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Influence of plant growth stage on resistance to anthracnose in Andean lupin (*Lupinus mutabilis*)

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Abstract. Anthracnose, caused by *Colletotrichum acutatum*, is the most destructive fungal disease of Andean lupin (*Lupinus mutabilis* Sweet) in Ecuador and of other lupin species around the world. Symptoms of necrotic spots occur throughout the main stem, and infection progresses to cause bending of the main stem and lateral branches, resulting in yield loss. Although there is no known anthracnose resistance, this study aims to assess tolerance of Andean lupin and investigate lupin–*C. acutatum* interactions. Two Andean lupin genotypes, I-450 Andino and I-451 Guaranguito, were inoculated on the meristematic section of the main stem, either by spraying or by pipetting *C. acutatum* spores on to an artificial wound. Although the two methods gave similar results, spraying is the preferred method because it mimics natural pathogen infection. Plant-pathogen interactions were assessed at five different phenological stages (leaf stages 2–3, 4–5, 6–7, 8–9, and 10–11) with three *C. acutatum* isolates by using a 0–5 scale to assess disease symptoms. In both genotypes, anthracnose symptoms were greater at early seedling stage (2–3-leaf stage), decreasing significantly in early vegetative phase (6–7-leaf stage) and increasing again when the flower stage began (10–11-leaf stage). However, the tolerance of these two Andean lupin genotypes to anthracnose was not equally expressed at all developmental stages. We recommend, in a breeding program, that screening for anthracnose first occurs at the 6–7-leaf stage (6 weeks old) and again when flowering starts at the 10–11-leaf stage (10 weeks old) so that the overall tolerance can be determined. This method could be used in lupin breeding programs for improving resistance to anthracnose.

Additional keywords: *Colletotrichum acutatum*, *Lupinus mutabilis*, plant phenology, resistance breeding, screening method.

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Introduction

The Andean lupin or 'tarwi' (*Lupinus mutabilis* Sweet) is domesticated and cultivated in South America. It is of agricultural importance in Ecuador (Peralta *et al*. 2012) and in Peru and Bolivia (Jacobsen and Sherwood 2002). Interest in Andean lupin has recently extended to Europe because of its nutritional value (Jacobsen and Sherwood 2002). Lupin seeds are a valuable source of protein, iron, and zinc. Disadvantages of the Andean lupin are indeterminate growth, high content of alkaloids and susceptibility to diseases. Research has been carried out on old world species of lupin but little on resistance screening and breeding with *L. mutabilis*, a species native to the inter-Andean region. A high level of genetic diversity exists among lupin species (Talhinhas *et al*. 2003). The principal biotic factor limiting production of *L. mutabilis* in Ecuador and other lupin species around the world is the fungal disease anthracnose (Elmer *et al*. 2001; Talhinhas *et al*. 2002; Thomas 2003), caused by *Colletotrichum acutatum* (Talhinhas *et al*. 2002; Falconi *et al*. 2013). The pathogen infects the main stem and lateral branches and produces necrotic lesions upon which orange conidial masses develop (Falconi 2012), resulting in significant yield reduction (Thomas and Sweetingham 2004).

The National Agricultural Research Institute (Instituto Nacional de Investigaciones Agropecuarias, INIAP) of Ecuador maintains a lupin collection of >500 accessions representing 17 species (Rivera *et al*. 1998), and of these, ~120 accessions are *L. mutabilis*. Researchers of the Legume and Andean Grain National Program (PRONALEG-GA/INIAP) have been conducting field studies for >15 years aiming at selecting Andean lupin genotypes with improved agronomic traits. In this selection process, I-450 Andino (INIAP-450 ANDINO 1999) and I-451 Guaranguito (Peralta *et al*. 2012) were chosen as the best genotypes because they are early maturing (6 months), have uniform white seed, and have almost twice the yield of other genotypes (INIAP-450 ANDINO 1999; Peralta *et al*. 2012). However, I-450 Andino and I-451 Guaranguito are susceptible to anthracnose.

