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Genetic diversity of *Colletotrichum lupini* and its virulence on white and Andean lupin

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Lupin cultivation worldwide is threatened by anthracnose, a destructive disease caused by the seed- and air-borne fungal pathogen *Colletotrichum lupini*. In this study we explored the intraspecific diversity of 39 *C. lupini* isolates collected from different lupin cultivating regions around the world, and representative isolates were screened for their pathogenicity and virulence on white and Andean lupin. Multi-locus phylogeny and morphological characterizations showed intraspecific diversity to be greater than previously shown, distinguishing a total of six genetic groups and ten distinct morphotypes. Highest diversity was found across South America, indicating it as the center of origin of *C. lupini*. The isolates that correspond to the current pandemic belong to a genetic and morphological uniform group, were globally widespread, and showed high virulence on tested white and Andean lupin accessions. Isolates belonging to the other five genetic groups were mostly found locally and showed distinct virulence patterns. Two highly virulent strains were shown to overcome resistance of advanced white lupin breeding material. This stresses the need to be careful with international seed transports in order to prevent spread of currently confined but potentially highly virulent strains. This study improves our understanding of the diversity, phylogeography and pathogenicity of a member of one of the world's top 10 plant pathogen genera, providing valuable information for breeding programs and future disease management.

The fungal genus *Colletotrichum* contains many important plant pathogenic species that cause anthracnose and other pre- and post-harvest diseases in a wide variety of hosts^{1–4}. Among potential hosts are important fruit, cereal and legume crops such as strawberry⁵, maize⁶ and soybean^{7,8}. Besides being of economic importance, *Colletotrichum* spp. have been widely used as model species to study plant–fungus interactions because of the diversity of lifestyles within this genus^{9–12}. *Colletotrichum* is listed in the top 10 of most important fungal plant pathogens worldwide¹³. Within the genus, members of the *Colletotrichum acutatum* species complex are notorious and cause disease in many important crops^{14,15}. The most important morphological characteristic for members of this species complex are the acute ends of its conidia¹⁴. Discrimination of *Colletotrichum* species solely based on morphological traits, however, is deemed unreliable due to the few and highly variable characteristics, the strong influence of environmental conditions and the high overlap between species¹⁶. Therefore, a polyphasic approach, combining morphological and genetic data is recommended^{17,18}. Multi-locus phylogeny revealed a high diversity within the *C. acutatum* species complex, showing at least 32 different species divided among five clades¹⁴. Although many species within the *C. acutatum* species complex have a broad host range, *Colletotrichum lupini*, belonging to clade 1, appears to be highly host specific on lupins (*Lupinus*)^{19,20}.

Lupin anthracnose caused by *C. lupini* is the most important disease in lupin cultivation worldwide, affecting all economically important lupin species such as blue (*Lupinus angustifolius* L.), white (*L. albus* L.), Andean (*L. mutabilis* Sweet.), yellow (*L. luteus* L.) and ornamental lupin (*L. polyphyllus* Lindl.)²⁰. The disease was first reported in 1912 in Brazil²¹, but the fungal pathogen was identified much later²². A first outbreak was reported in the 1940–1950s in North America and was followed by a more severe and globally widespread outbreak around the 1980s which is still persisting until this day²⁰. The disease is mainly dispersed via seeds, facilitating rapid spread through international seed transports, and within the crop by rain splash during the growing season²³. Even low amounts of initial inoculum can cause total yield losses making this disease highly destructive^{24,25}. Typical symptoms are stem twisting and necrotic lesions on stems and pods (Fig. 1)²⁶. Current disease management is focused on planting certified disease-free seed and chemical protection^{23,27}. However, crop resistance could offer a more sustainable alternative. In blue lupin, anthracnose resistance is controlled by single resistance

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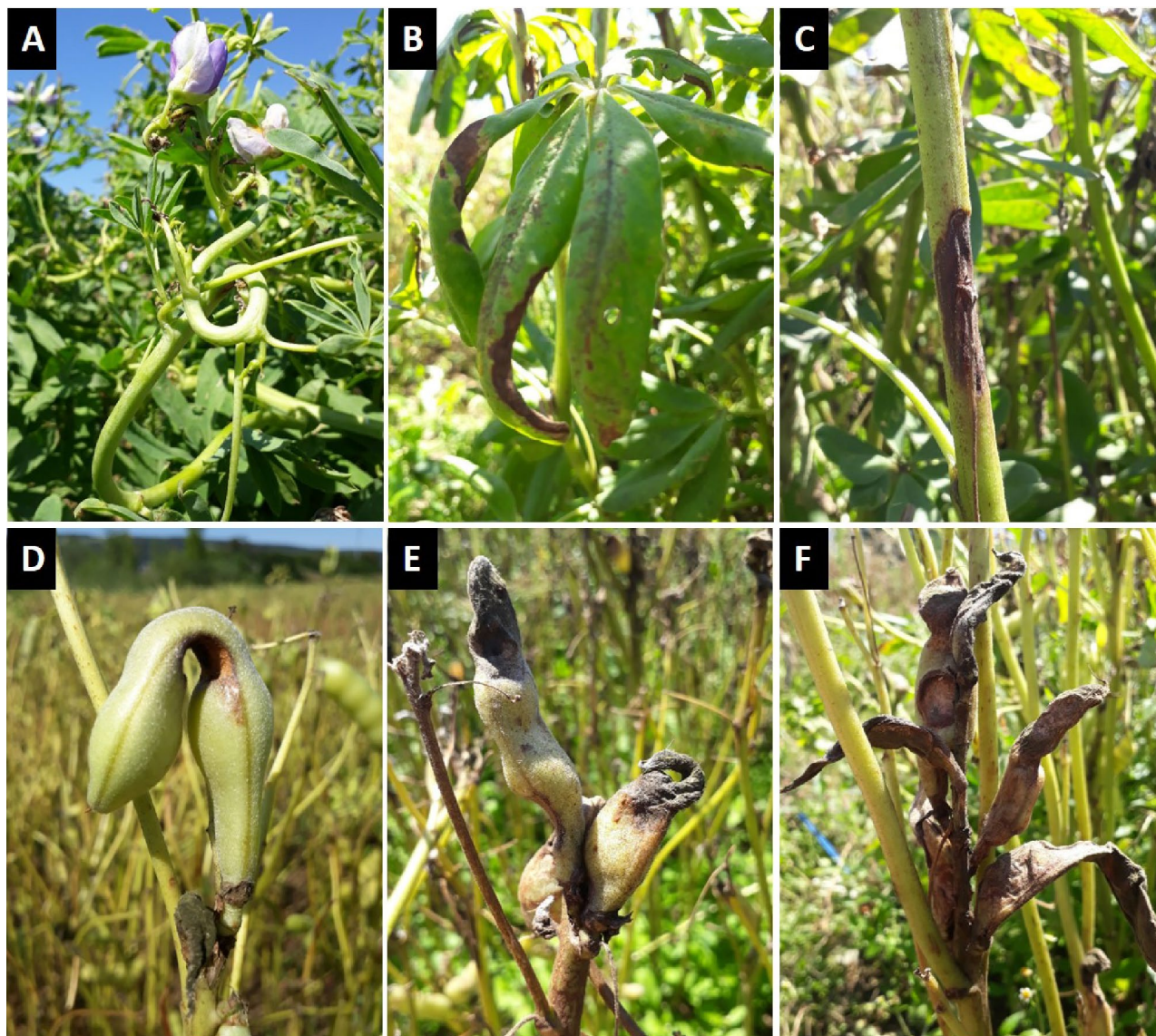


Figure 1. Symptoms on lupin tissue associated with *Colletotrichum lupini*. (A) typical stem twisting (*Lupinus mutabilis*); (B) on the leaves (*L. albus*); (C) on the main stem (*L. albus*); (D–F) on the pods (*L. albus*). Photos by Alkemade JA.

genes^{28–30}, whereas in white, Andean and yellow lupin no such single gene resistance is known and the observed quantitative resistance is considered to be polygenic^{31–33}. The increasing demand for plant-based protein is renewing the interest for lupins as a high quality protein crop^{34–36}, the current anthracnose pandemic, however, severely hampers cultivation.

