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RESEARCH PAPERS

Surveys of potato-growing areas and surface water in Lebanon for potato brown and ring rot pathogens

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Summary. Field surveys were carried out over three growing seasons (2013–2015), in the main potato growing areas of Lebanon, to assess the occurrence of potato brown rot caused by *Ralstonia solanacearum* and potato ring rot caused by *Clavibacter michiganensis* subsp. *sepedonicus*. A total of 232 potato samples were collected from Bekaa valley and 145 samples from Akkar plain, which are the largest Lebanese areas cropped with potatoes. Composite samples of 200 potato tubers were randomly collected from each field, following procedures laid down in EU legislation. Twelve potato demonstration fields were established in Akkar plain and designed for potato export to European markets: these were also surveyed using the same strategy. Furthermore, a network of 40 sampling sites in Bekaa and 19 sites in Akkar was established to collect surface water. GPS coordinates of potato fields and water sampling sites were recorded to map specific sampling points using Geographic Information System. All samples gave negative results for *R. solanacearum* and *C. michiganensis* subsp. *sepedonicus* in potatoes and *R. solanacearum* in water, as indicated using the official EU methods for detection and diagnosis for these pathogens. A monitoring system for *R. solanacearum* and *C. michiganensis* subsp. *michiganensis* has been set up in Lebanon. This will increase the phytosanitary quality of potatoes and provide access to broader international markets.

Key words: monitoring, potatoes, *Ralstonia solanacearum*, *Clavibacter michiganensis* subsp. *sepedonicus*.

Introduction

Potato is one of the main sources of carbohydrates in Mediterranean and European diets. In Lebanon, potato production is very important, both for food security as well as a source of revenue in rural areas. Hence, potato is a strategic crop for Lebanese agriculture, covering about 11,000 ha (Anonymous, 2012a) and with production of approx. 300,000 tonnes per year, and is the greatest field crop tonnage in this country. Potato is cultivated for fresh consumption and processing products, and a portion of production is exported. Potato cultivation in Lebanon is mainly concentrated in the Bekaa valley (central-eastern Lebanon) at 900–1000 m a.s.l. (70% of total potato cultivated area), and the Akkar plain (northern Lebanon, 25–30% of total potato cultivated area). The cropping is from March till November each year. The foreseen quota of 50,000 tonnes of potatoes is allowed to be exported to the EU, following the EU-Lebanon Association Agreement signed in 2002 and in force in 2006 (Anonymous, 2002). This represents an important source of additional income for potato farmers, with a potential estimated gross product between 17 and 22 million Euros (Anonymous, 2012b). Potato production in Akkar has good potential of

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exportation, due to early harvesting (early March to late April).

Despite its importance, potato production in Lebanon has not achieved full export potential because of a number of production constraints. These include: low soil fertility, inadequate supply of certified seed potatoes, use of low yielding varieties and occurrence of diseases, the most common being late blight, viral infections and bacterial soft rot (Choueiri *et al.*, 2004; 2012; Moretti *et al.*, 2016). The main constraint hindering the phytosanitary quality of Lebanese table potatoes is the lack of a certification scheme applied to tuber lots intended for export. Such a scheme requires field surveys, sampling of potatoes and surface water, and laboratory analyses for the possible detection of the bacterial pathogens *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus*.