Anthracnose resistance can be evaluated with parameters such as expression of symptoms, lesion expansion, and amount of sporulation (Thomas *et al*. 2008), and the interaction of these

parameters with plant age affects disease severity (Wastie 1991). Examples of such interactions include: potato plants and late blight (*Phytophthora infestans*), where young plants are susceptible, later becoming more resistant, and then older plants become susceptible again (Stewart 1990); bean cultivars inoculated with *C. lindemuthianum* showing differential resistance related to the developmental stages of the plants (Bigirimana and Hofte 2001); and wheat being susceptible to *Septoria tritici* at the seedling stage but resistant in the tillering and flag leaf stages (Gieco *et al*. 2004). Appropriate methodologies should be developed to evaluate disease resistance for each crop–disease system.

Screening experiments have been conducted to identify anthracnose-tolerant lupin genotypes in Ecuador (Peralta *et al*. 2004; Murillo *et al*. 2006). Visual observations of naturally infected adult plants suggested that lupin is most susceptible during bloom (Peralta *et al*. 2004). Some tolerance in *L. mutabilis* has been found after spraying with *C. acutatum* on 2-week-old plants (Murillo *et al*. 2006). In *L. angustifolius* and *L. albus*, two methods of inoculation were used under greenhouse conditions to identify anthracnose-tolerant plants (Talhinhas 2002). However, the study could not distinguish different levels of tolerance and there were differences between results for the field and greenhouse. A reliable method to evaluate anthracnose resistance in Andean lupin and knowledge about the appropriate phenological stage to evaluate for resistance are needed for breeding programs.

To our knowledge, this is the first study to evaluate lupin anthracnose symptoms, hypothesising that differences in resistance may be due to phenological stages. The objective of this study was to elucidate the significance of the lupin growth stage on anthracnose development caused by *C. acutatum*. Because there are no Andean lupin varieties with known anthracnose resistance, our protocol development was done with the susceptible I-450 Andino and I-451 Guaranguito genotypes. The appropriate inoculation method and phenological stage for testing these genotypes for tolerance should facilitate evaluation of anthracnose resistance in other genotypes of Andean lupin.

Materials and methods

Fungal isolates and inoculum production

Inoculation experiments were performed using three *C. acutatum* isolates (Lup1, Lup14, Lup18) collected from three areas with different environmental conditions in the province of Cotopaxi, Ecuador: Lup1 (EMBL ITS accession no. JN543059), Lup14 (EMBL ITS accession no. JN543061), Lup18 (EMBL ITS accession no. JN543063). Morphological and molecular characteristics of isolates are described in Falconi *et al*. (2013). Spores of each isolate were taken from potato dextrose agar (PDA; Difco, BD Diagnostics, Sparks, MD, USA) slants, and subcultures were grown on Petri plates containing autoclaved PDA. The isolates were incubated for 10 days at room temperature 20 ± 2°C. Conidial suspensions were prepared by flooding the surface with sterile distilled saline solution (NaCl 0.8% + Tween 80 0.1%) and gently scraping with a glass rod. The concentration of spores was determined using a hemocytometer and diluted with sterile distilled saline solution to 2.5×10^6 conidia mL^{-1}.

Andean lupin genotypes

The genotypes I-450 Andino and I-451 Guaranguito Andean lupin were selected for early maturity, productivity, and agro-morphological characters. They are described in more detail in Peralta *et al*. (2012).

Inoculation methods

To establish plants for inoculation, 12 seeds of each genotype were surface-disinfected in 0.5% NaClO for 5 min, rinsed with water, and sown, four of each in 22-cm diameter pots containing 4 kg of a mixture of sterilised soil–ground pumice–coconut fibre (1 : 1 : 1). After 15 days, one seedling was removed from each pot, leaving three plants per experimental unit. Seedlings were grown in a greenhouse with a temperature of 12 ± 2°C night, 20 ± 2°C day with a 12-h photoperiod and relative humidity of 70 ± 10%.

Two inoculation methods—spraying and micropipetting into a wound—were evaluated. For inoculation by spraying, ~500 µL of each *C. acutatum* isolate suspension was sprayed on the meristematic parts and young stems of lupin plants. A small, hand-held Venturi atomiser (aerograph) with an air pump was used. For the second method, an artificial wound was made with a hypodermic syringe at the apical main stem (the same depth of 2 mm for each wound). The spore suspension (25 µL) of each isolate was injected with a micropipette into each wound. The inoculation area of each plant was completely covered with a small, black plastic bag for 72 h. A piece of cotton drenched in sterile distilled water was added to the bags before sealing to maintain high relative humidity and to promote infection. Plants at the 4–5-leaf stage were inoculated by the two methods. A severity scale of 0–5 was used (see *Disease evaluation* below) and symptoms were evaluated on the main stem at 26 days after inoculation. The number of resistant plants was expressed as percentage. The experiment was conducted twice.

