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REVIEW

Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review

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This paper briefly reviews research on the causative agents of blackleg and soft rot diseases of potato, namely *Pectobacterium* and *Dickeya* species, and the disease syndrome, including epidemiological and aetiological aspects. It critically evaluates control methods used in practice based on the avoidance of the contamination of plants, in particular the use of seed testing programmes and the application of hygienic procedures during crop production. It considers the perspective of breeding and genetic modification to introduce resistance. It also evaluates the application of physical and chemical tuber treatments to reduce inoculum load and examines the possibility of biocontrol using antagonistic bacteria and bacteriophages.

Keywords: biocontrol, breeding for resistance, *Erwinia* spp., genetically modified potato, hygienic practices, physical and chemical control

Introduction

Potato (*Solanum tuberosum*) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*), in terms of both area cultivated and total production (Douches *et al.*, 1996). Potato does not require special growth conditions; it has been for a long time a major field crop in temperate regions, and increasingly in warmer regions (Haverkort, 1990).

Commercial cultivars are derived from a restricted number of potato clones introduced into Europe in the 16th century following the exploration of South America. This has resulted in a narrow genetic base with a limited range of resistance to many diseases that lower yields and tuber quality (Hooker, 1981). An estimated 22% of potatoes are lost per year to viral, bacterial and fungal diseases and pests, which is equivalent to an annual loss of over 65 million tonnes (Ross, 1986) (International Potato Center, Lima, Peru http://www.cipotato.org/; Food and Agriculture Organization, United Nations http://www.fao.org/).

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Bacteria belonging to the *Pectobacterium* and *Dickeya* genera are causal agents of blackleg and tuber soft rot of potato (Pérombelon & Kelman, 1980; Pérombelon, 2002). In seed potato production, these diseases are next in economic importance to bacterial wilt caused by *Ralstonia solanacearum*, ahead of ring rot and common scab caused by *Clavibacter michiganensis* subsp. *sepedonicus* and *Streptomyces scabies*, respectively (van der Wolf & De Boer, 2007).

During the last 40 years different aspects of blackleg and tuber soft rot and their pathogens have been reviewed. Attention has focused on taxonomy (Graham, 1964; Dye, 1969; Hauben *et al.*, 1998; Samson *et al.*, 2005), ecological and epidemiological characteristics (Starr & Chatterjee, 1972; Pérombelon & Kelman, 1980; Pérombelon & Salmond, 1995; Pérombelon, 2002; Charkowsky, 2006; Toth *et al.*, 2011), pathogenesis and regulation of extracellular enzyme synthesis (Pérombelon, 1982, 2002; Stanghellini, 1982; Barras *et al.*, 1994; Hugouvieux-Cotte-Pattat *et al.*, 1996; De Boer, 2003), genetics and molecular biology (Chatterjee, 1980; Robert-Baudouy, 1991), comparative genomics of pectinolytic bacteria (Toth *et al.*, 2003, 2006) and the biochemical basis of resistance of potato to blackleg and soft rot (Lyon, 1989). In contrast, there has been no comprehensive review of disease control to date.

This publication is a critical evaluation of past and current attempts to control blackleg and tuber soft rot, mainly from a European perspective. Disease control measures examined are: avoidance of contamination by the blackleg pathogen, role of fertilization, application of classical breeding and genetic modification to introduce resistance, the use of physical and chemical tuber treatments and research on biocontrol of blackleg and soft rot pathogens. The measures refer to both seed and ware crops to different extents; in the case of the former, the objectives are primarily to produce healthy crops by avoiding/reducing tuber contamination, whereas with the latter, the aim is to minimize yield loses by avoiding/preventing disease in the field and in storage.

Disease syndrome

Bacteria

The main bacteria causing blackleg, which affects the growing plant, and tuber soft rot of potato are the soft rot bacteria *Pectobacterium atrosepticum* (Pa), *P. carotovorum* subsp. *carotovorum* (Pcc) and *Dickeya* spp. (van der Wolf & De Boer, 2007), all formerly belonging to the genus *Erwinia* (*E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*) (Dye, 1969). They are pectinolytic Gram-negative, facultative anaerobic, nonsporing, motile, straight rods with peritrichous flagellae (Charkowsky, 2006). They belong to the *γ-Proteobacteria* subdivision and are clustered in the Enterobacteriaceae family (Charkowsky, 2006). They characteristically produce a variety of cell-wall-degrading enzymes that allow infiltration and maceration of plant tissues on which they feed (Barras *et al.*, 1994).

Whereas Pcc has a broad host range worldwide, Pa is restricted only to potato, principally in temperate regions. In contrast, *Dickeya* spp. affect a restricted number of host species in both temperate, subtropical and tropical regions (Ma *et al.*, 2007; Toth *et al.*, 2011).

