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The expansion of Brown Rot disease throughout Bolivia: Possible role of climate change

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Abstract.

Bacterial wilt is a devastating plant disease caused by the bacterial pathogen *Ralstonia solanacearum* species complex and affects different crops. Bacterial wilt infecting potato is also known as 'brown rot' (BR) and is responsible for significant economic losses in potato production, especially in developing countries. In Bolivia, BR affects up to 75% of the potato crop in areas with high incidence and 100% of stored potatoes. The disease has disseminated since its introduction to the country in the mid-80s mostly through contaminated seed tubers. To avoid this, local farmers multiply seed tubers in highlands because the strain infecting potatoes cannot survive near-freezing temperatures that are typical in the high mountains. Past disease surveys have shown an increase in seed tubers with latent infection in areas lower than 3000 m.a.s.l. of altitude. Since global warming is increasing in the Andes Mountains, in this work, we explored the incidence of BR in areas >3000 m.a.s.l. Results showed BR presence in the majority of these areas, suggesting a correlation between the increasing of disease incidence and increase of temperature and the number of irregular weather events resulting from climate change. However, it cannot be excluded the increasingly availability of latently infected seed tubers boosted the spread of BR.

Keywords: *Ralstonia solanacearum*, Brown Rot, climate change, Andean highlands, plant disease expansion

Cultivating potatoes is a challenging task since climatic factors and numerous diseases and pests impair the production. In terms of diseases caused by pathogenic bacteria, the disease called brown rot (BR) is one of the most serious for potatoes (Mansfield et al. 2012). Brown rot is caused by *Ralstonia solanacearum* (Smith, 1896, Yabuuchi et al. 1995), a complex of bacterial species that live in soil and enters the plant through wounds and secondary rootlets. Once inside the plant, the bacteria multiply, produce extracellular polysaccharides and effector proteins secreted by the type-three machinery, which causes wilting and subsequent death. *Ralstonia solanacearum* also attacks many other economically important crops such as tomatoes, eggplant, peanuts, bananas, ornamental and forest crops - collectively called bacterial wilt (Lebeau et al. 2011; Genin and Denny 2012).

R. solanacearum is considered a species complex formed by different strains, with wide genetic diversity and pathogenicity. Early efforts classified this species complex in 'races' and 'biovars' based on the host plant and the metabolic response to particular sugars and alcohols (French et al. 1995), however, modern phylogenetic studies have arranged it in four different phylogenetic groups called phylotypes (Fegan and Prior 2005). Recently, Safni and colleagues (2014) revisited the current taxonomy of this species complex splitting it in three different species: *Ralstonia solanacearum*, *Ralstonia syzygii* and *Ralstonia pseudosolanacearum*.

R. solanacearum is widespread in tropical and subtropical areas of the world because it grows at relatively high temperatures (around 28°C, Elphinstone 2005). However, the strain which preferentially infects potato is adapted to colder areas, probably because it originated in the northern Andean highlands (Clarke et al. 2015). This strain is commonly known as race 3 biovar 2 (R3bv2) and is responsible for considerable economic losses worldwide (about 1 billion US dollars per year, Elphinstone 2005). Direct yield losses have increased considerably in developing countries where no protective regulations exist or are not respected. To illustrate the damage that R3bv2 causes in developing countries, this work reviewed Bolivia, an Andean country, as an example.

In Bolivia, BR generates significant losses to potato production as it affects $\leq 75\%$ of potatoes in producing areas with high incidence and 100% of stored potatoes (Fernandez-Northcote and Alvarez 1993). This damage amounts to losses between US-\$300 and 1000 per hectare (Barea et al. 2008). Considering that potato production in Bolivia involves approximately 132,000 ha with an average yield of 6000 Kg per hectare (Balderrama 2009), the potential economic losses caused by BR are significant for Bolivia. Local Bolivian farmers reported the first potato plants with wilting symptoms in the mid-1980s, shortly after a major import of potato tubers by the national government from a neighboring country (Smith et al. 1998). The initial

outbreak occurred in the central Andes cordillera in the Chuquisaca region. From there BR spread throughout the country affecting many areas devoted to commercial potato production for human consumption (mostly lowlands, Figure 2). For several years, researchers from different institutions such as the Proinpa Foundation, Bolivia (Fernandez-Northcote and Alvarez 1993; Barea et al. 1999; 2008), the International Mycological Institute, United Kingdom (Smith et al. 1998) and the International Potato Center (CIP, Priou and Barea 2004) have performed inspections and diversity analyses of the *R. solanacearum* population in Bolivia. Most of the studies were focused on seed tuber propagation areas since potato seed tubers were distributed from this area to the rest of the country for commercial production. The prospecting studies performed on Chuquisaca region showed that 36% and 59% of the farm plots were infested with *R. solanacearum* during crop years 1992-93 and 1995-96 respectively (Barea et al. 1999). For the crop years 2001-2003, Barea and coworkers (2008) showed a BR increase of 18% in the same area, reaching about 70% of the land plots showing the disease. These results clearly indicated an important increase of the disease in propagation areas over the years. It is worth mentioning that these areas are located at an average altitude and temperature of 2300 m.a.s.l. and 11°C, respectively.