Globally, brown rot caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* (1995) (*Rsol*) is one of the most important, destructive and widespread diseases of numerous crops in tropical, subtropical and temperate regions of the world (Hayward, 1991). The pathogen differs in host range, geographical distribution, pathogenicity and biochemical properties (Horita and Tsuchiya, 2001). Brown rot has been estimated to affect about 1.7 million ha of potatoes in approx. 80 countries, with global loss estimates of over USD 950 million *per annum* (Champoiseau *et al.*, 2009). *Rsol* is a heterogeneous species (Hayward, 1994), which has been identified by its host range (defining races) (Buddenhagen *et al.*, 1962; He *et al.*, 1983) and metabolic properties (defining biovars) (He *et al.*, 1983; Hayward, 1994). A phylogenetic classification system was more recently proposed by Prior and Fegan (2005), consisting of four phylotypes, each further divided into sequevars. Since 1992, an increased number of outbreaks of potato brown rot caused by the bacterium *Rsol,* biovar 2 race 3 (phylotype II, sequevar I), has been reported in several countries of the European Union, including Belgium, Germany, France, the Netherlands, Sweden and the United Kingdom (Elphinstone, 1996; Janse, 1996; Anonymous, 1999). *Clavibacter michiganensis* subsp. *sepedonicus* (Speakerman & Kotthoff) Davis *et al*. 1984 (*Cms*), the causal agent of bacterial ring rot of potato, is also a quarantine organism which constitutes a worldwide threat to potato growers and industries. *Cms* can cause damage of different kinds – by direct crop loss during growth and storage, by rejection of infected seed tuber lots and the cost of control and quarantine measures, by loss of export markets or by difficulties experienced in opening new markets. In Europe, *Cms* annually causes an estimated Euro 15 million in economic damage (van der Wolf *et al.*, 2005), although present economic losses appear to be much less. Indirect losses are through the statutory measures taken against disease outbreaks, including restrictions on cropping, disinfection costs and lost export trade. Control within the EU is governed by EC Council Directive 93/85/EEC (Anonymous, 1993), and survey and testing methods are outlined in updates for this Directive (Anonymous, 2006b).

At present, Lebanon faces challenges to ensure excellent phytosanitary quality of its potatoes, especially if this is a requirement for their export. To ensure such a quality system, efforts are needed to develop survey strategies, to implement sampling methods and guarantee acceptable traceability. Additionally, in the past, Saad and Nienhaus (1969) reported infections by *Rsol* and *Cms* to be present in Lebanon, although they were rare and localized. *Rsol* was reported in Akkar, after visual field inspections, but was never confirmed by available diagnostic tests or by direct isolation from symptomatic plant material. Conversely, *Cms* was mainly reported in Bekaa valley, where it was presumably isolated from symptomatic plant material. Considering the phytosanitary risk posed by these quarantine pathogens for agriculture and trade, activities were undertaken to develop a sound system of surveys and traceability, in order to monitor the main areas of potato cultivation in Lebanon, and to determine the phytosanitary status of potato production. With an expected increase in demand for certified Lebanese potatoes and sustained interest from the potato growers to produce potatoes with acceptable phytosanitary quality, a need was identified for investigation of the bacterial quarantine potato diseases in existing and potential Lebanese potato-growing areas. This paper presents the results of extensive surveys in the main Lebanese potato production areas, accompanying sampling and laboratory analyses, conducted during three years, 2013–2015. This was to assess the possible presence (or confirm the absence) of *Rsol* and *Cms* in potato growing areas, and implement a traceability system able to locate possible infested locations, in case the results from the phytosanitary analyses were positives.

Materials and methods

Extensive surveys were planned during three consecutive potato growing seasons (2013–2015) by the Lebanese competent NPPO authority, to carry out official inspections and sampling during each growing season. Tubers were sampled, before and after harvesting, for official analyses, in order to detect the possible presence of *Rsol* and/or *Cms*. Surface water, used for irrigation in potato growing areas, was also sampled and subjected to official analyses for the detection of *Rsol*. Sampling and testing methodologies followed, as far as possible, procedures laid down in the EU legislation (Anonymous, 2006a; 2006b), also including specific training of phytosanitary inspectors and laboratory technicians, thus ensuring correct field monitoring, sampling and laboratory analyses. The 3-year survey covered: a) the Bekaa Mohafaza area, representing 72% of domestic potato production by approx. 500 farmers, and b) the Akkar Mohafaza area, representing 25% of domestic potato production by approx. 500–600 farmers.

Positive control bacterial strains and growth conditions

Control bacterial strains used in this study during the detection and identification procedures were obtained from the French Plant Pathogenic Bacteria Collection (CFBP, INRA-Angers, France). *Ralstonia solanacearum* strain "CFBP3857" was grown on modified selective medium SMSA (Engelbrecht, 1994; Elphinstone *et al*., 1998) at 27°C, and *Clavibacter michiganensis* subsp. *sepedonicus* strain "CFBP 3561" was grown on MTNA (Jansing and Rudolph, 1998) at 21°C. *Rsol* was maintained as suspensions in sterile distilled water at room temperature, whereas *Cms* was maintained on YDC agar slants at 4°C.