Lupin developmental stages

To investigate if phenological stages are associated with anthracnose severity, Andean lupin genotypes I-450 Andino and I-451 Guaranguito were inoculated with three *C. acutatum* isolates, Lup1, Lup14, and Lup18, and anthracnose symptoms from seedling to flower initiation stage were assessed. Phenological stages of lupins are referred to in terms of leaf number, for example, 4-leaf stage (Kettel *et al*. 2003). Inoculations were performed at the following developmental stages: early seedling (cotyledons turn green and 2 or 3 leaves above epicotyl, 2 weeks old); seedling (cotyledons remain green and 4 or 5 leaves above epicotyl, 4 weeks old); early vegetative (cotyledons absent, internode elongation on main stem, 6 or 7 leaves–internodes, 6 weeks old); vegetative (internode elongation, 8 or 9 leaves–internodes on main stem, 8 weeks old); and floral initiation (beginning of main stem flowering, 10 weeks old, 10 or 11 leaves–internodes on main stem).

Phenotypically healthy seeds of I-450 Andino and I-451 Guaranguito were selected, surface-disinfected and rinsed as above. The number of seeds per genotype, management, type

of substrate, and environmental conditions were as described as for inoculation methods. Sowing was repeated every 2 weeks to obtain the five phenological stages at the same time after 10 weeks. Pots with seedlings and plants were randomly distributed in the greenhouse before inoculation. In each experiment, three pots were used per genotype, each containing three plants at the five phenological stages. Different stages of genotypes I-450 Andino and I-451 Guaranguito were inoculated on the same day, and at the same point, by spraying with each of the *C. acutatum* isolates Lup1, Lup14, and Lup18. A non-inoculated treatment was included as a control. The experiment was performed twice. Experiments were conducted and managed as described above.

Disease evaluation and data analyses

Anthracnose was scored on individual plants by using a 0–5 scale: 0, no symptoms; 1, small injuries <1 mm at the inoculation point or scars at the meristem region where the pathogen was sprayed, sporulation absent; 2, central apical stem bent due to infection, small lesions <5 mm diameter, little sporulation; 3, lesions >5 mm at the inoculation point or at the meristem region, sporulation evident; 4, large lesion girdling more than half the circumference of the stem with abundant sporulation; 5, large lesion severing the stem (adapted from Thomas *et al*. 2008). In the present study, we considered small scars with sporulation absent in level 1 as a resistant reaction, and the bending of the main stem with sporulation present in level 2 as a susceptible response. Data were collected 26 days after inoculation.

Data were subjected to analysis of variance (ANOVA) for inoculation methods and repeated-measures ANOVA for the phenological stages, and Fisher's least significant (l.s.d.) difference test was performed when the ANOVA showed significance, using the statistical program InfoStat for Windows (National University of Córdoba, Spain).

Results

Inoculation methods

There were no significant differences between the two inoculation methods. For genotype I-450 Andino, the resistant reaction was low, at 9% with spraying and 18% with micropipetting. Genotype I-451 Guaranguito displayed 23% and 16% resistant reaction with spraying and micropipetting, respectively (Table 1). On the basis of these results, spraying was used in the next experiment.