The problem. As potatoes are mostly multiplied through seed tubers, vegetative propagation is the main source of dissemination of *R. solanacearum*. This is made possible because the R3bv2 strain survives inside tubers for a considerable time, even at 4°C, which is considered a low temperature for *R. solanacearum* (Scherf et al. 2010). However, R3bv2 is not adapted to survive frequent near-freezing temperature fluctuations even inside tuber tissues (Scherf et al. 2010). With little to no scientific/technological guidance, Bolivian farmers realized that low temperatures avoided R3bv2 growth; hence, farmers usually multiply their seed tubers in very cold areas located at elevated altitude in the mountains. Extreme minimum winter temperatures in the Andean highlands reach -3 to -5°C which is a restrictive condition for R3bv2 survival (Scherf et al. 2010). Subsequently seed tubers are multiplied and distributed throughout the country for commercial production. This strategy has been successful for many years; however, effects from global warming are increasing the risk of disseminating BR throughout the country.

The spread of BR across the country may be promoted due to temperature increases at higher elevation and the escalation of erratic weather events as a result of climate change in response to global warming. There is strong evidence that air temperature has increased on an average by +0.11°C per decade over the past 60 years along the tropical Andean region. This tendency has intensified during the last decades (+0.34°C per decade, Vuille and Bradley 2000) and might persist in the future as predictive models suggest that a substantial increase of 5–6 °C may take place in the Andes by the end of the 21st century (Bradley et al. 2006). Other foreseen changes include a slight increase of precipitation through variable and more intense events in the highlands and a higher number of extreme events (Anderson et al. 2011). These climatic changes could favor *R.*

solanacearum survival in the colder climates of the Bolivian highlands allowing it to adapt to the new and possibly friendlier conditions. In turn, this might increase the dispersion of the pathogen beyond the border areas where it did not survive previously. In order to confirm this hypothesis, we conducted an analysis focused to detect *R. solanacearum* in the coldest areas of the country (above 3000 m.a.s.l.) where usually local farmers multiply potato seed tubers and compared the results with data collected in previous decades.

We monitored BR in the highland areas (mainly potato seed zones) of Bolivia according to administrative divisions of the area as municipalities. Seed tubers were collected from 47 locations corresponding to 16 municipalities with an average altitude and temperature of 3,470 m.a.s.l. and 8.3°C respectively. In these municipalities, temperature falls below 0°C during the morning hours especially in winter (June, July, and August). Local farmers cultivate BR-susceptible varieties of potato (there is no broad and stable resistance to *R. solanacearum* in potato, Huet 2014). The selection criteria for municipalities were that they are seed producing areas for potato located above 3,000 m.a.s.l. During the winter of 2013, various farmers' fields were selected randomly per municipality to sample tubers according to availability (Table 1). The bacteria were isolated from seed tubers by means of culturing in Kellman semi-solid medium supplemented with 2,3,5-triphenyl tetrazolium chloride (0.005% w/v). After the incubation period (48 h, 28°C), the bacterial colonies showing typical appearance (*i.e.* fluidal with a pale red center and large white border) were diluted in sterile, distilled water (50 µl) and boiled for 5 min. Three microlitres of the boiled bacterial suspension was used to perform PCR reactions using specific and tested primers (759 and 760, Opina et al. 1997) to detect the pathogen. This strategy has proven useful for detection of *R. solanacearum* and for sanitary surveillance of planting material (Umesha et al. 2015). The results indicated that the incidence of BR is high since 75% of municipalities showed evidence of the presence of the pathogen; however, contaminated tuber percentage is low (on average 6.66 % of the tubers showed the pathogen out of 1085 tubers analyzed, see Table 1). These results indicated that although fewer tubers are infected, *R. solanacearum* is present in the majority of the coldest areas of the country. The biovar of each isolated strain was determined using Hayward's medium to assess acidification of various disaccharides and sugar alcohols as described previously (French 1995). As expected, R3bv2 (phylotype II) was the predominant group found in the seed tubers.

Results obtained in this work are alarming due to the presence of latent disease in potato seed tuber production areas. These areas are located at a higher altitude (over 3,000 m.a.s.l.) than those analyzed in previous studies (Fernandez-Northcote and Alvarez 1993, Barea et al. 1999; 2008; Priou et al. 2004). Barea and coworkers (2008) affirmed that the disease has never been found during surveys (2001-2003) in areas above 3000 m.a.s.l., but only in areas of lower altitude ($\leq 2,300$ m) as described previously. This implies that the pathogen is progressively more capable of survival at higher altitudes,

which alludes to a probable link with climate change. Recent studies have shown that in the Andean area, climate change is causing minimum (rather than maximum) temperatures to rise (Vuille et al. 2008), accompanied by irregular climate events (short periods of warmer temperatures in midwinter, reduction in the number of cold days, etc.; Anderson et al 2011). Under such conditions it would not be unusual to observe an increase of BR since it is well known that the disease increases with increasing temperatures (French et al. 1998).

