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Emergent Potato Leaf Spot Diseases in the Highland and Lowland Regions of Bolivia

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Abstract

In Bolivia, potato (*Solanum tuberosum* L.) leaf spot diseases have traditionally been regarded as of little importance especially in the Andean highlands. In recent years, however, new types of leaf spot disease have appeared, their distribution widened, and their incidence and severity increased. The present work identifies the main types of leaf spot present in the traditional highland growing regions of the Departments of La Paz (around 4350 m) and Cochabamba (2900-4100 m), and in the north of the Department of Santa Cruz, the new lowland area of potato production (around 235 m). Five causal agents were identified in the highland region: *Alternaria solani, Septoria lycopersici, Cercospora solanicola, Passalora concors* and *Botrytis cinerea*. These affected several types of native potato. In the lowland region, *A. solani* and *Stagonospora* spp. were found to cause leaf spot disease on cv. Desireé. In both agroecosystems, the diseases sometimes appeared alongside late blight (caused by *Phytophthora infestans*). The leaf spot disease caused by *A. solani*, was very destructive, while that caused by *S. lycopersici* was only destructive in the highlands.

Keywords: Native potatoes; Disease intensity; Destructive disease

Introduction

Potato (*Solanum tuberosum* L.) leaf spot diseases are caused by different necrotrophic phytopathogens. They are sometimes confused with late blight, which is caused by *Phytophthora infestans*. Leaf spot diseases are most frequently seen in Bolivia's cold, damp Andean highlands (they are far less common in the warmer regions of the country), usually affecting native crops such as *imillas* (*Solanum tuberosum* subsp. *andigena*), bitter (*Solanum×juzepczukii*) and *phurejas* (*Solanum phureja*) potatoes [1]. Leaf spot diseases have also been recorded in the highland regions of Peru, Ecuador and Colombia [2-4]. According to Hooker [2], the causal agents in high altitude areas include *Septoria lycopersici*, *Cercospora* spp., *Phoma andina*, *Ulocladium atrum* and *Botrytis cinerea*, while *Chaenophora cucurbitarum* affects plants in the lowlands. Leaf spot diseases in the highland regions have traditionally been regarded as of little importance. However, in recent years their distribution and intensity (incidence and severity) have increased, as has the impact of late blight [5]. Other types of leaf spot disease are caused by *Phoma andina* and *Phoma huancayense* [4]. The leaf spot caused by *S. lycopersici* is regionally important in the Venezuelan, Peruvian and Ecuadorian Andes, from where reports of over 60% leaf destruction have been made [6,7]. Indeed, "septoriosis" has long been known to have serious effects [8]. In Bolivia, where it is known as khasahui, it is not a devastating disease, although it is of moderate importance and can cause early leaf death [3].

The main production areas of the Andean highlands in the Departments of La Paz (>4000 m) and Cochabamba (>3500 m) are permanently cloudy, very damp, and cold - typical of the transition zone between the lowlands and the Andean Region or Cordillera Real (Figure 1). In recent years, *S. lycopersici* has caused damage to potato crops in these areas, especially in the native *imillas* and *Solanum×juzepczukii* forms [5]. The distributions of this and other forms of leaf spot disease are now expanding, and types of leaf spot new to the highlands of Cochabamba have been recorded [9]. The present work describes the main forms of leaf spot in the highland regions of the Departments of La Paz and Cochabamba, and in a new, milder, lowland potato production area of Bolivia.

Materials and Methods

In January and February of 2012, 2013 and 2014, survey expeditions were made to record the leaf spot diseases affecting potato plants in Bolivia's traditional highland growing areas of the Departments of La Paz (around 4350 m) and Cochabamba (2900-3300 m), and in the