Field surveys and potato tubers sampling

Field surveys were undertaken during three growing seasons in the major potato cultivation regions in Lebanon, covering early potatoes (winter-spring cycle) produced in the Akkar plain and sold to the local and Arab countries markets, summer potatoes (spring-summer cycle) produced in the Bekaa valley and marketed "fresh", or stored and marketed to foreign markets (Arab countries and Russia), and late potatoes (summer-autumn cycle), produced also in the Bekaa valley and marketed locally. In each region, fields were arbitrarily selected and inspected, in a network of representative locations. Two hundred and thirty two fields were selected in the Bekaa valley and 145 fields in the Akkar plain (Table 1). Fields were divided into three groups: A (< 5 ha), B (5–10 ha) or C (> 10 ha) (Table 2). The number of fields from each region was proportionate to the area cultivated with potato in that region. GPS coordinates of visited fields were recorded into a survey network, to ensure traceability of specific sampling points for following sampling seasons (Figures 1 and 2). During the growing seasons, fields were inspected by Ministry of Agriculture (MoA) phytosanitary inspectors for the presence of any indicative ring rot and/or brown rot symptoms: inspections for ring rot were done during flowering and inspections for brown rot were carried out 25–30 days after flowering and before maturity.

Table 1. Numbers of potato fields surveyed for brown rot and ring rot pathogens, and waterways surveyed for the brown rot pathogen, during 2013, 2014 and 2015 in the Bekaa valley and the Akkar district, Lebanon.

Year	Bekaa valley		Akkar district	
	Potato fields	Waterways	Potato fields	Waterways
2013	57	21	30	8
2014	94	9	60	11
2015	81	10	55	-

Table 2. Distribution and structure of selected potato fields in 2013-2015[a].

Year	Bekaa valley				Akkar district			
	A	B	C	Total	A	B	C	Total
2013	30	20	7	57	18	10	2	30
2014	47	33	14	94	32	23	5	60
2015	35	35	11	81	30	20	5	55

[a] A, farms with an acreage < 5ha; B, farms with an acreage 5-10 ha; C, farms with an acreage > 10 ha.

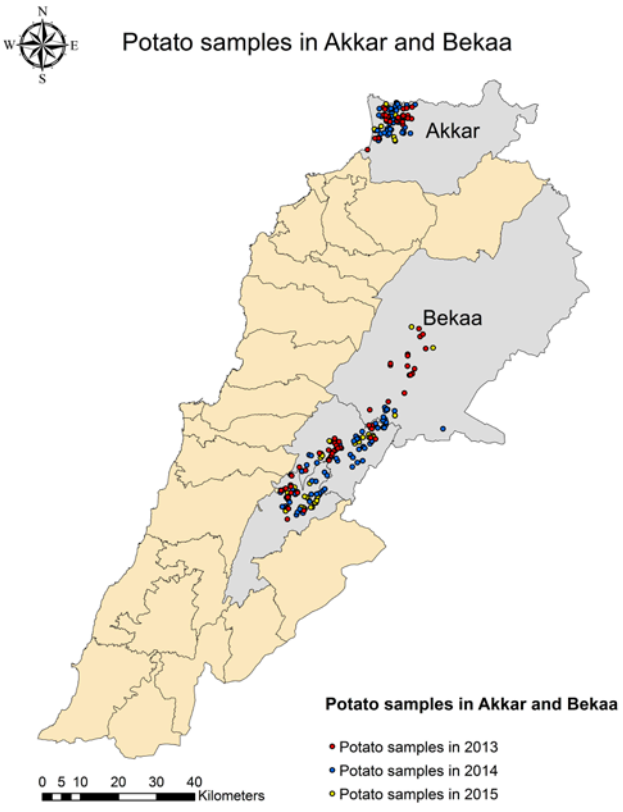

Figure 1. Potato sampling sites in the Akkar district and the Bekaa valley in Lebanon, during three growing seasons.