Influence of plant stage on resistance

In order to study whether the developmental stages affect the level of anthracnose resistance, isolates Lup1 and Lup14 were sprayed on plants from the two genotypes at five phenological stages. Most I-451 Guaranguito plants inoculated with isolate Lup1 were susceptible at all developmental stages. Two of 18 plants were resistant at the 2–3-leaf stage, increasing to three at the 4–5-leaf stage and five at the 6–7-leaf stage plants. However, the number of resistant plants decreased to two again at the 8–9-leaf and 10–11-leaf stages. I-450 Andino displayed the same trend. One plant was resistant at the 2–3-leaf stage, increasing to three at the 4–5-leaf and 6–7-leaf stages, and decreasing to two and nil of 18 plants at the 8–9-leaf and the 10–11-leaf stages (Fig. 1*a*). I-451 Guaranguito showed a similar trend when inoculated with *C. acutatum* isolate Lup14. There were two resistant plants at the 2–3-leaf stage, increasing to four at the 4–5-leaf stage and seven at the 6–7-leaf stage. However, the number of resistant main stems decreased at the 8–9-leaf and 10–11-leaf stages (three resistant plants of 18 plants at both stages). I-450 Andino displayed a similar tendency. One plant was resistant at the 2–3-leaf stage, increasing to three at the 4–5-leaf and five at the 6–7-leaf stages, decreasing to three at the 8–9-leaf stage and one of 18 plants at the 10–11-leaf stage (Fig. 1*b*). In general, more plants showed non-sporulating lesions at the 6–7-leaf stage than at other developmental stages. Anthracnose symptoms were particularly noticeable during flowering. Although I-450 Andino and I-451Guaranguito were susceptible genotypes, the 6–7-leaf stage showed several resistant main stems for both genotypes.

Additional screening tests were conducted to investigate whether phenological stages are associated with anthracnose symptom development in Andean lupin. I-450 Andino and I-451 Guaranguito were inoculated with the three *C. acutatum* isolates Lup1, Lup14, and Lup18. In general, disease severity was greater at the younger stage and at 10–11-leaf stage than at the 4–5-leaf, 6–7-leaf and 8–9-leaf stages. Disease severity was significantly ($P = 0.05$) higher on young plantlets at the 2–3-leaf stage and on plants that had begun flowering (10–11-leaf stage) than at the other phenological stages. The three isolates were least pathogenic at the 6–7-leaf stage (Table 2). This may suggest that resistant genes are expressed differentially at different plant ages, affecting pathogenicity of *C. acutatum*.

Table 1. Percentage of anthracnose-resistant plants of two Andean lupin genotypes

No significant differences by least standard difference at $P = 0.05$

Genotypes	Inoculation methods	
	Spraying	Micropipetting into a wound
I-450 Andino	9	8
I-451 Guaranguito	23	16

Discussion

Anthracnose resistance of lupin is not equally expressed at all developmental stages. Genotypes were susceptible in early stages (2–3-leaf and 4–5-leaf stages) and resistant at the 6–7-leaf stage, followed by a susceptible reaction at the vegetative and beginning of flowering stages (8–9-leaf and 10–11-leaf stages, respectively). Studies by Pastor-Corrales *et al*. (1995) separated CIAT's bean (*Phaseolus vulgaris* L.) collection into four different groups: cultivars for which seedlings are highly anthracnose-susceptible but adult plants are resistant; cultivars resistant at all stages of growth; cultivars moderately resistant; and cultivars moderately susceptible at all stages of growth. In our study, differences in disease severity were observed for the two lupin genotypes inoculated with three isolates at the five phenological stages (Table 2), consistent with the findings of Bigirimana and Hofte (2001) that certain bean cultivars (e.g. Prelude) were resistant to anthracnose after the primary leaf stage but susceptible in young, 8-day-old seedlings. In all our experiments conducted under the same environmental conditions, 6–7-leaf plants (6 weeks old) exhibited lower disease severity, supporting