The pathogen was first described as *Gloesporium lupini*, followed by *C. gloeosporioides* and *C. acutatum* until it was fully described as *C. lupini*^{14,37,38}. Currently two genetic groups (I and II) are distinguished within *C. lupini* based on vegetative compatibility groups (VCG)³⁸, the ITS (internal transcribed spacer) region³⁷ and multi-locus phylogeny of the ITS, GAPDH (glyceraldehyde-3-phosphate dehydrogenase), CHS-1 (chitin synthase), HIS3 (histone), ACT (actin), TUB2 (β -tubulin 2), HMG (HMG box region) and APN/MAT1 (Apn2-Mat1-2-1 intergenic) loci³⁹. The TUB2 and GAPDH loci were shown to be the most informative within the *C. acutatum* species complex and APN/MAT1 the most informative within *C. lupini*, whereas classification based on the ITS region can be problematic due to low resolution within the complex^{14,39}. Although only two groups within *C. lupini* have been distinguished, with most of the reported strains belonging to group II³⁹, intraspecific diversity is thought to be greater as a high diversity was found in a Chilean *C. lupini* collection using random amplified polymorphic DNA (RAPD) markers⁴⁰ and a distinct lupin infecting *C. acutatum* group was identified in Ecuador based on the ITS region⁴¹. This suggests that highest intraspecific diversity is found in South America, which is believed to be the center of origin of members belonging to clade I of the *C. acutatum* species complex^{10,15}.

The overall aim of this study was to assess a worldwide collection of lupin-infecting *Colletotrichum* isolates through (i) multi-locus phylogeny, (ii) morphology and (iii) virulence on white and Andean lupin. Insights into

C. lupini diversity, phylogeography and plant-*C. lupini* interactions will improve our understanding of the current lupin anthracnose pandemic and support future disease management strategies and lupin breeding programs.

Results

***Colletotrichum lupini* comprises of six genetic groups supported by morphology.** From the 50 sequenced isolates, 39 belonged to *C. lupini* (Table 1). A globally representative subset of 28 *C. lupini* isolates was characterized based colony morphology (form, aerial mycelium, margin type and color of the reverse side) and 18 of those were further characterized for growth rate and conidial shape and size, revealing ten distinct morphotypes (A–J; Fig. 2, Table 2, Supplementary Figs. S1, S2). Despite certain variability, all observed conidia shared features typical for *C. lupini* (hyaline, smooth-walled, aseptate, straight and with one acute end) as described by Damm et al.¹⁴. Morphotype A was the most common and was observed for isolates from across the world (Europe, Australia, North- and South America), all belonging to genetic group II. Morphotypes B, C and G were observed for isolates from South Africa and morphotypes D, E, G, I and J were observed for isolates from South America.

Multi-locus phylogenetic analyses of 50 *Colletotrichum* isolates identified six distinct genetic groups within *C. lupini* (I–VI; Fig. 3, Supplementary Fig. S3). The combined sequence dataset contained 2251 characters (ITS: 1–496, GAPDH: 497–745, TUB2: 746–1200, APN/MAT1: 1201–2251) including alignment gaps. The APN/MAT1 locus showed the highest variability across the nucleotide data set, with 75.8% conserved sites for the whole data set (including out-groups) and 97.4% within *C. lupini* (Supplementary Table S1). The TUB2 and GAPDH loci showed 89.9% and 81.1% identical sites for the entire dataset and 97.8% and 98.4% identity within *C. lupini*, respectively. The ITS region showed the lowest variability with 97% identical sites across the whole dataset and 99.2% within *C. lupini*. As shown in Fig. 3, most *C. lupini* strains clustered with a high bootstrap support (BS) value of 79 and posterior probability (PP) of 1 with reference strains representing genetic group II (CBS 109221, IMI 375715 and RB221). Strains within group II showed a high identity among each other (>99.9%) and showed morphotype A, except for Chilean strain JA15 showing morphotype D (Fig. 2). South African strain JA10 and Peruvian strain JA20, with morphotypes G and F, respectively, clustered together with a BS of 84 and PP of 1, forming a highly supported group (III). South African strains JA11 and JA12, with morphotypes C and B, respectively, clustered together with a BS of 98 and PP of 1, forming a highly supported group (IV). Ecuadorian strains JA18 and JA19 with distinct morphotypes I and J, respectively, showed 99.7% identity with reference strains of group II and clustered together with a BS of 60 (Fig. 3, Supplementary Fig. S3) and a PP of 1 in (Fig. 3), forming a distinct group (V). The reference strains for group I (CBS 109225 with morphotype H, CBS 109226 and CBS 509.97) are clustered together with a BS of 99 and PP of 1 and show 100% identity with each other and 99.6% identity with reference strains of group II. South American strains JA21, JA22 and CBS 109216, with morphotype E, cluster together with a BS of 98 and PP of 1 (Fig. 3) and a BS of 54 (Supplementary Fig. S3) forming a highly supported group (VI). JA21 and JA22 showed 99.8% and CBS 109216 showed 99.7% identity with reference strains of group I and 99.4% and 99.2% identity with reference strains of group II, respectively.

Distinct virulence patterns on white and Andean lupin. Virulence assays performed on two white lupin (*L. albus* L.) accessions (Feodora and Blu-25) and two Andean lupin (*L. mutabilis* Sweet.) accessions (LUP 17 and LUP 100) with strains representing the different morphotypes and genetic groups indicated in Fig. 3, revealed strong strain ($p < 0.0001$), lupin species ($p < 0.0001$) and strain \times lupin species interaction effects ($p < 0.0001$). A strong accession effect was found within white lupin ($p < 0.0001$), whereas for Andean lupin there was no significant accession effect ($p = 0.43$). Strain ($p < 0.0001$) and strain \times accession ($p < 0.0001$) interaction effects were found for both species. Strains belonging to genetic group II with morphotype A, caused severe disease on white lupin accession Feodora and both Andean lupin accessions (Supplementary Fig. S4), showing standardized area under the disease progress curve (sAUDPC) means ranging from 3.95 to 5 (Fig. 4). On the more tolerant white lupin accession Blu-25, sAUDPC means for strains of group II with morphology A were more variable, with JA01 and IMI 375715 showing moderate (2.7–2.9) and Chilean strains JA16 and 17 showing high (3.8–4.1) virulence. Chilean strain JA15, also belonging to genetic group II but with a different morphology (D), caused low disease on LUP 100 and Blu-25 (1.9), showing a different virulence spectrum compared to the other tested strains of genetic group II. South African strains JA11 and JA12, belonging to genetic group IV with morphotypes C and B, respectively, showed a similar virulence spectrum on white lupin as strains of group II. JA10 and JA20, representing group III and morphotype G and F, respectively, were overall avirulent (<2), with the exception of JA10 on Feodora, showing moderate virulence (2.95). Peruvian strain JA21, representing genetic group VI and morphotype E, caused low disease on white lupin (1.4–1.8), but severe disease on Andean lupin (4.25–5). A similar observation was found for the two Ecuadorian strains JA18 and JA19 of genetic group V and morphotypes I and J, respectively. These two strains caused low disease on white lupin and high disease on Andean lupin LUP 100. On Andean lupin LUP 17, however, a severe disease phenotype was only found for JA18 (3.6), whereas JA19 barely caused any disease symptoms (1.25). Similar to the observations for JA19, the Ukrainian strain CBS 109225 (genetic group I, morphotype H) caused severe disease on Andean lupin LUP 100 (3.36) and low disease on Andean lupin LUP 17 and white lupin (1.2–2). The *C. tamarilloi* and *C. acutatum* strains were avirulent across the lupin accessions (<1.26).

