agronomy

MDPI

Article

Elucidating the Response of Diverse Faba Bean Genotypes to Powdery Mildew Infection

Ángel M. Villegas-Fernández *, Lucía García, Eleonora Barilli, Nicolas Rispail and Diego Rubiales

Institute for Sustainable Agriculture, CSIC, Avd. Menéndez Pidal s/n, 14005 Córdoba, Spain; ebarilli@ias.csic.es (E.B.); nrispail@ias.csic.es (N.R.); diego.rubiales@ias.csic.es (D.R.)
* Correspondence: avillegas@ias.csic.es

Abstract: Faba bean (*Vicia faba*) is a temperate grain legume of major importance for food and feed. Powdery mildews are an important group of diseases in many crops, although in faba bean, it is still considered to be of only minor and local relevance. Here, we report the occurrence of powdery mildew in southern Spain, which was identified through ITS sequencing as *Erysiphe trifolii*. Resistance screenings allowed the identification of a wide range of responses to the disease, with accessions BPL-710 and ILB-4708 outstanding due to their high levels of resistance. Histological studies showed that the mechanisms of resistance may involve the inhibition of germination and impairment of fungal development, as shown by a limited number of primary and secondary hyphae compared to those of the susceptible accessions. This work permitted a better understanding of the interaction of faba bean and powdery mildew, laying the ground for breeding programs for resistance if needed in the future.

Keywords: *Vicia faba*; *Erysiphe trifolii*; management; resistance; breeding

Citation: Villegas-Fernández, Á.M.; García, L.; Barilli, E.; Rispail, N.; Rubiales, D. Elucidating the Response of Diverse Faba Bean Genotypes to Powdery Mildew Infection. *Agronomy* **2024**, *14*, 663. https://doi.org/10.3390/agronomy14040663

Academic Editor: Robert P. Larkin

Received: 31 December 2023
Revised: 4 March 2024
Accepted: 21 March 2024
Published: 25 March 2024

1. Introduction

Faba bean (*Vicia faba*) is an annual temperate grain legume crop of importance for human food and animal feed. Like other legumes, igurecontributes to sustainable agriculture by fixing atmospheric nitrogen in symbiosis with the soil bacteria *Rhizobium leguminosarum*. This unique ability reduces the dependence of farmers on extensive use of chemical fertilizers, protecting soil and water quality [1]. Faba bean cultivation is particularly important in Mediterranean countries, the Middle East, and China but extends worldwide, with its cultivation covering nearly three million hectares and there being an overall production of 7,786,000 tons. After some decades of regression of the crop, there is a renewed interest, with spectacular increases in production in Ethiopia, Canada, and Australia, among others [2].

There are several important fungal diseases that affect faba bean, such as chocolate spot, ascochyta blight, and rust, which may reduce yields under favorable conditions for the development of the pathogens. The first two are diseases caused by necrotrophic pathogens (*Botrytis* spp. and *Ascochyta fabae*, respectively) that may be very harmful in the flowering stage of the plants if mild temperatures and high relative humidity concur [3]. The causal agent of rust is the biotrophic pathogen *Uromyces viciae-fabae*, which produces orange pustules on the leaves and stems of faba bean plants and may be a threat to crop yields in the pod-filling stage if temperature and humidity are appropriate [4]. Although management practices may help mitigate the damage caused by these pathogens, the most effective way to control them is through the application of fungicides. This option, however, increases costs for farmers and may be damaging to the environment. A more efficient alternative is the use of resistant cultivars, which requires the development of breeding programs for disease resistance, and there has been success in obtaining cultivars with high levels of resistance to different diseases [5].

Powdery mildews are biotrophic pathogens that infect a wide array of plant species, including important crops, becoming, under some circumstances, an important limitation for their cultivation [6]. They belong to Ascomycota with the class Leotiomycetes and the order Erysiphales [7]. When infecting a plant, they produce typical symptoms of a white powder covering the surface of the aerial tissues of the plant. In the case of legumes, powdery mildew may be a serious problem for peas [8]. However, it is not regarded as a major problem in faba bean, although there are reports of occurrence in Canada, China, India, the United Kingdom, Korea, Armenia, Pakistan and Sudan [9–16]. Identification of the causal agent in these cases is confusing, with references to *Eysiphe polygoni* [10], *Microsphaera penicillata* [9,17], *E. pisi* f.sp. *pisi* [11], or just *Oidium* sp. [12].

In Southern Spain, like in most Mediterranean climates with mild winters, spring faba bean types are typically sown in autumn or winter under rain-fed conditions and are harvested by late spring [18]. By doing so, terminal drought and high summer temperatures are avoided. Powdery mildew is seldom a problem under these conditions. However, as part of a breeding program, faba bean is often grown under small cages covered with insect-proof nets to avoid pollinators with drip irrigation, which results in a higher temperature and relative humidity. Under these conditions, which might be extended to horticultural faba beans, powdery mildew may occur at the end of the season in late-sown plants. In the context of a changing climate, the challenges for crops in the near future may be very different from those of today. This includes emerging diseases, which increase their incidence by reaching new areas or affecting new hosts [19]. Then, it cannot be disregarded that powdery mildew may become a serious problem for faba bean cultivation in the short term, especially considering that the crop is extending to very diverse areas where the conditions for the development of disease might be encountered. However, the knowledge about this disease in faba bean is very limited, with very little information on the causal agents, mechanisms of interaction between plants and pathogens, and potential sources of resistance. If the disease becomes widely extended in faba bean, serious outbreaks could endanger crop yields, given that no resources to counteract its effects are available at the moment.

The aims of this work were, on the one hand, to identify the causal agent responsible for the occurrence of powdery mildew in faba bean plants in Córdoba (South of Spain) and, on the other hand, to identify and characterize sources of resistance to be used in the future to breed cultivars resistant to the disease.