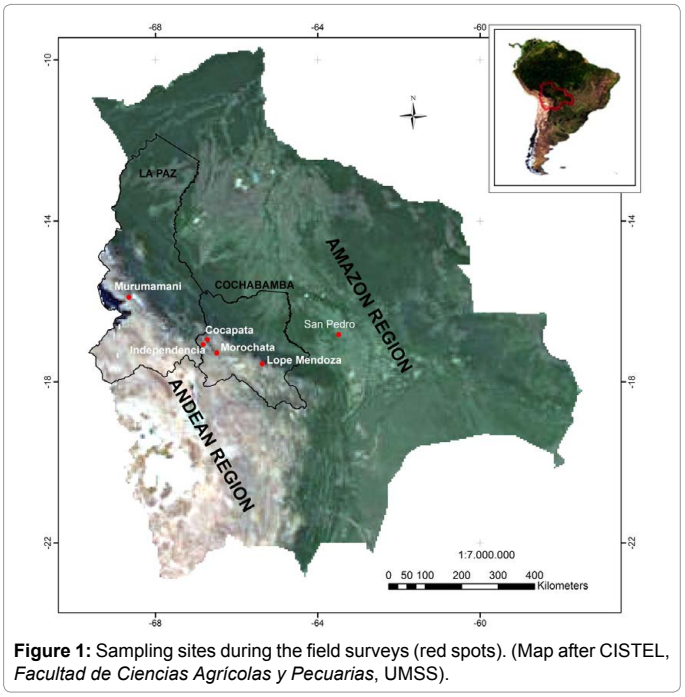

Figure 1: Sampling sites during the field surveys (red spots). (Map after CISTEL, *Facultad de Ciencias Agrícolas y Pecuarias*, UMSS).

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Department	Province	Area of influence	Locality	Altitude	Latitude	Longitude
				(m)	S	W
La Paz	Omasuyos	Murumamani	Murumamani	4300	15°54'0.00"	68°39'0.00"
			Humanata	4250	15°52'41.15"	68°38'49.37"
	Camacho	Humanata	Humanata	4150	15°28'28.82"	69° 7'20.24"
Cochabamba	Carrasco	Lope Mendoza	Escalante	2950	17°31'55.49"	65°22'55.32"
			Chullchungani	3050	17°33'39.15"	65°20'6.75"
			Phuyuhuasi	3150	17°34'14.46"	65°20'26.32"
			Montepunku	2950	17°35'10.76"	65°18'27.14"
			Laimetoro	3100	17°39'4.00"	65° 6'30.52"
	Ayopaya	Morochata	Piucilla	3320	17° 13' 34"	66o 30' 17"
	Ayopaya	Independencia	Sensei	3200	17° 4'49.18"	66°50'11.56"
		Cocapata	Choro	4100	16°58'42.23"	66°42'54.20"
	Chapare	Coranipampa	Coranipampa	2650	17° 7'20.68"	65°56'2.58"
			Pairumani	2600	17° 7'54.35"	65°56'8.47"
Santa Cruz	Cordillera	Chane	San Pedro	500	16°49'37.61"	63°28'52.86"

Table 1: Locations where leaf samples were taken.

Locality	Variety	Potato species	Common name of disease	Causal agent
Murumamani	Waych'a	*Solanum tuberosum* subsp. *andigena*	Khasahui (septoriosis)	*Septoria lycopersici*
Humanata	Rosita	*Solanum tuberosum* subsp. *andigena*	Khasahui (septoriosis)	*Septoria lycopersici*
Humanata	Waych'a	*Solanum tuberosum* subsp. *andigena*	Khasahui (septoriosis)	*Septoria lycopersici*
Escalante	Desireé	*Solanum tuberosum* subsp. *tuberosum*	EBLS	*Alternaria solani*
Chullchungani	Desireé	*Solanum tuberosum* subsp. *tuberosum*	Mancha negra (EBLS)	*Alternaria solani*
Phuyuhuasi	Desireé	*Solanum tuberosum* subsp. *tuberosum*	Mancha negra (EBLS)	*Alternaria solani*
Montepunku	Desireé	*Solanum tuberosum* subsp. *tuberosum*	Mancha negra (EBLS)	*Alternaria solani*
Laimetoro	Puka (*)	*Solanum andigena* x *tuberosum*	Manchón (leaf blotch)	*Cercospora spp.*
Piucilla	Waych'a	*Solanum tuberosum* subsp. *andigena*	Mancha plateada (CLB)	*Passalora concors*
Sensei	Waych'a	*Solanum tuberosum* subsp. *andigena*	Mancha negra (EBLS)	*Alternaria solani*
Choro	Waych'a	*Solanum tuberosum* subsp. *andigena*	Khasahui (septoriosis)	*Septoria lycopersici*
Coranipampa	Phureja	*Solanum phureja*	Gray mold	*Botrytis sp.*
San Pedro	Desireé	*Solanum tuberosum* subsp. *tuberosum*	Mancha negra (EBLS)	*Alternaria solani*
San Pedro	Desireé	*Solanum tuberosum* subsp. *tuberosum*	Mancha marrón (BLS)	*Stagonospora sp.*

(*)=Puka Toralapa, an improved variety that shows inherent resistance, developed by the *Estación Experimental Toralapa, Instituto Boliviano de Tecnología Agropecuaria* in 1980-1982. EBLS=Early Blight Leaf Spot. CLB=Cercospora Leaf Blotch. BLS=Brown Leaf Spot.