Discussion

This study compared 39 *C. lupini* and 11 *Colletotrichum* spp. isolates collected from across the world to explore intraspecific diversity of *C. lupini* and to better understand the dynamics of the current lupin anthracnose pandemic and potential implications of further migrations of distinct pathogenic strains. Based on multi-locus phylogeny supported by isolate morphology, we identified four distinct genetic groups additional to previously

Strain ^a	Alternative code(s)	Species	Host	Origin	Year	GenBank no. ^b			
						ITS	GAPDH	TUB2	APN/MAT1
JA01		<i>Colletotrichum lupini</i>	<i>Lupinus albus</i>	Switzerland, Melikon	2018	MT741840	MW342515	MW342537	MW342559
JA02		<i>C. lupini</i>	<i>L. albus</i>	Switzerland, Feldbach	2019	MW342494	MW342516	MW342538	MW342560
JA03		<i>C. lupini</i>	<i>L. albus</i>	Germany, Hattenhofen	2019	MW342495	MW342517	MW342539	MW342561
JA04		<i>C. lupini</i>	<i>L. albus</i>	Germany, Witzenhausen	2018	MW342496	MW342518	MW342540	MW342562
JA05		<i>C. lupini</i>	<i>L. albus</i>	Germany, Westerau	2018	MW342497	MW342519	MW342541	MW342563
JA06		<i>C. lupini</i>	<i>L. albus</i>	Russia, Saint Petersburg	2018	MW342498	MW342520	MW342542	MW342564
JA07	BRIP 63850, WAC 12994	<i>C. lupini</i>	<i>L. angustifolius</i>	Australia, WA, Dongara	2004	MW342499	MW342521	MW342543	MW342565
JA08	BRIP 63851, WAC 12995	<i>C. lupini</i>	<i>L. luteus</i>	Australia, WA, Mingenew	2004	MW342500	MW342522	MW342544	MW342566
JA09	BRIP 63857, WAC 13001	<i>C. lupini</i>	<i>L. albus</i>	Australia, WA, Yandooka	2004	MW342501	MW342523	MW342545	MW342567
JA10	CMW 9930, SHK 788	<i>C. lupini</i>	<i>L. albus</i>	South Africa, Bethlehem	1994	MW342502	MW342524	MW342546	MW342568
JA11	CMW 9931, SHK 1033	<i>C. lupini</i>	<i>L. albus</i>	South Africa, Stellenbosch	1995	MW342503	MW342525	MW342547	MW342569
JA12	CMW 9933, SHK 2148	<i>C. lupini</i>	<i>L. albus</i>	South Africa, Malmesbury	1999	MW342504	MW342526	MW342548	MW342570
JA13		<i>C. lupini</i>	<i>L. mutabilis</i>	USA, Florida, Martin County	2013	MW342505	MW342527	MW342549	MW342571
JA14		<i>C. lupini</i>	<i>L. hartwegii</i>	USA, Florida, Martin County	2013	MW342506	MW342528	MW342550	MW342572
JA15	A-02	<i>C. lupini</i>	<i>L. albus</i>	Chile, Cajón	2009	MW342507	MW342529	MW342551	MW342573
JA16	A-10	<i>C. lupini</i>	<i>L. angustifolius</i>	Chile, Cajón	2009	MW342508	MW342530	MW342552	MW342574
JA17	A-24	<i>C. lupini</i>	<i>L. albus</i>	Chile, Temuco	2015	MW342509	MW342531	MW342553	MW342575
JA18	Lup1	<i>C. lupini</i>	<i>L. mutabilis</i>	Ecuador, Juan Montalvo	2007	MW342510	MW342532	MW342554	MW342576
JA19	Lup18	<i>C. lupini</i>	<i>L. mutabilis</i>	Ecuador, Pujili	2007	MW342511	MW342533	MW342555	MW342577
JA20		<i>C. lupini</i>	<i>L. mutabilis</i>	Peru, Carhuaz	2019	MW342512	MW342534	MW342556	MW342578
JA21		<i>C. lupini</i>	<i>L. mutabilis</i>	Peru, Carhuaz	2019	MW342513	MW342535	MW342557	MW342579
JA22		<i>C. lupini</i>	<i>L. mutabilis</i>	Peru, Carhuaz	2019	MW342514	MW342536	MW342558	MW342580
CBS 109216	BBA 63879	<i>C. lupini</i>	<i>L. mutabilis</i>	Bolivia		JQ948156	JQ948486	JQ949807	MW342581
CBS 109221	BBA 70352, RB172	<i>C. lupini</i>	<i>L. albus</i>	Germany		JQ948169	JQ948499	JQ949820	MK478328
CBS 109225	BBA 70884	<i>C. Lupini</i>	<i>L. albus</i>	Ukraine		JQ948155	JQ948485	JQ949806	MK478329
CBS 109226	RB121, IMI 504884, HY09, BBA 71249	<i>C. lupini</i>	<i>L. albus</i>	Canada, Nova Scotia		JQ948158	JQ948488	MK478189	MK478316
CBS 509.97	RB235, LARS 178	<i>C. lupini</i>	<i>L. albus</i>	France		JQ948159	JQ948489	JQ949810	MK478355
IMI 375715	96A4	<i>C. lupini</i>	<i>L. albus</i>	Australia, WA, Perth	1997	JQ948161	JQ948491	JQ949812	MK478341
RB020	PT30	<i>C. lupini</i>	<i>L. albus</i>	Portugal, Azores	1999	MK463722	KM252117	MK478186	MK478308
RB042	CBS 129944, CMG12	<i>C. lupini</i>	<i>Cinnamomum zeylanicum</i>	Portugal, Lisbon	1996	MH865693	JQ948508	JQ949829	MK478310
RB116	CSL 1294	<i>C. lupini</i>	<i>L. polyphyllus</i>	UK, York		MK463723	KM252194	KM251944	MK478313
RB122	BBA 71310, C3	<i>C. lupini</i>	<i>L. luteus</i>	Poland		MK463726	MK463750	MK478190	MK478317
RB123	IMI 504885, SHK788	<i>C. lupini</i>	<i>L. albus</i>	South Africa, Bethlehem	1994	MK463727	MK463751	MK478191	MK478318
RB124	BBA 70555	<i>C. lupini</i>	<i>L. albus</i>	Chile		MK463728	MK463752	MK478192	MK478319
RB125	CBS 109224, BBA 70399	<i>C. lupini</i>	<i>L. albus</i>	Austria		JQ948172	JQ948502	JQ949823	MK478320
RB127	PT702	<i>C. lupini</i>	<i>Olea europaea</i>	Spain		MK463729	MK463753	MK478193	MK478321
RB147	IMI 350308	<i>C. lupini</i>	<i>Lupinus</i> sp.	UK, Kent	1991	MK463730	KM252203	KM251951	MK478322
RB221	IMI 504893	<i>C. lupini</i>	<i>Lupinus</i> sp.	France, Brittany	2016	MK463733	MK463756	MK478196	MK478345
RB226		<i>C. lupini</i>	<i>Lupinus</i> sp.	France, Brittany	2016	MK463738	MK463761	MK478201	MK478350
CBS 129814	T.A6	<i>C. tamarilloi</i>	<i>Solanum betaceum</i>	Colombia, Gundi-namarca	2012	JQ948184	JQ948514	JQ949835	MW342584
CBS 129955	RB018, Tom-12	<i>C. tamarilloi</i>	<i>Solanum betaceum</i>	Colombia, Antioquia, Santa Rosa	1998	JQ948189	JQ948519	JQ949840	MK478307
CBS 211.78	IMI 309622, RB184	<i>C. costaricensis</i>	<i>Coffea</i> sp.	Costa Rica, Turrialba		JQ948181	JQ948511	JQ949832	MK478333
CBS 134730	RB237	<i>C. melonis</i>	<i>Malus domestica</i>	Brazil, Rio Grande do Brazil		KC204997	KC205031	KC205065	MK478357

Continued

Strain ^a	Alternative code(s)	Species	Host	Origin	Year	GenBank no. ^b			
						ITS	GAPDH	TUB2	APN/MAT1
IMI 304,802	RB216	<i>C. cuscutae</i>	<i>Cuscuta</i> sp.	Dominica		JQ948195	JQ948525	JQ949846	MK478340
IMI 384185	CPC 18937, RB218	<i>C. paranaense</i>	<i>Caryocar brasiliense</i>	Brazil		JQ948191	JQ948521	JQ949842	MK478342
CBS 130239	Frag NL-1	<i>C. nymphaeae</i>	<i>Fragaria x ananassa</i>	The Netherlands	2011	JQ948250	JQ948580	JQ949901	MW342583
IMI 360928	CPC 18926, RB163	<i>C. nymphaeae</i>	<i>Fragaria x ananassa</i>	Switzerland, Zürich	1993	JQ948243	JQ948573	JQ949894	MK478326
CBS 122122	BRIP28519, RB179	<i>C. simmondsii</i>	<i>Carica papaya</i>	Australia	1987	JQ948276	JQ948606	JQ949927	MK478332
CBS 369.73	NRCC 10081	<i>C. acutatum</i>	<i>L. angustifolius</i>	New Zealand, Kumeu	1968	JQ948350	JQ948681	JQ950001	MW342582
CBS 370.73	NRCC 10088, RB187	<i>C. acutatum</i>	<i>Pinus Aridata</i>	New Zealand, Tokoroa	1965	JQ948351	JQ948682	JQ950002	MK478335

Table 1. Isolation details and GenBank accessions of *Colletotrichum* strains used in this study. *JA* strains from the FiBL culture collection characterized in this study, *RB* personal collection of Riccardo Baroncelli described in Dubrulle et al.³⁹, *CBS* collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, *IMI* Culture collection of CABI Europe UK Centre, Egham, UK, *ITS* internal transcribed spacers 1 and 2 together with 5.8S nrDNA, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *TUB2* β -tubulin 2, *APN/MAT1* Apn2-Mat1-2-1 intergenic. Codes in bold were used for morphology analysis in this study. Accession numbers in bold are newly determined in this study.

described genetic groups I and II. Highest intraspecific diversity was identified among *C. lupini* isolates collected from across the South American Andes region. This is in line with reports of Falconí et al.⁴¹ and Riegel et al.⁴⁰ showing high diversity in Ecuador and Chile, respectively. In those regions, Andean lupin has been cultivated for more than 2000 years⁴² growing alongside numerous wild lupin species⁴³. Isolates collected in South Africa showed a distinct morphology and virulence spectrum, indicating higher diversity than previously shown⁴⁴. Although lupins form a significant part of the local agriculture and have been researched there since at least 1897⁴⁵, they are not native to South Africa and lupin anthracnose was not reported in South Africa until 1993⁴⁶. Taking into account the relatively recent reports of anthracnose in South Africa, the low diversity in Europe and Australia and the center of origin for species within clade 1 of the *C. acutatum* species complex being in South America^{10,15}, we consider the South American Andes to be the center of origin of *C. lupini*.

