Dickeya and Pectobacterium species: consistent threats to potato production in Europe

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Abstract

This brief review highlights the current status of the bacterial species of Dickeya and Pectobacterium and the blackleg and soft rot complex in potato. The changes in the pathogen and disease profile over the past years, in Finland and the rest of Europe are discussed. Evaluation of the commonly practiced control measures is briefly presented and significance of the High Grade (HG) status from the viewpoint of blackleg management strategy and sustainable potato production and the project initiative of MTT Oulu towards that goal is cited as one example. The scope of this review is limited to selected topics of practical importance and presented in a way understandable to readers who are not necessarily experts in Dickeya and Pectobacterium but are aware of the problem of blackleg and soft rot. These include potato producers, disease inspection agents and agricultural advisers. Details of the biology of the pathogens are not included. However, readers who are interested in such advances of the pathogens are referred to the most up to date reviews cited in this article.

Introduction

The International Potato Center (CIP) ranks potato to be the third most important food crop in the world after rice and wheat in terms of human consumption. More than a billion people worldwide eat potato, and global total production exceeds 300 million metric tons (http://cipotato.org/potato/facts). However, this important crop which holds a promise as a potential future food of the world is affected by about 160 different diseases and disorders of which 50 are fungal, 10 bacterial, 40 viral origin. The rest are disorders of unknown origin (). Diseases could affect the leaf, stems and tubers at all stages of development and could occur both in the field and storage. From historical records we find that one of the tragic experiences in human history, the Irish famine during 1846-1850 which caused the death and emigration of
millions of people, in Ireland, was caused by the potato disease commonly known as the ‘late bight of potato (Phytophthora infestans). Late blight of potato remains to be one of the unsolved plant disease problems even in the 21st century. Potato production in Europe requires repeated application of fungicides against late blight during the growing season, resulting in high production costs and risk of environmental hazards. It is, therefore, important that effective and sustainable potato disease control practices are in place in order to exploit the potential of the potato crop.

Among the pathogens affecting potato bacteria are one of the most serious problems. Blackleg and soft rot of potato caused by Dickeya and Pectobacterium species are the most harmful and destructive diseases causing heavy losses in seed and table potato production. In the Netherlands alone the annual losses in seed potato production due to Dickeya and Pectobacterium spp. is estimated to be up to 30 million euro. The economic importance of blackleg and soft rot has been increasing since recently because of the spread of an aggressive new clade of Dickeya, now classified as new species, Dickeya solani (Toth et al., 2011). The species is reported from France, Finland, Poland, The Netherlands and Israel (Tsror et al., 2008, Slawiak et al., 2009, Laurila et al., 2010, Degefu et al., 2013) and the major outbreaks of blackleg recorded during warm summers in Finland were caused by D. solani (Degefu et al., 2013). All strains isolated from the countries reported are clonal suggesting that they have a common origin or are the result of single introduction event. The same strain has been isolated from the ornamental plant hyacinth initiating an argument that the strain might have been introduced from hyacinth to potato through contaminated irrigation water (Slawiak et al., 2009). There are reports that the bacteria are isolated from river water (Laurila et al., 2008).

Furthermore, two species of Pectobacterium are reported to cause blackleg in potato. Pectobacterium brasiliensis, a highly aggressive species, is responsible for the blackleg in potato in Brazil (Duarte et al., 2004) and South Africa (van der Merwe et al., 2010). There are some unofficial reports that the species is already detected in potato in Switzerland (Patrice de Werra, Personal communication).

Pectobacterium wasabiae which was first reported in Japan associated with Japanese horseradish a (Gardan et al., 2003, Pitman et al., 2010) and was later isolated from potato in New Zealand (Pitman et al., 2010) and the USA and Canada (Ma et al. 2007; De Boer et al., 2012) and Europe (Nabhan et al., 2012). According to recent studies (Waleron et al., 2013; Nabhan et al., 2012; Nykyri et al., 2012) P. wasabiae has been unnoticed but present in potato fields worldwide. Recent genomic studies proved that some strain collections since 1960 from Finland, Ireland and Germany were misidentified and can now be accurately classified as P. wasabiae. Previous identifications were based on biochemical tests but according to a recent study (Waleron, et al., 2013) the biochemical tests which have been routinely used for identification of Pectobacterium species did not allow the identification of P. wasabiae. Only molecular methods could reveal the differences.
Shift in diversity of causal agents. What does it mean?