Table 2: Types of leaf spot diseases detected, where they were detected, and potato varieties affected.

north of the Department of Santa Cruz, the country's new, lowland growing region (San Pedro locality, around 500 m) (Figure 1) (Table 1). In the Department of La Paz, leaf samples were collected in the communities of Murumamani, Humanata (both in the Province of Omasuyos) and Humanata (in the Province of Camacho) (Table 1). Given their proximity to the Andean peaks of the Cordillera Real mountain range, these communities are under permanent cloud, damp, and cold. In the Department of Cochabamba, samples were collected in the communities within the areas of influence of Lope Mendoza (in the Province of Carrasco), Morochata and Independencia (Province of Ayopaya), and Coranipampa (Province of Chapare) (Table 1). These areas have the same kind of climate as described above, a consequence of their proximity to the transition zones to the Andean region to Amazon region (Figure 1). In the Department of Santa Cruz, samples were collected in the area of Chané to the north of Santa Cruz (Province of Cordillera) (Table 1). Soybean (*Glycine soja*) and sugar cane (*Saccharum officinarum*) are extensively grown here; potatoes are grown during the winter (March to August). The climate at this time is mild to cold and damp, a consequence of cold winter winds arriving from Argentina.

In the highlands of the Departments of La Paz and Cochabamba, leaves showing signs of leaf spot disease (total N=10-20 depending on the year) were collected from different plants growing in small holdings sown with native varieties such as Waych'a (a variety of *imilla*) (*S. tuberosum* subsp. *andigena*), and phureja blanca (a variety of phureja) (*S. phureja)*, and bitter potato (*S. x juzepzukii*). In the lowlands of Santa Cruz, similar samples (total N=10-20) were collected from plants growing in extensive plots sown with variety Desireé (*S. tuberosum subsp. tuberosum*).

The collected samples were taken to the laboratory in plastic bags inside preservation boxes within two days, and the fungal reproductive structures stained with lactophenol methylene blue [10] and examined under a stereomicroscope. The fungi causing the leaf spot symptoms were identified using the taxonomic keys and indicators of different authors as indicated below.

Results

Six types of leaf spot were found affecting the native *S. t.* subsp. *andigena* and introduced *S. t.* subsp. *tuberosum* (Table 2).

Early blight leaf spot caused by *Alternaria solani* sorauer

This was detected in the highlands of Cochabamba near Escalante, Chullchungani, Phuyuhuasi and Montepunku (in the Lope Mendoza area, Province of Carrasco). It was also detected in the lowland area of the Department of Santa Cruz at San Pedro (in the Chane area) (Table 2). In both agroecosystems the climate during the growing period is

damp and largely cold (8-18ºC). In the highlands it affected the native variety Waych'a (*S. tuberosum* subsp. *andigena*), while in the lowlands if was found on variety Desireé (*S. tuberosum* subsp. *tuberosum*). In all the places it was detected alongside (i.e., on the same plant or even the same leaf) late blight (caused by *P. infestans*). Incidence was generalized in both agroecosystems, and the disease more severe in cv. Desireé.

The characteristic symptom was the formation of dark brown leaf spots surrounded by a halo that becomes more obvious with time (Figures 2A and 2B). In Waych'a they were more angular and irregular, limited by the veins and the interior of the spots showed discontinuous, irregular rings (Figure 2A). In cv. Desireé, the spots were more circular and regular, larger, and the inner rings more notable (Figure 2B).

Within the spots, small, dark brown, superficially growing structures were seen, formed from small clusters of conidiophores, straights and, septate (Figure 2C). Dark conidia formed at the apices of the latter, solitary, slightly flexuous with the body of the conidium ellipsoidal tapering to a beak which is same length as longer than the body, pale or dark brown, smooth, with 9 or more transverse and 0 longitudinal or oblique septa; beak is flexuous (Figure 2D). Using the keys of Ellis [11], the fungus was identified as *A. solani.*

Septoria leaf spot (khasahui) caused by *Septoria malagutii* ciccar. and Boerema ex E.T. Cline, sp. nov. (Syn. *Septoria lycopersici speg.*)