2. Materials and Methods

2.1. Species Determination with ITS (rDNA Internal Transcribed Spacer) Sequencing

Conidia from naturally infected plants of faba bean harvested in the field (isolate CO-18) were collected and maintained on susceptible faba bean plants (accession V-404) under controlled conditions. Faba bean seeds were sown on 1 L plastic pots (2 seeds per pot) containing a mixture of sand and peat (1:1) and were grown in a growth chamber at 20 °C with a photoperiod of 14 h of visible light (150 µmol m^{-2} s^{-1} photon flux density) and 10 h of darkness until the faba bean plants had 3 fully expanded leaves. A single-colony isolate was obtained from these plants and used to inoculate new healthy plants. The single-colony isolate CO-18 was allowed to grow and then used for the following screening and ITS analysis. Powdery mildew was obtained from several plants by scratching the surface of several leaves with a razor and collecting the tissue. Then, DNA was obtained from the isolate using the CTAB method as reported in [20]. ITS sequences were amplified using the *Erysiphe*-specific primers EryF (5′ TACAGAGTGCGAGGCTCAGTCG 3′) and EryR (5′ GGTCAACCTGTGATC CATGTGACTGG 3′), as reported earlier in [21], and two clones were sequenced. The PCR mix and amplification conditions were as described in [21].

Two independent PCR products were purified and sequenced directly from both strands using an ABI3730XL sequencer. The sequences obtained were used as queries in BLAST (http://www.ncbi.nlm.nih.gov/BLAST, accessed on 14 March 2021) searches to identify the most similar sequences available in the GenBank databases.

2.2. Screenings under Controlled Conditions

Two different experiments were carried out in growth chambers under controlled conditions. In the first one, a collection of 19 germplasm accessions of faba bean were evaluated for their response to powdery mildew infection. In Table S1 (Supplementary Data), the origin of all evaluated accessions are given. This set of accessions were selected for their differential responses to chocolate spot and rust [22,23]. Pea cv. Messire, which is susceptible to pea powdery mildew (*E. pisi*) [24], was also included to assess its response to the powdery mildew isolated from faba bean. One seed of each accession was sown in 1 L plastic pots containing a mixture of sand and peat (1:1) and grown in a growth chamber at 20 °C with a photoperiod of 14 h of visible light (150 µmol m^{-2} s^{-1} photon flux density) and 10 h of darkness until the faba bean plants had 3 fully expanded leaves.

The powdery mildew isolate used (CO-18) was collected from naturally infected plants of faba bean in the field and maintained on infected faba bean plants (accession V-404) growing in a growth chamber as described above; fresh inoculum was available two weeks after infecting the plants. For the experiment, heavily sporulating leaves were detached from these plants, and spores were blown into a settling tower, containing the accessions to be evaluated. The conidium density was around five conidia per mm^2. After inoculation, the plants were transferred back to the growing chamber under the same conditions. The experiment was designed to be completely randomized with three replications consisting of a pot with a plant.

Plants were evaluated 12 days after inoculation when symptoms (sporulating mycelium of the plant growing over the surface of the leaves) were clear and extended on susceptible accessions. For evaluation, a "corrected severity" (CS) was defined, which included both the surface covered by disease symptoms and the extension of the disease all across the plant. It was calculated as follows:

$$CS = (S + IL)/2$$

CS: Corrected severity.
S: Severity (% of plant surface covered by symptoms).
IL: % of leaves with symptoms against the total number of leaves per plant.

In the second experiment, nine accessions from experiment 1 were selected, and 8 additional commercial cultivars were studied (Table S1, Supplementary Data). All of the procedures and experimental design were the same as those in the first experiment, with the only difference being that plants were inoculated when they had 6–8 fully expanded leaves.

2.3. Characterization of the Responses to Infection

An experiment under controlled conditions was carried out in order to characterize the different responses to infection found in the previous experiments. Five accessions were selected from the previous experiments, namely, BPL 710 and ILB 4708 (resistant), 132-1 (intermediate), and V404 and cv. Alameda (susceptible). Again, plants were grown as in the previous experiments. Inoculation in this case was performed on detached leaves as in [25]. When plants presented 3–5 fully expanded leaves, the first and second leaves (from the top) were detached and placed on a square Petri dish (15 × 15 cm) containing an agar–water medium (0.4% p/v); each Petri dish contained only the leaves from a single plant. The experiment was designed to be completely randomized with four replications (one replication being one plant growing in a pot). Inoculation was performed as in the experiments described above, and the uncovered Petri dishes were placed inside the settling tower.

For sampling, one leaflet of each leaf was randomly selected two days after inoculation, and the tip was cut out and processed for histological observations as described in [25]. In brief, two slices of the central area of each leaf were placed with the adaxial surface up on filter paper dipped in fixative (1:3, absolute ethanol/glacial acetic acid, *v*/*v*). The fixative was changed several times until the tissues were bleached; they were transferred to filter paper dipped in tap water for a minimum of 2 h and then moved again to filter paper in

lactoglycerol (1:1:1, lactic acid/glycerol/water, *v*/*v*/*v*) for at least another 2 h. After this, the sample was carefully laid with the adaxial surface down on a cover glass on which a drop of trypan blue in lactoglycerol (0.1%, *w*/*v*) had been placed, and then it was mounted in lactoglycerol on a microscope slide. Then, at least 100 spores were counted per leaf at 20× magnification with a light microscope. Germination was recognized by the presence of at least a primary germ tube. To assess further development, 100 germinated sporelings were examined (when available), and the numbers of primary hyphae, secondary hyphae, and secondary appressoria were counted (Figure 1).

