Symptomatology associated with "Purple top", an emerging disease of solanaceous fruit species

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ABSTRACT

"Purple top" is an emerging disease that affects solanaceous crops, reported mainly on *Solanum tuberosum* (potato). Additionally, *Bactericera cockerelli* has been reported as the insect vector of *Candidatus* Liberibacter solanacearum (*Ca*Lso), a pathogen that has been associated with this disease. However, the information about this disease in Andean fruit species such as *Solanum betaceum* (tree tomato), *S. quitoense* (naranjilla) and *Physalis peruviana* (cape gooseberry) is almost nil. This study was carried out in the Tumbaco Experimental Farm of INIAP to describe the phenotypic symptoms caused by this disease. Molecular diagnosis by PCR method was carried out to diagnose the causal agent associated with the symptomatology. The main symptoms observed were purple color leaves, smaller apical leaves, inward curvature edge leaves, over-sprouting, yellowish and stopped plant growth; however there also were asymptomatic plants. *P. peruviana* was the most susceptible species because showed 100% of incidence. *Ca*Lso was identified as the causal agent associated with this disease. These results contribute to understanding the expression of this disease in Andean fruit crops. However, more studies related to epidemiology, diagnostic methods, mixed infections, vector-pathogen interaction and disease control are required in order to generate information that allows a complete knowledge of this pest.

Keywords: diagnosis; *Physalis peruviana*; *Solanum betaceum*; *Solanum quitoense*; vector.

INTRODUCTION

"Purple top" is a worldwide emerging disease that affects Solanaceae crops, mainly potato crops (*Solanum tuberosum*), and has recently been reported in Ecuador (Castillo, 2019). This disease is associated with the potato psyllid *Bactericera cockerelli* (Šulc) (Hemiptera: Triozydae), an insect vector of North American origin, which has migrated from Mexico, Guatemala, Honduras, Nicaragua (Rubio *et al.*, 2006) and is already in Ecuador (Castillo *et al.*, 2019; EPPO, 2020). Their eggs are ovoid in shape with a shiny chorion, presenting at one end a small filament with which they adhere to the surface of the leaves (CABI, 2022). Adult males have a length between 2.8 and 2.9 mm (including wings); whereas adult females have between 2.8 and 3.2 mm (Department of Agriculture Australian Government, 2012). *B. cockerelli*, when feeding, injects its saliva that contains toxins that induce yellowing and curling of the leaves, and stunted growth (Li & Trumble, 2006) and can also introduce pathogens into the plant.

In potato, purple top has been related to infection caused by a phytoplasma (Caicedo *et al.*, 2015; Castillo *et al.*, 2018). Since phytoplasmas could not be isolated

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and cultivated in artificial media, a special condition was created in them in which taxonomists give them a category of '*Candidatus*' (Reveles *et al.*, 2014). Due to this fact, as of 2004 the scientific name to refer to phytoplasmas is established as *Candidatus* Phytoplasma (Firrao *et al.*, 2004).

On the other hand, *Candidatus* Liberibacter solanacearum (*Ca*Lso) is an emerging pathogenic bacterium that causes significant losses in crops worldwide (Hajri *et al.*, 2017). *B. cockerelli* is considered the vector of this pathogen (Hansen *et al.*, 2008; Vallejo, 2020) and it has been reported affecting potatoes (Liefting *et al.*, 2008a) and Solanaceae fruit crops (Liefting *et al.*, 2008b; Caicedo *et al.*, 2020).

Due to the complexity of the causative agents and their vector, this disease is difficult to control and detect, and has caused damage to crop and significant yield losses. These emerging diseases caused by new organisms such as those above mentioned, have become of increasing importance in many production areas worldwide, and lately increasingly in Latin America (Pérez-López *et al.*, 2016).

Currently in Ecuador, symptoms of this disease has been observed in plants of fruit crops such as tree tomato (*S. betaceum*) and cape gooseberry (*Physallis peruviana*); however, the information is almost nil and there is nothing about naranjilla (*S. quitoense*) and their related species. For this reason, the objective of this study was to describe the symptoms associated to this pathological problem.

MATERIALS AND METHODS

Location of the experimental site

This research was carried out at the Tumbaco Experimental Farm of the National Institute of Agricultural Research (INIAP), located in the province of Pichincha, latitude 00°12′ South, longitude 78° 24′ West, altitude 2348 m a.s.l., annual rainfall of 800 mm, average temperature of 17 °C and average relative humidity of 75%.