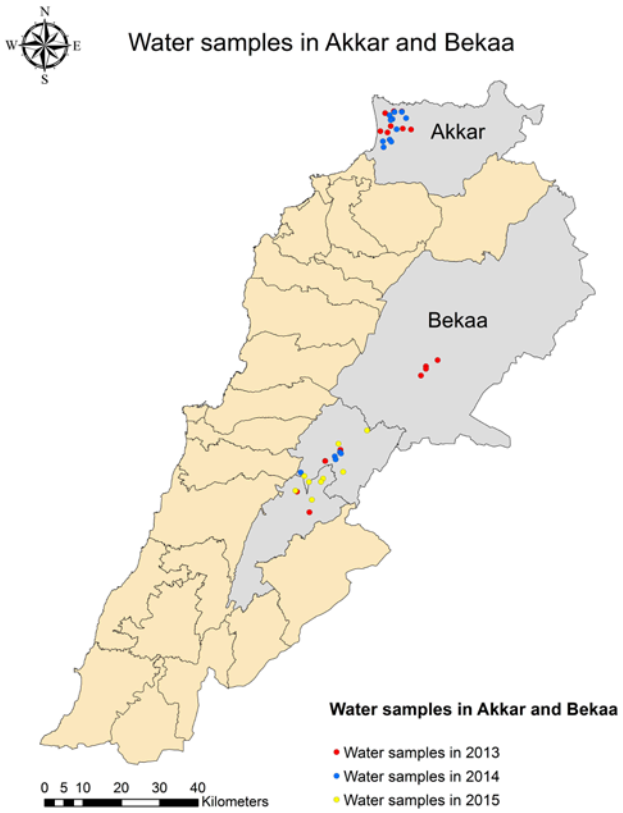

Figure 2. Water sampling sites in the Akkar district and the Bekaa valley in Lebanon during three growing seasons.

At harvest, in agreement with the official EU methods for detection and diagnosis of *Rsol* and *Cms* in potatoes (Anonymous, 2006a and 2006b), composite samples consisting of 200 tubers were collected at random from each field in the survey network. The tubers from each field were sampled following a 'W' pattern as the sampling procedure. They were placed in jute bags with capacity up to 50 kg. The samples were then transported to the LARI Plant Protection analysis laboratories in the Tal Amara Station near Zahlé (Bekaa valley) and stored at 4°C until testing, which was normally carried out after 2-3 days. Each sample bag carried two labels, one inside and one on the outside, containing relevant identification information.

Potato demonstration fields designated for exportation

For brown and ring rot monitoring, particularly from the Akkar district, in the area designated for export of early potatoes to Europe, demonstration fields of farmers specialized in the production of potatoes for export were designated at some private farms, under two projects funded by the Danish Refugee Council (DRC) in 2014 and the International Labor Organization (ILO) during 2015. The aims of these projects were to ensure the necessary interactions among the Lebanese public institutions and the private sector, and represent concrete examples for companies which could adequately abide with all the requirements of EU countries for potato trade. During the first project (in 2014), the number of farmer beneficiaries was 20, so 20 demonstration fields were established using 'Synergy' as the potato cultivar. Through the second project (in 2015), 65 farmers were trained according Global-GAP guidelines for potato production. This included taking account of the demands for food and environmental security. Four demonstration plots were planted at each site with three potato culti-

vars ('Vivaldi', 'Annabelle' or 'Colomba') required by the European market. The demonstration farms were registered and the farmers carried out all the necessary operations to enable traceability of their products. They followed the indications given by the MoA to regularly perform all field inspections and samplings during the growing season and during harvesting. Samples of 200 potato tubers were randomly collected from each demonstration field and sent to the Bacteriology Laboratory of the Department of Plant Protection, where they were analysed as described above.