This was identified in samples from the Departments of La Paz and Cochabamba collected at an altitude of around 4100 m (Table 2). In both of these highland agroecosystems, the conditions are damp and cold (7-15ºC) during the growing season (November to May). In the Department of La Paz, it was detected in the Murumamani area in the communities of Murumamani, Paconi and Umanata, where it affected the native varieties Waych'a and the bitter potato (variety Luk'ys) (*Solanum x juzepzukii*) (Table 2). In the Department of Cochabamba it was identified in the areas of Cocapata and Choro (Province of Ayopaya), and in the community of Phuyuhuasi within the Lope Mendoza area (Province of Carrasco), where it affected the variety Waych'a (Table 2). In all these areas the disease appeared alongside (i.e., on the same plant or leaf) late blight. In Murumamani (Dept. of La Paz) and Choro (Dept. of Cochabamba), the disease was sometimes destructive.

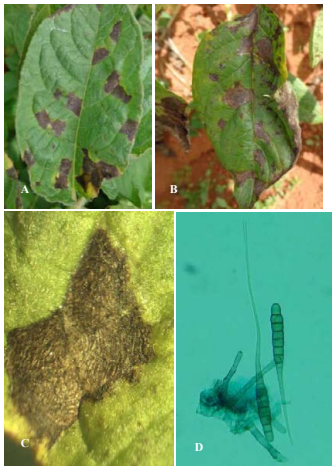

Figure 2: Leaf spot caused by *Alternaria solani* on the native and introduced potatoes varieties. A: Angular form of lesion on the variety Waych'a (*S. tuberosum* subsp. *andigena*). B: Circular to irregular lesions on variety Desireé (*S. tuberosum* subsp. *tuberosum*). C: Formation of irregular rings in the lesions (Mag. 75X). D: Conidiophores in small clusters, straights and conidia are ellipsoidal tapering to a beak same length as longer than the body (Mag. 400X).

Figure 3: Leaf spot caused by *Septoria lycopersici* in the native variety Waych'a (*S. tuberosum* subsp. *andigena*). A: Small circular lesions on the leaf. B: Formation of irregular, concentric rings in the lesion (Mag. 50X). C: Formation of pycnidia (Mag. 100X). D: Pycnidia immersed in the tissue releasing elongated, hyaline-colored conidia through the ostiole (Mag. 400X). The conidia are hyaline, filiform, or occasionally straight.

The spots were small (1-5 mm), circular to irregular, dark brown, and with concentric, irregular rings (Figures 3A and 3B). At first these spots appear isolated from one another, but over time coalesce, giving rise to lesions similar to those seen in late blight. Small (although visible with the naked eye) blackish pycnidia were seen at the center of the spots (Figure 3C). The pycnidial conidiomata were epigenous, solitary, scattered with several per lesion, immersed in the epidermis, and just visible (<100 µm in diameter). The ostiole was brown-black and some 45-70 µm diameter. The conidia were hyaline, filiform, slightly curved, occasionally straight or sigmoid, sharply pointed at both ends, and sometimes slightly rounded at the base. The apex was rounded when the conidia were still attached (90 ± 4.4 [50-122] µm long×1.9 ± 0.03 [1.0-2.0] µm wide). There were 4-7 septa with no constrictions (Figure 3D). Using the keys of Cline and Rossman [1], the fungus was identified as *Septoria malagutii.*

Leaf blotch caused by *Cercospora spp.* Atk.

This was detected in plants in the Laimetoro area (Province of Carrasco) in the Department of Cochabamba (Table 2), affecting (in isolation) the native varieties Waych'a and Chaucha blanca (*Solanum chaucha*) and the improved variety Puka toralapa (Hybrid *Solanum tubersoum* variety with R genes). Cloud cover is permanent in this area, the humidity is high, and the temperatures between 5 and 15°C. Where the humidity is very high the disease can become destructive.

The characteristic symptoms included damp-looking angular and circular-to-irregular spots, sometimes coalescing to form blotches similar to those caused by late blight (Figure 4A). The disease affected both the leaves and shoots. The spots themselves showed just-visible, irregular, concentric rings. The center of the leaf on both sides had a whitish felt-like covering (Figures 4B and 4C) formed by the fruiting structures of the fungus, i.e., clusters of conidiophores (Figure 4D) and acicular conidia (hyaline in color) (Figure 4D). The conidiophores emerged through the stomata, were unbranched, subcylindrical to geniculate-sinuous, appeared in small, dense fascicles, and showed dimensions of up to 70×5.5 µ. The conidia were slightly curved, pale olivaceous, smooth, with 6-8 septa, and with dimensions of up to 82×5.5 µ. The keys of Chupp [12], Ellis [11] and Braun and Crous [13] identified the causal agent as *Cercospora* spp.