Plant material

Independent experimental plots were established with fruit crops of the Solanaceae family, which are of economic importance in Ecuador. The crops studied were tree tomato (*S. betaceum*), naranjilla (*S. quitoense*), and cape gooseberry (*P. peruviana*). The first plot was constituted by segregants of *S. betaceum* x *S. unilobum* backcrossed by *S. betaceum* (20 individuals). The second plot was formed by segregants of *S. quitoense* (5 individuals), segregants from the cross *S. quitoense x S. hyporhodium* (5 individuals), from the cross *S. quitoense x S. hirtum* (5 individuals), and segregants of *S. hirtum* (10 individuals). The third plot was constituted by segregants of *P. peruviana*, the plot was constituted by segregatns (20 individuals) of this species.

In the nursery, a batch of 50 nursery plants of *P. peruviana* (2 months-old after transplanting to plastic bags) was also evaluated because they showed purplish colour leaves; seedlings of the other species did not show any symptom at this stage thus they were not analysed.

The plants of the three Solanaceae crops were planted at a distance of 2 x 2 m. Fertilization was done with urea (46% N) applying 81 g plant^{-1} divided into four applications, P_2O_5 applying 98 g plant^{-1} divided into two applications, K_2O applying 87 g plant^{-1} divided into three applications and weekly watering (21 L plant^{-1}) were given. Preventive phytosanitary controls were carried out in all plants in the three experimental plots with copper to avoid the appearance of foliar diseases and no insecticide was applied to avoid affecting the presence of *B. cockerelli.*

Bactericera cockerelli identification

Three months after the experimental plots were established, the presence of *B. cockerelli* was monitored through the capture of five adult insects with an entomological mesh in each plot, in this way the existence of the vector in the research place was confirmed. They were analysed in the Entomology Laboratory of the Phyto and Zoosanitary Regulation and Control Agency (Agrocalidad) and the identification was done by taxonomic keys and specialized literature (Hodkinson & White, 1979; OIRSA, 2015).

Description of symptoms

There is not a scale to evaluate the presence or severity of purple top on fruit crops; for this reason, the results of this research were used to elaborate a scale to qualify the occurrence of symptoms in the three species for further studies.

At 12 months after field transplanting of *S. betaceum* and *S. quitoense* and hybrids, and at 8 months in the case of *P. peruviana*, the incidence of symptoms associated to the disease was recorded by visual inspection of the individuals and described by relating them to those reported in the literature (Butler & Trumble, 2012; Caicedo *et al.*, 2020; Castillo, 2019).

Molecular diagnosis

Molecular diagnosis to identify the causal agent associated to the disease was carried out in symptomatic (purple

color leaves, smaller apical leaves, inward curvature edge leaves, over-sprouting, yellowish and stopped plant growth) and asymptomatic samples. The analysis included the diagnosis of *Phytoplasma* sp. and *Ca*Lso in the Molecular Biology Laboratory of Agrocalidad.

Five to ten leaves (symptomatic or asymptomatic depending on the case) were selected randomly from the field sample. They were ground using liquid nitrogen and a 100 mg sample of leaf ribs and leaf area were taken. The sample was homogenized grinding it with liquid nitrogen. The total DNA was extracted using the commercial kit DNeasy Plant Mini Kit ® (Qiagen). The total DNA concentration was estimated with a NanoDrop 2000.

For the case of *Phytoplasma* sp., the protocol published by Christensen *et al.* (2013) was used. This protocol uses the real-time PCR method with the following primers: 16S-F 5′-CGTACGCAAGTATGAAACTTAAAGGA-3′ (forward), first reverse 16S-R 5’- TCTTCGAATTAAACAACATGATCCA- 3’ (reverse), and 16S-P 5’-FAM-TGACGGGACTCCGCACAAGCG-BHQ1-3’ (probe) based on the 16S region of rDNA. The reaction was carried out under the following conditions: 1X Probe Master Mix (Roche), 0.3 µM of each primer and 0.2 µM of probe. Real-time PCR amplification was carried out on a CFX 96 (Biorad) with 1 cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 sec, 60 °C for 1 min, and a cycle of 40 °C for 10 sec.

In the case of *Ca*Lso, a protocol based on the conventional PCR method and described by IPPC (2017) was used. In this protocol, an 1163 bp fragment of the 16s rRNA gene was amplified by the primer LsoF 5′-GTC GAG CGC TTA TTT TTA ATA GGA-3′ (forward) (Li *et al.*, 2009) and OI2c 5′-GCC TCG CGA CTT CGC AAC CCA T-3′ (reverse) (Jagoueix *et al.*, 1996). The PCR reaction contained 1X of PCR buffer, 1.5 mM of MgCl2, 0.2 mM of dNTPs mix, 1.25 units of GoTaq® G2 Hot Start Polymerase (Promega) and 2 µl of DNA. PCR amplification was performed in 1 cycle at 95 °C for 3 min, 35 cycles at 94 °C for 30 sec, 62 °C for 30 sec and 72 °C for 1 min, and one cycle of 72 °C for 10 min, using a T100 thermal cycler (Biorad). The PCR products were visualized on a 1.5% agarose gel and stained with Syber Safe (Invitrogen) with a molecular weight marker of 1kb and 100bp.