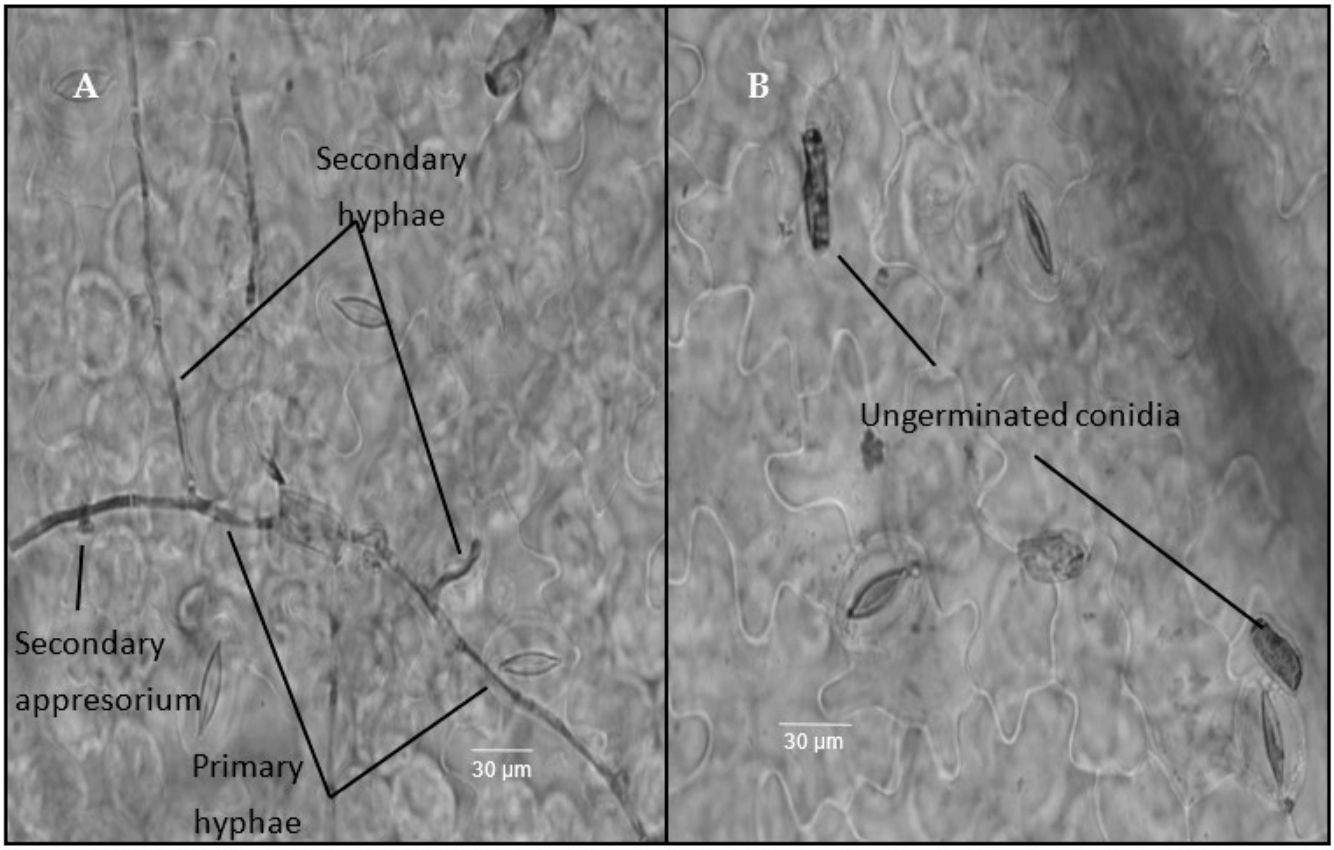

Figure 1. Germinated powdery mildew conidium (isolate CO-18) growing on the surface of a leaf of cv. Alameda and showing various structures (**A**). Ungerminated conidia on the leaf surface of accession BPL 710 (**B**).

2.4. Response of Faba Bean to Infection by E. pisi

Given that powdery mildew incited by *E. pisi* is widespread in areas with peas but is not seen on neighboring faba bean crops, an additional experiment was conducted under controlled conditions to confirm and exclude the pathogenicity of *E. pisi* on faba bean. Four faba bean plants (cv. Alameda, which was shown to be susceptible to *E. trifolii* in our experiments) and four pea plants (cv. Messire susceptible to both *E. trifolii* and *E. pisi*) were grown as described in the previous experiments. When plants had four fully expanded leaves, they were inoculated with isolate CO-23 of *E. pisi*, as in the above experiments. Leaflets were detached from all leaves 48 h after inoculation, and histological procedures were carried out as previously described.

2.5. Statistical Analysis

Analyses of variance (ANOVAs) were carried out for the different parameters evaluated in each experiment, and the means were compared by using the Tukey test. The software employed was Statistix 8 (AnalyticalSoftware, Tallahassee, FL, USA).

3. Results

3.1. Species Determination with ITS (rDNA Internal Transcribed Spacer) Sequencing

The direct sequencing of PCR products obtained using the EryF and EryR primers yielded sequences from 667 to 678 bp that were identical to each other and showed 100% similarity to seven GenBank sequences belonging to different *E. trifolii* (syn. *E. trifoliorum*) isolates growing on legumes and deposited in GenBank under the accession numbers OP256907.1 [26], LC010015.1 and LC010014.1 [27], MZ265172.1 [28], LC270860.1 [29], and FJ378884 and FJ378874 [30].

3.2. Screenings under Controlled Conditions

The first symptoms appeared 6–7 days after inoculation, and they were clearly visible on all plants 12 days after inoculation. A wide range of responses to infection by powdery mildew was found in the 19 genotypes of faba bean studied in the first experiment (Figure 2), with accessions BPL-710, ILB-5284, IC-158-1, 095-1, 135-1, BPL-261, and 132-1 appearing as the most resistant ones with CS < 10% and with V-404 as the most susceptible with a CS of 49.2% (V-404). Pea cv. Messire was even more susceptible with a CS of 64.9%.