The three species of bacteria can all cause tuber soft rot, but for a time, only Pa was believed to cause blackleg in temperate and *Dickeya* spp. in warmer regions (Pérombelon & Salmond, 1995). Recently, however, Pcc has been shown to infect potato plants causing typical blackleg symptoms (de Haan *et al.*, 2008). This may reflect the warmer summers prevailing in Europe lately or the possibility of newly adapted forms having arisen. It is notable that in Colorado and Arizona in the USA, with hot summers, Pcc was regarded for a long time as a true blackleg pathogen together with Pa (Molina & Harrison, 1977).

Although *Dickeya* spp. have long been associated with blackleg in tropical and subtropical regions, only strains of *Dickeya dianthicola* have been isolated from blackleg-diseased plants in Western Europe in the past two or three decades. These so-called 'cold-tolerant' strains showed a lower growth temperature optimum than other *Dickeya* strains (Janse & Ruissen, 1988). However, since 2005, a new genetic clade, probably representing a highly virulent *Dickeya* species belonging to biovar 3, has been detected in Europe (Tsror *et al.*, 2008; Sławiak *et al.*, 2009). Strains belonging to this clade were isolated from seed potatoes in France, Finland, Poland, the Netherlands and Israel. In many of these countries the pathogen was introduced via the international trade of seed potatoes. All isolates were clonal, which suggests a common origin and possibly a single introduction event. The same genetic clade has also been found in hyacinth. One might speculate that in the recent past this genetic clade was introduced from hyacinth into potato, possibly via the use of contaminated irrigation water (Sławiak *et al.*, 2009).

Recently, two new subspecies of *P. carotovorum* were described as organisms causing potato blackleg. *Pectobacterium carotovorum* subsp. *brasiliensis*, a highly aggressive bacterium, is responsible for the majority of blackleg incidences in Brazil (Duarte *et al.*, 2004) and South Africa (van der Merwe *et al.*, 2010). *Pectobacterium carotovorum* subsp. *wasabiae* has been described as a new potato pathogen in New Zealand responsible for high blackleg levels (Pitman *et al.*, 2010). Until then, *P. c.* subsp. *wasabiae* was found only in association with soft rot on Japanese horseradish (Gardan *et al.*, 2003; Pitman *et al.*, 2010).

Symptoms

The most characteristic symptom of potato blackleg caused by both *Pectobacterium* and *Dickeya* species is a slimy, wet, black rot lesion spreading from the rotting mother tuber up the stems, especially under wet conditions. However, when conditions are dry, symptoms tend to be stunting, yellowing, wilting and desiccation of stems and leaves (Pérombelon & Kelman, 1980). Late in summer, under persistent rainy conditions, extensive stem rot, starting from the top and progressing downwards to the base, can develop and can be confused with the symptoms of blackleg, but is usually caused only by Pcc (Pérombelon & Kelman, 1980).

Tuber soft rot is initiated at lenticels, the stolon end and/or in wounds under wet conditions. The lesion can spread to the whole tuber and thence to neighbouring tubers in storage. Tuber tissue is macerated to a creamy consistence which turns black in the presence of air, developing an evil smell when invaded by secondary organisms. When seed tubers start rotting in the field before emergence, blanking occurs. In inadequately ventilated cool stores, rotting can spread to adjoining tubers as liquid from the rotting tubers percolates onto others, sometimes leading to massive rotting pockets in the stored tuber lot.

Epidemiology

Knowledge on this topic is derived from studies usually carried out in temperate countries involving mainly Pcc and Pa, and rarely *Dickeya* spp., which only recently have become more economically important. Care must be taken when extrapolating past results to *Dickeya* spp.

The soft rot bacteria do not overwinter in soil. Survival in soil is restricted to 1 week to 6 months, depending on environmental conditions such as soil temperature, moisture and pH. Survival can be longer in association with plant material, including volunteers. In any event, the bacteria cannot survive in the soil in a crop rotation system of 3–8 years (Anilkumar & Chakravarti, 1970; Rangarajan & Chakravarti, 1970; Lim, 1975; Pérombelon & Hyman, 1988).

It is now generally accepted that the major source for blackleg infection is the latently infected seed (mother) tubers (Pérombelon, 1974). When the mother tuber rots, the bacteria are released into the soil and are transmitted by soil water to contaminate neighbouring progeny tubers. Czajkowski *et al.* (2010) showed that the bacteria in soil can also colonize potato roots and subsequently move via the vascular system into progeny tubers. Once in the stems, the bacteria do not necessarily cause stem rot (blackleg) and can survive in latent form.

Crop contamination can also occur from airborne sources (Graham, 1976; Pérombelon *et al.*, 1979; Harrison *et al.*, 1987; Pérombelon, 1992). Soft rot bacteria can be carried from diseased plants by airborne insects over long distances to contaminate other potato crops. Also, they can be present in aerosols produced by rain impaction on blackleg plants and by haulm pulverization prior to harvest. Air sampled in Scotland, even away from potato crops, contained both Pcc and Pa, more on rainy than dry days. Although they remain viable for 5–10 min only, they can be blown away for several hundred metres before deposition, mainly by the scrubbing action of rain.