RESULTS

Bactericera cockerelli

All five specimens collected in each plot were confirmed as *B. cockerelli.* Adults showed a blackish coloration, with long wings that exceed the body, presenting two white stripes on the dorsal part of the abdomen which is the main trait of this species (Figure 1 A, B and C).

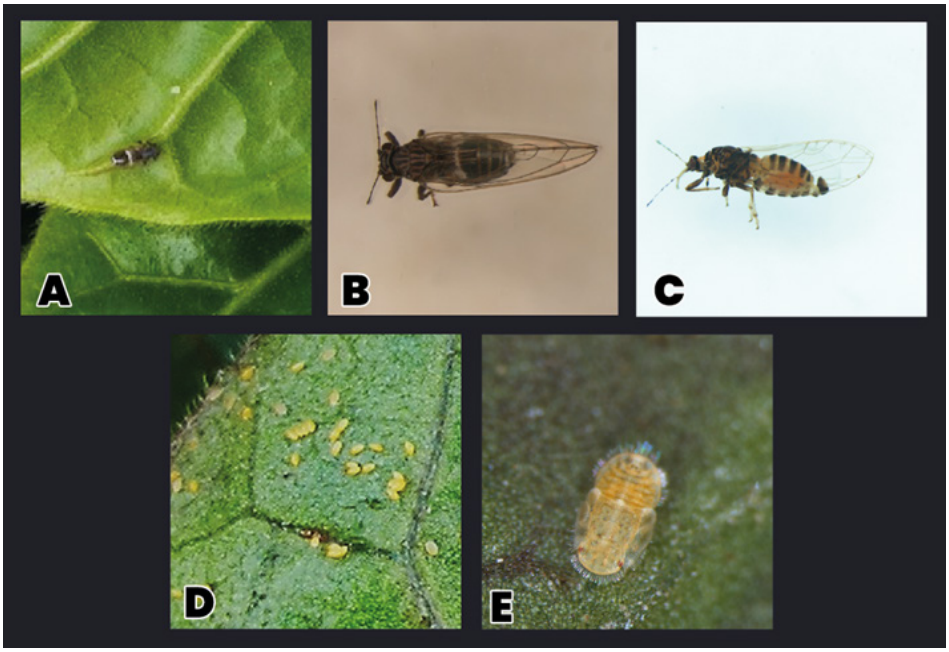

Figure 1: *Bactericera cockerelli* present on leaf (A), adult insect (B-C), eggs and nymphal stages on the underside leave (D), and nymphal of fourth stage (E).

Abundant egg positions were found on the underside of the leaves, they were whitish and yellowish, oval and had a short filament that was attached to the leaf. The nymphal forms of the insect were yellowish and flat (Figure 1D and E).

Symptoms in tree tomato (Solanum betaceum)

Healthy tree tomato plants had larges leaves and the apical leaves have purple color which is the normal grown in this crop and it is not caused by the disease in study (Figure 2A and B). Furthermore, fruiting was normal. There was an 85% incidence of plants with symptoms related to purple top, one segregant had no symptoms and the other two showed a severe virus affection.

In plants that showed symptoms different from normal, it was observed that the lower leaves remain normal, but in the apex there was a change in the purple color of the smaller apical leaves to more reddish-pinkish tints and slight inward curvature edge (Figure 2C and D). In this case, just leaves showing this kind of coloration has to be taken to carry out a correct sampling to do the molecular diagnosis and avoid false negatives. On the other hand, the larger apical leaves showed an inter-rib yellowing in the bundle, which stands out more when viewed through a bright light (Figure 2E and F).

In other cases, a proliferation of apical shoots with reduced leaves was observed, a symptom known as witch's broom (Figure 2G). As the disease progresses, the apical leaves take pink or reddish colors (Figure 2H), the older leave.s begin to take a yellow color and deform, this problem causes the detention of the general growth of the plant.

In some plants, an over-sprouting of stems and a stopping of plant growth was observed (Figure 2I and J). In some cases, there was also yellowing of the leaves.

There were two plants showing severe blistering and curling on the leaves (Figure 2K) which suggested a virus infection possibly by potato leaf roll virus (PLRV) (Sivaprasad *et al.*, 2016; Espinoza, *et al.*, 2017). These plants were negative for *Ca*Lso in the molecular analysis (Table 2); it has been suggested an antagonism between virus and some *Candidatus* species when are both infected plants (Ebadi *et al.*, 2020); however further studies are required to confirm this hypothesis.