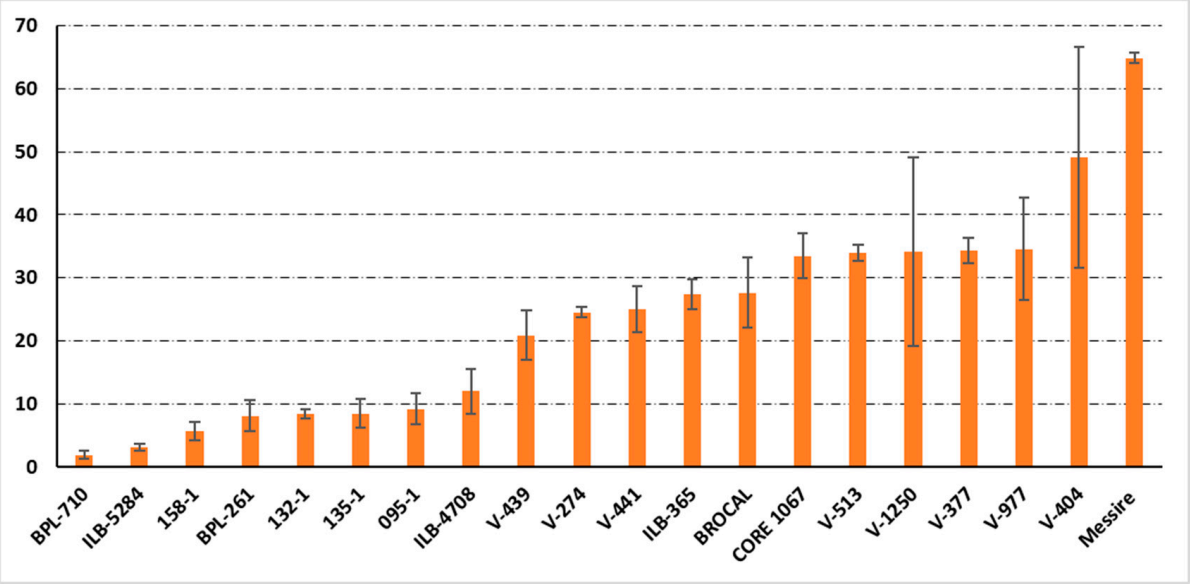

Figure 2. Responses to powdery mildew of 19 accessions of faba bean and a pea check (cv. Messire) under controlled conditions. The corrected severity (CS) was evaluated 12 days after inoculation. Vertical lines show the standard error of the mean for n = 3.

The results of the second experiment confirmed the resistance of BP-710 and ILB-4708 and the susceptibility of V-404 (Figure 3). A high variation in responses was also identified among the commercial cultivars, with cvs. Arrechana, Borjana, and Prothabon being the most resistant ones (CS < 20%) and Brocal and Quijote being the most susceptible (CS > 40%). As an example, the differences in the CS between BPL-710 and cv. Alameda are illustrated in Figure 4.

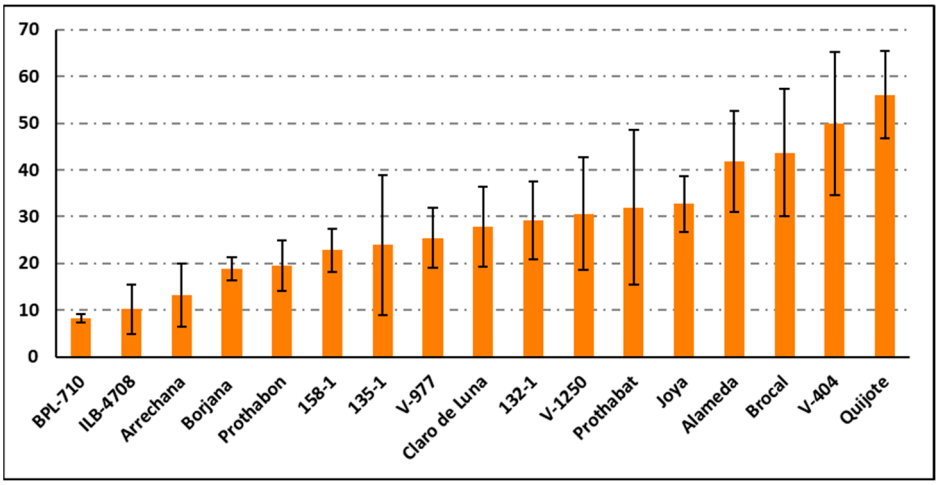

Figure 3. Responses to powdery mildew of 17 accessions of faba bean under controlled conditions. The corrected severity (CS) was evaluated 12 days after inoculation. Vertical lines show the standard error of the mean for n = 3.

Figure 4. Resistant accession BPL-710 (**left**) compared to susceptible cv. Alameda (**right**) 12 days after inoculation with *E. trifolii CO-18*.

These differences in CS values between accessions in both experiments were found to be significant in the respective ANOVAs ($p < 0.001$). Pearson's correlation analysis between the results of the nine accessions that were common to both experiments was significant ($p < 0.02$) with r = 0.76.

The original datasets of the evaluations of both experiments are given in the Supplementary Data (Tables S2 and S3).

3.3. Characterization of the Responses to Infection

The analyses of variance identified significant differences between the five tested accessions for all evaluated parameters (Table 1). Spore germination was significantly lower (less than 20%) on leaves of the two resistant accessions BPL-710 and ILB-4708, and it was particularly high on the susceptible cv. Alameda. Equally, the numbers of primary and secondary hyphae and the number of secondary appressoria formed by germinated spores, which were indicative of colony growth, were much lower on the resistant accessions BPL-710 and ILB-4708 than on the susceptible cv. Alameda. The result of this can be seen

microscopically in Figure 5, with only germinated spores on BPL-710 and fully developed colonies on cv. Alameda.

Table 1. Values of the different parameters studied in the histological studies of five accessions of faba bean infected by powdery mildew (*E. trifolii*): percentage of conidium germination and numbers of appresoria and of primary and secondary hyphae per colony (germinated spores). Different letters in each column indicate significant differences (Tukey test, $p < 0.05$).

