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Detection and identification of *Candidatus* Liberibacter solanacearum in potato and tomato

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A new bacterial species '*Candidatus* Liberibacter solanacearum' has been found in association with serious diseases of tomatoes, potatoes and other solanaceous crops observed in the U.S. In particular, it was found associated with a potato disease called 'zebra chip' which can cause significant economic losses, by reducing both yield and quality of potato. *Candidatus* Liberibacter species are obligate intracellular parasites that inhabit the phloem cells of infected plant host species and are transmitted by the tomato/potato psyllid *Bactericera cockerelli*. *Ca.* L solanacearum may also be transmitted through infected seed potato pieces. **This disease has not yet been detected in Florida.**

Diseases associated with *Ca.* L. solanacearum include zebra chip in potatoes (Leifting et al., 2008) and psyllid yellows in tomatoes and peppers (Munyaneza et al., 2009). *Ca.* L. solanacearum has also been reported to cause dieback and leaf curling in tamarillo *(Solanum betaceum*) (Leifting, et al., 2008, Watson, 2009) and secondary root proliferation in carrots (Munyaneza et al., 2010). *Ca.* L. solanacearum has also been detected in several solanaceous weeds with psyllid yellows symptoms including *Solanum ptychanthum* (black nightshade), *Solanum elaeagnifolium* (silver leaf nightshade) and *Lycium barbarum* (wolfberry) (Wen et al., 2009).

Symptoms associated with *Candidatus* Liberibacter solanacearum in potato

1. Yellowing or purpling of leaves and shoots (Fig. 1 & 2)
2. Curling or rolling of leaves (Fig. 1 & 2)
3. Stunted shoots with shortened and swollen internodes (Fig. 2)
4. Aerial tuber formation (Fig. 3)
5. Scorched potato tops that collapse prematurely (Fig. 4)
6. Early senescence
7. Tubers may have enlarged lenticels, necrotic flecking of the vascular tissue and streaks along the medullary rays that are enhanced when slices of the potatoes are fried, and brown purplish stolons (Fig. 5)

Fig. 1: Shoots of zebra chip affected potato plants infected with *Candidatus* Liberibacter solanacearum. Leaves on younger shoots display purpling, and older leaves are chlorotic. The leaves are also rolled. (Photos courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.).

Fig. 2. Shoots of a zebra chip affected potato plant infected with *Candidatus* Liberibacter solanacearum. Leaves are chlorotic, curled and rolled. Leaves on younger shoots display mild purpling. The shoots are stunted and swollen (arrows) and swelling is occurring at the nodes. (Photo courtesy: Dr. Lia Liefting NZ. Images from National Diagnostic Protocol, Australian Govt.)

Fig. 3: Aerial tuber formation on zebra chip affected potato plant infected with *Candidatus* Liberibacter solanacearum. (Image courtesy: Dr. Lia Liefting NZ. Images from National Diagnostic Protocol, Australian Govt.)

Fig. 4: Shoots of a zebra chip affected potato plant infected with *Candidatus* Liberibacter solanacearum. Leaves are chlorotic and rolled and some are necrotic or scorched. (Photo courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.).