Detection and identification of *R. solanacearum* and *C. michiganensis* subsp. *sepedonicus* from potato tubers

Immunofluorescence test (IF test)

On the standard sample size of 200 tubers, a small core of tissue (containing vascular bundles) was removed from the stolon end of each tuber. The cores were pooled in a disposable plastic cup, and then covered with a sufficient volume (approx. 40 mL) of extraction buffer (50 mM Na/K phosphate buffer, pH = 7) and placed on a rotary shaker (80-100 rpm) for 16 to 24 h in a refrigerated room (4–8°C). The supernatant was filtered through sterile gauze into a sterile centrifuge tube. The extract was then concentrated by centrifugation at 10,000 × *g* for 10 min at 4°C, and the pellet was resuspended in 1.5 mL of pellet buffer. IF tests were performed on the sample extracts following the instructions described in PM 7/97 "Indirect immunofluorescence test for plant pathogenic bacteria" (EPPO, 2009), and using the specific antibodies for *Rsol* and *Cms* manufactured by Loewe Biochemica GmbH. The positive control bacterial strains for both pathogens (see above) were included in the assays. Negative controls were those provided with the commercial kits used.

Detection and identification of *R. solanacearum* in water

Surveys of irrigation water and sewage

Surveys of surface water used for crop irrigation were carried out at potato-growing sites in the traditional potato growing regions of the Bekaa valley and the Akkar plain. Sampling sites were selected and mapped to ensure wide coverage of potato-growing areas, and their GPS coordinates were recorded to ensure that specific sampling points could be relocated. A total of 40 sampling sites were identified, mapped and visited over three growing seasons in the Bekaa valley, while 19 sampling sites were identified, mapped and visited in Akkar. All potato production areas in Bekaa valley are irrigated (about half from surface water and the rest from wells in the middle season, and approx. 90% from wells and 10% from surface water in the late season). Irrigation canals were present all around each potato field, some with fast running water, and some with slow streaming water. Water was sampled from these canals (Figures 3a and 3b). This was because possible outbreaks of brown rot may result from contaminated irrigation water, and the pathogen (*Rsol*) may be spread around the area. Survey of surface water was also carried out at different sites, such as Lake Taanayel and other riverbeds along the Litani River that irrigate the main potato-growing area. In the Akkar plain, surface water was collected from streams around the potato cropping area. In addition, south of Zahlé, MoA inspectors visited the largest and most important potato store, processing and distribution plant in Lebanon. Potato lots coming from Akkar, Ba'albek (north Bekaa) and Zahlé (central and south Bekaa) are brought there to be stored, washed, selected and packaged for distribution. Water samples from two particularly significant sites were taken for *Rsol* analysis: the first was from under the rolling cylinder, where potato tubers just coming out of refrigerated stores and prior to selection are washed with water sprays (Figure 3c), and the second from outside the distribution plant, where water and sewage are collected after floor washing (Figure 3d).

Surface water used to irrigate potato crops was monitored and sampled during the period from August to late September of each year, when water temperatures exceeded 15°C. This was done to optimize the possibility of detecting and/or isolating *Rsol*.

At each selected sampling site, three duplicates of 50 mL water samples were collected into centrifuge tubes, at approx. 2–3 m spacing in the water body at a depth of around 30 cm. For processing and sewage effluents, samples were collected from the point of effluent discharge, again in three duplicates of 50 mL each. The capped centrifuge tubes were transported to the laboratory in refrigerated boxes (5–10°C) and kept refrigerated overnight

Figure 3. Examples of sites for monitoring the possible presence of *Rsol*: Irrigation canal (a) and irrigation basin (b) near potato fields, where surface water was sampled. Potato washing machines (c), where potatoes are washed prior to storage, and where water samples were taken for analyses. Waste water container (d), located just outside a potato processing factory, where waste water was collected for analysis.

before testing (Anonymous, 1998). Water samples were concentrated by centrifuging each 50 mL sample at 10,000 × *g* for 15 min at 4°C. After discarding the supernatant, the pellet was resuspended in 1.0 mL sterile 10 mM phosphate buffer, pH 7.2 ($Na_2HPO_4 \times 12H_2O$, 2.7 g, $NaH_2PO_4 \times 2H_2O$, 0.4 g, distilled water up to 1.0 L). Ten and 100-fold serial dilutions were made of this suspension and duplicate 50 µL aliquots from the original suspension and their dilutions were uniformly spread onto plates of SMS agar medium (Engelbrecht, 1994 as modified by Elphinstone *et al.* 1998) using sterile spreaders. Thirty µL aliquots from positive control cultures were also spread on the same SMSA medium. All plates were incubated at 28°C and, when colonies with typical morphology of *Rsol* were observed after 48–72 h, they were selected, purified and identified.

