A high-throughput phenotyping tool to identify field-relevant anthracnose resistance in white lupin

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Abstract

The seed- and air-borne pathogen *Colletotrichum lupini*, the causal agent of lupin anthracnose, is the most important disease in white lupin (*Lupinus albus*) worldwide and can cause total yield loss. The aims of this study were to establish a reliable high-throughput phenotyping tool to identify anthracnose resistance in white lupin germplasm and to evaluate a genomic prediction model, accounting previously reported resistance QTLs, on a set of independent lupin genotypes. Phenotyping under controlled conditions, performing stem inoculation on seedlings, showed to be applicable for high-throughput and its disease score strongly correlated with field plot disease assessments ($r = 0.95$, $p < 0.0001$) and yield ($r = -0.64$, $p = 0.035$). Traditional 1-row field disease phenotyping showed no significant correlation with field plot disease assessments ($r = 0.31$, $p = 0.34$) and yield ($r = -0.45$, $p = 0.17$). Genomically-predicted resistance values showed no correlation with values observed under controlled or field conditions, and the parental lines of the RIL population used for constructing the prediction model exhibited a resistance pattern opposite to that displayed in the original (Australian) environment used for model construction. Differing environmental conditions, inoculation procedures or population structure may account for this result. Phenotyping a diverse set of 40 white lupin accessions under controlled conditions revealed eight accessions with improved resistance to anthracnose. The standardized area under the disease progress curves (sAUDPC) ranged from 2.1 to 2.8 compared to the susceptible reference accession with a sAUDPC of 3.85. These accessions can be incorporated into white lupin breeding programs. In conclusion, our data supports stem inoculation-based disease phenotyping under controlled conditions as a time-effective approach to identify field-relevant

38 resistance which can now be applied to further identify sources of resistance and their underlying
39 genetics.

40

41 **Keywords:** *Lupinus albus*, *Colletotrichum lupini*, wound inoculation, field resistance, breeding,
42 genomic prediction

The current demand for plant-based protein is high and is expected to increase even further with the growing world population (Henchion et al. 2017). Most of the global demand is met by soybean produced on a large-scale in the USA and Brazil (Pagano and Miransari 2016). As lupins have a similar protein content and better digestibility (Monteiro et al. 2014), they offer great potential to complement soybean as an alternative protein source (Lucas et al. 2015). The genus *Lupinus*, belonging to the family *Fabaceae*, consists of about 170 species worldwide, with blue (*Lupinus angustifolius* L.), Andean (*L. mutabilis*), yellow (*L. luteus* L.) and white lupin (*L. albus* L.) being of agricultural importance (Gresta et al. 2017). Their unique symbiosis with *Bradyrhizobium* sp. (*lupini*) makes lupins highly efficient atmospheric nitrogen fixers (Fernández-Pascual et al. 2007; Peix et al. 2015). Additionally, white lupin is capable of forming specialized cluster roots which release carboxylates to mobilize poorly available phosphorus sources (Gallardo et al. 2019; Lambers et al. 2013). White lupin is recognized for its high protein content (36 to 38%), starch-free white seeds, outstanding fatty acid quality and additional health benefits, making its grains ideal for the food and feed industry (Arnoldi et al. 2015; Boschini et al. 2008; Monteiro et al. 2014).

Despite its potential for low-input agriculture as highlighted above, the cultivation of white lupin has been severely limited by anthracnose disease caused by the seed- and air-borne ascomycete *Colletotrichum lupini*, which is a member of the *Colletotrichum acutatum* species complex (Damm et al. 2012; Nirenberg et al. 2002). Contrary to the broad host range seen for most members of the *C. acutatum* species complex, *C. lupini* is host-specific to members of the *Lupinus* genus (Talhinhas et al. 2016). Infected seeds are the primary source of inoculum and small amounts of infected seeds (0.1%) can cause severe yield loss (Thomas and Sweetingham

2004). Little is known about the disease cycle and interaction between *C. lupini* and its host but gene expression and protein synthesis during white lupin infection shared similarities to those of hemibiotrophic pathogens (Dubrulle et al. 2020a), which is the most common lifestyle within the *C. acutatum* species complex (De Silva et al. 2017; Peres et al. 2005). At the start of the growing season, *C. lupini* is believed to colonize the host endophytically, causing no to minor disease symptoms. Upon flowering, disease incidence rapidly increases, causing the typical disease symptoms of stem elongation and pod twisting, followed by the formation of necrotic lesions containing orange masses of conidia which are rain-splash dispersed within the crop (Thomas and Sweetingham 2004, White et al. 2008). At first, the disease occurred predominantly in humid areas but it has quickly spread around the globe, making anthracnose the most notorious disease for lupin cultivation worldwide (Talhinhas et al. 2016). The current disease epidemic in Europe started in the 1970's, coinciding with a strong decline in lupin cultivated area (FAOSTAT 2017). Currently, disease management of white lupin is focused on planting pathogen-free seed and chemical control (Thomas et al. 2008a; White et al. 2008).

Breeding efforts for anthracnose resistance mainly took place under field conditions in New Zealand, Australia, Chile and Germany (Adhikari et al. 2009; Cowling et al. 2000; Jacob et al. 2017; von Baer 2009). These efforts have led to the discovery of resistance in the Ethiopian landraces P27174 and P27175 (Adhikari et al. 2009; Cowling et al. 2000), which form a distinct genetic group within white lupin (Raman et al. 2014). Analysis of a recombinant inbred line (RIL) population of P27174 and the susceptible cultivar Kiev Mutant revealed that the resistance was under polygenic control (Yang et al. 2010), which is also the case for yellow lupin (Adhikari et al. 2011), and unlike blue lupin, where single dominant genes, *Lanr1*, *AnMan* or *LanrBo*, control

anthracnose resistance (Fischer et al. 2015; Yang et al. 2004; Yang et al. 2008). Quantitative trait locus (QTL) mapping of the RIL population between P27174 and Kiev Mutant revealed three major anthracnose resistance QTLs, antr04/05_1, antr04/05_2 and antr05_3, on linkage groups ALB02 (LG4), ALB04 (LG17) and ALB10 (LG2), respectively, and jointly explained up to 49% of the phenotyping variation observed under field conditions in 2004 and 2005 in Western Australia (Phan et al. 2007; Yang et al. 2010). The same QTLs, were recently identified with greater precision by Książkiewicz et al. (2017) using a large number of SNP markers identified from genotyping-by-sequencing (GBS; Elshire et al. 2011). Despite the high synteny between blue and white lupin, QTLs conferring anthracnose resistance in white lupin did not correspond to those found in the latter species (Książkiewicz et al. 2017). The high number of markers made available by GBS not only facilitated QTL mapping but also opened the way for genomic selection, by which a statistical model is constructed from phenotypic and genotypic data of a representative population to predict breeding values for a target (polygenic) trait of inbred lines or genetic resources (Heffner et al. 2009). A genomic prediction model, proposed to aid in marker assisted selection based on the RIL population used to identify the major QTLs, showed a predictive ability of 0.56 for anthracnose resistance and also took into account variation not explained by the observed QTLs (Rychel-Bielska et al. 2020).