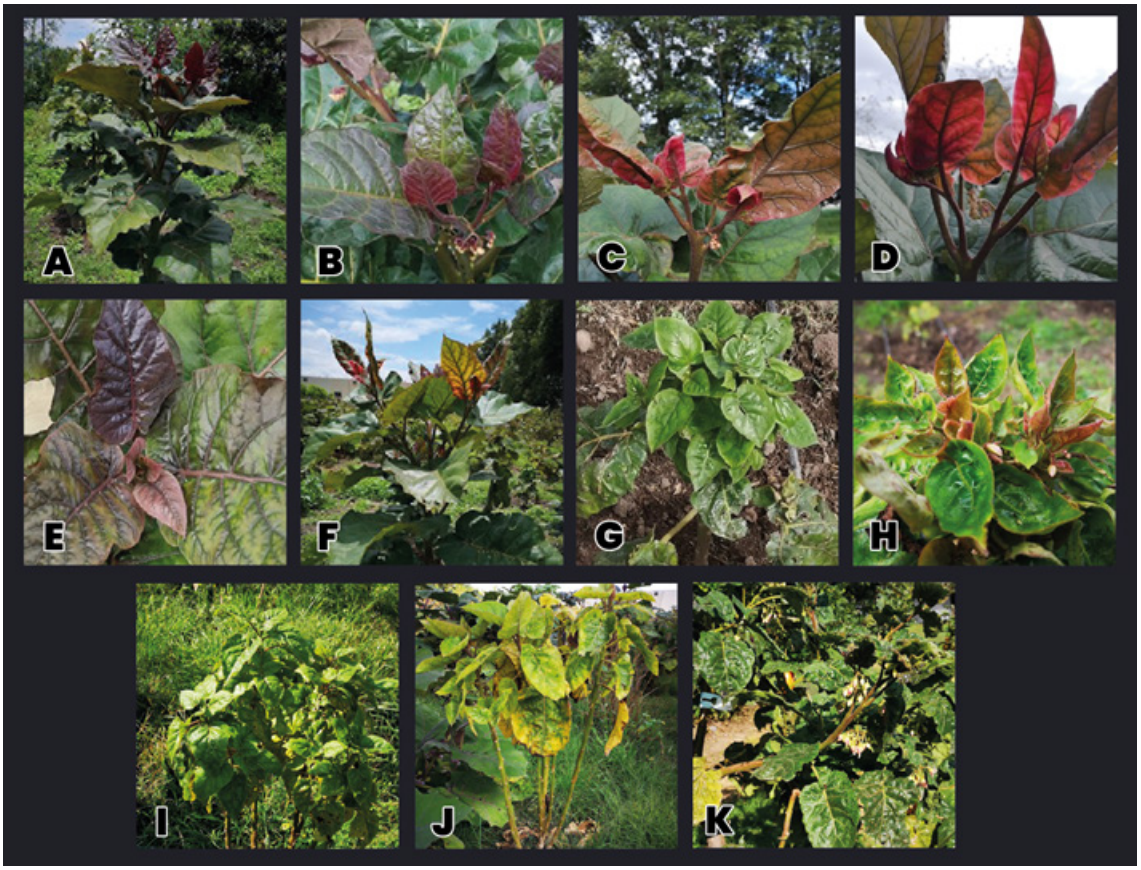

Figure 2: Symptomatology observed in tree tomato (*Solanum betaceum*). Plant with normal growth and leaves in the lower part (A), apical leaves with the characteristic purple color (B), color change of apical leaves with curling symptoms and reddish-pinkish tints in the leaves (C, D), presence of inter-rib yellowing (E) which stand out when observed through light (F), proliferation of apical shoots and reduction of the size of the leaves (G), pink and reddish leaves (H), over-sprouting of stems, stopping of plant growth and yellowing (I, J), and plant with symptoms of severe virus infection such as blistering and deformation of leaves (K).

Symptoms in naranjilla (Solanum quitoense)

S. quitoense and some of its hibrids with *S. hyporhodium* and with *S. hirtum* did not show visual symptoms in all plants, consequently there were asymptomatic plants; just *S. hirtum* segregants showed clear symptomatology (over-sprouting) in all plants. The 100% of *S. quitoense* plants were asymptomatic; while hybrids with *S. hirtum* showed 80% of symptomatology incidence, hybrids with *S. hyporhodium* showed 40%, and *S. hirtum* segregants showed 100%.