	Germination (%)	No. Primary Hyphae	No. Secondary Hyphae	No. Secondary Appresoria
BPL-710	13.44 a	0.03 a	0.00 a	0.00 a
ILB-4708	16.81 b	0.08 a	0.00 a	0.00 a
132-1	27.87 c	0.24 b	0.00 a	0.00 a
V-404	29.94 c	0.39 c	0.20 b	1.20 b
Alameda	41.50 d	2.59 d	3.05 c	3.88 c

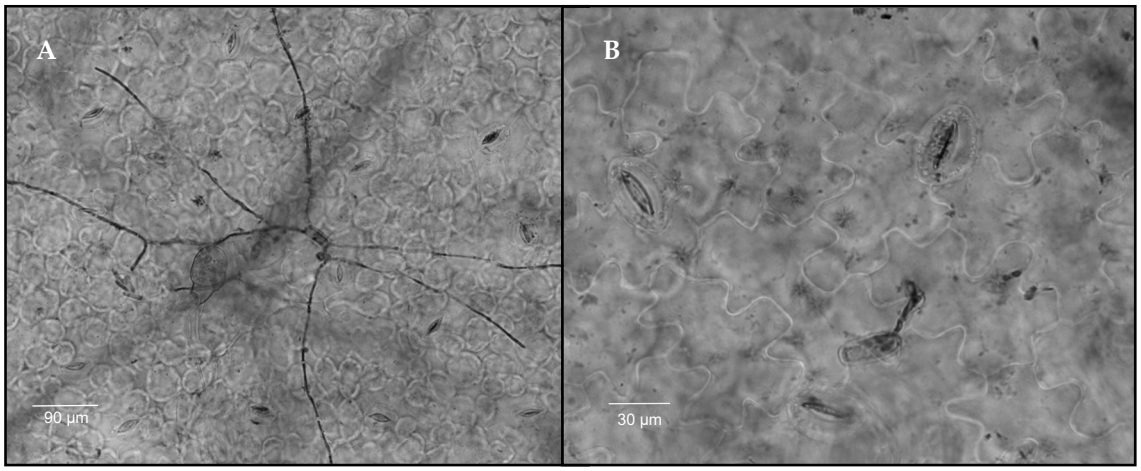

Figure 5. A fully developed colony of powdery mildew (isolate CO-18) from a spore on cv. Alameda (**A**) with several primary and secondary hyphae and secondary appressoria. A germinated spore on accession BPL-710 (**B**).

3.4. Response to Infection by E. pisi

Powdery mildew symptoms were macroscopically visible on pea plants already by 9 dai, reaching 68% CS by 14 dai. However, no powdery mildew symptoms developed at any time on the faba bean plants inoculated with *E. pisi*. This was confirmed histologically (Table 2), since the spore germination and formation of primary and secondary hyphae of *E. pisi* were markedly reduced on faba bean compared to those on peas.

Table 2. Values of the different parameters from the histological studies of five accessions of faba bean infected by powdery mildew: percentage of conidium germination, percentage of primary hyphae, and percentage of secondary hyphae per germinated spore. Different letters indicate significant differences (Tukey test, $p < 0.05$).

	Germination (%)	No. Primary Hyphae	No. Secondary Hyphae
Faba bean	26.2 a	1.0 a	0.00 a
Pea	49.8 b	2.58 b	0.75 b

4. Discussion

In this paper, we report the occurrence of powdery mildew on faba bean in Spain for the first time. Powdery mildew has been described on faba bean in several countries, being ascribed to *E. pisi* in the United Kingdom [11], to *Microsphaera penicillata* in Canada [17], and to *E. polygoni* in Sudan [15], Korea [12], China [10], India [16], and Pakistan [14]. As far as we know, no occurrence has been reported before in the Mediterranean region. It is also the first time that *Erysiphe trifolii* has been identified as the causal agent of powdery mildew on this crop. Previous identifications were based on morphological traits in some cases, but never before had the species of powdery mildew infecting faba bean been determined with ITS.

E. trifolii has been reported on clovers, grass peas, peas, and lentils in the field [21,30,31]. *E. trifolii* occurs yearly in Cordoba, late in the season by profusely infecting annual medics (unpublished). It has also been reported that *E. trifolii* causes powdery mildew in peas and *Lathyrus* spp. It is severe only in late-planted nurseries in Cordoba and, normally, in India with higher temperatures during the crop season [24,32–35]. This is also what we found on faba beans, with infection only occurring in late-planted nurseries. Hence, the environmental conditions seem to be critical for the development of powdery mildew on faba bean, with higher temperatures favoring *E. trifolii*.

Emergent diseases are a challenge for agricultural production around the world. Major diseases of the most important crops are kept at bay by a combination of different strategies, such as the employment of pesticides, resistant cultivars, and agronomic tools. However, the outbreak of an unexpected disease may cause important trouble, jeopardizing yields for a long time before practical solutions are implemented. There have been many examples of the occurrence of unexpected diseases in different crops in the last decades, as in the cases of java rose apple, common bean, monkshood, snapdragon, sunflower, Japanese persimmon, and soybean [36–42]. The appearances of powdery mildew in carrot caused by *Erysiphe heraclei* in Brazil [43] and in faba bean gall in Ethiopia [44] are recent examples of novel outbreaks for both the disease and the host of our study. This risk is even higher in the context of a changing climate [45,46]. This is why it is important to anticipate, and, among the different available options, it would be very useful to have a repository of genes that confer resistance to potential serious diseases. The south of Spain is experimenting a shortening of the cool season and a general increase in temperatures in spring, which might favor the early development of powdery mildew in faba bean, as we have seen. Thus, it would be very useful to have a germplasm presenting resistance to powdery mildew in case it is necessary to carry out a breeding program of faba bean for resistance to that disease. Our experiments under controlled conditions proved to be an efficient tool for screening collections of accessions for their responses to powdery mildew, which facilitated this activity without being conditioned by the environmental conditions of field trials. The high correlation between the common accessions in the two experiments that we performed confirms the solidness and reproducibility of our protocol. These experiments revealed a wide diversity of responses to *E. trifolii* among the tested genotypes, pointing to a rich gene pool for the interaction with this disease. It is remarkable that the commercial varieties that are among the most grown in the area (Alameda, Brocal, Prothabat, and Claro de Luna) rank high in susceptibility, which might be a problem in a hypothetical outbreak of powdery mildew in faba bean. Only Prothabon presents a moderate response to the disease, while Arrechana and Borjana are newly obtained cultivars that are not cultivated in the area. All of this stress the risks of a potential outbreak of powdery mildew in the region and the need for sources of resistance among faba bean germplasms. In our study, BPL 710 stood out as the most resistant accession to the disease in both experiments, with low levels of severity. This accession also presented high levels of resistance to chocolate spots and rust in previous studies [22], so it may work as a good source of resistance for commercial cultivars. There are also other interesting accessions that may provide additional resistant genes for breeding.