Disease phenotyping under field conditions is subject to unpredictable environmental conditions. Therefore, reliable screening tools for anthracnose resistance are essential to further exploit genetic diversity within white lupin. This study describes a reproducible and time-effective phenotyping tool for anthracnose resistance breeding, performing stem inoculation on seedlings under controlled conditions. For this, a diverse collection of white lupin landraces that

109 was shown to include outstanding material for key traits such as grain yield in different climatic
110 zones (Annicchiarico et al. 2010) and drought tolerance (Annicchiarico et al. 2018), which likely
111 holds new sources of anthracnose resistance, was used. The objectives of this study were to (i)
112 compare the developed high-throughput phenotyping tool and traditional 1-row field
113 assessments with field plot assessments obtained over two years at two sites, (ii) identify
114 anthracnose resistance in a collection of 40 white lupin accessions and (iii) evaluate the
115 consistency of resistance values predicted according to the Rychel-Bielska et al. (2020) genomic
116 prediction model with resistance values observed under controlled and field conditions for an
117 unrelated germplasm set.

Materials and Methods

***Colletotrichum lupini* strain characterization.** *C. lupini* strain JA01, used as inoculum in this study, was isolated from pod tissue of an infected white lupin plant in 2018 in Mellikon, Switzerland (47°34'05.7"N 8°21'18.7"E). Mycelium from a pure JA01 culture, grown for 10 days on PDA (potato dextrose agar, Carl Roth®, Karlsruhe, Germany) at 22°C in the dark, was harvested with a sterile spreader after flooding the Petri dish with 2 ml sterile ddH₂O. Genomic DNA was isolated with a CTAB extraction protocol as described by Minas et al. (2011). The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS) was amplified by performing PCR in a S1000 Thermal Cycler (Bio-Rad, CA, USA) using the ITS5 “GGAAGTAAAAGTCGTAACAAG” and ITS4 “TCCTCCGCTTATTGATATGC” (White et al. 1990) primers as described by Dubrulle et al. (2020b). PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) and sent for sequencing to Eurofins Genomics (Ebersberg, Germany). The consensus sequence was assembled using BioEdit v. 7.2.5 (Hall 2004) and identified as *C. lupini* through BLAST analysis and deposited at GenBank under accession number MT741840. Australian *C. lupini* strain IMI 375715 (genetic group 2; Dubrulle et al. 2020b) was used in one experiment to compare with strain JA01 for virulence on white lupin.

Genomic prediction. The genomic prediction model was based on results published by Rychel-Bielska et al. (2020). The germplasm used for genomic prediction modelling included 191 F₈ individuals issued from the RIL population of Kiev Mutant x P27174. Phenotypic data was collected in Perth (Western Australia) from two experiments in 2004 and 2005 (three replicates), under field conditions in a 1-row setup, using spray-inoculated (*C. lupini* strain IMI 375715) disease spreader rows (Adhikari et al. 2009; Yang et al. 2010). The same material underwent

genotyping-by-sequencing (GBS) characterization according to Elshire et al. (2011) method as described in Książkiewicz et al. (2017). A genomic prediction model was set up on the basis of intra-population predictions estimated via on cross-validations with 10% validation fraction, averaged across 20 repetitions outputted by two possible models, rrBLUP and Bayesian Lasso, using the R package GROAN (Nazzicari and Biscarini 2017). The best-predictive model, as correlation between predicted and observed values based on cross-validation for the RIL population, was applied to an independent set of 384 landrace accessions originating from across the Mediterranean, East Africa, Madeira and the Canaries islands, selected from a diverse collection described in Annicchiarico et al. (2010). SNP-generated markers were obtained for this material using the same procedure described for the model training set. Concurrently, we used Książkiewicz et al.'s (2017) information on SNP markers closely linked to the three QTLs for anthracnose resistance, namely, marker TP222136 for antr04/05_1 on linkage group ALB02, markers TP61122 and TP26007 for antr04/05_2 on linkage group ALB04, and markers TP251482, TP252015 and TP1928 for antr05_3 on linkage group ALB10, to infer the allelic value of these QTLs in the landrace genotypes.

Plant material. A total of 40 white lupin (*L. albus*) cultivars, breeding lines and landraces were collected from different sources and will be referred to as accessions throughout this publication (**Table 1**). Fifteen accessions were selected based on their contrasting predicted disease scores and because they represented different allelic combinations with three useful QTLs (two accessions), two useful QTLs (antr04/05_1 + antr05_3 for three accessions; antr04/05_1 + antr04/05_2 for one accession), one useful QTL (antr04/05_1 for three accessions; antr05_3 for three accessions), or no useful QTL (three accessions). The rest of the accessions

was selected based on their field performance in 2015 to 2017 (C. Arncken, personal communication). All accessions were phenotyped for anthracnose resistance under controlled conditions and 26 accessions were phenotyped under field conditions in a 1-row setup, from which eleven accessions were also phenotyped in field plots. The commercial cultivar Feodora served as reference throughout all phenotyping assays.

Controlled condition experiments. For high-throughput resistance phenotyping under controlled conditions, one seed per pot was sown in 0.2 liter pots with a diameter of 6 cm, containing non-sterilized peat soil (Einheitserde® Classic, Einheitserdewerke Werkverband e.V., Sinnatal-Altengronau, Germany) and vermiculite (ISOLA Vermiculite AG, Bözen, Switzerland) in a ratio of 5 to 1. Pots were placed in a growth chamber ($25 \pm 2^{\circ}\text{C}$, 16 h light and ~70% relative humidity). *C. lupini* JA01 (or IMI 375715) was grown on PDA for 6 to 8 days at 22°C in the dark. Spores were harvested with a sterile spreader after flooding the Petri dish with 2 ml sterile ddH₂O. The concentration was determined using a haemocytometer (0.1 mm; Marienfeld, Lauda-Königshofen, Germany) and adjusted to 10^5 spores/ml. Stem inoculation, inspired by protocols described by Falconi et al. (2015) and Dubrulle et al. (2020b), was performed on 14 day old seedlings (3-4 leaf stage) by carefully puncturing the apical main stem with a sterile syringe needle followed by application of 5 μl spore suspension. Control plants were inoculated with 5 μl sterile ddH₂O. After inoculation, plants were incubated for 48 hours at 100% relative humidity (16 h light, 22°C) before being returned to the growth chamber. Plants were watered every three days with 30 ml per plant, and fertilized twice with 100 ml Universal-Flüssigdünger (Gebr. Mayer Produktions- und Vertriebs. GmbH, Wahrenholz, Germany) at two and eight days post

183 inoculation (dpi). The experiments were performed in a randomized complete block design with
184 a minimum of five replicates per experiment.