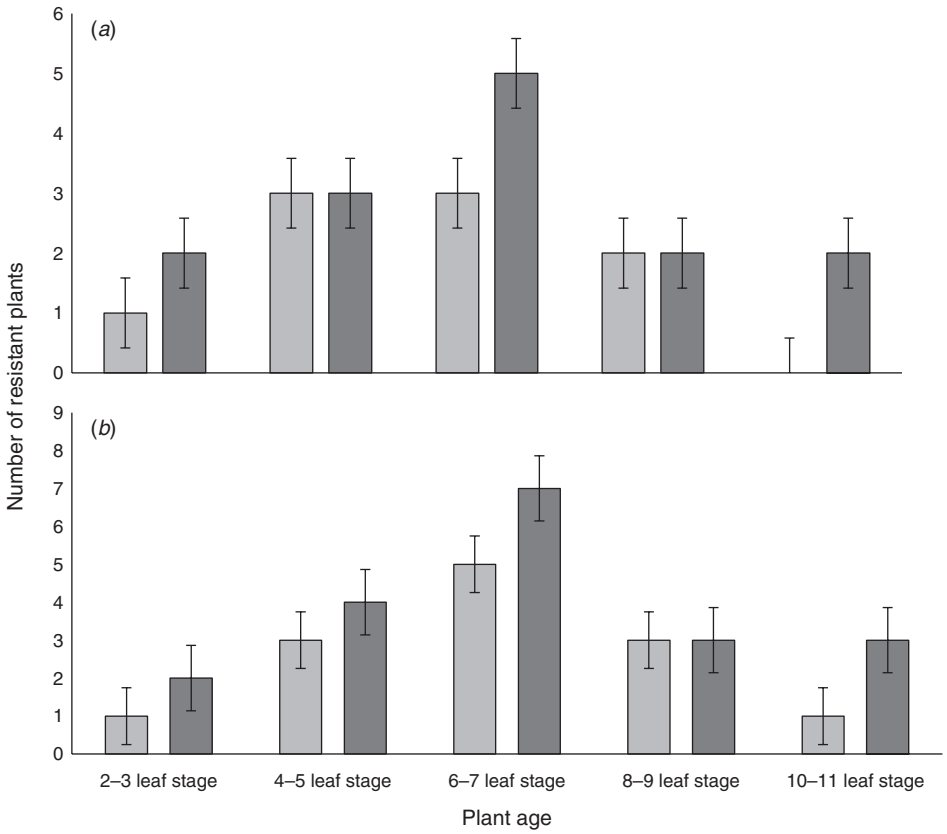

Fig. 1. Effect of developmental stages on resistance to anthracnose in two Andean lupin genotypes, I-450 Andino (light grey bars), I-451 Guaranguito (dark bars). Bars represent the average l.s.d. ($P = 0.05$) at each stage: (*a*) *C. acutatum* isolate Lup1, (*b*) *C. acutatum* isolate Lup14; $n = 18$.

Table 2. Anthracnose disease severity at five phenological stages of Andean lupin following inoculation by three isolates of *Colletotrichum acutatum*

Within columns, means followed by the same letter are not significantly different by least standard difference at $P = 0.05$

Plant stage	Lup1	Lup14	Lup18	Av. anthracnose
2–3-leaf	3.21c	2.33bc	2.33b	2.62
4–5-leaf	2.13a	1.80ab	1.76ab	1.89
6–7 leaf	1.09a	1.07a	1.24a	1.13
8–9-leaf	1.12a	1.12a	1.89ab	1.37
10–11-leaf	3.60c	2.85c	3.71c	3.38
Mean anthracnose	2.23	1.83	2.18	2.07

the hypothesis that inoculated 6-week-old plants are less susceptible to *C. acutatum* infection than plants at other phenological stages.

This plant response reflects the current situation of susceptible genotypes of Andean lupin in Ecuador (Peralta *et al*. 2004) and resistant and susceptible cultivars of *L. angustifolius* in Australia (Thomas and Sweetingham 2004; Thomas *et al*. 2008), where epidemics of lupin anthracnose progressed more slowly in plots of intermediate-aged plants, dramatically increasing during flowering. This might be caused by stage-specific resistance genes. Some of these genes might not be expressed in late developmental stages. Our results regarding changes in disease susceptibility during the life cycle of the plant contribute to a viable management option for Andean lupin production in areas of high anthracnose risk in Ecuador and to efficient management of lupin anthracnose around the world. Early growth, vegetative growth, and the beginning of flowering are the stages when lupin is most susceptible to anthracnose, and during these times, the plants need protection through the application of chemical fungicides.

Each plant pathosystem requires the development of its own methodologies for assessing disease symptoms in order to identify resistant cultivars. Most studies on anthracnose resistance either in young seedlings (Thomas *et al*. 2008) or at the time of podset (Adhikari *et al*. 2008) assess the disease severity on the lupin main stem. Because the apical main stem is the most susceptible organ (Talhinhas 2002; Thomas 2003), the observed differences in this study are mainly due to the response of the lupin apical stem organs in which inoculation took place. Similar disease symptoms in the two Andean lupin genotypes were observed by using the two inoculation methods tested (Table 1). The most common means by which *Colletotrichum* species penetrate plant surfaces is directly through plant cuticles or via natural openings (Bailey *et al*. 1992). Spraying mimics the natural situation where spores are splashed by rain and wind from infected lupin seedlings or stubble (Thomas 2003) and the spores penetrate directly or through hydathodes and stomata. The infection process in *Colletotrichum* is characterised by a short