The majority of the *C. lupini* isolates (26 out of 39) belong to the highly virulent genetic group II, showing morphotype A, and were collected in Europe, Australia, South Africa, the USA and Chile. This result confirms previous reports classifying most *C. lupini* strains from across the world in the same genetic group^{14,39,47–49}. The low genetic diversity among strains of group II, the uniform morphology and non-observed sexual morph¹⁴ indicates clonality as suggested by Talhinhas et al.²⁰. Pathogenicity of group II strains has also been shown on blue²⁸, yellow³² and various other lupin species across the world²⁰, indicating a broad host range within the genus *Lupinus*. Reports from South Korea and China indicate that group II strains also cause disease in those regions^{50,51}, highlighting that these strains are globally widespread and are the cause of the current anthracnose pandemic in lupin. The group II strain RB221 can be used as reference, as it is now fully sequenced⁵² and tested on both Andean and white lupin⁵³.

The stem-wound inoculation assay used in this study was previously described to be highly reproducible and strongly correlated to field performance under natural infection pressure²⁶. In the present study, virulence assays based on stem-wounding showed strong strain x accession interaction effects for white and Andean lupin, suggesting a strain-dependent host spectrum and the existence of different physiological races within *C. lupini*. Similar observations were described by Falconí et al.⁴¹, showing a *C. lupini* strain x Andean lupin accession interaction effect. The existence of physiological races has been observed for various *Colletotrichum* species, such as for *C. lindemuthianum* on common bean⁵⁴, *C. sublineola* on sorghum⁵⁵ and *C. truncatum* on lentil⁵⁶, but, in general, this is not common within the genus *Colletotrichum*. The similar virulence levels of isolates belonging to group II observed on Andean and white lupin accessions are in line with Alkemade et al.²⁶, in which equal virulence was observed for IMI 375715 (Australia) and JA01 (Switzerland) when inoculated on six different white lupin accessions. However, an exception within group II is Chilean strain JA15, which, besides having a distinct morphology, was less virulent on Andean lupin LUP 100 and white lupin Blu-25. Further, Chilean strains JA16 and JA17 (also group II) overcame resistance of the resistant advanced breeding line Blu-25, which has been specifically bred for anthracnose resistance in Chile and was shown resistant under Swiss field conditions²⁶. These results indicate that new introductions of highly virulent foreign strains can have severe consequences as seen for many other crops^{57–59} and it should be investigated if this high virulence is also affecting other resistant (white) lupin material^{26,31,60}. Although disease development after stem-wounding of seedlings correlated strongly to field disease scores of mature plants²⁶, we cannot exclude the possibility that conclusions drawn on virulence level might differ for secondary infection processes (e.g. via rain splash).

This study provides first solid evidence that, based on multi-locus phylogeny and morphology, genetic diversity within *C. lupini* is higher than previously shown. High-resolution genome-wide sequencing and an increased sampling density from especially the South American Andes region are now necessary to increase genetic resolution and to better understand *C. lupini* phylogeny and phylogeography. This could provide the basis for in-depth comparative genomic studies to identify effector gene clusters within the *C. lupini* genome. This study confirms that the current lupin anthracnose pandemic is caused by a genetically uniform group of highly virulent strains. The identification of strains with an increased virulence on tolerant white lupin breeding material and the observation of strain-specific virulence patterns should be taken into account in lupin resistance breeding programs.

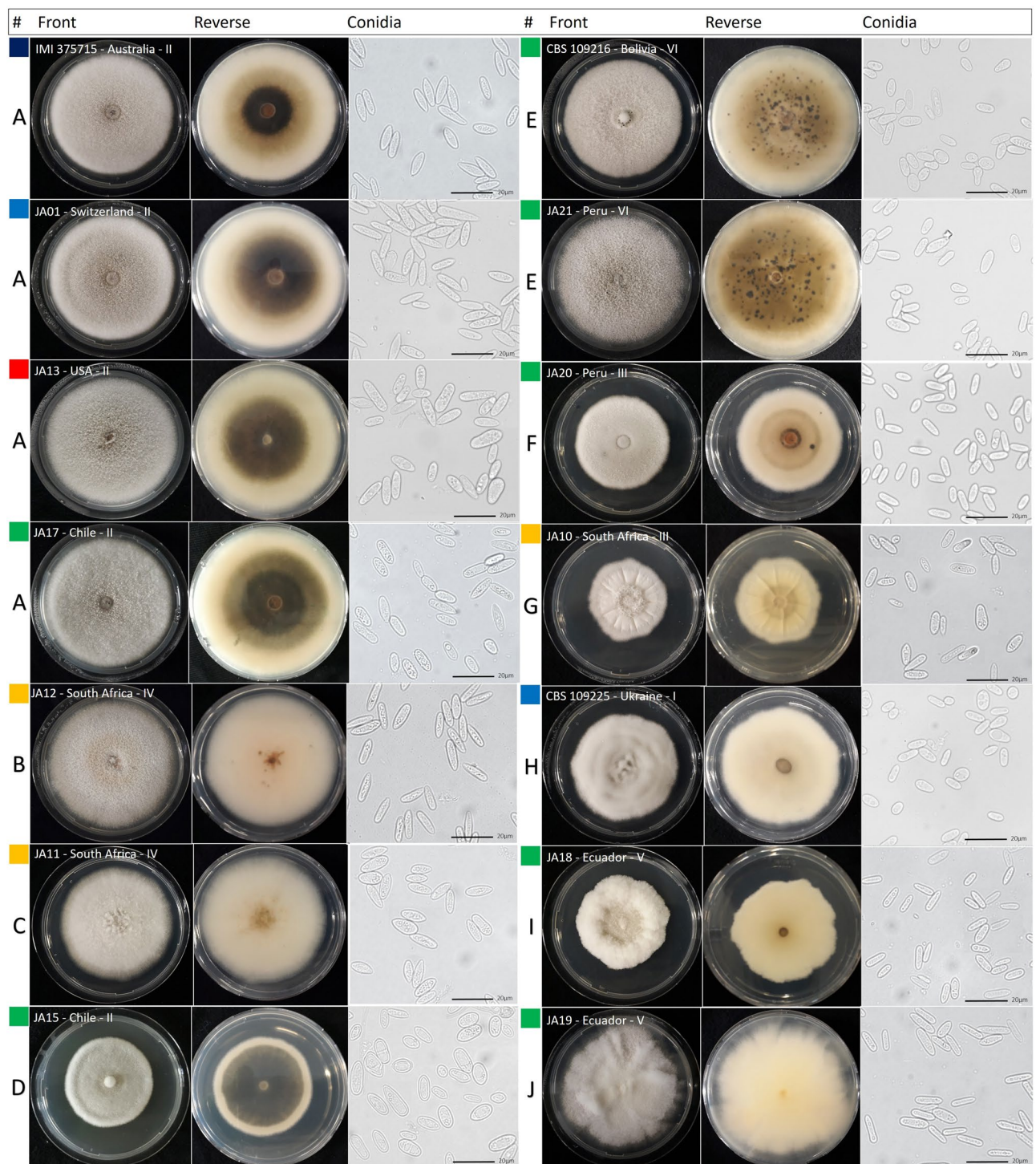


Figure 2. *Colletotrichum lupini* morphology. Capital letters (A–J) indicate the different morphology types based on conidia shape and size and colony growth rate and morphology (see Table 2). Strain codes are followed by country of origin and roman numbers (I–VI) indicate genetic groups. Plates show the front and reverse of 14 day old colonies on PDA. Scale bars indicate 20 μ m. Colors indicate strain origin: blue = Europe, green = South America, red = North America, orange = Southern Africa, dark blue = Australia.