185 **Field experiments.** Field experiments were performed in Switzerland at site F (Feldbach,
186 47°14'20.0"N 8°47'18.8"E) and site M (Mellikon) in 2018 and 2019 within 6-row plots. Plot sizes
187 in Mellikon were 1.5 x 5 m and in Feldbach 1.5 x 2.7 m with a seed density of 65 seeds/m². Total
188 field trial size in Mellikon 2019 was 608 m², in 2018 450 m², in Feldbach 2019 146 m² and in 2018
189 243 m². Plot experiments relied on natural infection and were performed in a randomized
190 complete block design with three replicates in 2019 and eight replicates in 2018. In addition, a
191 high-throughput 1-row setup was used at both sites in 2019 with two replicates in a randomized
192 complete block design according to a protocol described by Adhikari et al. (2009). Accessions
193 were sown in 1 m rows containing eight plants, with every second row sown with the susceptible
194 cultivar Amiga containing natural seed infection as disease spreader rows. Seeds were sown at
195 the end of March and harvested in mid-August. Before sowing, seeds were inoculated with
196 *Bradyrhizobium* sp. *lupini* (HiStick®L, BASF, UK) and no fertilizer was applied during the growing
197 season.

198 **Disease assessment.** Under controlled conditions, disease phenotypes were assessed at
199 4, 8 and 12 dpi (18, 22 and 26 days after sowing) using a 1 to 9 disease score index (DSI) based
200 on a protocol described by Thomas et al. (2008b): 1 = healthy plant, lesion size is 0 to 5% of stem
201 length; 2 = minor disease symptoms, minor petiole elongation and slight yellowing of older
202 leaves, lesion size is 5 to 15% of stem length; 3 = minor disease symptoms, increased petiole
203 elongation and yellowing of older leaves, lesion size is 15 to 30% of stem length; 4 = moderate
204 disease symptoms, main stem and petiole elongation, minor leaf and stem twisting, leaves are

yellowing, lesion size is 30 to 45% of stem length; 5 = moderate disease symptoms, whole plant starts to wilt, leaves and stem start twisting, main stem on the verge of collapsing, lesion is starting to sporulate and its size is 45 to 60% of stem length; 6 = severe disease symptoms, plant has collapsed, severe leaf wilting but plant still has a few green leaves, lesion is sporulating and the size is 60 to 70% of stem length; 7 = severe disease symptoms, plant has completely wilted and only the youngest leaves have not dried out, the lesion is sporulating and the size is 70 to 80% of stem length; 8 = very severe disease symptoms, plant has completely wilted and dried out, lesion shows abundant sporulation and its size is 80 to 90% of stem length and 9 = complete plant decay, lesion size is 90 to 100% of stem length (illustrated in **Figure 1A**). The controlled conditions standardized area under the disease progress curve (sAUDPC_{cc}) was calculated to assess and compare disease progression (Jeger and Viljanen-Rollinson 2001; Madden et al. 2007). Although disease scores obtained at single time points gave similar results as sAUDPC (**Table S1**), the sAUDPC was preferred to account for potential biases. At 12 dpi, lesion size, stem length and shoot dry weight were determined. Lesion size is expressed relative to overall stem length ($Lesion_{rel}$) and shoot dry weight is expressed relative to control (SDW_{rel}).

Disease assessments in the field were performed using a 1 to 9 DSI based on a protocol described by Jacob et al. (2017): 1 = no disease symptoms (0%), 2 = very minor disease symptoms (>0 to 2% diseased plants), 3 = minor disease symptoms (>2 to 5% diseased plants), 4 = low to moderate disease symptoms (>5 to 8% diseased plants), 5 = moderate disease symptoms (>8 to 14% diseased plants), 6 = moderate to severe disease symptoms (>14 to 22% diseased plants), 7 = severe disease symptoms (>22 to 37% diseased plants), 8 = severe to extremely severe disease symptoms (>37 to 61% diseased plants), 9 = extremely severe disease symptoms (>61% to 100%

diseased plants). In field plots anthracnose occurs in disease foci as illustrated in **Figure 1B**. Disease assessments were performed at least 4 times per growing season, (60 to 70 days 80 to 90 days, 100 to 110 days and 120 to 130 days after sowing) and sAUDPC for field plots (sAUDPC_{Field}) and for 1-rows (sAUDPC_{Row}) was calculated to assess and compare disease progression. Yield (dt/ha) was assessed for field plots at grain harvest in August. The two high-throughput phenotyping systems, controlled condition and 1-row field, were compared with field plot disease assessments.

Statistical analysis of experimental data. Statistical analyses were performed with R 4.0.3 (R Core Team 2020) using the packages *lme4* (Bates et al. 2014), *lmerTest* (Kuznetsova et al. 2017) and *emmeans* (Lenth et al. 2019), following a mixed model with factors of interest (i.e. accession, strain) as fixed and environment, environment x accession and replicated block nested in environment as random factor, after confirming the assumptions of normality of residuals and homogeneity of variance. To achieve a normal distribution, data was transformed with a square root or logit transformation. Data are presented as estimated least-squares means using the aforementioned mixed model. Mean separation between lupin accessions and the reference cultivar Feodora was analyzed using Dunnett's test ($p \leq 0.05$). Estimated means of controlled and field conditions were correlated using the Pearson correlation coefficient. Broad sense heritability (H^2) was estimated as: genotypic variance/phenotypic variance, according to (Toker 2004). The number of replicates (n) needed to obtain a least significant difference (LSD) of 1 for $\alpha=0.05$ was calculated according to (Williams and Abdi 2010), with the formula below with n =replicates, MSE =mean square error, df =degrees of freedom of residuals, t =t-test distribution and α =critical p-value: $n = (t_{\alpha, df})^2 * 2MSE$

Results

Evaluation of phenotyping under controlled conditions. BLASTn analysis of the ITS region confirmed that Swiss strain JA01 (GenBank accession MT741840) belongs to the species *Colletotrichum lupini*, showing 100% sequence identity with *C. lupini* strains corresponding to genetic group 2 (var. *setosum*; GenBank accessions JQ948169, JQ948161, MK463733) and 99.80% (494/495 bp) sequence identity with *C. lupini* strains corresponding to genetic group 1 (var. *lupini*; GenBank accessions JQ948155, JQ948159, JQ948158) at 100% query coverage. Comparing the three disease parameters, the highest broad-sense heritability (H^2) was found for standardized area under the disease progress curve under controlled conditions (sAUDPC_{CC}) with a H^2 of 0.82 followed by relative shoot dry weight (SDW_{rel}) with a H^2 of 0.78 and relative lesion size (Lesion_{rel}) with a H^2 of 0.75. Stem inoculation proved to be feasible for high-throughput phenotyping as up to 108 plants per m² could be phenotyped by one person within 26 days. To detect a least significant sAUDPC_{CC} difference of 1 between two genotypes, a minimum of 4 replications was required, in contrast to single-row analysis with a H^2 of 0.48 which required 15 replicates.