As for the mechanisms explaining the reduced progression of disease in the least susceptible accessions, reduced spore germination it appears to be one of the key elements for this, presenting values that are at least 40% lower in accessions BPL 710 and ILB 4708 than those in the other ones. This is not a very common mechanism of resistance against powdery mildew, although it has been reported in some accessions of *Pisum* spp. when infected with *E. pisi* [47]. Spore germination may be suppressed by morphological features, such as leaf surface properties and plant architecture [48]. Additionally, leaf wax chemistry may play a role in inhibiting germination [48–50]. In our experiment, fungal development seemed to be hampered in resistant accessions even for spores that succeeded in germinating, with limited growth that was reflected in the numbers of primary and secondary hyphae and secondary appressoria (indicative of colony growth) compared to those in the susceptible accessions, where bigger colonies were established. No visible macroscopic necrosis or signs of hypersensitive resistance were observed.

Additional studies will be needed to discern the genetic basis of the identified resistances. In the case of pea, three single genes have been reported conferring resistance to powdery mildew [8], but polygenic resistance is also suggested although not yet characterized [47]. Existence of both monogenic and polygenic resistances is a common fact needing detailed studies to discern among them [8,35,48,51].

Something similar happened with the response of faba ban to infection by *E. pisi*, with no macroscopically visible powdery mildew symptoms developing at all on faba bean plants, confirming the results reported in [34]. The resistance *to E. pisi* was seen as a marked reduction in *E. pisi* spore germination and development of hyphae compared to those in pea. This seems to point to nonhost resistance, which reflects a lack of adaption of the pathogen to a particular species, which is considered a nonhost [52]. This type of resistance might provide long-term protection and appears to be associated with basal plant resistance. Nonhost resistance may include different obstacles to infection by the plant, such as the presence or absence of signals, preformed barriers, and induced defense responses [52]. Interestingly, although the isolate of *E. trifolli* in our study is capable of infecting faba bean, this crop appears to be highly resistant to the isolates of *E. trifolii* ex *L. sativus*, ex. *M. truncatula,* and ex. *V. articulata*, confirming the complexity of *E. trifolii*, which should be regarded as a species complex composed of host-specialized isolates [34].

5. Conclusions

In conclusion, this work allowed the identification of the species of powdery mildew infecting faba bean in our area, as well as the selection of potential sources of resistance to the disease, and it described some of the mechanisms of resistance involved. All of these are the first steps towards a better understanding of this pathosystem, which will be necessary if powdery mildew becomes a problem for faba bean cultivation in the future. Future work should focus on widening the scope of studies to other faba bean genotypes and species of powdery mildew, as well as on identifying the most interesting genes of resistance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14040663/s1, Table S1: Origin of the different accessions evaluated in the experiments under controlled conditions for their response to infection by powdery mildew. IFAPA: Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (Spain). ICARDA: International Center for Agricultural Research in the Dry Areas (Lebanon); Table S2: Original dataset of corrected severity for the first experiment under controlled conditions for the evaluation of 19 accessions of faba bean and 1 accession of pea to infection by powdery mildew; Table S3: Original dataset of corrected severity for the second experiment under controlled conditions for the evaluation of 18 accessions of faba bean to infection by powdery mildew.

Author Contributions: Conceptualization, D.R. and Á.M.V.-F.; formal analysis, Á.M.V.-F., E.B. and N.R.; investigation, Á.M.V.-F., L.G., E.B. and N.R.; writing—original draft preparation, D.R. and Á.M.V.-F.; writing—review and editing, D.R., N.R. and Á.M.V.-F.; funding acquisition, D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish project PID2020-114668RB-I00 (MCIN/AEI/10.13039/501100011033) and by the HORIZON EUROPE—BELIS Project 101081878.