Previous and current data allowed recreating BR spread throughout Bolivia since the first outbreak and a projection for the future (Figure 2). The predicted increase of BR spread in areas of potato production were based on data from 1983, 2003 and 2013 and an estimate of the future scenario was projected applying the ecological niche model (calculated using the Ecocrop tool from the Food and Agriculture Organization, <http://ecocrop.fao.org>). The predictive study for 2050 suggests that virtually each potato producing area of the country would be affected by BR if control measures are not implemented.

Disease Management. How could the spread of BR be controlled in Bolivia? Generally, BR is difficult to eradicate, however some alternatives are available. Some potato cultivars show moderate resistance (tolerance) to *R. solanacearum* strains; however, tubers frequently harbor latent infection and, therefore, are not useful for seed tuber multiplication programs. Consequently, agricultural practices rely mostly on utilization of disease-free tubers as starting material for seed tuber multiplication programs, use of amendments (manures), crop rotation with non-host plants, control of weeds and nematode populations, discard plant debris and residues, prevent flow of contaminated water, work on adequate soil drainage, selection of appropriate planting time to avoid heat and other agricultural practices (Saddler, 2005). Biological control agents have a proven, wide-range biocontrol efficacy; therefore a combined application with different organic matter (manures, animal waste, etc.) was more effective in controlling or suppressing the disease (Yuliar et al. 2015). A program for permanent alerts and monitoring should be established to minimize risk that includes the use of a method for early diagnosis of the disease and implements participatory mechanisms of technological diffusion among farmers. Precise and highly sensitive methods of diagnosis are based on molecular and immunology technologies which offer faster results than conventional methods at a cost equivalent to these techniques (Miller and Martin 1988). Agricultural institutions (such as the National Institute for Agriculture and Forestry, the Proinpa Foundation and others) should identify affected areas in the country, provide healthy potato seed tuber to farmers and train farmers in control practices. The achievements should be disseminated among producers and national institutions involved in plant health (National Agricultural Health and Food Safety Office, called SENASAG for its acronym in Spanish).

Conclusion. Results generated in this work suggest that climate change may be influencing the expansion of the BR frontier in the Bolivian highlands. However, it cannot be excluded that the pathogen is adapting or evolving as a result of selective pressures of potato cropping at higher altitude where climate change would impose additional selective forces. Alternatively, the spread of the pathogen could be due to an increasing availability of latently infected seed tubers to the previously pathogen-free areas. Thus, the results shown in this work should be considered as a starting point for more extensive studies to understand the possibility of and extent of climate change impacting on the spread of BR and other plant diseases.

Considering that BR is one of the most devastating diseases and the significant losses that it causes, the results shown in this work imply a serious threat to the production of potatoes and other crops (*e.g.* tomato) in Bolivia. Immediate and resolute control actions by agricultural and plant protection authorities to reduce the impact of BR would have significant short and long term benefits for Bolivian producers.

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Figure and table captions.

Figure 1. Map of Bolivia showing the specific municipalities that were surveyed in this work. Sample locations (gray dots) and positive presence of BR (dots with black thick edge).

Figure 2. Distribution of BR in Bolivia: past stages (1993, 2003), current (2013) and a projection (2050) based on a scenario using the Ecocrop tool from the Food and Agriculture Organization, <http://ecocrop.fao.org>. BR is denoted as a gray smear on the maps.

Table 1. Results of detection of *Ralstonia solanacearum* using standard PCR techniques in six main geographic regions and subregions of Bolivia devoted to the production of potato seed tuber.

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Table 1.

Departament ^a	Municipality	Locations	Number of analyzed tubers	Number of positive tubers	Percentage of positive tubers
Cochabamba	Tiraque	5	18	1	5.56
	Colomi	2	28	3	10.71
	Vacas	2	98	11	11.22
	Morochata	7	242	18	7.44
	Cocapata	1	67	6	8.96
	Pocona	2	12	2	16.67
	Totora	1	12	1	8.33
Chuquisaca	Tarabuco	6	160	10	6.25
	San Lucas	1	12	0	0.00
	Villa Serrano	3	30	0	0.00
La Paz	Patacamaya	2	28	1	3.57
	Achacachi	1	38	0	0.00
Oruro	Challapata	1	36	0	0.00
	San Pedro de Totora	3	24	4	16.67
Potosí	Villazón	7	40	3	7.50
Tarija	El Puente	3	240	9	3.75
Total	16	47	1085	58	
Average					6.66

^a Department is the biggest political and administrative division of Bolivian territory.