Increased levels of anthracnose resistance identified in white lupin. Disease phenotyping under controlled conditions revealed quantitative, rather than qualitative disease resistance as none of the 40 phenotyped white lupin accessions showed complete resistance to anthracnose. Eight accessions (Leb01, Eth01, Ares, NL01, Blu-25, Frieda, Eth02 and Kiev Mutant) showed significantly lower sAUDPC_{CC} means compared with the susceptible reference cultivar Feodora, with differences ranging between -1.8 (Leb01) and -1 (Kiev Mutant; **Figure 2A**). The accessions Frieda, Eth01 and Blu-25 also were more resistant under field conditions (**Figure 2C**).

The difference between the sAUDPC_{cc} means of accession Hetman and Feodora tended towards significance ($p=0.08$). Five of the accessions mentioned above, Frieda, Kiev Mutant, NL01, Blu-25, and Eth01 also showed significantly higher SDW_{rel} means compared with Feodora, with differences ranging between 0.36 (Frieda) and 0.15 (Eth01; **Figure S1A**). The assessment of Lesion_{rel} did not reveal significant differences compared to Feodora (**Figure S2A**).

Strong correlation between controlled condition phenotyping and field performance. A subset of 11 white lupin accessions was screened in field plots to verify the phenotypes expressed under controlled and 1- row field conditions. Significant genotype ($p=0.01$), field site ($p=0.001$) and genotype x field interaction ($p<0.001$) effects were found for the field plot experiments. Spatial within-trial variation was accounted for by a block effect ($p>0.001$). No significant effects were detected for the 1-row experiments. Disease pressure gradually increased over the growing season and was on average lower in 2018 (3.95) compared to 2019 (4.77). Growing conditions in 2018 were warmer (18.35°C) and drier (267 mm) on average, compared to 2019 (16.35°C and 572 mm; **Figure S4**). Significant Pearson correlation coefficients were found between sAUDPC_{Field} averaged across all four environments and sAUDPC_{cc} ($r=0.95$, $p<0.0001$), SDW_{rel} ($r=-0.80$, $p=0.003$) and Lesion_{rel} ($r=0.89$, $p<0.0001$; **Table 2, Figure 2, S1 and S2**). For each environment individually, correlations with sAUDPC_{Field} ranged between 0.95 and 0.64 for sAUDPC_{cc}, -0.86 and -0.52 for SDW_{rel} and 0.95 and 0.44 for Lesion_{rel}. When comparing controlled condition parameters with harvested yield (dt/ha) of the field plots averaged across all four environments, a significant correlation of -0.64 ($p=0.03$) was found for sAUDPC_{cc}. Correlations between yield and SDW_{rel} ($r=0.62$, $p=0.04$) and Lesion_{rel} ($r=-0.58$, $p=0.064$) were or tended towards significance. For each environment individually, correlations with yield ranged between -0.79 and -0.22 for sAUDPC_{cc},

0.71 and 0.19 for SDW_{rel} and -0.86 and -0.25 for $Lesion_{rel}$. High-throughput disease phenotyping within a 1-row field setup ($sAUDPC_{Row}$) showed Pearson correlation coefficients of 0.33 ($p=0.43$) and -0.23 ($p=0.56$) with $sAUDPC_{Field}$ and yield averaged across all four environments, respectively, when replicated once at one site (**Table 2**). When replicated twice at two sites, Pearson correlations of 0.31 ($p=0.34$) and -0.45 ($p=0.17$) between $sAUDPC_{Field}$ and yield, respectively, were found. Comparing 1-row experiments to each environment individually showed no significant correlations.

Poor genomic predictions. The best-predicting model was rrBLUP, with a value of 0.50 for intra-populations predictive ability. The predicted disease scores ranged from 2.12 to 4.70. The useful QTL *antr04/05_2* on linkage group ALB04 was particularly rare and was only found in eight genotypes. This included two genotypes from Ethiopia, one of which was included in this study (Eth04) and which combined a very low predicted susceptibility score (2.165) with the simultaneous presence of all three QTLs (**Table 1**). Genomic prediction (GP) values generated by rrBLUP (**Table 1**) revealed no significant correlation with $sAUDPC_{CC}$ (-0.31, $p=0.26$) or $sAUDPC_{Row}$ values (-0.46, $p=0.085$; **Table 2**). When comparing GP with SDW_{rel} and $Lesion_{rel}$, significant Pearson correlation coefficients of 0.60 ($p=0.02$) and -0.55 ($p=0.03$), respectively, were revealed. Importantly, the parental lines of the RIL population that was used for the QTL study and the genomic prediction model, i.e., P27174 and Kiev Mutant, exhibited an opposite anthracnose resistance pattern to that displayed in their evaluation in Australia (Rychel-Bielska et al. 2020). The resistant parent P27174 ($n=15$) showed a $sAUDPC_{CC}$ of 3.7 and an SDW_{rel} of 0.5, while the susceptible parent Kiev Mutant ($n=23$) showed a $sAUDPC_{CC}$ of 2.9 and an SDW_{rel} of 1 (**Figure 2**, **Figure S1**). This observation was confirmed by a follow-up experiment where Kiev Mutant,

315 P27174, Feodora and four additional lupin accessions, were inoculated with either the Australian
316 *C. lupini* strain IMI 375715 or the Swiss *C. lupini* strain JA01 (**Figure S3**). This experiment also
317 indicated that both *C. lupini* strains exhibit a similar virulence pattern on white lupin as no strain
318 ($p=0.64$) or strain x accession interaction ($p=0.96$) effects were observed.

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Discussion

Considering the lack of resistance to anthracnose disease in white lupin, a reliable high-throughput phenotyping tool to screen global white lupin germplasm is required. The described anthracnose disease phenotyping protocol, inoculating the stems of 14 day old white lupin seedlings grown under controlled conditions with a spore suspension of the pathogen, was useful to identify resistant genotypes, feasible for high-throughput phenotyping and the data corresponded with field performance. Similar stem inoculation approaches have proven to be a reliable tool to speed up selection in resistance breeding for various crops such as cotton (Bolek et al. 2005), sunflower (Schwanck et al. 2016), various *Brassicaceae* (Atri et al. 2019), and different legumes such as pea (Chang et al. 2018), soy (Twizeyimana et al. 2012) and Andean lupin (Falconí 2012). This study showed strong significant correlations ($r > 0.8$) between controlled condition phenotyping and replicated multi-environment field evaluations. This is in line with an anthracnose resistance study performed in Canada, showing that disease evaluations of stem inoculated seedlings of six white lupin cultivars under controlled conditions corresponded to disease evaluations of a 3 year field trial (Bhaskara Reddy et al. 1996). High correlations between stem inoculation under controlled conditions and field evaluations were also reported for other crops such as soybean (Twizeyimana et al. 2012), pea (Li 2018), canola (Devey and Rosielle 1986) and grapevine (Poolsawat et al. 2012). Thus, stem inoculation of white lupin seedlings under controlled conditions can be considered a reliable tool to speed up white lupin resistance breeding.