In parallel, detection of *Rsol* was attempted by enrichment-PCR, using approx. 100 µL from the original final concentrate in 15 mL of SMS broth and kept for 72 h at 28°C on a rotary shaker at 75 rpm. Each sample was then concentrated by centrifugation at 10.000 × *g* for 10 min, then the supernatant was discarded and the pellet was resuspended in phosphate buffer. Ten and 100-fold serial dilutions were made from the suspension. DNA was extracted from each sample dilution by cell lysis as follows: pipetting 5 µL of 1M NaOH into an Eppendorf vial containing 95 µL of bacterial suspension, followed by incubation of the sample at 100°C in a heating block for 10 minutes, then 5 min in ice. Molecular detection of *Rsol* in samples was achieved using a specific primer pair, as described in Seal *et al.* (1993). The PCR reaction was carried out in a final volume of 25 µL containing 15 mM $MgCl_2$, 200 µM of each dNTP, 1 mM of each primer, 0.5 U of Taq polymerase (Promega) and 2 µL of DNA template. The PCR products were added with 2 µL of UView 6× Loading Dye (Bio-Rad) and run on 2% agarose gels in TAE buffer. The specific amplicons were observed under UV light with a transilluminator.

Results and discussion

Potato fields surveys and sampling

No visible symptoms of brown rot or ring rot were ever observed during the extensive surveys carried out over three growing seasons (2013–2015). Sampling and laboratory analyses focused on local ware potato production directed for local and export markets. Overall, the number of samples analyzed was satisfactory, since it covered most of the areas where potatoes are grown. Sampling sites visited in both the Bekaa valley and the Akkar plain during the 2013–2015 potato seasons are shown in Figures 1 and 2. Over this period, a total 377 samples was tested, including approx. 125 samples per year, covering an area of approx. 788 ha (Table 1). From the Bekaa valley, the larger area cropped with potatoes, 232 samples were collected. *Rsol* or *Cms* were not detected in any of the samples assayed. Likewise, neither of the quarantine pathogens was detected in the 145 samples collected from the Akkar district.

The demonstration fields established in Akkar and designated for potato export were first inspected to check for specific symptoms of bacterial diseases. Although a few symptoms related to mosaic or mottling of leaves were occasionally observed (probably associated with virus infections), no specific symptoms were found that indicated bacterial infections. Additionally, the representative samples taken for laboratory analysis confirmed the absence of *Rsol* and *Cms* by IF and PCR tests.

Under Lebanese climatic conditions, symptoms related to brown rot/ring rot are expected to be found in the field and often only at the end of the season, especially when the crop is repeatedly irrigated during the dry season. Occasionally, leaves showing apical wilting were seen in some fields, but they were associated with high temperatures and other diseases, such those caused by *Verticillium dahliae* and/or *Fusarium* spp., or were due to senescence or mechanical damage. Visual inspections of all tubers collected from different fields and originating from different seed potato lots showed that no brown rot or ring rot symptoms were present in tubers grown in the surveyed fields. In all cases, the laboratory analyses carried out on sampled potatoes, which were carrying some unspecific symptoms, confirmed that those samples were free from bacterial infections.

Water surveys and sampling

Most sampling sites in the Bekaa valley were in areas where potatoes were grown throughout the year. No contamination of surface waters by transient populations of *Rsol* was detected during the surveys of the Litani River and its irrigation canals. This indicates that the pathogen was not present in the vicinity of traditional potato production areas. Additionally, the pathogen was not detected in the surface waters of Taanayel, Gzail and Amiq sites, supplying negative detection data on the areas adjacent to fields in which potatoes were under cultivation. No *Rsol* contamination was detected in water samples collected inside the potato processing site south of Zahlé and its neighbourhood. In the Akkar district, *Rsol* was not detected from small canals adjacent to potato fields. In those canals, the water is usually slow-flowing or stationary, and the volume is often low, so that contaminated water would persist with high, localized, concentrations of the pathogen. Laboratory analyses did not detect the presence of pathogenic bacteria in any of water samples tested, while PCR produced only a DNA fragment of 288 bp from the positive pathogen control. The absence of target bacteria in surface water flowing through the agricultural areas indicates that possible reservoirs of the pathogen in roots of semi-aquatic weeds lining the canals are not present. This is contrary to the situation in Europe, where submerged roots of the riparian solanaceous weed *Solanum dulcamara* provide protected niches for the pathogen in some rivers (Olsson, 1976a, 1976b; Janse, 1996; Elphinstone *et al.*, 1998; Persson, 1998).