Strain	Morphotype	Genetic group	Growth rate (mm/day) ^a	Conidia L × W (μm) ^a	Conidia shape ^{bc}	Colony morphology ^c
IMI 375715, JA01, -06, -07, -13, -16, -17	A	II	6.2 ± 0.1	12 ± 2.1 × 4 ± 0.7	Cylindrical to elliptical, occasionally clavate	Flat, circular, with entire margins, white-greyish cottony aerial mycelium, pale to orange on reverse, dark in center
JA12	B	IV	5.6 ± 0.1	13.3 ± 1.4 × 3.4 ± 0.5	Cylindrical to elliptical, occasionally clavate	Flat, circular, with entire margins, white-brownish cottony aerial mycelium, pale on reverse
JA11	C	IV	5.5 ± 0.1	12 ± 1.7 × 4.5 ± 0.7	Cylindrical to elliptical, occasionally clavate	Flat, circular, slightly filiform margins, white-greyish cottony aerial mycelium, pale on reverse, orange in center
JA15	D	II	5 ± 0	9.7 ± 2.4 × 4.2 ± 1.0	Cylindrical, occasionally roundish	Flat, circular, with entire margins, white-greyish cottony aerial mycelium, dark on reverse, pale at margins
CBS 109216, JA21, -22	E	VI	5.4 ± 0.3	8.5 ± 2.1 × 3.5 ± 0.7	Cylindrical to clavate	Flat, circular, with entire margins, white-greyish cottony aerial mycelium, pale orange on reverse with black dots
JA20	F	III	4.2 ± 0.3	8.7 ± 1.1 × 3.2 ± 0.6	Cylindrical, occasionally clavate	Flat, circular, with entire margins, sparse white-greyish aerial mycelium, pale on reverse
JA10	G	III	4.9 ± 0.2	9.2 ± 1.7 × 3.6 ± 0.7	Cylindrical to elliptical, occasionally clavate	Irregular and radially sulcate with aerial mycelia growth in the center, pale on reverse
CBS 109225	H	I	5.2 ± 0.1	8.5 ± 1.7 × 3.8 ± 0.8	Cylindrical to clavate	Slightly irregular and thickly covered with wooly white-greyish aerial mycelia, pale on reverse
JA18	I	V	4.1 ± 0	10 ± 1.8 × 2.9 ± 0.7	Cylindrical	Irregular, wooly white areal mycelia on the margins, pale on reverse
JA19	J	V	6 ± 0.2	12.1 ± 1.8 × 2.4 ± 0.7	Cylindrical	Irregular, white-greyish wooly aerial mycelium, pale on reverse with occasional black/orange dots

Table 2. Growth rate, conidial size and shape, and colony morphology for the different morphotypes observed within *Colletotrichum lupini*. L length, W width. ^aMean ± SD, see also Supplementary Fig. S2. ^bObserved conidia were rather variable in shape and size, but all conidia were hyaline, smooth-walled, aseptate, straight, with one end round and one end acute as described for *Colletotrichum lupini* in Damm et al.¹⁴. ^cSee also Fig. 2.

Due to its seed-borne nature, caution should be taken when importing seeds, especially from South America, to prevent further introductions of potentially virulent strains across the world.

Methods

Fungal and plant material. A diverse collection of 39 *Colletotrichum lupini* and 11 closely related *Colletotrichum* spp. isolates, originating from Europe, Australia, Southern Africa and South and North America, was analyzed (Table 1). Nine isolates were collected from symptomatic lupin plants in this study, whereas the rest of the isolates was already identified as *C. lupini* or as other members of the *C. acutatum* species complex representing clades 1, 2 and 4. The *C. lupini* strains CBS 109225 (Ukraine), CBS 509.97 (France) and CBS 109226 (Canada) were chosen as reference for genetic group I, strains CBS 109221 (Germany), IMI 375715 (Australia) and RB221 (France) served as reference for genetic group II and the *C. acutatum* strains CBS 369.73 and CBS 370.73 were used as outgroup in the phylogenetic analysis. Inoculations were performed on two white lupin (*Lupinus albus* L.) accessions: Feodora (susceptible; breeder: Jouffrai Drillaud, France) and Blu-25 (tolerant; breeder: Semillas Baer, Chile), and two Andean lupin (*L. mutabilis*) accessions: LUP 17 and LUP 100 (genebank: IPK, Germany). Plant material can be requested at mentioned breeders and genebanks, who performed formal identification and gave permission to use the material for research purposes. The experimental research of the plant material used in this study complies with relevant institutional, national, and international guidelines and legislation.

Fungal isolation and culture conditions. Symptomatic (dried) lupin stem or pod tissue (Fig. 1) of 1–3 cm was surface sterilized (after rehydration in sterile ddH₂O for dried samples) for 5 s with 0.25% sodium hypochlorite solution and rinsed thrice for 5 s in sterile ddH₂O. Thin slices of 1 mm were cut and placed on PDA (potato dextrose agar, Carl Roth, Karlsruhe, Germany) amended with Tetracycline (0.02 g/l, Carl Roth) for 3 to 4 days at 22 °C in the dark. Single cultures were selected and grown on fresh PDA plates amended with Tetracycline for 4 to 6 days at 22 °C in the dark and suspected *Colletotrichum* species were sub-cultured. Single

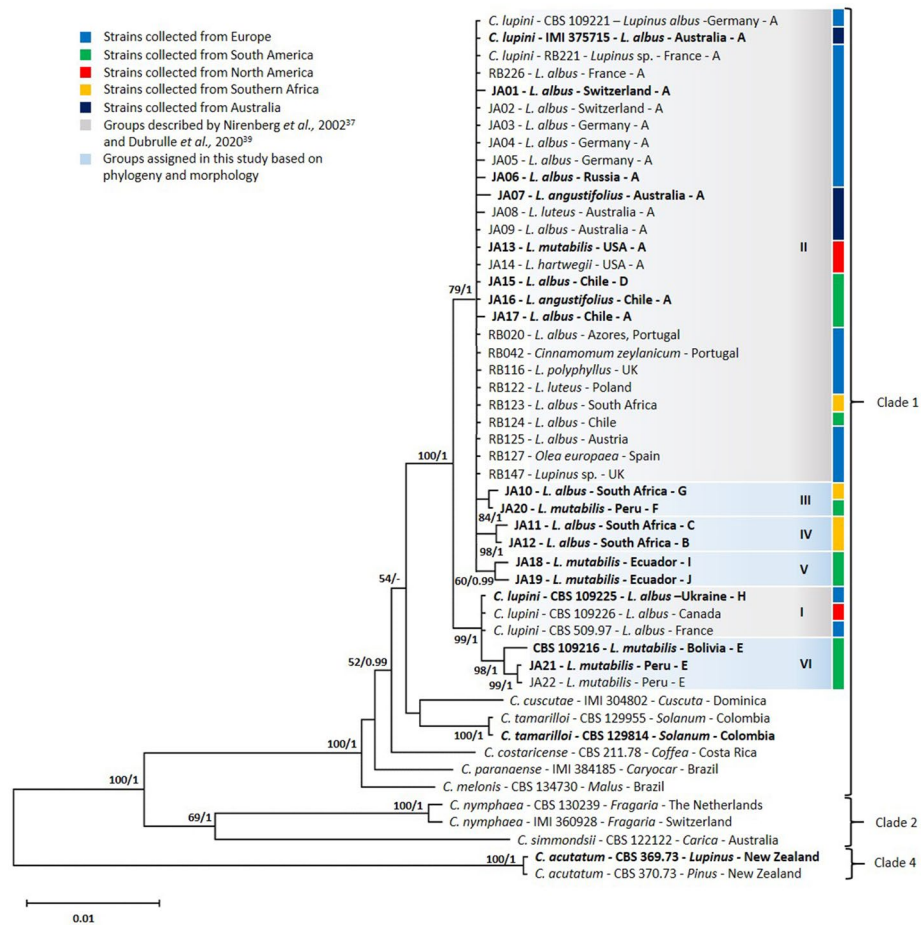


Figure 3. Multi-locus phylogeny of *Colletotrichum lupini*. Bayesian analysis tree inferred from the combined ITS, TUB2, GAPDH and APN/MAT1 sequence datasets of 50 *Colletotrichum* strains used in this study. Bootstrap support values (> 50) and Bayesian posterior probabilities (> 0.95) are given at each node. The tree is rooted to *C. acutatum* (CBS 369.73 and CBS 370.73). Strain codes are followed by host, country of origin and morphology (A–J). Grouping (I–VI) is based on phylogeny and morphology. Strains used for virulence assays are highlighted in bold. Clades indicate the different clades within the *C. acutatum* species complex.

spore cultures were obtained and transferred to PDA and maintained at 22 °C in the dark as working cultures and stored at – 80 °C in 25% glycerol for long-term storage.