An asymptomatic healthy plants of *S. quitoense* showed normal growth, green colored leaves and normal fruiting (Figure 3A); however, it resulted positive for *Ca*Lso (Table 2). One hybrid with *S. hirtum* (Figure 3B) was asymptomatic but it also resulted positive for *Ca*Lso and the others showed curling leaves and yellowish (Figure 3G and H), which could indicate that these hybrids had more genetic contribution from *S. hirtum* because this species was susceptible to the disease. In the case of hybrids with *S. hyporhodium*, most of them were asymptomatic showing green leaves and normal fruiting but they result positive to *Ca*Lso; nevertheless, a few showed inward curve edge leaves and yellowing of the edges (Figure 3C and D), but these symptoms could also be associated to the presence of virus (Ramos *et al.*, 2020) thus more research to determine mixed infections is need. Although this disease has been named as purple top, in the case of *S. quitoense* hybrids, no purple coloration was observed in the foliar area, and because the fruiting was normal, it could infer they act like host plant how was mentioned in other studies in fruit crops (Liefting *et al.*, 2008b). In *S. hirtum* segregants an over-sprouting of stems and apical leaves was observed, which showed deformed edges and reduction in size (Figure 3E and F).

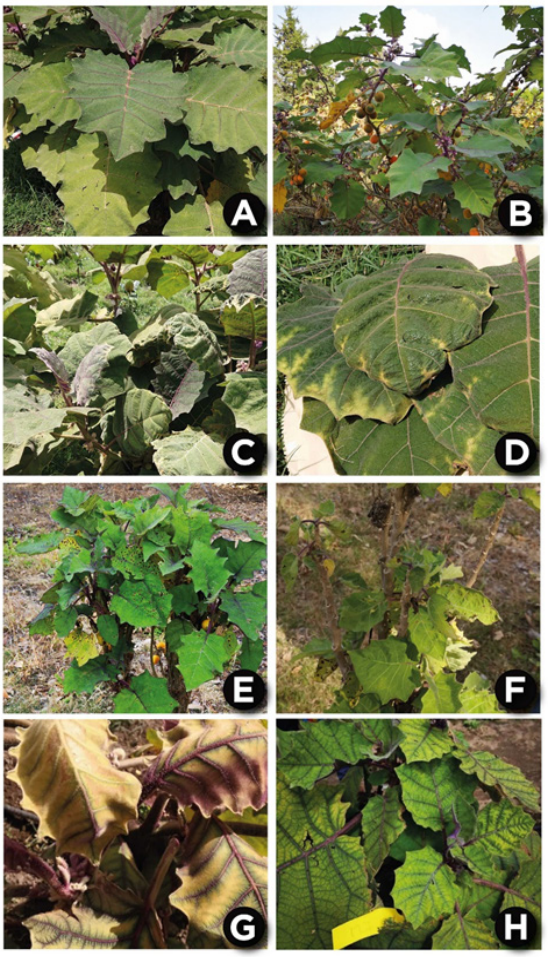

Figure 3: Symptomatology observed in naranjilla (*Solanum quitoense*). Asymptomatic *S. quitoense* plant with normal growth (A); asymptomatic hybrid of *S. quitoense* x *S. hirtum* (B), hybrid of *S. quitoense* x *S. hyporhodium* showing inward curvature edge leaves (C) and yellowish edges (D); *S. hirtum* showing over-sprouting (E) and size reduction and edge deformation of the apical leaves (F), hybrid of *S. quitoense* x *S. hirtum* showing inward curvature edge leaves (G) and inter-rib yellowing (H).

Symptoms in cape grooseberry (Physalis peruviana)

In the field, it was observed plants without symptoms (Figure 4A) at the 6 months after transplanting; however, after 8 months all plants showed symptoms (Figure 4B) (100% of incidence) but in different grade which could indicate that segregants had distinct response to the disease severity.

Plants with symptoms showed a purple coloration in the leaves, although in the beginning this coloration was observed in only one part of the plant and with the time it progressed, consequently this symptom spread throughout the plant and produced total withering and death (Figure 4C). For this reason, in plants where there was healthy and affected plant tissue (Figure 4D and E), samples were taken from both and their results were opposite (Table 2) even though they were from the same plant. This result would indicate that the accuracy of the molecular analysis is associated to symptomatic samples, consequently this condition has to be take into consideration to avoid false negatives because of field sampling.

On the other hand, 64% cape gooseberry seedlings from the nursery showed leaves with purplish discoloration (Figure 4F) and the rest had green leaves (Figure 4G) that showed a normal appearance of the seedling. The molecular diagnosis of both types of plants showed a negative result for both *Phytoplasma* sp. as for *Ca*Lso (Table 2); the diagnosis analysis was repeated after 15 days, obtaining the same negative results. This allowed to conclude that this case of purplish discoloration was due to a possible deficiency of phosphorus and boron according to the similar symptomatology described by Martínez *et al.* (2009). Therefore, a foliar fertilization (NPK 25-16-12 + B, Cu, Co, Fe, Mn, Mo and Zn) was carried out in these seedlings, and 30 days later leaves lost the purplish discoloration, and a green leaves were observed again in 100% of the plants in the batch (Figure 4H)

In base of the different type of symptoms, it is proposed the following scale (Table 1) to qualify the occurrence of this disease in the field. The scale considers slight symptoms for lower values and severe symptoms for the highest.