Grey mold caused by *Botrytis cinerea* Pers.:Fr.

This was identified in leaves (only) of plants in the Coranipampa area (Province of Chapare, Municipality of Colomi) in the Department of Cochabamba (Table 2). The climate here is temperate to warm temperate (foothill climate). The disease affected the phureja blanca variety (*S. phureja*), but with just a few spots, and appeared alongside late blight. The disease appeared as isolated, damp-looking, blackish spots, circular to irregular in shape. The tissue bordering the spots was slightly chlorotic (Figures 5A and 5B). On the underside of the leaves, a lead-gray woolly carpet was seen (Figure 5B), made up of sporangiophore clusters and spores (Figures 5C and 5D). The conidiophores were 1.5-2.2 mm long, branched, with a stipe, and had a rather open head. They are smooth, clear brown below, paler near the apex, and with the ends of the branches quite colorless. The conidia were ellipsoid or obovoid in shape, with a slightly protuberant hilum, colorless to pale brown, smooth, and of dimensions 8-12×6-10 µ. using the keys of Ellis [11], the fungus was identified as *Botrytis cinerea* Pers.

Cercospora leaf blotch caused by *Passalora concors* (Casp.) Sacc. (Syn. *Cercospora concors* (Casp.) Sacc.

This disease affected the leaves of native Waych'a Paceña plants (*S. tuberosum subsp. andigena*), commonly alongside late blight. The severity of infection ranged from 10-20% (determined using Image Analysis software). The lower to middle leaves were preferentially affected, with symptoms starting as yellowish-green, circular to irregular blotches on the upper leaf surface (Figure 1A). Gradually the centers of these blotches become grey to black with a soft yellow halo (Figure 1B). A fluffy grey layer of conidiophores and conidia eventually forms on the abaxial surface of the leaves, (Figures 1C and 1D). Hyphae were observed growing over the leaf hairs (Figures 1D and 1E). The conidiophores occurred in dense fascicles above the stomata; these were irregular in width, grayish, and highly branched (Figure 1F). The conidia were variable in size, catenate and slightly curved, obclavate, pale olivaceous, smooth, non-septate, and 12-16 µ in length×3-6 µ in width (Figure 1F). Using the keys of Ellis [11] and Crous and Braun [14], the fungus was identified as *Passalora concors*, a synonym of *Cercospora concors* (Casp.) (Crous). This is the first report of this pathogen causing Cercospora leaf blotch in the Andean highlands of Bolivia.

Figure 4: Leaf spot caused by *Cercospora solanicola* in the variety *Puka toralapa* (an improved variety that shows resistance to *P. infestans*). A: Circular to irregular lesions of damp, blackish appearance (Mag. 50X). B: Lesion showing irregular rings and the growth of a felt-like, whitish carpet in the central area. C: Conidiophore in cluster formation (Mag. 400X). D: Elongated, septate conidia stained with methylene blue (Mag. 400X).

Figure 5: Leaf spot caused by *Botrytis* spp. in the native variety phureja blanca (*Solanum phureja*). A: Dark lesion on the upper leaf surfauce. B: Lesion on the underside of the leaf showing a gray carpet-like growth. C: Conidiophores showing spore cluster formations (under the stereomicroscope, Mag. 100X); D: Conidiophore with clustering conidia (Mag. 400X).

Figure 6: Leaf spot caused by *Passalora concors* in the native variety Waych'a (*S. tuberosum* subsp. *andigena*). A: Circular to irregular lesion light colored lead on the upper side of the leaf. B: Lead-gray carpet of fungus on the underside of the leaf as downy mildew (under the stereomicroscope, Mag. 50X). C: Trichome on the underside of a leaf showing fungal growth (Mag. 400X). D: Conidiophore in the stromatic body and elongated hyaline conidia (Mag. 400X).