Morphology. A globally representative subset of 28 *C. lupini* isolates was characterized based on colony morphology (form, aerial mycelium, margin type and color of the reverse side). From those, a subset of 18 isolates was further characterized for growth rate (mm/day), and conidial shape and size¹⁹. Isolates were sub-cultured by placing a droplet of 5 µl spore suspension in the middle of three PDA plates and grown for 14 days at 22 °C in the dark. Culture diameter was recorded every 3 days. Photographs were taken from the front and reverse sides of the PDA plates after 14 days of incubation. Conidia were collected with a sterile spreader after flooding the Petri plate with 2 ml sterile ddH₂O, the spore suspension was filtered with sterile cheese cloth and microscopic slides were prepared with sterile ddH₂O. Conidia morphology was observed using light microscopy (DM2000-LED, Leica Microsystems, Wetzlar, Germany) equipped with a high definition camera (Gryphax Subra, Jenoptik AG, Jena, Germany). A minimum of at least 50 measurements were performed to determine conidia length and width. A principal component analysis (PCA) was performed on a subset of 17 representative *C. lupini* isolates, based on average conidia length and width, length width ratio, colony growth rate, form (circular = 1, most irregular = 4), aerial mycelia (no aerial mycelia = 1, most aerial mycelia = 4), color (palest = 1, darkest = 4) and filiform margin (yes = 1, no = 0), using R 4.0.3⁶¹ and the *FactoMineR* package⁶².

DNA extraction, PCR amplification and sequencing. Mycelium from single-spore cultures was collected after 7–10 days on PDA at 22 °C with a sterile spreader after flooding the Petri dish with 2 ml sterile ddH₂O. Genomic DNA was isolated with a CTAB extraction protocol⁶³. Partial gene sequences were determined for the internal transcribed spacer (ITS) region using primers ITS5 and ITS4⁶⁴, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene using primers GDF1 and GDR1⁶⁵, the β-tubulin 2 (TUB2) gene using primers

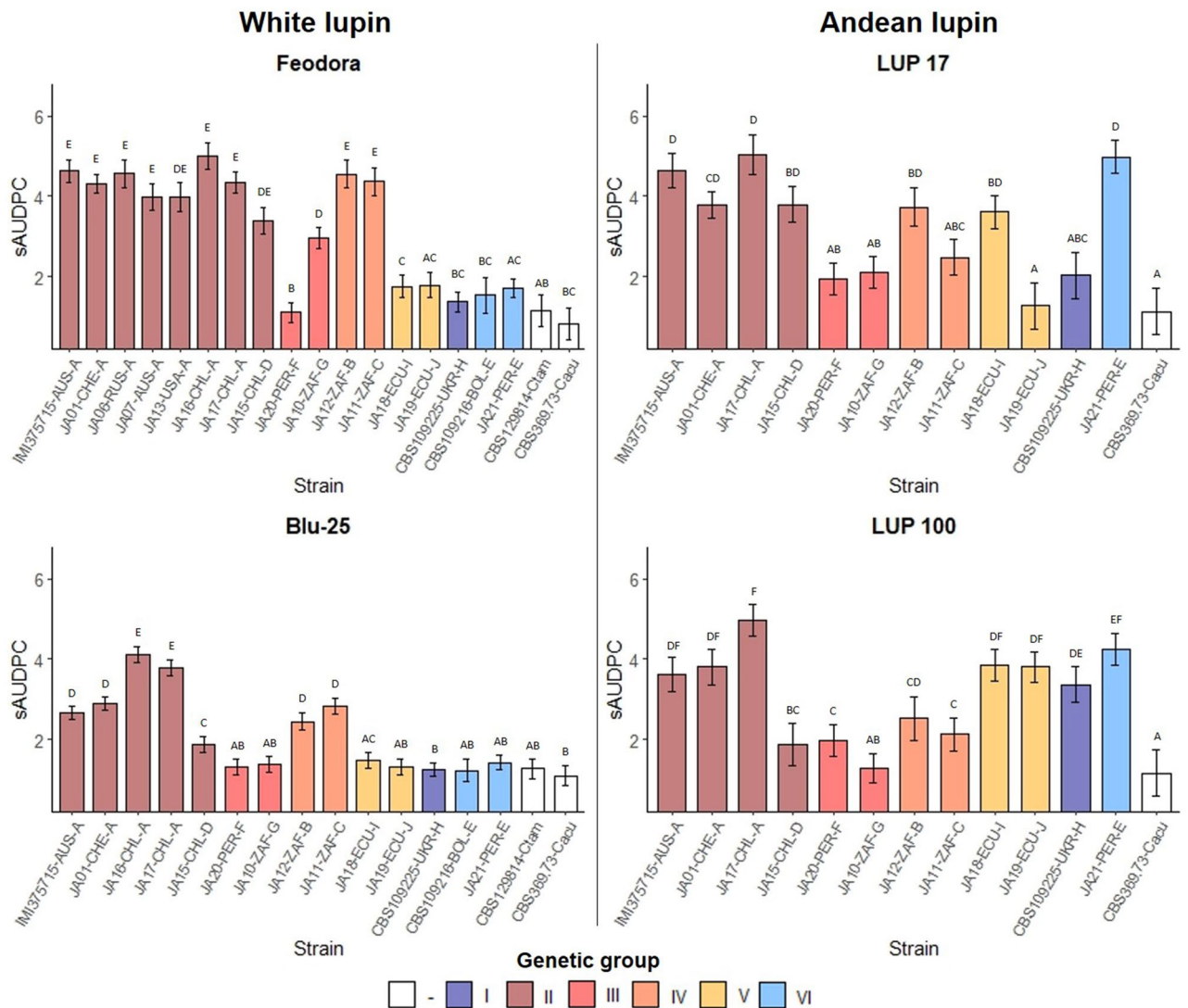


Figure 4. Virulence of *Colletotrichum lupini* strains on white (*Lupinus albus*) and Andean lupin (*L. mutabilis*). Anthracnose severity is expressed in standardized area under the disease progress curve (sAUDPC) and estimated means are shown. Strain codes are followed by abbreviated country of origin and morphotype (A–J). Different capital letters above bars indicate significant differences between strains (Tuckey-HSD, $p < 0.05$). Error bars indicate the standard error of the estimated mean.

Btub2Fd and Btub4Rd⁶⁶ and the Apn2-Mat1-2-1 intergenic (APN/MAT1) spacer and partial mating type gene using Apnmat1F and Apnmat1R³⁹. PCR was performed in a S1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to conditions described in Dubrulle et al.³⁹ PCR products were verified by gel electrophoresis, purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), quantified with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and sent to Eurofins Genomics (Ebersberg, Germany) for sequencing. The obtained DNA sequences were analyzed and consensus sequences were generated using BioEdit v. 7.2.5⁶⁷.

Phylogenetic analyses. Alignments for each of the four loci, including sequences obtained in this study and downloaded from GenBank (Table 1), were performed with ClustalW using MEGA X⁶⁸. Obtained multiple alignments were manually corrected and trimmed to obtain comparable sequences. Best-fit substitution models were determined for each locus separately and for the concatenated multi-locus alignment (ITS, TUB2, GAPDH and APN/MAT1). Phylogenetic analyses of the multi-locus alignment were based on Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analysis was performed using RAXML v. 8⁶⁹ through the CIPRES science gateway portal⁷⁰ using default parameters and 1000 bootstrap iterations. The BI analysis was performed with MrBayes v. 3.2.7⁷¹ using a Markov Chain Monte Carlo (MCMC) algorithm using four chains and starting from a random tree topology. Substitution models for each locus were included for each partition. The analysis ran for 500,000 generations with trees sampled every 1000 generations to reach average standard deviations of split frequencies below 0.01. The first 25% of saved trees were discarded at the ‘burn-in’ phase and the 50% consensus trees and posterior probabilities (PP) were determined from the remaining trees. Bootstrap

support values (BS) from the ML analysis were plotted on the Bayesian phylogeny. Further phylogenetic analyses were performed with the unweighted pair group method with arithmetic mean (UPGMA) with 10,000 replicates in Mega X. All generated sequences were deposited in GenBank (Table 1) and alignments and trees in TreeBASE.