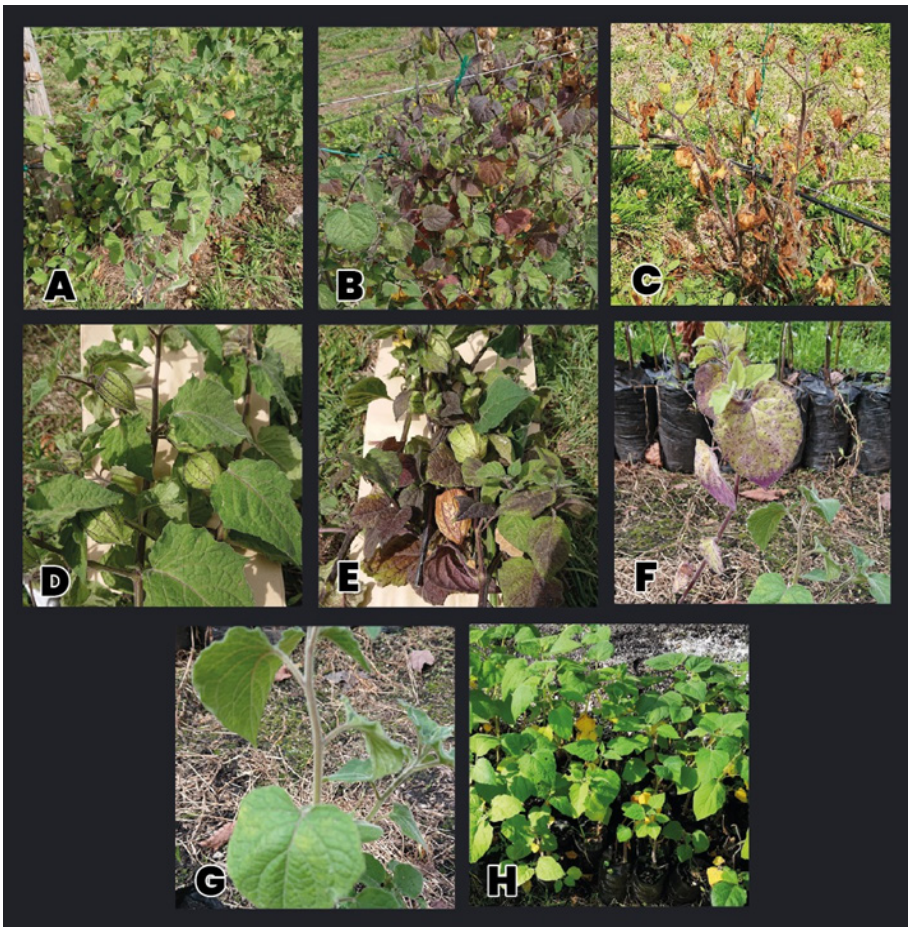

Figure 4: Symptomatology observed in uvilla (*Physalis peruviana*). Plant without symptoms (A), purple leaves on the 50% of the plant (B), death plant (C), leaves without symptoms (D), leaves with purple coloration (E), nursery plant with purple leave coloration (F), nursery plant without symptoms (G), and batch after foliar fertilization (H).

Table 1: Scale to qualify the occurrence of symptoms in the three species of Solananceae fruit crops

Symptom Type*	Scale	Figure
Tree tomato (*Solanum betaceum*)		
Plants without symptoms (negative molecular diagnosis is required to fit in this value)	0	2 A
Reddish-pinkish apical leaves	1	2 H
Reddish-pinkish apical leaves and inward curvature edge	3	2 C
Presence of inter-rib yellowing	5	2 E, 2 F
Proliferation of apical shots and stopping of plant growth	7	2 G
Proliferation of apical shots (over-sprouting) and yellowing	9	2 I, 2 J
Solanum quitoense (Naranjilla) and relative species		
Plants without symptoms (negative molecular diagnosis is required to fit in this value)	0	3A
Asymptomatic plant but positive result in molecular diagnosis	1	3 B
Inward curvature edge leaves and slight yellowing	3	3 C, 3 D
Presence of inter-rib yellowing	5	3 H
Severe inward curvature edge leaves and yellowish	7	3G
Oversprouting, size reduction and edge deformation of the apical leaves	9	3 E, 3 F
Uvilla (*Physalis peruviana*)		
Plants without symptoms showing green leaves (negative molecular diagnosis is required to fit in this value)	0	4 A
Plants showing less than 50% of purple leaves	1	na
Plants showing more than 50% of purple leaves	3	4 B
Withered and dead plant	5	4 C

* Symptoms can be observed independently in different plants into the same plot.
na= Figure no available.