Data Availability Statement: Data is contained within the article or Supplementary Material.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rubiales, D. Faba beans in sustainable agriculture. *Field Crops Res.* **2010**, *115*, 201–202. [CrossRef]
2. Abou-Khater, L.; Maalouf, F.; Rubiales, D. Status of Faba Bean (*Vicia faba* L.) in the Mediterranean and East African Countries. In *Developing Climate Resilient Grain and Forage Legumes*; Springer: Singapore, 2022; pp. 297–327.
3. Tivoli, B.; Baranger, A.; Avila, C.M.; Banniza, S.; Barbetti, M.; Chen, W.D.; Davidson, J.; Lindeck, K.; Kharrat, M.; Rubiales, D.; et al. Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica* **2006**, *147*, 223–253. [CrossRef]
4. Sillero, J.C.; Fondevilla, S.; Davidson, J.; Patto, M.C.V.; Warkentin, T.D.; Thomas, J.; Rubiales, D. Screening techniques and sources of resistance to rusts and mildews in grain legumes. *Euphytica* **2006**, *147*, 255–272. [CrossRef]
5. Rubiales, D.; Khazaei, H. Advances in disease and pest resistance in faba bean. *Theor. Appl. Genet.* **2022**, *135*, 3735–3756. [CrossRef] [PubMed]
6. Takamatsu, S. Studies on the evolution and systematics of powdery mildew fungi. *J. Gen. Plant Pathol.* **2018**, *84*, 422–426. [CrossRef]
7. Mieslerová, B.; Cook, R.T.; Wheater, C.P.; Lebeda, A. Ecology of Powdery Mildews–Influence of Abiotic Factors on their Development and Epidemiology. *Crit. Rev. Plant Sci.* **2022**, *41*, 365–390. [CrossRef]
8. Fondevilla, S.; Rubiales, D. Powdery mildew control in pea. A review. *Agron. Sustain. Dev.* **2012**, *32*, 401–409. [CrossRef]
9. Kharbanda, P.D.; Bernier, C.C. Powdery mildew of *Vicia faba* in Manitoba. *Can. J. Plant Sci.* **1977**, *57*, 745–749. [CrossRef]
10. Yu, T. Powdery mildew of broad bean caused by *Erysiphe polygoni* in Yunan, China. *Phytopathology* **1946**, *36*, 370–376.
11. Cook, R.; Fox, R. Powdery mildew on faba beans and other legumes in Britain. *Plant Pathol.* **1992**, *41*, 506–512. [CrossRef]
12. Kwon, J.; Kang, S.; Park, C. Powdery mildew on broad bean (*Vicia faba*) caused by *Oidium* sp. in Korea. *Res. Plant Dis.* **2001**, *7*, 120–122.
13. Papoyan, F. Susceptibility of various varieties of forage broad bean to Asochyta, rust and powdery mildew. *Sb. Tr. NII Zashchity Rast. ArmSSR* **1970**, *1*, 251–261.
14. Iqbal, S.; Ghafoor, A.; Bashir, M.; Baksh, A. Reaction of faba bean genotypes to various diseases in Pakistan. *Fabis Newsl.* **1988**, *21*, 40–42.
15. Hussein, M.M. Major disease problems of faba beans in Sudan. In *Faba Bean Improvement: Proceedings of the Faba Bean Conference, Cairo, Egypt, 7–11 March 1981*; Springer: Dordrecht, The Netherlands, 1982; pp. 227–232.
16. Kalia, P.; Sood, S.; Sharma, A. Reaction of faba bean (*Vicia faba*) to powdery mildew (*Erysiphe polygoni*). *Indian J. Agric. Sci.* **2002**, *72*, 681.
17. Morrall, R.; McKenzie, D. Susceptibility of five faba bean cultivars to powdery mildew disease in Western Canada. *Can. J. Plant Sci.* **1977**, *57*, 281–283. [CrossRef]
18. Mínguez, I.; Rubiales, D. Faba bean. In *Crop Physiology: Case Histories for Major Crops*; Daniel, F., Sadras, V.C., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 453–482.
19. Ristaino, J.B.; Anderson, P.K.; Bebber, D.P.; Brauman, K.A.; Cunniffe, N.J.; Fedoroff, N.V.; Finegold, C.; Garrett, K.A.; Gilligan, C.A.; Jones, C.M. The persistent threat of emerging plant disease pandemics to global food security. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2022239118. [CrossRef] [PubMed]
20. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef]
21. Attanayake, R.N.; Glawe, D.A.; Dugan, F.M.; Chen, W. *Erysiphe trifolii* causing powdery mildew of lentil (*Lens culinaris*). *Plant Dis.* **2009**, *93*, 797–803. [CrossRef] [PubMed]
22. Villegas-Fernández, A.M.; Sillero, J.C.; Erneran, A.A.; Flores, F.; Rubiales, D. Multiple-disease resistance in *Vicia faba*: Multi-environment field testing for identification of combined resistance to rust and chocolate spot. *Field Crops Res.* **2011**, *124*, 59–65. [CrossRef]
23. Villegas-Fernández, A.M.; Sillero, J.C.; Rubiales, D. Screening faba bean for chocolate spot resistance: Evaluation methods and effects of age of host tissue and temperature. *Eur. J. Plant Pathol.* **2012**, *132*, 443–453. [CrossRef]
24. Fondevilla, S.; Chattopadhyay, C.; Khare, N.; Rubiales, D. *Erysiphe trifolii* is able to overcome *er1* and *Er3*, but not *er2*, resistance genes in pea. *Eur. J. Plant Pathol.* **2013**, *136*, 557–563. [CrossRef]
25. Fondevilla, S.; Carver, T.L.W.; Moreno, M.T.; Rubiales, D. Macroscopic and histological characterisation of genes *er1* and *er2* for powdery mildew resistance in pea. *Eur. J. Plant Pathol.* **2006**, *115*, 309–321. [CrossRef]
26. Zhang, Z.-K.; Zhang, W.-X.; Kou, Z.-A.; Wang, X.-F.; Wang, Y.-L.; Islam, R.; Liu, L.; Tian, Y.-Q. First Report of Powdery Mildew Caused by *Erysiphe polygoni* on *Trifolium repens* in China. *Plant Dis.* **2022**, *106*, 3215. [CrossRef] [PubMed]
27. Takamatsu, S.; Ito, H.; Shiroya, Y.; Kiss, L.; Heluta, V. First comprehensive phylogenetic analysis of the genus *Erysiphe* (Erysiphales, Erysiphaceae) I. The *Microsphaera* lineage. *Mycologia* **2015**, *107*, 475–489. [CrossRef] [PubMed]

