td>-0.908 (0.000)</td>
<td>-0.898 (0.000)</td>
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<tr>
<td>DSf 2008</td>
<td>0.208</td>
<td>-0.146 (0.540)</td>
<td>-0.905 (0.000)</td>
<td>-0.906 (0.000)</td>
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<td>DSf 2007</td>
<td>0.620</td>
<td>0.410 (0.073)</td>
<td>0.293 (0.210)</td>
<td>-0.529 (0.016)</td>
<td>-0.866 (0.000)</td>
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<tr>
<td>DSf 2008</td>
<td>0.008</td>
<td>0.049 (0.836)</td>
<td>-0.385 (0.094)</td>
<td>-0.620 (0.004)</td>
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Actual P values are between brackets.

Discussion

Intercropping is useful for agriculture in terms of yield stability, increase in total yield, pest and disease management, weed management, erosion control, and soil fertility amongst others (Hauggard-Nielson et al., 2006). The results of this study, obtained over two growing seasons in two countries, confirm that intercropping faba bean with cereals is an effective strategy for controlling faba bean rust. The experiment was designed based on replacing half of the host plant species (faba bean) with the associated cereal crop. Reducing host plant density and increasing the distance between faba bean plants resulted in reduction of the amount of susceptible plant tissue per field area unit leading to reduction in the likelihood of spore deposition (Fernández-Aparicio et al., 2011).
The reduction in rust severity due to intercropping with cereals observed in this study is in agreement with a number of occasional reports of reductions on faba bean rust in Denmark (Fernández-Aparicio et al., 2006), highland Ethiopia (Terefe et al., 2016; Eshetu et al., 2018) and Spain (Villegas-Fernández et al., 2017). On the contrary Zhang et al. (2019) reported a reduction in other faba bean diseases, but not significant for rust. Reduction has been reported for other aerial diseases of faba bean such as chocolate spot and alternaria spot (Sharaiha et al., 1989; Sahile et al., 2008b; Fernández-Aparicio et al., 2011). The cereals in the intercropping system might work as a natural barrier that intercept rust urediospores carried by wind from infecting faba bean plants resulting in reducing the amount of available effective inoculums to infect other faba bean plants. This hypothesis of barrier effect of the accompanying crops is supported by the significant association of the reduction of rust severity on faba bean with the plant stature and fresh biomass of the accompanying crop. Nutritive status of the plant is known to affect disease severity, with increased application of nitrogen fertilizer favouring rust and other diseases. Luo et al. (2021) found that the relative control effect of intercropping on diseases of wheat intercropped with faba bean increased as application of N increased, suggesting that rational application of nitrogen fertilizer in intercrops contributes to disease control. As long as we focus on low input agriculture, we did not study fertilization effects, but following the studies of Luo et al. (2021) we hypothesise that faba bean rust reduction effects of intercrops would increase with nitrogen fertilization.

The stratified dispersal of faba bean rust was modelled by Sapoukhina et al. (2010) to identify the spatial patterns of cultivar mixtures that could slow epidemic spread, showing that mixtures creates a natural barrier to the progression of a smooth epidemic wave. Besides reduction in dispersal phase of rust, additional effects on rust life cycle other than dispersal could be derived from alterations in faba bean nutritive status and changes in microclimate inside canopy promoted by the cereal component (Boudreau and Mundt, 1992). Similar effects
have been reported for pea ascochyta blight spores in pea intercropped with cereal (Schoeny et al., 2010), chocolate spot reduction in faba bean-maize and faba bean-barley intercrops (Sahile et al., 2008 a,b; Guo et al., 2020) and powdery mildew reduction in pea-cereals intercrops (Villegas-Fernández et al., 2021).

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

Acknowledgements

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