Table 2: Results of the molecular analysis for the diagnosis of Candidatus Liberibacter solanacearum in the different species of Solanaceae

Crop	Symptomatology	Figure	Result
Solanum betaceum	Plant without symptoms	2 A	Negative
	Apical leaves with reddish-pinkish coloration	2 C, 2 D, 2 E	Positive
	Over-sprouting	2 G, 2 I, 2 J	Positive
	Plants showing severe virus symptoms (blistering and curling leaves)	2 K	Negative
Solanum quitoense	Plants without symptoms (asymptomatic)	3 A	Positive
S. quitoense x *S. hirtum* (hybrid)	Plant without symptoms	3 B	Positive
	Plants showing inward curvature edge leaves and inter-rib yellowing	3 G, 3 H	Positive
S. quitoense x *S. hyporodium* (hybrid)	Plants without symptoms* and showing inward curvature or yellowing edge leaves	3 C, 3 D	Positive
Solanum hirtum	Over-sprouting	3 E, 3 F	Positive
Physalis peruviana	Leaves without symptoms (6 month old plant)	4 A	Negative
	Leaves with purple color (8 month old plant)	4 B	Positive
	Branch with leaves without symptoms**	4 D	Negative
	Branch with leaves showing purple coloration**	4 E	Positive
	Seedlings from nursery without symptoms (green leaves)	4 G	Negative
	Seedlings from nursery showing leaves with purple coloration	4 F	Negative

* Figure not available but can be referenced with 3B (hybrid). ** Samples from the same plant.

Diagnosis by molecular analysis

Table 2 shows the results obtained from the diagnosis by molecular analysis, most of the samples amplified at 1163 bp thus they were positive for the presence of *Ca*Lso (Figure 5). Regarding to the diagnosis of *Phytoplasma* sp., a negative result was obtained in the PCR-real time for all analyzed samples (Ct = 0).

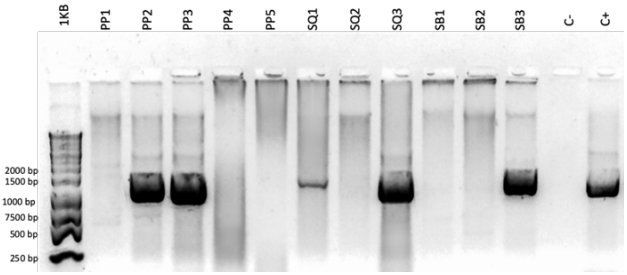

Figure 5: Electrophoresis gel showing the amplification of some samples of Solanaceas at 1163 bp (*Ca*Lso positive). PP= Physalis peruviana, SQ= Solanum quitoense and hybrids2, and SB= Solanum betaceum.

DISCUSSION

Phytoplasmas are bacteria that inhabit the phloem of plants and the hemolymph of a high diversity of insects (IRPCM, 2004). They are associated with a wide variety of symptoms such as apical foliar curl, short internodes, purple coloration of the leaflets, chlorosis, proliferation of axillary shoots (witch's broom), stem thickening, virescence (development of green flowers and loss of pigments), phyllodia (conversion of flowers to leaves) and specifically in potatoes the formation of aerial tubers (Himeno *et al.*, 2014; Kumari *et al.*, 2019).

Although *Phytoplasma* sp. was not detected in the analyzed samples in this study, phytoplasmas associated with purple top have been reported in other Solanaceae such as *S. tuberosum* (Caicedo *et al.*, 2015; Castillo *et al.*, 2018); therefore, more studies on methods for molecular detection (specific primers) are needed to explore the possibility of more pathogens associated to this disease.

The mechanism responsible for the purple coloration in the leaves called 'purple top' corresponds to the activation of anthocyanin biosynthesis (Teng *et al.*, 2005) as a defense response of the plant against infection. In this study, different symptoms such as pinkish apical foliar coloration, inward curvature edge leaves, small apical leaves, yellowing, interveinal thinning, over-sprouting and stopped plant growth were mainly expressed in *S. betaceum*; while the predominant characteristic in *S. hirtum* plants was over-sprouting; and the purple coloration of the leaves was in *P. peruviana*. *S. quitoense* was the only species that presented a positive diagnosis but having asymptomatic plants, and in the main symptomatology in its hybrids with *S. hirtum* and *S. hyporhodium* was basically the inward curvature edge leaves and yellowing of the edges of the leaves. The symptoms described concur with those reported by Caicedo *et al.* (2020) in tree tomato and cape gooseberry crops. However, research related to the symptoms caused by the vector has been also done because it has been reported that *B. cockerelli*´s toxins can also induce yellowing and curling leaves (Li & Trumble, 2006).