In Western Europe and North America and, the main causal agent of potato blackleg has been *Pectobacterium atrosepticum*. It was even once believed that a single serotype of the species is able to cause blackleg in potato (De Boer *et al.*, 1987). The number of species and subspecies of *Pectobacterium* has increased over recent years and, as indicated above *Dickeya* spp. has become an important blackleg pathogen in Europe. This change in the blackleg and soft rot etiology from a disease of single serotype of *P. atrosepticum* (formerly called *Erwinia carotovorum* subsp. *atrosepticum*) to a disease complex caused by several distinct species has become challenging especially with their differentiation and identification by classical microbiological tests. (De Boer *et al.*, 2012) *P. atrosepticum* and *P. carotovorum* were the most common species in soft rot disease of plants and were readily distinguished by biochemical tests. With the additional species, *P. wasabiae*, and *P. brasiliensis* becoming important in potato, it has become more difficult to make accurate identifications based on biochemical tests alone (Waleron *et al.*, 2013) because carbohydrate utilization patterns and other phenotypic characteristics vary among strains of the same species (De Boer, *et al.*2012)

Moreover, the presence of multiple *Pectobacterium* spp. even in the same plant has been noted. The diagnostic PCR based approaches for detection of the pathogens from tubers and infected plants revealed the more complex soft-rotting and blackleg bacterial population than the conventional culture isolation method. The presence of multiple *Pectobacterium* and *Dickeya* spp. in the blackleg and soft rot complex raises the question how do these species, when acting simultaneously interact in natural or plant environment. Whether there exists synergism among genotypes that might magnify the incidence or severity of disease or some degree of antagonism that lower disease severity. The question of possible synergistic or antagonistic interactions among the blackleg and soft rot bacteria is yet to be fully addressed. However, some preliminary observation in which both *P. atrosepticum* and *P. wasabiae* were vacuum inoculated into seed tubers, disease incidence in the subsequent plant stand was not greater than when either pathogen was inoculated alone (De Boer, *et al.*, 2012). In addition, according to unpublished observation, a reduced blackleg severity was also noted when potato was co inoculated with *Dickeya* spp. and *P. carotovorum* (van der Wolf, unpublished).

There is a clear difference in the adaptation to whether or especially to temperature regimes between *Dickeya* and *Pectobacterium* species. *P. atrosepticum* and *P. wasabiae* cause blackleg under cooler summer conditions whereas *Dickeya solani* causes severe outbreaks of blackleg in warm summers when temperatures are around 25ºC (Degefu *et al.*, 2013). Under such warm conditions *D. solani* often takes over the other species (Fig.1). *P. brasiliensis*, according to
current understanding, is confined to tropical and sub tropical climates. It is, therefore, apparent that the occurrence of species adapted to different weather conditions makes blackleg as a likely phenomenon in a given cropping season. Our survey results during the five years indicated that the prevailing weather condition (Degefu, Unpublished) modulates the blackleg bacterial species that predominates in a given season.

Figure 1. Prevalence of *Dickeya solani* and *Pectobacterium* spp. in potato with typical blackleg symptoms during two seasons representing different weather conditions; 2008 (cooler summer) and 2010 (warm summer). (Degefu *et al.*, 2013)

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**Symptoms**

Blackleg begins from a contaminated seed tuber, but the symptoms can occur at several stages of plant development. In severe cases, entire seed tuber and developing sprouts may rot in the
ground prior to emergence, resulting in a poor stand. Blackleg often develops later in the plant development even during flowering. In this case, stem bases of diseased plants typically show an inky-black to light-brown decay that originates from the mother tuber extending up the stem. Leaves of infected plants tend to roll upward (Fig 2A) at the margins, become yellow, wilt, and often die (Fig 2B). Stem infection can occur through wounds or through natural openings such as leaf scars. Lesions on diseased stems first appear as irregular brownish to inky-black areas which enlarge into a soft, mushy rot that causes the entire stems to wilt and die (Fig 2B) Potato tubers with soft rot have tissues that are very soft and watery, and have a slightly granular consistency. The diseased tissue is cream-to tan-colored, and often has a black border separating diseased from healthy areas. At early stages, soft-rot decay is generally odorless, but stinking odor usually develops as secondary decaying bacteria invade infected tissues. Most internal tuber tissues may be consumed by the decaying organisms (Fig. 2C), at times leaving only a shell of potato skin in the soil. Up to as much as 200 ml of bacterial slime could be released from a single rotten tissue to the seed environment contaminating healthy seeds during grading or planting.