From the three disease parameters, sAUDPC_{CC} showed the strongest correlation with field performance ($r = 0.95$, $p < 0.0001$) and the highest heritability ($H^2 = 0.82$). In other plant-

342 pathosystems, using sAUDPC as a disease parameter was also highly correlated to field
 343 performance (Bradley et al. 2006; Li 2018; Trapero et al. 2013). The disease parameters SDW_{rel}
 344 and $Lesion_{rel}$ also showed high heritability and correlation to field performance, although these
 345 were lower than for $sAUDPC_{CC}$. With $lesion_{rel}$, no accessions could be identified that significantly
 346 showed less infection than Feodora. Besides resistance, SDW_{rel} has the advantage of also
 347 encompassing tolerance (Maya and Matsubara 2013). It should be taken into account, however,
 348 that a distinct symptom of lupin anthracnose is shoot elongation, potentially leading to a slight
 349 increase in biomass in minor to moderately infected plants at early stages, and therefore
 350 potentially leading to a misinterpretation of SDW_{rel} results. Based on $sAUDPC_{CC}$, eight out of the
 351 forty accessions were significantly more resistant to anthracnose than the susceptible reference
 352 cultivar Feodora (**Figure 2A**). Among those are three released cultivars, Frieda, Ares and Kiev
 353 Mutant, the breeding line Blu-25 and four genetic resources, Eth01, Eth02, NL01 and Leb01,
 354 which are thus valuable resources for white lupin breeding and cultivation. The developed
 355 phenotyping tool should now be applied on a larger set of accessions to find more sources of
 356 resistance in white lupin to counter yield loss caused by anthracnose.

357 Disease phenotyping within a 1-row field setup has been widely applied for resistance
 358 breeding (Niks et al. 2011), including lupins (Adhikari et al. 2009; Ruge-Wehling et al. 2009). In
 359 this study, 1-row disease evaluations based on $sAUDPC_{Row}$, replicated once at one site, showed
 360 no significant correlations with field plot evaluations ($sAUDPC_{Field}$). With four replicates over two
 361 sites, correlation with $sAUDPC_{Field}$ improved. Compared to stem inoculation under controlled
 362 conditions, 1-row disease phenotyping showed a weaker correlation to field performance and
 363 results should be interpreted with caution. However, with sufficient replicates, 1-row disease

phenotyping could be an alternative approach to identify anthracnose resistance in white lupin, especially when environmental variation has to be considered.

Predicted resistance values based on the genomic prediction model reported by Rychel-Bielska et al. (2020), taking into account the three major QTLs and possible minor ones, showed no positive correlations with sAUDPC_{CC} and sAUDPC_{Row}, suggesting no association of the reported QTLs with anthracnose resistance in the current study. As discussed by Rychel-Bielska et al. (2020), different inoculation methods, environmental variation and *C. lupini* strain-specific virulence can influence plant resistance and therefore should be taken into account. In this study, stem inoculation through wounding was performed under controlled conditions and might have contributed to the observed lack of correlation and contrasting resistance levels observed for Kiev Mutant and P27174. This, however, does not explain the absence of positive correlations between 1-row field evaluations under natural infection and the predicted values. In blue lupin, a similar unexpected observation occurred when material with strong anthracnose resistance identified under Australian conditions (Yang et al. 2008) turned out to be susceptible in Germany (Fischer et al. 2015). Warmer temperatures up to 25°C and higher rainfall (mm) favour anthracnose disease pressure in lupin (Dubrulle et al. 2020b; Thomas and Sweetingham 2004), highlighting the relevance of varying environmental conditions. The average growing conditions during the Swiss (16.1 to 18.7°C, 195 to 694 mm, **Figure S4**) and Australian field experiments (12.9 to 13.1°C, 441 to 566 mm, BOM 2005) indicate the lower temperature of 3 to 6 °C on average at the Australian site may have contributed to the observed inconsistencies. Differences in *C. lupini* strain virulence are unlikely as no strain or accession x strain interaction effects were found and *C. lupini* is suggested to be clonal (Dubrulle et al. 2020b; Talhinas et al. 2016).

Although QTL mapping and genomic prediction studies for quantitative traits are considered valuable (de Ronne et al. 2020; Haile et al. 2020; Lyra et al. 2020) and are being applied for key traits in white lupin (Annicchiarico et al. 2020; Annicchiarico et al. 2019), the method encounters various limitations (Wang et al. 2018; Xu et al. 2017). Genomic prediction models could effectively be applied to less related populations (Hao et al. 2019), but optimal predictions are achieved with related training populations (Akdemir and Isidro-Sánchez 2019; Albrecht et al. 2011; Berro et al. 2019). We suggest the main reason for poor genomic predictions of anthracnose tolerance in this study was the seemingly completely inconsistent response within the current testing conditions of the two parent genotypes that represented resistance and susceptibility in the RIL population evaluated in the Australian site, whose data were used for constructing the genomic model.

This study provides a high-throughput phenotyping tool to identify field-relevant resistance against lupin anthracnose under controlled conditions to support white lupin resistance breeding. Phenotyping white lupin germplasm within a 1-row setup under field conditions was deemed to be less reliable as lower correlations with field plot disease assessments were found compared with controlled condition phenotyping. The resistant accessions identified in this study, Frieda, Blu-25, Eth01, Eth02, NL01, Leb01 and Ares, should be incorporated into current breeding programs after field validation. As predicted resistance values could not be validated, further insight on the effect of environmental conditions, infection pathways and the genetic basis underlying anthracnose resistance in white lupin is required. Current research focuses on phenotyping and genotyping a diverse set of 200 white lupin

407 genotypes, including Kiev Mutant, P27174 and the resistant accessions identified in this study
408 using the described phenotyping tool.

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642 **Table 1.** Origins and characteristics of white lupin (*Lupinus albus*) accessions phenotyped for
643 anthracnose (*Colletotrichum lupini*) resistance in this study.