*Ca*Lso has been reported in *S. tuberosum* causing zebra chip (Liefting *et al.*, 2008a; Workneh *et al.*, 2020), and yellowing and wilting in *S. betaceum* and *P. peruviana* plants in Ecuador (Caicedo *et al.*, 2020); besides Liefting *et al.* (2008b) found this pathogen in asyntomatic *S. betaceum* and *P. peruviana* plants grown in New Zealand. In this study, the results in *S. betaceum* and *P. peruviana* agree with that mentioned by Caicedo *et al.* (2020); in addition, it was also found the presence of this pathogen in other solanaceous species of importance in Ecuador such as *S. quitoense* and its hybrids, and *S. hirtum* (rootstock for *S. quitoense*).

Phylogenetic analysis of *Ca*Lso strains from *S. tuberosum* has shown that Ecuadorian strains are related to strains from Canada and New Zealand (Castillo *et al.*, 2021). Vallejo (2020) carried out a phylogenetic analysis with samples of *S. tuberosum*, *Capsicum annum*, *S. betaceum* and *P, peruviana*, founding that the haplotype A of *Ca*Lso is present in Ecuador; this haplotype is present in South, Central and North America (EPPO, 2020). However, more studies using different strains obtained from solanaceous fruit species grown in different sites of production are needed to know the relationship with foreign strains and their geographical distribution.

According to the experience of this study, it is recommended to take samples (mainly leaves) showing clear symptomatology (described in this paper) for the molecular diagnose of *Ca*Lso; otherwise false negative diagnosis could occur because this bacterium is phloem-limited (IPPC, 2017) and it seems that an accurate analysis can be carried out only with adequate pathogen concentrations in the sample. In addition, it is recommended to carry out more studies using different types of specific primers for improving *Ca*Lso PCR detection (Ravindran *et al.*, 2011).

B. cockerelli was collected at the study site, and it

would be associated with the transmission of *Ca*Lso that was identified by molecular diagnosis, which is consistent with what reported by Hansen *et al.* (2008) and Vallejo (2020). List (1939) reported that the temperature of 26.6 °C is very favorable for the development and survival of these insects; while temperatures below 15.5 °C and above 32.2 °C are not favorable. The Tumbaco Experimental Farm has an average temperature of 17 °C but rises to 27 °C during the morning, having adequate conditions for the presence of this vector at this site.

The management of purple top in open field has been based mainly on the control of the insect vector through systemic insecticides and other practices based on periodic monitoring (Butler & Trumble, 2012). The most suitable system for monitoring the populations of immature stages (eggs and nymphs) is the direct weekly sampling of leaves, which must be cut from the middle part of the plant and carefully checked to determine the numbers of eggs and nymphs of different sizes. In the case of adult populations, yellow traps with glue, water traps (yellow trays) and/or the use of an entomological net are used. It is necessary to eradicate plants with initial symptoms to avoid reinfection and eliminate weeds (Vereijssen *et al.*, 2018) because *B. cockerelli* has a wide range of hosts. In addition, Calvo *et al.* (2016), Ramírez-Ahuja *et al.* (2017) and Vereijssen *et al.* (2018) have reported that there are some parasitoids that can carry out biological control of this insect. On the other hand, it is recommended that inside greenhouses there should be no broken plastic and mesh that allow the vector to enter; in addition, yellow or neon orange traps should be placed to carry out the monitoring (AlJabr, 2007).

CONCLUSIONS

Purple top is a disease that has not been widely studied in Andean fruit species of the Solanaceae family. The main symptoms related to the presence of this disease in the studied fruit crops were purple color leaves, smaller apical leaves, inward curvature edge leaves, over-sprouting, yellowish and stopped plant growth. *S. quitoense* and some of its hybrids were asymptomatic but positive for the disease. The fruit crop more susceptible to purple top was *P. peruviana* because had 100% of incidence (presence of symptoms). In addition, *Ca*Lso was identified as one of the pathogens associated to purple top. These symptoms are of great value to identify this pathogenic problem and look for control strategies; however, molecular techniques are always required to have a hundred percent certainty in the diagnosis.

The information generated constitutes a contribution to begin to describe this new emerging disease; however, more studies about other specific primers for sample analysis, new molecular diagnostic methodologies to identify mixed infections, disease epidemiology, disease and vector control are needed. Consequently, this study is a start point for further research about this disease that is strongly affecting the Andean fruit crops.

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