Brown leaf spot caused by *Stagonospora* spp. *(Sacc.) Sacc.*

This was detected in cv. Desireé plants growing in the area of Chane (Province of Cordillera) in the north of the Dept. of Santa Cruz (Table 2) during the winter (March-August). The climate in this area is damp and temperate to temperate-cold (10-20°C), a consequence of cold winter winds blowing in from Argentina. Spots were few in number, and appeared alongside those caused by *A. solani*. The characteristic symptom is the formation of dark brown irregular spots with marked brown edges, that become elongated by coalescence (Figure 6A) and which may then acquire a yellowish halo (Figure 6A). Small, light or dark brown fruiting bodies (pycnidia) were visible within the spots (Figure 6B), partially embedded in necrotic tissue (Figure 6B). The conidia, released from the ostiole, were elongated (Figure 6C), hyaline to clear brown in color, and with several transverse septs (Figure 6D) and (Figure 7).

Discussion

The six causal agents of leaf spot diseases reported in this work are *A. solani* [11], *S. malagutii* [1], *Cercospora* spp. [12], Ellis [11], *P. concors* [14]; Ellis [11], *B. cinerea* [15], and *Stagonospora* spp. [16]. All have been reported in other Andean countries and indeed other regions of the world [2-4,8,9,17-20]. *Stagonospora* spp. is a new causal of agent of leaf spot in lowland Bolivia.

Most of the leaf spot diseases affecting Bolivia's potato crops were

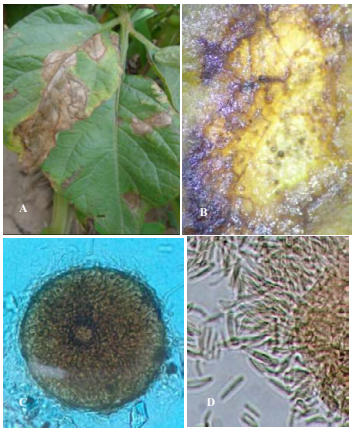

Figure 7: Leaf spot caused by *Stagonospora* spp. in the variety Desireé (*S. tuberosum* subsp. *tuberosum*). A: Irregular, clear-brown, elongated lesion on the upper side of the leaf. B: Formation of small pycnidia on the leaf surface (red arrow). C: Microscopic view of the pycnidia (Mag. 400X). D: Conidia with 2-4 septs (Mag. 600X).

found in the damp and cold highland areas of the Departments of La Paz and Cochabamba. This agrees with that reported by Turkensteen [4], Hoopes and Sage [3] and Hooker [2], who indicate them to be appear in Andean Bolivia, Peru, Ecuador and Colombia. In the lowland area examined leaf spot disease was also found, but much less commonly; this agrees with that reported by Hooker [2]. A relationship appears to exist between the appearance of disease and altitude/environmental conditions (dampness and cold temperatures). Note that khasahui, caused by *Septoria malagutii* (a synonym of *S. lycopersici*) was the only disease at altitudes of over 4000 m. Coca-Morante [9] indicates that this disease can cause 40% losses of production in high areas of the Department of La Paz. In addition, Torres [20] indicates that the spots caused by this disease appear in cold, wet areas at altitudes of 3800-400 m. However, Hooker [2], indicates that in Venezuela the disease occurs at 1600-2500 m, while in Ecuador it is reported to be seen mostly at over 3000 m [7].

The diseases caused by *A. solani, Cercospora* spp., *P. concors* and *B. cinerea* appeared in highland areas between 2600 and 3300 m in the Departments of La Paz and Cochabamba. That caused by *Stagonospora* spp. appeared at 250 m in the lowlands of Santa Cruz. Early blight caused by *A. solani* is found worldwide wherever potatoes are grown [2]. Neither leaf blotch caused by *Cercospora spp.*, nor gray mold caused by *B. cinerea*, have been previously reported in the Bolivian Andes. Grey mold is usually considered of minor economic importance in the region [2].

In the present work, leaf blotch caused by *P. concors* (Casp.) Sacc (Syn. *C. concors* [Casp.] Sacc.) was destructive under high humidity and cool temperatures. It has been previously reported in the highlands of Cochabamba [9]. It is also known in cool areas of Europe, the former Soviet Union, North America, in some restricted areas of Africa and Asia, and has recently been reported from China [21]. This disease is considered of minor importance, and may occur simultaneously with late blight (caused by *P. infestans*) or early blight (caused by *A. solani*).

Until now, *Stagonospora* spp. has never been reported to cause disease in Bolivia. For the time being it is of minor importance, and normally occurs simultaneously with other potato leaf disease such as late blight.

The present results suggest that leaf spot diseases in Bolivia should be monitored in order to prevent possible losses of production.

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