28. Bradshaw, M.; Braun, U.; Götz, M.; Jurick, W., II. Phylogeny and taxonomy of powdery mildew caused by *Erysiphe* species on *Lupinus* hosts. *Mycologia* **2022**, *114*, 76–88. [CrossRef] [PubMed]
29. Abasova, L.; Aghayeva, D.; Takamatsu, S. Notes on powdery mildews of the genus *Erysiphe* from Azerbaijan. *Curr. Res. Environ. Appl. Mycol.* **2018**, *8*, 30–53. [CrossRef]
30. Attanayake, R.; Glawe, D.; McPhee, K.; Dugan, F.; Chen, W. *Erysiphe trifolii*—A newly recognized powdery mildew pathogen of pea. *Plant Pathol.* **2010**, *59*, 712–720. [CrossRef]
31. Baiswar, P.; Ngachan, S.; Verma, V.; Kumar, R.; Jha, A.; Chandra, S. Molecular evidence of *Erysiphe pisi* on pea and *E. trifoliorum* on white clover in northeast India. *Australas. Plant Dis. Notes* **2015**, *10*, 12. [CrossRef]
32. Fondevilla, S.; González-Bernal, M.J.; Omri Ben Youssef, N.; Rubiales, D. Development of Quantitative Real-Time PCR Assays to Quantify Erysiphe pisi and Erysiphe trifolii and Its Implementation for Monitoring Their Relative Prevalence in Pea Crops in Spain and Tunisia. *Agronomy* **2022**, *12*, 334. [CrossRef]
33. Rubiales, D.; Barilli, E.; Rispail, N. Breeding for Biotic Stress Resistance in Pea. *Agriculture* **2023**, *13*, 1825. [CrossRef]
34. Rubiales, D.; Moral, A.; Rispail, N. Sulla Powdery Mildew: Phylogeny and Host Range. *Agronomy* **2022**, *12*, 1852. [CrossRef]
35. Santos, C.; Martins, D.; Rubiales, D.; Patto, M.C.V. Partial Resistance Against *Erysiphe pisi* and *E. trifolii* Under Different Genetic Control in *Lathyrus cicera*: Outcomes from a Linkage Mapping Approach. *Plant Dis.* **2020**, *104*, 2875–2884. [CrossRef]
36. Maharachchikumbura, S.; Guo, L.-D.; Chukeatirote, E.; McKenzie, E.; Hyde, K. A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*. *Trop. Plant Pathol.* **2013**, *38*, 227–235. [CrossRef]
37. Navas-Castillo, J.; Sánchez-Campos, S.; Díaz, J.A.; Sáez-Alonso, E.; Moriones, E. Tomato Yellow Leaf Curl Virus-Is Causes a Novel Disease of Common Bean and Severe Epidemics in Tomato in Spain. *Plant Dis.* **1999**, *83*, 29–32. [CrossRef]
38. Tomioka, K.; Hirooka, Y.; Nagai, T.; Sawada, H.; Aoki, T.; Sato, T. Plectosporium blight of monkshood caused by *Plectosporium tabacinum*. *J. Gen. Plant Pathol.* **2011**, *77*, 266–268. [CrossRef]
39. Tomioka, K.; Nishikawa, J.; Moriwaki, J.; Sato, T. Anthracnose of snapdragon caused by *Colletotrichum destructivum*. *J. Gen. Plant Pathol.* **2011**, *77*, 60–63. [CrossRef]
40. Tomioka, K.; Sato, T. Gray mold of yacon and sunflower caused by *Botrytis cinerea*. *J. Gen. Plant Pathol.* **2011**, *77*, 217–219. [CrossRef]
41. Yamamoto, J.; Tanaka, K.; Ohtaka, N.; Sato, T. Black leaf spot of Japanese persimmon (*Diospyros kaki*), a new disease caused by *Adisciso kaki* sp. nov. *J. Gen. Plant Pathol.* **2012**, *78*, 99–105. [CrossRef]
42. Yamaoka, Y. Recent outbreaks of rust diseases and the importance of basic biological research for controlling rusts. *J. Gen. Plant Pathol.* **2014**, *80*, 375–388. [CrossRef]
43. Boiteux, L.; Reis, A.; Fonseca, M.E.; Lourenço, V., Jr.; Costa, A.; Gonçalves, A.; Borges, R. Powdery Mildew Caused by *Erysiphe heraclei*: A Novel Field Disease of Carrot (*Daucus carota*) in Brazil. *Plant Dis.* **2017**, *101*, 1544. [CrossRef]
44. Hailu, E.; Damessa, G.; Gela, T.S.; Tadesse, N.; Bitew, B.; Boydom, A.; Kassa, D.; Tolessa, T. Faba Bean Gall; A New Threat for Faba Bean (*Vicia faba*) Production in Ethiopia. *Adv. Crop Sci. Technol.* **2014**, *2*, 144. [CrossRef]
45. Johnson, M.A.; Vinatzer, B.A.; Li, S. Reference-Free Plant Disease Detection Using Machine Learning and Long-Read Metagenomic Sequencing. *Appl. Environ. Microbiol.* **2023**, *89*, e0026023. [CrossRef] [PubMed]
46. Singh, B.K.; Delgado-Baquerizo, M.; Egidi, E.; Guirado, E.; Leach, J.E.; Liu, H.; Trivedi, P. Climate change impacts on plant pathogens, food security and paths forward. *Nat. Rev. Microbiol.* **2023**, *21*, 640–656. [CrossRef]
47. Fondevilla, S.; Carver, T.L.W.; Moreno, M.T.; Rubiales, D. Identification and characterization of sources of resistance to *Erysiphe pisi* Syd. in *Pisum* spp. *Plant Breed.* **2007**, *126*, 113–119. [CrossRef]
48. Niks, R.E.; Rubiales, D. Potentially durable resistance mechanisms in plants to specialised fungal pathogens. *Euphytica* **2002**, *124*, 201–216. [CrossRef]
49. Gniwotta, F.; Vogg, G.; Gartmann, V.; Carver, T.L.W.; Riederer, M.; Jetter, R. What do microbes encounter at the plant surface? Chemical composition of pea leaf cuticular waxes. *Plant Physiol.* **2005**, *139*, 519–530. [CrossRef] [PubMed]
50. Prats, E.; Llamas, M.J.; Jorrin, J.; Rubiales, D. Constitutive coumarin accumulation on sunflower leaf surface prevents rust germ tube growth and appressorium differentiation. *Crop Sci.* **2007**, *47*, 1119–1124. [CrossRef]
51. Marone, D.; Russo, M.A.; Laidò, G.; De Vita, P.; Papa, R.; Blanco, A.; Gadaleta, A.; Rubiales, D.; Mastrangelo, A.M. Genetic basis of qualitative and quantitative resistance to powdery mildew in wheat: From consensus regions to candidate genes. *BMC Genom.* **2013**, *14*, 562. [CrossRef]
52. Gill, U.S.; Lee, S.; Mysore, K.S. Host versus nonhost resistance: Distinct wars with similar arsenals. *Phytopathology* **2015**, *105*, 580–587. [CrossRef]