<i>Accession</i>	<i>Origin</i>	<i>Genbank code</i>	<i>Institution</i>	<i>Type</i>	<i>GP</i>	<i>QTL</i> <i>b</i>	<i>n</i>
Mut28^a	-	LUP 6445	IPK (Germany)	Genetic Resource	-	-	10
Cro01^a	Yugoslavia	LUP 237	IPK (Germany)	Genetic Resource	-	-	15
Eth01^a	Ethiopia	LUP 2078	IPK (Germany)	Genetic Resource	-	-	10
Frieda^a	Germany	Frieda	DSV (Germany)	Cultivar	-	-	19
Sulimo^{*a}	France	Sulimo	Jouffrai-Drillaud (France)	Cultivar	-	-	11
Blu-25^{*a}	Chile	Blu-25	Semillas Baer (Chile)	Breeding line	-	-	26
Feodora^{*a}	France	Feodora	Jouffrai-Drillaud (France)	Cultivar	-	-	37
Amiga^{*a}	France	Amiga	Florimond-Desprez (France)	Cultivar	-	-	20
Figaro^{*a}	France	Figaro	Jouffrai-Drillaud (France)	Cultivar	-	-	8
Zulika^{*a}	Czech Republic	Zulika	Oseva s.r.o. (Czech Republic)	Cultivar	-	-	7
Dieta^{*a}	UK	Dieta	Soya UK (UK)	Cultivar	-	-	8
KievMutant	Ukraine	95413	Polish Genebank (Poland)	Cultivar	-	-	23
P27175	Ethiopia	P27175	DPIRD (Australia)	Landrace	-	-	18
P27174	Ethiopia	P27174	DPIRD (Australia)	Landrace	-	-	15
Alg01	Algeria	La 572	CREA (Italy)	Genetic Resource	-	-	10
Eth02	Ethiopia	LUP 258	IPK (Germany)	Genetic Resource	-	-	11
Eth03	Ethiopia	LUP 2076	IPK (Germany)	Genetic Resource	-	-	6
NL01 ^c	The Netherlands ^c	LUP 2079	IPK (Germany)	Genetic Resource	-	-	10
Egy01	Egypt	503	Vavilov Research Institute (Russia)	Genetic Resource	-	-	6
MuPI	-	LUP 2104	IPK (Germany)	Genetic Resource	-	-	6
EdwBra	The Netherlands	V11-12-6	Louis Bolk Institute (NL)	Breeding line	-	-	6
Bianca	Italy	LUP 238	IPK (Germany)	Cultivar	-	-	6

Andro	Australia	Andromeda	DPIRD (Australia)	Cultivar	-	-	10
Hetman	-	LUP 6449	IPK (Germany)	Cultivar	-	-	12
Spn01	Spain	-	Local market (Spain)	-	-	-	6
Eth04 ^a	Ethiopia	LAP0084	CREA (Italy)	Genetic	2.1	1,1,1	7
				Resource	7		
Isr01 ^a	Israel	LAP0071	CREA (Italy)	Genetic	3.5	1,1,1	7
				Resource			
Egy02 ^a	Egypt	LAP0093	CREA (Italy)	Genetic	3.3	1,0,1	7
				Resource	1		
Can01 ^a	Canaries	LAP0063	CREA (Italy)	Genetic	3.4	1,0,1	8
				Resource	6		
Leb01 ^a	Lebanon	LAP0070	CREA (Italy)	Genetic	3.5	1,0,1	8
				Resource	6		
Ita01 ^a	Italy	LAP0098	CREA (Italy)	Genetic	2.6	1,1,0	8
				Resource	9		
Ita02 ^a	Italy	LAP0101	CREA (Italy)	Genetic	3.5	1,0,0	6
				Resource	1		
Ita03 ^a	Italy	LAP0099	CREA (Italy)	Genetic	3.6	1,0,0	6
				Resource	9		
Ita04 ^a	Italy	LAP0107	CREA (Italy)	Genetic	3.1	1,0,0	8
				Resource	7		
Sud01 ^a	Sudan	LAP0082	CREA (Italy)	Genetic	3.5	0,0,1	7
				Resource	7		
Mor01 ^a	Morocco	LAP0055	CREA (Italy)	Genetic	3.8	0,0,1	8
				Resource	6		
Ares ^a	France	Ares	CREA (Italy)	Cultivar	4.6	0,0,1	7
Ita05 ^a	Italy	LAP0097	CREA (Italy)	Genetic	3.7	0,0,0	8
				Resource	9		
Leb02 ^a	Lebanon	LAP0069	CREA (Italy)	Genetic	4.0	0,0,0	8
				Resource	8		
Ludet ^a	France	Ludet	CREA (Italy)	Cultivar	3.5	0,0,0	7
					9		

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645 GP stands for Genomic prediction, QTL stand for quantitative trait locus and n indicates total
646 observations obtained with stem inoculation under controlled conditions. Accession names
647 highlighted in bold were tested in field plots in 2019 and accession names followed by an *
648 were also tested in in 2018. ^a = phenotyped with 1-rows under field conditions in 2019. ^b =

649 presence/absence (1/0) of resistance QTL antr04/05_1 (ALB02), antr04/05 _2 (ALB04) and
650 antr05_3 (ALB10). ^c = accession NL01 might have an Ethiopian origin based on plant
651 morphology in the field.
652

Table 2. Pearson correlation coefficients of two high-throughput phenotyping systems, 1) under controlled conditions and 2) with 1-rows under field conditions, with field plot phenotyping and genomic prediction values.

Field plot phenotyping, natural infection			Controlled condition phenotyping, stem inoculation			1-row field phenotyping, disease spreader rows ^a	
2018 n = 7 2019 n = 11		Mean	sAUDPC cc	SDW _{rel}	Lesion _{rel}	sAUDPC _R ow, 1 rep, 1 site ^b	sAUDPC _{Ro} w, 2 reps, 2 sites
sAUDPC _{Field}	F 2018	3.99	0.64	-0.52	0.75*	0.003	-0.19
	F 2019	4.15	0.69*	-0.64*	0.44	0.23	0.48
	M 2018	3.92	0.95***	-0.86*	0.95***	0.19	0.30
	M 2019	5.38	0.77**	-0.68*	0.56*	0.22	0.44
	Average		0.95***	-0.80**	0.89***	0.33	0.31
Yield (dt/ha)	F 2018	42	-0.22	0.19	-0.25	-0.03	-0.26
	F 2019	20	-0.43	0.41	-0.35	-0.18	-0.37
	M 2018	26	-0.79*	0.71*	-0.86*	-0.05	-0.31
	M 2019	14	-0.72*	0.66*	-0.58*	-0.29	-0.54
	Average		-0.64*	0.65*	-0.58*	-0.23	-0.45
Genomic prediction (n=15)			-0.31	0.60*	-0.55*	-0.38	-0.46*

M = Mellikon and F = Feldbach, sAUDPC = standardized area under disease progress curve, SDW_{rel} = shoot dry weight relative to control, Lesion_{rel} = Lesion size relative to total stem length, n = number of genotypes. * = p≤0.1, * = p≤0.05, ** = p≤0.01 and *** = p≤0.001. ^a = correlations of the genomic predictions are based on 2 replicates of environment M 2019, ^b = correlations are comprised of the average of obtained correlation coefficients of all available replications

Figure 1. Disease assessment under controlled conditions and field plots. A: Representative disease symptoms of white lupin after *C. lupini* stem inoculation under controlled conditions (12 dpi). Disease score index of 1 = healthy, 3 = minor disease symptoms, 5 = average disease symptoms. 7 = severe disease symptoms and 9 = complete plant decay. Plant shown is the reference accession Feodora. B: Illustration of disease foci (brown) occurring in field plots. Disease score index: 1 = no disease symptoms (0%), 3 = minor disease symptoms (>2 to 5% diseased plants), 5 = moderate disease symptoms (>8 to 14% diseased plants), 7 = severe disease symptoms (>22 to 37% diseased plants), 9 = extremely severe disease symptoms (>61% to 100% diseased plants).