Virulence. Virulence tests were performed on white and Andean lupin with representative *C. lupini* strains (see Fig. 3), *C. tamarilloi* strain CBS 129814 and *C. acutatum* strain CBS 369.73 through stem-wound inoculation as described by Alkemade et al.²⁶, which was shown to highly correspond to field performance in Switzerland ($r=0.95$). Disease scores ranging from 1 (non-pathogenic), 2 (low virulence) to 9 (highly virulent) were taken 4, 7 and 10 days post inoculation (dpi) and the standardized area under the disease progress curve was calculated (sAUDPC)²⁶. All inoculations were performed in a growth chamber (25 ± 2 °C, 16 h light and ~70% relative humidity) in a completely randomized block design with a minimum of six replicates per experiment.

Statistical analysis. Statistical analyses were performed with R 4.0.3 using the packages *lme4*⁷², *lmerTest*⁷³ and *emmeans*⁷⁴, following a mixed model with factors of interest (i.e. strain, lupin species, lupin accession) as fixed and replicated block nested in experiment as random factor. Datasets that did not follow assumptions of normality of residuals and homogeneity of variance were log10 transformed. Data are presented as estimated least-squares means using the aforementioned mixed model. A Tukey-HSD test ($p \leq 0.05$) was applied for pairwise mean comparison of the different *Colletotrichum* strains within each lupin accession.

Data availability

The data that support the findings of this study are shown in this manuscript or, in the case of new sequences data, are openly available in Genbank at <https://www.ncbi.nlm.nih.gov/genbank/> (for reference numbers see Table 1) and in Treebase at <http://purl.org/phylo/treebase/phyloids/study/TB2:S27356?x-access-code=260136f8e6416a0614b93528ddbfe0ef&format=html>.

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References

- Lenné, J. Some major plant diseases. In *Plant Pathologist's Pocketbook* (eds Waller, J. M. et al.) 4–18 (CABI Publishing, 2002).
- Cannon, P., Damm, U., Johnston, P. & Weir, B. *Colletotrichum*—Current status and future directions. *Stud. Mycol.* **73**, 181–213 (2012).
- Udayanga, D., Manamgoda, D. S., Liu, X., Chukeatirote, E. & Hyde, K. D. What are the common anthracnose pathogens of tropical fruits? *Fungal Divers.* **61**, 165–179 (2013).
- Shivas, R. G. et al. *Colletotrichum* species in Australia. *Australas. Plant Pathol.* **45**, 447–464 (2016).
- Baroncelli, R. et al. Molecular diversity of anthracnose pathogen populations associated with UK strawberry production suggests multiple introductions of three different *Colletotrichum* species. *PLoS ONE* **10**, e0129140 (2015).
- Frey, T., Weldekidan, T., Colbert, T., Wolters, P. & Hawk, J. Fitness evaluation of *Rcg1*, a locus that confers resistance to *Colletotrichum graminicola* (Ces.) GW Wils. using near-isogenic maize hybrids. *Crop Sci.* **51**, 1551–1563 (2011).
- Rogério, F. et al. Genome sequence resources of *Colletotrichum truncatum*, *C. plurivorum*, *C. musicola* and *C. sojae*: Four species pathogenic to soybean (*Glycine max*). *Phytopathology* **110**, 1497–1499 (2020).
- Bouffleur, T. R. et al. Soybean anthracnose caused by *Colletotrichum* species: Current status and future prospects. *Mol. Plant Pathol.* **22**, 393–409 (2021).
- Perfect, S. E., Hughes, H. B., O'Connell, R. J. & Green, J. R. *Colletotrichum*: a model genus for studies on pathology and fungal–plant interactions. *Fungal Genet. Biol.* **27**, 186–198 (1999).
- Baroncelli, R. et al. The *Colletotrichum acutatum* species complex as a model system to study evolution and host specialization in plant pathogens. *Front. Microbiol.* **8**, 2001 (2017).
- De Silva, D. D., Crous, P. W., Ades, P. K., Hyde, K. D. & Taylor, P. W. Life styles of *Colletotrichum* species and implications for plant biosecurity. *Fungal Biol. Rev.* **31**, 155–168 (2017).
- Yan, Y. et al. *Colletotrichum higginsianum* as a model for understanding host–pathogen interactions: A review. *Int. J. Mol. Sci.* **19**, 2142 (2018).
- Dean, R. et al. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **13**, 414–430 (2012).
- Damm, U., Cannon, P. F., Woudenberg, J. H. C. & Crous, P. W. The *Colletotrichum acutatum* species complex. *Stud. Mycol.* **73**, 37–113 (2012).
- Bragança, C. A., Damm, U., Baroncelli, R., Júnior, N. S. M. & Crous, P. W. Species of the *Colletotrichum acutatum* complex associated with anthracnose diseases of fruit in Brazil. *Fungal Biol.* **120**, 547–561 (2016).
- Cannon, P. F., Bridge, P. & Monte, E. Linking the past, present and future of *Colletotrichum* systematics. In *Colletotrichum: Host Specificity, Pathology and Host-Pathogen Interaction* (eds Prusky, D. et al.) 1–20 (APS Press, 2000).
- Johnston, P. The importance of phylogeny in understanding host relationships within *Colletotrichum*. In *Colletotrichum: Host Specificity, Pathology, and Host-Pathogen Interaction* (eds Prusky, D. et al.) 21–28 (APS Press, 2000).
- Cai, L. et al. A polyphasic approach for studying *Colletotrichum*. *Fungal Divers.* **39**, 183–204 (2009).
- Lardner, R., Johnston, P., Plummer, K. & Pearson, M. Morphological and molecular analysis of *Colletotrichum acutatum sensu lato*. *Mycol. Res.* **103**, 275–285 (1999).
- Talhinhas, P., Baroncelli, R. & Le Floch, G. Anthracnose of lupines caused by *Colletotrichum lupini*: A recent disease and a successful worldwide pathogen. *J. Plant Pathol.* **98**, 5–14 (2016).
- Bondar, G. Tremoco branco e suas molestias. *Bol. Agric. Sao Paulo* **13**, 427–432 (1912).
- Weimer, J. Anthracnose of lupines. *Phytopathology* **33**, 249–252 (1943).
- White, P., French, B. & McLarty, A. Grains research and development corporation. In *Producing Lupins* (eds White, P. et al.) 48–49 (Department of Agriculture and Food, 2008).
- Thomas, G. & Sweetingham, M. Cultivar and environment influence the development of lupin anthracnose caused by *Colletotrichum lupini*. *Australas. Plant Pathol.* **33**, 571–577 (2004).
- Shea, G. et al. Case study: Industry response to the lupin anthracnose incursion in Western Australia. In *Lupins for Health and Wealth. Proc. 12th International Lupin Conference* (eds Palta, J. A. & Berger, J. B.) 425–431 (International Lupin Association, 2008).
- Alkemade, J. A. et al. A high-throughput phenotyping tool to identify field-relevant anthracnose resistance in white lupin. *Plant Dis.* <https://doi.org/10.1094/PDIS-07-20-1531-RE> (2021).