biotrophic phase. At this point, cells of the two organisms are in close contact, followed by a necrotrophic phase (Esquerré-Tugayé *et al*. 1992). However, for some diseases, infection through wounds is essential (Agrios 2005); thus, the inoculum was pipetted in to an artificial wound. When using the artificial wounding method, the biotrophic phase of the pathogen does not exist. Vargas *et al*. (2012) hypothesised that the switch from biotrophic to necrotrophic lifestyle of *C. graminicola* in maize enables the fungus to evade the response of the plant immune system and allows full fungal pathogenicity. In our study, spraying and pipetting the inoculum produced similar disease symptoms, suggesting that wounding is not essential for infection by *C. acutatum*. Therefore, we consider that inoculation by spraying may be more appropriate to investigate lupin anthracnose because it better reflects natural infection. Andean lupin breeding has benefited from the extensive collection of genetic material in areas of traditional cultivation. Most breeding efforts have focused on earliness, productivity, and adaptation to different environmental zones; however, developing anthracnose-resistant Andean lupin varieties is also important. Our data provide basic information to assist a breeding program to develop anthracnose-resistant genotypes in the Andean lupin collection. In our opinion, the most reliable protocol is by spraying a mixture of *C. acutatum* isolate as a preliminary selection of the large population. We recommend scoring at the most resistant stage (6–7-leaf stage, 6 weeks old) and confirm at the most susceptible stage, 10–11-leaf stage (10 weeks old) to identify material with improved response. Ten-week-old plants that are still alive may be considered tolerant. Selection of plants based on the evaluation of disease resistance at only one stage could result in the loss of progeny that, at other stages, could possess favourable resistant genes. Resistant plants must be selfed to promote homozygosity and anthracnose assessed on the offspring. Because of the high level of outcrossing of Andean lupin, no homozygous cultivars exist in Ecuador, but efforts should be made to create homozygous resistant cultivars. If homozygous material and good levels of resistance were available, it would be feasible to do quantitative trait locus analysis and create mapping populations.

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References

Adhikari K, Thomas G, Buirchell B, Sweetingham M (2008) Identification of anthracnose resistance in yellow lupins and its incorporation into breeding lines. In 'Lupins for health and wealth. Proceedings 12th International Lupin Conference'. Fremantle, W. Aust. (Eds JA Palta, JB Berger) pp. 251–254. (International Lupin Association: Canterbury, New Zealand)

Agrios GN (2005) 'Plant pathology.' 5th edn (Elsevier Academic Press)

Bailey JA, O'Connell RJ, Pring RJ, Nash C (1992) Infection strategies of *Colletotrichum* species. In '*Colletotrichum*: Biology, pathology and control'. (Eds JA Bailey, MJ Jeger) pp. 88–119. (CAB International: Wallingford, UK)

Bigirimana J, Hofte M (2001) Bean anthracnose: Inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. *Journal of Phytopathology* **149**, 403–408. doi:10.1046/j.1439-0434.2001.00645.x

Elmer WH, Yang HA, Sweetingham MW (2001) Characterization of *Colletotrichum gloeosporoides* isolates from ornamental lupines in Connecticut. *Plant Disease* **85**, 216–219. doi:10.1094/PDIS.2001.85.2.216

Esquerré-Tugayé MT, Mazau D, Barthe JP, Touzé A (1992) Mechanisms of resistance to *Colletotrichum* species. In '*Colletotrichum*: Biology, pathology and control'. (Eds JA Bailey, MJ Jeger) pp. 121–133. (CAB International: Wallingford, UK)

Falconi CE (2012) *Lupinus mutabilis* in Ecuador with special emphasis on anthracnose resistance. PhD Thesis, Plant Breeding Laboratory, Wageningen University, Wageningen, The Netherlands.

Falconi CE, Visser RGF, van Heusden AW (2013) Phenotypic, molecular, and pathological characterization of *Colletotrichum acutatum* associated with lupine and tamarillo in the Ecuadorian Andes. *Plant Disease* **97**, 819–827. doi:10.1094/PDIS-02-12-0175-RE

Gieco JO, Dubcovsky J, Aranha LE (2004) Interaction between resistance to *Septoria tritici* and phenological stages in wheat. *Scientia Agricola* **61**, 422–426. doi:10.1590/S0103-90162004000400011

INIAP-450 ANDINO (1999) Variedad de chocho (*Lupinus mutabilis*). FUNDACYT, PIB-206 Project. Brochure. INIAP, Quito, Ecuador.