Figure 2. Anthracnose severity under controlled conditions expressed in sAUDPC and its correlation with field performance. A: Estimated means of standardized area under the disease progress curve (sAUDPC) of white lupin accessions phenotyped under controlled conditions (CC). The green dots indicate accessions tested in the field, blue indicates the susceptible reference accession Feodora and red dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars indicate standard error of the estimated mean. * $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ difference with Feodora (Dunnett's test). **B & C:** Correlation plots of CC sAUDPC and field sAUDPC of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

Supplementary

Table S1. Pearson correlation coefficients of sAUDPC and disease score at single time point with field plot phenotyping under natural conditions across four environments. •= $p\leq0.1$, * = $p\leq0.05$, ** = $p\leq0.01$ and *** = $p\leq0.001$.

Controlled condition	H ²	sAUDPC, Field	Yield (dt/ha), Field
<i>4dpi</i>	0.76	0.48	-0.10
<i>8dpi</i>	0.79	0.93***	-0.66*
<i>12dpi</i>	0.82	0.96***	-0.67*
<i>sAUDPC</i>	0.82	0.95***	-0.64*

Figure S1. Anthracnose severity under controlled conditions expressed in Relative SDW and its correlation with field performance. A: Estimated means of relative shoot dry weight (SDW) of white lupin accessions phenotyped under controlled conditions (CC). The green dots indicate accessions tested in the field, blue indicates the susceptible reference accession Feodora and red dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars indicate standard error of the estimated mean. * $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ difference with Feodora (Dunnett's test). **B & C:** Correlation plots of CC sAUDPC and field sAUDPC of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

721 **Figure S2. Anthracnose severity under controlled conditions expressed in relative lesion size**
722 **(%) and its correlation with field performance. A:** Estimated means of relative lesion size of white
723 lupin accessions phenotyped under controlled conditions (CC). The green dots indicate
724 accessions tested in the field, blue indicates the susceptible reference accession Feodora and red
725 dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted
726 lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars
727 indicate standard error of the estimated mean. * $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$
728 difference with Feodora (Dunnett's test). **B & C:** Correlation plots of CC sAUDPC and field sAUDPC
729 of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018
730 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

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734 **Figure S3. Virulence of *C. lupini* strain JA01 and IMI 37515 on different white lupin (*L. albus*)**
735 **accessions.** Box plot showing the standardized area under the disease progress curve (sAUDPC)
736 of different white lupin accessions inoculated under controlled conditions with the Swiss *C. lupini*
737 strain JA01 (red) or Australian strain IMI 375715 (blue). No strain effect ($F\text{-value}_{1-6}=0.22$, $p=0.64$)
738 and no strain x accession interaction effect ($F\text{-value}_{1-6}=0.23$, $p=0.96$) was observed.

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Figure S4. Anthracnose development and weather data throughout growing season in Mellikon and Feldbach in 2018 and 2019. Mean DSI indicates overall mean of disease score index across all accessions, mean T and mean P indicate mean temperature (°C) and mean precipitation (mm), respectively, from sowing to harvest. Error bars indicate standard error of the mean. B indicates blue precipitation bar plots and R indicate the red temperature line. Source weather data is Agrometeo 2019.

<i>Accession</i>	<i>Origin</i>	<i>Genbank code</i>	<i>Institution</i>	<i>Type</i>	<i>GP</i>	<i>QTL b</i>	<i>n</i>
Mut28^a	-	LUP 6445	IPK (Germany)	Genetic Resource	-	-	10
Cro01^a	Yugoslavia	LUP 237	IPK (Germany)	Genetic Resource	-	-	15
Eth01^a	Ethiopia	LUP 2078	IPK (Germany)	Genetic Resource	-	-	10
Frieda^a	Germany	Frieda	DSV (Germany)	Cultivar	-	-	19
Sulimo^{*a}	France	Sulimo	Jouffrai-Drillaud (France)	Cultivar	-	-	11
Blu-25^{*a}	Chile	Blu-25	Semillas Baer (Chile)	Breeding line	-	-	26
Feodora^{*a}	France	Feodora	Jouffrai-Drillaud (France)	Cultivar	-	-	37
Amiga^{*a}	France	Amiga	Florimond-Desprez (France)	Cultivar	-	-	20
Figaro^{*a}	France	Figaro	Jouffrai-Drillaud (France)	Cultivar	-	-	8
Zulika^{*a}	Czech Republic	Zulika	Oseva s.r.o. (Czech Republic)	Cultivar	-	-	7
Dieta^{*a}	UK	Dieta	Soya UK (UK)	Cultivar	-	-	8
KievMutant	Ukraine	95413	Polish Genebank	Cultivar	-	-	23
P27175	Ethiopia	P27175	DPIRD (Australia)	Landrace	-	-	18
P27174	Ethiopia	P27174	DPIRD (Australia)	Landrace	-	-	15
Alg01	Algeria	La 572	CREA (Italy)	Genetic Resource	-	-	10
Eth02	Ethiopia	LUP 258	IPK (Germany)	Genetic Resource	-	-	11
Eth03	Ethiopia	LUP 2076	IPK (Germany)	Genetic Resource	-	-	6
NL01 ^c	The Netherlands ^c	LUP 2079	IPK (Germany)	Genetic Resource	-	-	10
Egy01	Egypt	503	Vavilov Research Institute (Russia)	Genetic Resource	-	-	6
MuPI	-	LUP 2104	IPK (Germany)	Genetic Resource	-	-	6
EdwBra	The Netherlands	V11-12-6	Louis Bolk Institute (NL)	Breeding line	-	-	6
Bianca	Italy	LUP 238	IPK (Germany)	Cultivar	-	-	6
Andro	Australia	Andromeda	DPIRD (Australia)	Cultivar	-	-	10
Hetman	-	LUP 6449	IPK (Germany)	Cultivar	-	-	12
Spn01	Spain	-	Local market (Spain)	-	-	-	6
Eth04 ^a	Ethiopia	LAP0084	CREA (Italy)	Genetic Resource	2.1 7	1,1,1	7
Isr01 ^a	Israel	LAP0071	CREA (Italy)	Genetic Resource	3.5	1,1,1	7
Egy02 ^a	Egypt	LAP0093	CREA (Italy)	Genetic Resource	3.3 1	1,0,1	7
Can01 ^a	Canaries	LAP0063	CREA (Italy)	Genetic Resource	3.4 6	1,0,1	8

Leb01 ^a	Lebanon	LAP0070	CREA (Italy)	Genetic Resource	3.5 6	1,0,1	8
Ita01 ^a	Italy	LAP0098	CREA (Italy)	Genetic Resource	2.6 9	1,1,0	8
Ita02 ^a	Italy	LAP0101	CREA (Italy)	Genetic Resource	3.5 1	1,0,0	6
Ita03 ^a	Italy	LAP0099	CREA (Italy)	Genetic Resource	3.6 9	1,0,0	6
Ita04 ^a	Italy	LAP0107	CREA (Italy)	Genetic Resource	3.1 7	1,0,0	8
Sud01 ^a	Sudan	LAP0082	CREA (Italy)	Genetic Resource	3.5 7	0,0,1	7
Mor01 ^a	Morocco	LAP0055	CREA (Italy)	Genetic Resource	3.8 6	0,0,1	8
Ares ^a	France	Ares	CREA (Italy)	Cultivar	4.6	0,0,1	7
Ita05 ^a	Italy	LAP0097	CREA (Italy)	Genetic Resource	3.7 9	0,0,0	8
Leb02 ^a	Lebanon	LAP0069	CREA (Italy)	Genetic Resource	4.0 8	0,0,0	8
Ludet ^a	France	Ludet	CREA (Italy)	Cultivar	3.5 9	0,0,0	7