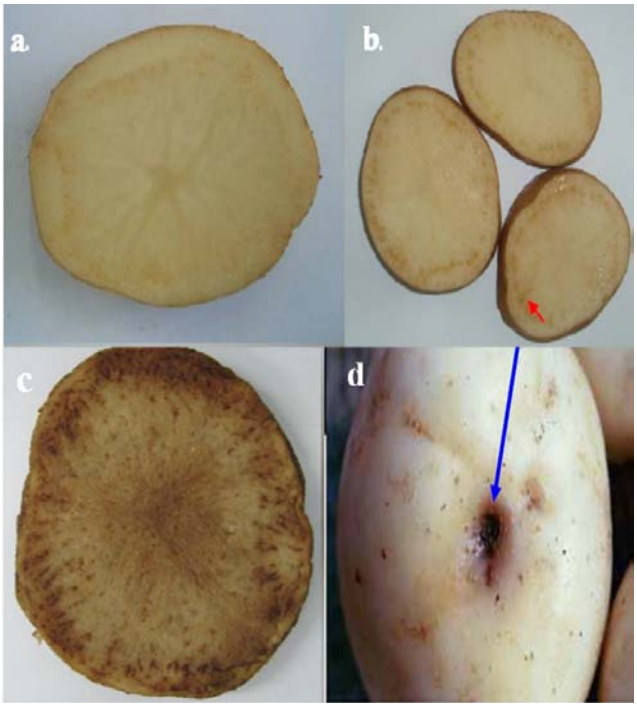

Fig. 5: Fresh tuber slices of zebra chip affected potato plants infected with *Candidatus* Liberibacter solanacearum. The images show mild (a), moderate (b) and (c) severe discoloration and flecking of the vascular tissue and streaking of the medullary rays of tuber slices after frying and (d) brown to pinkish collapsed stolons. (Images courtesy: Dr. Lia Liefting (a,b,c : Images from National Diagnostic Protocol, Australian Govt.) and Munyaneza (d, Images adopted from http://www.colostate.edu/Depts/SLVRC/disease/SRMAC2011/MunyanezaSRMAC2011.pdf)

II. Symptoms associated with *Candidatus* Liberibacter solanacearum in tomato

1. Stunting (Fig. 6)
2. Apical growth can be spiky and chlorotic (Fig.6 & 7)
3. Leaflets may be distorted and curled (Fig. 6, 7 & 9)
4. Leaves may be mottled or chlorotic (Fig. 9)
5. Some varieties have interveinal chlorosis and vein greening of the leaves (Fig. 6)
6. Purpling of the mid vein and leaves in some cultivars (Fig. 10)
7. Some tomato varieties may have fruit deformation (Fig. 8)

Fig. 6: Shoots of psyllid yellows affected tomato plants infected with *Candidatus* Liberibacter solanacearum. The images show stunting (a and b) and chlorosis of the apical growth (a, b, c, and d), which can be spiky (c). The leaflets on some affected shoots are also distorted and curled (a and c). Some varieties may have interveinal chlorosis and vein greening (d) (Images courtesy: Dr. Lia Liefting, Images from National Diagnostic Protocol, Australian Govt.)

Fig. 7: A psyllid yellows affected tomato plant infected with *Candidatus* Liberibacter solanacearum. The image shows chlorotic and spiky apical growth and mottling and distortion and curling of the leaves (Image courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.)

Fig. 8: Fruit deformation of a psyllid yellows affected tomato plant that was also infected with *Candidatus* Liberibacter solanacearum (Image courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.).

Fig. 9: Leaves of psyllid yellows affected tomato plants infected with *Candidatus* Liberibacter solanacearum. The images show chlorosis and mottling (a and b). The leaflets may also be distorted and curled (c) (Images courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.).

Fig. 10: Shoot tip of psyllid yellows affected tomato plant infected with *Candidatus* Liberibacter solanacearum showing purpling of the leaflets and petioles (Images courtesy: Dr. Lia Liefting, NZ. Images from National Diagnostic Protocol, Australian Govt.).

III. Potato/Tomato Psyllids vector

Fig. 12: Different stages of growth of the Potato/Tomato Psyllid vector. (A and B) adult psyllids (Munyaneza, 2012), C) eggs (Photo courtesy: J. Kelly Clark, University of California, IPM for Potatoes in the Western United States, ANR publication) and D) nymph (Image source: http://www.ipm.ucdavis.edu/PMG/r607300811.html.)

Sample selection

Candidatus Liberibacter solanacearum is phloem limited and hence vascular tissue is the best source that should be sampled for successful PCR detection. Higher concentrations of the bacterium were observed in root, tuber and stolon tissue compared to aerial tissue of potato plants (Wen *et al.*, 2009).

Potato: Yellowing or purpling leaves and the shoots of the infected symptomatic plants as well as aerial tubers needs to be sampled which are more reliable.

Tomato: Tissue from symptomatic shoots should be used, which can include yellow coloured leaves, stems, leaf petioles, the peduncles attached to fruit and the portion of affected fruit to which the peduncle is attached.

Collection and Shipping plant materials to the UF Plant Diagnostic Center:

- Samples (1-2 g) should be collected from the above mentioned symptomatic tissues, should be covered with a moist paper towel and placed in a ziptop bag. The tubers should be kept as such without the moist paper towel in the ziptop bag.
- The bags should be legibly labelled with following details; type of tissue, symptoms noticed on the plant, location (county/city), date of collection and the name of the grower/extension agent.
- The samples needs to shipped overnight through FEDEX to the Southern Plant Diagnostic Clinic, with the address mentioned below. If funding for shipping is problematic, please call the lab to discuss options. All the shipments/deliveries should be prior notified by email to: clharmon@ufl.edu

Address for shipment:
Dr. Carrie Harmon, UF Plant Diagnostic Center
2570 Hull Road, Building 1291, University of Florida,
Gainesville, FL 32611-0830. Telephone No: 352-273-4645

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