27. Thomas, G., Sweetingham, M. & Adcock, K. Application of fungicides to reduce yield loss in anthracnose-infected lupins. *Crop Prot.* **27**, 1071–1077 (2008).
28. Yang, H., Boersma, J. G., You, M., Buirchell, B. J. & Sweetingham, M. W. Development and implementation of a sequence-specific PCR marker linked to a gene conferring resistance to anthracnose disease in narrow-leaved lupin (*Lupinus angustifolius* L.). *Mol. Breed.* **14**, 145–151 (2004).
29. Yang, H., Renshaw, D., Thomas, G., Buirchell, B. & Sweetingham, M. A strategy to develop molecular markers applicable to a wide range of crosses for marker assisted selection in plant breeding: A case study on anthracnose disease resistance in lupin (*Lupinus angustifolius* L.). *Mol. Breed.* **21**, 473–483 (2008).
30. Fischer, K. *et al.* Characterization and mapping of *LanrBo*: A locus conferring anthracnose resistance in narrow-leaved lupin (*Lupinus angustifolius* L.). *Theor. Appl. Genet.* **128**, 2121–2130 (2015).
31. Adhikari, K., Buirchell, B., Thomas, G. J., Sweetingham, M. W. & Yang, H. Identification of anthracnose resistance in *Lupinus albus* L. and its transfer from landraces to modern cultivars. *Crop Pasture Sci.* **60**, 472–479 (2009).
32. Adhikari, K. N., Thomas, G., Buirchell, B. J. & Sweetingham, M. W. Identification of anthracnose resistance in yellow lupin (*Lupinus luteus* L.) and its incorporation into breeding lines. *Plant Breed.* **130**, 660–664 (2011).
33. Falconi, C. *Lupinus mutabilis* in Ecuador with special emphasis on anthracnose resistance. PhD thesis, Wageningen University, the Netherlands (2012).
34. Lucas, M. M. *et al.* The future of lupin as a protein crop in Europe. *Front. Plant Sci.* **6**, 705 (2015).
35. Van de Noort, M. Lupin: An important protein and nutrient source. In *Sustainable Protein Sources* (eds Nadathur, S. R. *et al.*) 165–183 (Academic Press, 2017).
36. Gulisano, A., Alves, S., Neves Martins, J. & Trindade, L. M. Genetics and breeding of *Lupinus mutabilis*: an emerging protein crop. *Front. Plant Sci.* **10**, 1385 (2019).
37. Nirenberg, H. I., Feiler, U. & Hagedorn, G. Description of *Colletotrichum lupini* comb. nov. in modern terms. *Mycologia* **94**, 307–320 (2002).
38. Shivas, R., McClements, J. & Sweetingham, M. Vegetative compatibility amongst isolates of *Colletotrichum* causing lupin anthracnose. *Australas. Plant Pathol.* **27**, 269–273 (1998).
39. Dubrulle, G. *et al.* Phylogenetic diversity and effect of temperature on pathogenicity of *Colletotrichum lupini*. *Plant Dis.* **104**, 938–950 (2020).
40. Riegel, R., Veliz, D., von Baer, I., Quitral, Y. & Munoz, M. Genetic diversity and virulence of *Colletotrichum lupini* isolates collected in Chile. *Trop. Plant Pathol.* **35**, 144–152 (2010).
41. Falconi, C. E., Visser, R. G. & van Heusden, A. W. Phenotypic, molecular, and pathological characterization of *Colletotrichum acutatum* associated with Andean lupine and tamarillo in the Ecuadorian Andes. *Plant Dis.* **97**, 819–827 (2013).
42. Atchison, G. W. *et al.* Lost crops of the Incas: Origins of domestication of the Andean pulse crop tarwi, *Lupinus mutabilis*. *Am. J. Bot.* **103**, 1592–1606 (2016).
43. Nevado, B., Atchison, G. W., Hughes, C. E. & Filatov, D. A. Widespread adaptive evolution during repeated evolutionary radiations in New World lupins. *Nat. Commun.* **7**, 1–9 (2016).
44. Lotter, H. & Berger, D. Anthracnose of lupins in South Africa is caused by *Colletotrichum lupini* var. *setosum*. *Australas. Plant Pathol.* **34**, 385–392 (2005).
45. Van Der Mey, J. A. Crop development of *Lupinus* species in Africa. *S. Afr. J. Sci.* **92**, 53–56 (1996).
46. Ghebremariam, D., Koch, S. & Swart, W. Susceptibility of lupin cultivars to South African isolates of *Colletotrichum gloeosporioides* associated with lupin anthracnose. *Afr. Plant Prot.* **8**, 51–56 (2002).
47. Yang, H. & Sweetingham, M. The taxonomy of *Colletotrichum* isolates associated with lupin anthracnose. *Aust. J. Agric. Res.* **49**, 1213–1224 (1998).
48. Elmer, W. H., Yang, H. A. & Sweetingham, M. W. Characterization of *Colletotrichum gloeosporioides* isolates from ornamental lupines in Connecticut. *Plant Dis.* **85**, 216–219 (2001).
49. Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. & Oliveira, H. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology* **92**, 986–996 (2002).
50. Han, K., Kim, B., Choi, I., Park, J. & Shin, H.-D. First report of anthracnose caused by *Colletotrichum lupini* on yellow lupin in Korea. *Plant Dis.* **98**, 1158–1158 (2014).
51. Zou, M. *et al.* First report of *Colletotrichum lupini* causing anthracnose on lupin in China. *Plant Dis.* **103**, 767 (2019).
52. Dubrulle, G. *et al.* Deciphering the infectious process of *Colletotrichum lupini* in lupin through transcriptomic and proteomic analysis. *Microorganisms* **8**, 1621 (2020).
53. Guilengue, N., Neves-Martins, J. & Talhinhas, P. Response to anthracnose in a tarwi (*Lupinus mutabilis*) collection is influenced by anthocyanin pigmentation. *Plants* **9**, 583 (2020).
54. Falleiros, M. O., Mota, S. F., Ferreira, A. N. & de Souza, E. A. Mixture of *Colletotrichum lindemuthianum* races in anthracnose resistance screening and its implication for common bean breeding. *Trop. Plant Pathol.* **43**, 271–277 (2018).
55. Xavier, K., Mizubuti, E., Queiroz, M., Chopra, S. & Vaillancourt, L. Genotypic and pathogenic diversity of *Colletotrichum sublineola* isolates from sorghum (*Sorghum bicolor*) and johnsongrass (*S. halepense*) in the southeastern United States. *Plant Dis.* **102**, 2341–2351 (2018).
56. Armstrong-Cho, C., Wang, J., Wei, Y. & Banniza, S. The infection process of two pathogenic races of *Colletotrichum truncatum* on lentil. *Can. J. Plant Pathol.* **34**, 58–67 (2012).
57. Ordonez, N. *et al.* Worse comes to worst: Bananas and Panama disease—When plant and pathogen clones meet. *PLoS Pathog.* **11**, e1005197 (2015).
58. Godfray, H. C. J., Mason-D'Croz, D. & Robinson, S. Food system consequences of a fungal disease epidemic in a major crop. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150467 (2016).
59. Lidwell-Durnin, J. & Laphorn, A. The threat to global food security from wheat rust: Ethical and historical issues in fighting crop diseases and preserving genetic diversity. *Glob. Food Sec.* **26**, 100446 (2020).
60. Jacob, I., Feuerstein, U., Heinz, M., Schott, M. & Urbatzka, P. Evaluation of new breeding lines of white lupin with improved resistance to anthracnose. *Euphytica* **213**, 236 (2017).
61. R Core Team. *R: A Language and Environment for Statistical Computing*. (R Foundation for Statistical Computing, 2020). <http://www.R-project.org/>. Accessed 10 Oct 2020.
62. Lê, S., Josse, J. & Husson, F. FactoMineR: An R package for multivariate analysis. *J. Stat. Softw.* **25**, 1–18 (2008).
63. Minas, K., McEwan, N. R., Newbold, C. J. & Scott, K. P. Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures. *FEMS Microbiol. Lett.* **325**, 162–169 (2011).
64. White, T. J., Bruns, T., Lee, S. & Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (eds Innis, M. A. *et al.*) 315–322 (Elsevier, 1989).
65. Guerber, J. C., Liu, B., Correll, J. C. & Johnston, P. R. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycologia* **95**, 872–895 (2003).
66. Woudenberg, J., Aveskamp, M., De Gruyter, J., Spiers, A. & Crous, P. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **22**, 56 (2009).
67. Hall, T. A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.* **41**, 95–98 (1999).

68. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).
69. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).
70. Miller, M. A., Pfeiffer, W. & Schwartz, T. The CIPRES science gateway: A community resource for phylogenetic analyses. In *Proc. 2011 TeraGrid Conference: Extreme Digital Discovery* 1–8 (2011).
71. Ronquist, F. *et al.* MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
72. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
73. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **82**, 1–26 (2017).
74. Lenth, R., Singmann, H., Love, J., Buerkner, P. & Herve, M. *emmeans: Estimated Marginal Means, aka Least-Squares Means (Version 1.3.4)* (2019). <https://CRAN.R-project.org/package=emmeans>. Accessed 5 Oct 2020.

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Author contributions

J.A.A., P.H. and M.M.M. conceived the original idea for this study. J.A.A. conducted the experiments and took the lead in manuscript writing. J.A.A. analyzed the data with contributions from P.H. and M.M.M. All authors significantly contributed to data interpretation and provided critical feedback that shaped the final version. J.A.A. designed the figures and tables with input from P.H., M.M.M., R.T.V. and M.R.F.

Competing interests

The authors declare no competing interests.

Additional information

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