Whilst typical blackleg and soft rot are easily distinguishable from other diseases on the basis of visual inspection, symptoms caused by *Dickeya* spp. under warm dry conditions may be confused with those of other wilting diseases. Since wilting can occur with no obvious symptoms of blackleg. In Israel (Fig. 3), symptoms caused by *Dickeya* spp. were indistinguishable from those of wilt caused by *Verticillium dahliae* or those due to natural plant senescence.
Figure 2. Symptoms of blackleg and soft rot in potato. Initial wilting (A), advanced wilting and typical blackleg (B) and soft rot (C) on potato caused by Dickeya and Pectobacterium species. (Photographs: Yeshitila Degefu, MTT Oulu)
Figure 3. Disease symptoms in dry weather conditions in Israel showing initial wilt in upper leaves followed by wilt and desiccation in the lower leaves and tuber soft rot (different from what is commonly observed in cool and humid condition) (photographs courtesy of L.Tsror, Gilat Research Centre, Israel).

**Economic impact**

Blackleg or stem rot occurs in field and soft rot of tubers could occur in field, transit or in storage. Therefore, losses could be encountered at all stages but it could more severe especially if tuber soft rot progresses in the store because all other costs, the cost of production, harvesting, storage etc, are already included in the value of the crop. Losses from infection by *Dickeya* and *Pectobacterium* spp. include yield reduction and the downgrading of seed lots at the time of field inspection. Because of differences in the national certification tolerances, the economic impact differ from country to country (Toth *et al*., 2014). Although not documented, losses worldwide probably reaches hundreds of millions of euro. In the Netherlands the annual losses in seed potato production as a result of down grading seed lots alone is estimated to be up to 30 million euro.
Sources of inoculum and survival

The survival of the blackleg and soft rot bacteria is very short. According to previous knowledge and results our current studies, the bacteria survives in soil is not longer than 3 to 6 months. It is, therefore, apparent that soil does not play important role as a source of infection in situations where many years of crop rotation is commonly practiced. A two year study (Degefu, unpublished) indicated that the pathogen did not survive the Finnish winter in infected tubers (Fig.3) and plant debris brought to the surface during autumn plowing. The bacteria have been occasionally detected from daughter tubers of potato originating from deeply buried mother tubers. In practice the role of volunteer potato in the seasonal carryover of Dickeya and Pectobacterium is less pronounced in the conventional practice of crop rotation system of potato production. In addition, it is often observed that the volunteer potato plants are killed by herbicides especially when the following crop is a cereal crop and a selective herbicide against broadleaf plants is used for weed control.

Figure 4. Overwintering tubers brought to the surface during autumn tilling and buried under the snow during winter and collected early in the spring for checking the presence of live Dickeya
and *Pectobacterium* spp. Tubers are mushy and watery. (Photograph: Yeshitila Degefu, MTT Oulu).

In general the role of weeds in survival of *Dickeya* and *Pectobacterium* spp. is not known. There are only few studies conducted and the reports are less conclusive. Recently (Tsror *et al*., 2010) detected and isolated latent *Dickeya* spp. from the weed *Cyperus rotundus*, a weed which is prevalent in warm tropical climate. One study indicated that *P. carotovorum* was isolated from the rhizosphere of some weed species (McCarter-Zorner *et al*., 1985). In Finland *Dickeya* spp. was detected by PCR from roots of a crucifer weed (Degefu, unpublished) collected from infected potato field but the plant showed neither any disease symptom nor the bacteria isolated from its roots suggesting that the bacteria was only present in the root zone as surface contaminant without infecting the roots.