GP stands for Genomic prediction, QTL stand for quantitative trait locus and n indicates total observations obtained with stem inoculation under controlled conditions. Accession names highlighted in bold were tested in field plots in 2019 and accession names followed by an * were also tested in in 2018. a = phenotyped with 1-rows under field conditions in 2019. b = presence/absence (1/0) of resistance QTL antr04/05_1 (ALB02), antr04/05_2 (ALB04) and antr05_3 (ALB10). c = accession NL01 might have an Ethiopian origin based on plant morphology in the field.

Field plot phenotyping, natural infection			Controlled condition phenotyping, stem inoculation			1-row field phenotyping, disease spreader rows ^a	
2018 n = 7 2019 n = 11		Mean	sAUDPC _{cc}	SDW _{rel}	Lesion _{rel}	sAUDPC _R ow ₁ 1 rep, 1 site ^b	sAUDPC _{Ro} w ₁ 2 reps, 2 sites
sAUDPC _{Field}	F 2018	3.99	0.64	-0.52	0.75*	0.003	-0.19
	F 2019	4.15	0.69*	-0.64*	0.44	0.23	0.48
	M 2018	3.92	0.95***	-0.86*	0.95***	0.19	0.30
	M 2019	5.38	0.77**	-0.68*	0.56*	0.22	0.44
	Average		0.95***	-0.80**	0.89***	0.33	0.31
Yield (dt/ha)	F 2018	42	-0.22	0.19	-0.25	-0.03	-0.26
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	M 2018	26	-0.79*	0.71*	-0.86*	-0.05	-0.31
	M 2019	14	-0.72*	0.66*	-0.58*	-0.29	-0.54
	Average		-0.64*	0.65*	-0.58*	-0.23	-0.45
Genomic prediction (n=15)			-0.31	0.60*	-0.55*	-0.38	-0.46*

M = Mellikon

and F = Feldbach. sAUDPC = standardized area under disease progress curve, SDW_{rel} = shoot dry weight relative to control, Lesion_{rel} = Lesion size relative to total stem length, n = number of genotypes. * = p ≤ 0.1, * = p ≤ 0.05, ** = p ≤ 0.01 and *** = p ≤ 0.001. ^a = correlations of the genomic predictions are based on 2 replicates of environment M 2019, ^b = correlations are comprised of the average of obtained correlation coefficients of all available replications

Figure 1. Disease assessment under controlled conditions and field plots. A: Representative disease symptoms of white lupin after *C. lupini* stem inoculation under controlled conditions (12 dpi). Disease score index of 1 = healthy, 3 = minor disease symptoms, 5 = average disease symptoms. 7 = severe disease symptoms and 9 = complete plant decay. Plant shown is the reference accession Feodora. B: Illustration of disease foci (brown) occurring in field plots. Disease score index: 1 = no disease symptoms (0%), 3 = minor disease symptoms (>2 to 5% diseased plants), 5 = moderate disease symptoms (>8 to 14% diseased plants), 7 = severe disease symptoms (>22 to 37% diseased plants), 9 = extremely severe disease symptoms (>61% to 100% diseased plants).

281x208mm (150 x 150 DPI)

Figure 2. Anthracnose severity under controlled conditions expressed in sAUDPC and its correlation with field performance. A: Estimated means of standardized area under the disease progress curve (sAUDPC) of white lupin accessions phenotyped under controlled conditions (CC). The green dots indicate accessions tested in the field, blue indicates the susceptible reference accession Feodora and red dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars indicate standard error of the estimated mean. • $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ difference with Feodora (Dunnett's test). B & C: Correlation plots of CC sAUDPC and field sAUDPC of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

387x347mm (150 x 150 DPI)

Controlled condition	H ²	sAUDPC, Field	Yield (dt/ha), Field
<i>4dpi</i>	0.76	0.48	-0.10
<i>8dpi</i>	0.79	0.93***	-0.66*
<i>12dpi</i>	0.82	0.96***	-0.67*
<i>sAUDPC</i>	0.82	0.95***	-0.64*

Figure S1. Anthracnose severity under controlled conditions expressed in Relative SDW and its correlation with field performance. A: Estimated means of relative shoot dry weight (SDW) of white lupin accessions phenotyped under controlled conditions (CC). The green dots indicate accessions tested in the field, blue indicates the susceptible reference accession Feodora and red dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars indicate standard error of the estimated mean. • $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ difference with Feodora (Dunnett's test). B & C: Correlation plots of CC sAUDPC and field sAUDPC of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

383x344mm (150 x 150 DPI)

Figure S2. Anthracnose severity under controlled conditions expressed in relative lesion size (%) and its correlation with field performance. A: Estimated means of relative lesion size of white lupin accessions phenotyped under controlled conditions (CC). The green dots indicate accessions tested in the field, blue indicates the susceptible reference accession Feodora and red dots indicate the RIL population parental lines, Kiev Mutant and P21714. Dashed line and dotted lines indicate the mean and estimated standard error, respectively, of Feodora. Error bars indicate standard error of the estimated mean. • $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ difference with Feodora (Dunnett's test). B & C: Correlation plots of CC sAUDPC and field sAUDPC of each of the four different environments Feldbach (F) 2018 and 2019 and Mellikon (M) 2018 and 2019 individually (B) and combined (C). R indicates Pearson correlation coefficient.

385x345mm (150 x 150 DPI)

Figure S3. Virulence of *C. lupini* strain JA01 and IMI 375715 on different white lupin (*L. albus*) accessions. Box plot showing the standardized area under the disease progress curve (sAUDPC) of different white lupin accessions inoculated under controlled conditions with the Swiss *C. lupini* strain JA01 (red) or Australian strain IMI 375715 (blue). No strain effect ($F\text{-value}_{1-6}=0.22$, $p=0.64$) and no strain $\times$ accession interaction effect ($F\text{-value}_{1-6}=0.23$, $p=0.96$) was observed.

177x119mm (150 x 150 DPI)

Figure S4. Anthracnose development and weather data throughout growing season in Mellikon and Feldbach in 2018 and 2019. Mean DSI indicates overall mean of disease score index across all accessions, mean T and mean P indicate mean temperature (°C) and mean precipitation (mm), respectively, from sowing to harvest. Error bars indicate standard error of the mean. B indicates blue precipitation bar plots and R indicate the red temperature line. Source weather data is Agrometeo 2019.

225x204mm (150 x 150 DPI)