Jacobsen SE, Sherwood S (2002) 'Cultivo de granos andinos en Ecuador: Informe sobre los rubros quinua, chocho y amaranto.' (FAO, CIP, CRS: Quito, Ecuador)

Kettel K, Tuck B, Payne WA, Chen C, Machado S, Karow R (2003) Narrow-leaf lupin. Dryland Cropping Systems, Oregon State University Extension Service, Corvallis, OR, USA.

Murillo A, Rivera M, Peralta E, Mazon N, Vargas F (2006) Avances preliminares en el mejoramiento genético del choco (*Lupinus mutabilis* Sweet) para resistencia a antracnosis. In 'Memorias del XII Congreso Internacional de cultivos andinos'. 23–27 Julio. p.26. (INIAP: Quito, Ecuador)

Pastor-Corrales MA, Otoya MM, Molina A (1995) Resistance to *Colletotrichum lindemuthianum* isolates in different common bean races. *Plant Disease* **79**, 63–67. doi:10.1094/PD-79-0063

Peralta E, Murillo A, Mazon N, Rivera M, Estrella P, Falconi E, Vargas F (2004) Informe técnico sobre el cultivo del chocho. INIAP, Ecuador.

Peralta E, Mazon N, Murillo A, Rivera M, Rodriguez D, Lomas L, Monar C (2012) 'Manual agrícola de granos Andinos, chocho, quinua, amaranto y ataco.' Miscellaneous Publication No. 69. (INIAP, PRONALEG-GA: Quito, Ecuador)

Rivera M, Pinzón J, Caicedo C, Murillo A, Mazón N, Peralta E (1998) Catálogo del Banco de Germoplasma de chocho (*Lupinus mutabilis* Sweet) y otras especies de lupinos. Estación Experimental Santa Catalina, Programa Nacional de Leguminosas, Departamento Nacional de Recursos Filogenéticos, Quito, Ecuador.

Stewart HE (1990) Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaflets. *Mycological Research* **94**, 823–826. doi:10.1016/S0953-7562(09)81385-3

Talhinhas P (2002) Lupinus genus germplasm characterisation, anthracnose resistance evaluation and study of diversity and taxonomy of the causal agent (*Colletotrichum acutatum* Simmonds ex Simmonds). PhD Thesis in Agronomy, Instituto Superior de Agronomia (UTL), Lisboa, Portugal.

Talhinhas P, Sreenivasaprasad S, Neves-Martins J, Oliveira H (2002) Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology* **92**, 986–996. doi:10.1094/PHYTO.2002.92.9.986

Talhinhas P, Neves-Martins J, Leitao J (2003) AFLP, ISSR, and RAPD markers reveal high level of genetic diversity among *Lupinus* spp. *Plant Breeding* **122**, 507–510. doi:10.1111/j.1439-0523.2003.00892.x

Thomas GJ (2003) Lupin anthracnose—identification and management. Department of Agriculture and Food, Western Australia, Farmnote No. 15. Available at: www.agric.wa.gov.au (accessed 24 January 2014).

Thomas GJ, Sweetingham MW (2004) Cultivar and environment influence the development of lupin anthracnose caused by *Colletotrichum lupini*. *Australasian Plant Pathology* **33**, 571–577. doi:10.1071/AP04060

Thomas GJ, Sweetingham MW, Yang HA, Speijers J (2008) Effect of temperature on growth of *Colletotrichum lupini* and on anthracnose infection and resistance in lupins. *Australasian Plant Pathology* **37**, 35–39. doi:10.1071/AP07075

Vargas WA, Sanz Martín JM, Rech GE, Rivera LP, Benito EP, Díaz-Mínguez JM, Thon MR, Sukno SA (2012) Plant defense mechanisms are activated during biotrophic and necrotrophic development of *Colletotrichum graminicola* in maize. *Plant Physiology* **158**, 1342–1358. doi:10.1104/pp.111.190397

Wastie RL (1991) Breeding for resistance. In 'Advances in plant pathology, *Phytophthora infestans*: the cause of late blight of potato'. (Eds DS Ingram, PH Williams) pp. 193–224. (Academic Press: London)