Other weeds may also play roles in persistence of the pathogen in waterways (Wenneker *et al.*, 1999; Cruz *et al.*, 2012; Janse, 2012). In Lebanese potato production areas, many of the irrigation canals feeding potato growing areas are concrete-lined and free from weeds growing. This reduces the possibility of reservoir plants harbouring *Rsol* bordering potato fields. The use of herbicides to keep the waterways free from weeds would help to avoid the presence of possible reservoir plants. Nonetheless, in case of an outbreak of brown rot, monitoring of waterways should include selected weeds as well, especially those wild *Solanaceae* reported in Lebanon, like *Solanum dulcamara* and *S. luteum*, whereas *Portulaca* sp. is not reported to be present (www.lebanon-flora.org). In Lebanon, the normal daily surface water temperature varies from approx. 10ºC in winter to approx.

30ºC in summer, which coincides with the optimum temperature range for survival of *Rsol*. Therefore, it is likely that the pathogen could persist in Lebanese surface waters for long periods. However, in spite of these suitable conditions, *Rsol* was never detected in surveyed water sites, either by direct isolation or with molecular analyses. In addition, more than 50% of potato fields are irrigated from wells in the middle of the growing season, and 90% by wells in the late season, thus limiting the survival of the brown rot pathogen in surface water. Moreover, an effect similar to soil solarization during the hot, dry and sunny Lebanese summers may reduce survival of the pathogen under field conditions. The abundant presence of green algae in waterways may also have antagonistic effects on *Rsol* (Wenneker *et al*., 1999; Van Elsas *et al.*, 2001.

Potato processing plant surveys and sampling

The largest potato storage, processing and distribution facility in Lebanon was monitored and water samples collected for laboratory analyses. In this facility, potatoes are washed with 1% chlorine disinfected water prior to packaging into bags and refrigerated storage. Samples were taken directly under the washing rolling cylinder during the processing of potatoes, and additional samples were taken from waste water tanks located outside the processing plant. All samples taken and assayed using the standard the EU detection procedure were negative for the detection of *Rsol*.

Rsol is a complex species and has long been recognized as a number of phenotypically diverse strains, originally placed into five pathogenic races and five biovars (Buddenhagen *et al*., 1962; Hayward, 1964). Following a subdivision of this complex into four phylotypes (Prior and Fegan, 2005), taxonomy now recognises Phylotype II as *Rsol*, Phylotypes I and III as *R. pseudosolanacearum*, and Phylotype IV as *R. syzygii* (Safni *et al*., 2014). *Rsol* can survive for long periods where suitable hosts are cultivated. Survival may occur in plant debris, ensuring the survival of sufficient inoculum from season to season, but also in reservoir plants, soil and surface water (Hayward, 1991). This pathogen can also show saprophytic behaviour similar to other plant pathogenic bacteria (e.g. *Pectobacterium* spp.). It is particularly important that potato growing areas are regularly monitored, with acceptable and officially recognized survey strategies, to provide sensitive and accurate pathogen detection and prevent potential disease outbreaks. In case of potato ring rot, *Cms* is not reported to have good survival in soil from one potato crop to the next, especially in subtropical conditions (Manzer and Genereux, 1981). The areas considered in our study are climatically different from each other: the Akkar district is a coastal area characterized by warm dry summers and mild wet winters, whereas Bekaa valley, with its elevation, resembles some central-eastern European regions, with a more continental climate. Saad and Nienhaus (1969) divided Lebanon into eight climatic regions, based on temperature and rainfall. They reported isolation of *Cms*, although no clear diagnosis using pure cultures was recorded from symptomatic potatoes grown in the Bekaa valley, but they did not indicate if *Rsol* was isolated and identified in any area, so their diagnosis was possibly based only on observation of symptoms (wilts). In Akkar, they also reported *Xanthomonas solanacearum* on tobacco (together with *Pseudomonas solanacearum* on potato). Because both names now refer to *Rsol*, and because wilting symptoms were seen on tobacco and potato, this indicates that, if *Rsol* was really present in the 1960s, it was probably race 1. This race notoriously affects both host plants in tropical areas. Nonetheless, this report is doubtful, as no identified isolate was obtained.