There are several reports from many countries that *Dickeya* and *Pectobacterium* have been isolated from rivers and water courses (Laurila *et al*., 2008; Toth *et al*., 2010 and references therein). Therefore, water could be a potential source of inoculum and means of dissemination of the pathogens under circumstances where irrigation is used. The bacteria is found in rather low number in water samples making detection and isolation difficult with without enrichment culturing. So far *Dickeya* and *Pectobacterium* have been detected from the rivers and water courses in Southern Finland. In North Finland *Dickeya* spp. was detected from a river Lestijoki and water courses around Himanka area where mostly table potato is produced and irrigation is practiced more often. So far neither *Dickeya* nor *Pectobacterium* has been detected from rivers and water courses around the HG areas of Finland.

Crop contamination can also occur in field and storage from airborne sources such as rain aerosols and insects (Pérombelon, 1992; Czajkowski *et al*., 2011 and references there in). Although the bacteria are carried to long distances, the survival of airborne inoculum was found to be very short and the impact on disease epidemics may comparatively be less pronounced.

It is, therefore, generally known that *Dickeya* and *Pectobacterium* survive poorly outside the host plant (potato) and latently infected potato tubers are the major source of blackleg infection. Bacteria from rotting mother tuber can colonize potato roots and move via the vascular system into progeny tubers where it may cause stem rot or blackleg or survive latent in the progeny tubers (Czajkowski *et al*., 2010). Healthy seed tubers could be contaminated from rotting progeny tubers during harvesting, grading and planting. It is estimated that a single rotting tuber can release up to 200 ml of slimy bacterial fluid mass which deliver inoculum for contamination of up to 100 kg of seed tubers.
Control strategies.

The control of blackleg and soft rot has been very challenging. Many approaches have been considered but did not lead to good success. Chemotherapy and thermotherapy are not practical, and potato varieties resistant to the disease syndrome are lacking. Cultural measures such as avoiding poorly drained fields, excessive irrigation and hygienic measures involving cleaning farm implements help to minimize the risk of disease. Since it is widely recognized that seed tubers latently infected by the bacteria are the main source of infection, disease control strategies have focused mainly on the seed system- use of disease free seed multiplied from minitubers produced in disease free environment and practice of seed certification schemes. Whereas the production of minitubers in hydroponic and aeroponics cultures has contributed to the prevention of seeds from early generation field infections, circumstances of infection of high class first field generation are also observed (Degefu, unpublished). While it is unclear where infection of this high class first generation seed could occur, it is evident that a stringent testing of tissue culture material and minitubers for Dickeya and Pectobacterium is necessary to guarantee healthy planting material.

The obligations of protecting the High Grade (HG) area

The lack of healthy seed is one of the limiting factors of potato production. Major outbreaks of blackleg are often encountered when producers use non certified or home saved seeds (Degefu et al., 2013). The High Grade status is an EU granted status to ensure the supply of healthy seed potato for sustainable potato production. The rationale of the HG seed area provision is to maintain the high plant health status of the national potato crop. It prevents the import of seed potatoes from areas where certain harmful organisms are present. Areas included in the HG seed areas in the EU include, the municipalities Tynävä and Liminka in North Finland, Northern Ireland, Scotland, Cumbria and Northumberland in England, parts of Germany, and the Azores. Together with the HG status, comes an obligation to keep these areas free from dangerous pathogens and pests. Therefore, the concepts and practices of the High Grade status hold high promises to the production of high grade or healthy seeds in the countries that hold the status. It is, therefore, important that countries holding the status practice stringent measures to protect those areas from invasion by emerging aggressive species of Dickeya and Pectobacterium as one means of combating the ever increasing problem blackleg and soft rot in Europe. The Agrifood Research (MTT) Oulu has taken a step to work out a working model for the protection of the HG zone of the country by launching a specific project involving the major players of seed potato production and distribution in the country. The project aims at monitoring the activities in the potato production chain and focused in the HG area.
The HG status holds promises for the supply of healthy seed potato which is basic for sustainable potato production. It is also a privilege to for the countries holding the status to boost their seed potato export and develop their economy. Thus, a close observation of the rationale and obligation of HG is important step to prevent the introduction and spread of alien species and emerging disease problems which threaten potato production and global food security. In addition to safeguarding potato production from the menace of heavy losses from *Dickeya* and *Pectobacterium* in wider scale, it also creates economic advantages and growth of the seed potato sector in countries holding the status and a good reason for maintaining the privilege.

**References**