During the last 12 years, *Rsol*, was intercepted 51 times in ware potatoes imported from Egypt into the European Union (European Commission, 2016). Later, Abou-Jawdah *et al.* (2001), during a 2 year field survey of potato viruses in Akkar and Bekaa, did not report the presence of both of the quarantine bacteria, and only a few cases of soft rot were observed.

The present survey, undertaken in the various potato growing areas in Lebanon during three growing seasons, provides a clear picture of the phytosanitary situation of potato related to brown rot and ring rot. This is the first large-scale survey of potato fields targeting both *Rsol* and *Cms*, which used a specified survey strategy and validated diagnostic tests. Accordingly, a specified and systemic field and waterway monitoring and traceability for bacterial diseases were carried out, which aimed to allow the Akkar region to be ready to produce potatoes under internationally recognized phytosanitary standards. In the Akkar plain all farmers used certified seed tubers coming from outside Lebanon, and only one growing season is possible.

The Italian-Lebanese Project EuLebPot intended to achieve a European standard and conformity for Lebanese potatoes and required considerable effort by the MoA in carrying out the surveys for both bacteria, based on survey programmes prepared and updated every year in collaboration with the Department of Plant Protection of LARI (DPP-LARI). Surveys are conducted by way of regular field inspections during the crop growth period and near harvest (including tuber sampling and cutting for visual inspections), followed by laboratory analyses of all collected samples. Staff responsible for carrying out surveys and export controls have been subject to regular training, provided with relevant technical information, and given detailed instructions for ware potato plant quarantine sampling. An inspectors' handbook has also been issued through the project (Abou Zeid *et al*., 2013). In order to ensure the correct application of validated detection methods, the laboratory of DPP-LARI upgraded its equipment and the diagnosticians have been specifically trained. Therefore, laboratories of DPP-LARI can provide good diagnostic support for Lebanese phytosanitary field inspectors in relation to seed and ware potato testing for brown and ring rot.

Despite the implementation of field surveys and sampling for detecting *Rsol* and *Cms* in the main potato growing areas, and the establishment of good communication and consultation procedures with stakeholders involved in the potato sector, including potato exporters, weaknesses in traceability and appropriate control inspection methods for potato warehouses are still present. The MoA should work more and make a greater effort to improve these procedures. In cases where brown rot and/or ring rot are detected, as has occurred in other countries (Katayama and Kimura, 1984; Tuin *et al.*, 1994; Bénard, 1996; Janse, 1996; 2012; Stead *et al.*, 1996; Pánková *et al.*, 2007), the main strategies for implementing contingency plans will be the availability of field data and full traceability of infected samples. These will ensure urgent application of control and hygiene measures for a rapid eradication of possible disease foci. Disease management strategies will involve different methods, including: accurate identification of infection sources, early detection and diagnosis of *Rsol* and *Cms*, exclusion of contaminated fields from potato production for a number of years, disinfection of machines, stores, etc. using chlorine or quaternary ammonium compounds, control of surface water, volunteer plants and weed hosts, especially *S. dulcamara*. These are considered to be key components in successful eradication, and a detailed plan for implementation should be always ready. Since potato production is carried out all over the Bekaa plain and the Akkar district, frequently without following adequate crop rotation systems, continuous efforts should be made to ensure regular monitoring of possible *Rsol* and *Cms* outbreaks in potato production fields and potato industrial premises. This will allow continuing certification of freedom from these quarantine pathogens in potato lots intended for international markets and for domestic use.

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