Surface water in the USA and Scotland was found to be contaminated with Pcc and to a lesser extent with Pa (McCarter-Zorner *et al.*, 1984; Harrison *et al.*, 1987). Recently, in Europe, a biovar 3 *Dickeya* spp. genetic clade, not previously described, was found in river water and at the same time in seed potatoes in Finland (Laurila *et al.*, 2008, 2010). Hence, surface water used for irrigation purposes is likely to be a source for the pathogen, and may also be a source of new variants of the pathogen.

Contamination of crops can also occur during mechanical flailing (Pérombelon *et al.*, 1979). Most importantly, contamination of tubers can occur during harvesting and handling (grading) in store via the disintegration of rotting tubers and the spread of rotting tissue on machinery into wounds inflicted during handling (Elphinstone & Pérombelon, 1986; Pérombelon & van der Wolf, 2002). Therefore, there is a high risk that one or several soft rot bacteria will contaminate the commercially produced tubers, where they can survive from one generation to the next (Pérombelon, 1974, 1992; Pérombelon & Kelman, 1980; van Vuurde & de Vries, 1994).

Aetiology

Blackleg develops after rotting of seed (mother) tubers, but it is not a necessary sequel to mother-tuber rotting. Conditions which favour decay also favour blackleg development (Pérombelon, 1992). An important environmental factor for blackleg development is soil water level. Presence of a water film on the tuber surface induces development of anaerobic conditions in the mother tubers, thereby favouring bacterial multiplication and initiation of rotting (Pérombelon *et al.*, 1989b). Anaerobiosis affects oxygen-dependent host resistance, allowing unhindered bacterial multiplication and production of cell-wall-degrading enzymes, resulting in a rotting lesion (Fuqua *et al.*, 2001).

Another critical condition in disease development is the level of seed contamination, as shown in the case of Pa. The higher the bacterial density, the more likely the pathogen will predominate in the incipient lesion and the sooner rotting is initiated (Bain *et al.*, 1990). Progeny tuber contamination is related to seed tuber contamination as well as blackleg disease (Toth *et al.*, 2003). Although only limited data concerning *Dickeya* spp. are available, it seems that level of seed contamination is less important for blackleg development, possibly because they are more aggressive than *Pectobacterium* spp. (Velvis & van der Wolf, 2009).

Competition within rotting mother tubers, modulated by environmental conditions, temperature especially, determines which pathogen will predominate if more than one is present (Pérombelon, 2002). The soft rot bacteria can also interact with other pathogens, especially vascular ones such as *Ralstonia solanacearum*, *Fusarium* spp., *Verticillium* spp. and *Rhizoctonia solani* (Tsror *et al.*, 1990; Pérombelon, 2002). Weakening of host resistance by one pathogen may favour the development of another.

Control Strategies

Several approaches have been studied to control blackleg and tuber soft rot, but the degree of success has been variable. Methods based on avoiding contamination and reliance on seed certification schemes are widely used and have been partially successful. Improved store management can reduce bacterial load on tubers and tuber rotting. Both physical (especially hot water treatment) and chemical methods have been explored, but with limited success. The use of biological control has been and is still being attempted, but it is too soon to say how successful it will be. Finally, breeding for resistance has so far failed, but the use of genetic modification is promising, if politically feasible.

Avoiding contamination

When it was realized that blackleg is not a soilborne disease, the blackleg-affected plant was thought to be the main source of the pathogen. Therefore, disease control would be achieved by an indexing (certification) system of seed obtained from disease-free crops. In Europe, plant inspection services under national jurisdiction are responsible for certification of seed potatoes. The European Plant Protection Organization (http://www.eppo.org) provides standardized protocols and guidance for certification of plant material. Inside the European Union, the Phytosanitary Directive (2000/29/EG) describes general regulation on crop production requirements and guidance for member states in respect of good cultivation practices (for example, in the Netherlands, NAK provides guidance for certification).

For more than half a century, certification of seed potatoes has been the traditional approach to ensure that this objective is achieved. However, the degree of control achieved is erratic and heavily dependent on the weather prevailing during growth of the seed crop. Greatly improved understanding of blackleg epidemiology and aetiology can now explain why this approach has met with little consistent success. These measures cannot detect widespread latent infection of progeny tubers (the next generation of seed) from symptomless plants, as discussed above. Moreover, depending on weather conditions, heavily contaminated seed can give rise to little or no disease, and the converse is also true. Despite this, the measures can do some good. For example, roguing at an early stage of crop growth, which entails the removal of diseased plants, including daughter tubers, no doubt contributes to reducing an important source of the pathogen. Rotting progeny tubers are common on plants with symptoms, from which bacteria can spread during mechanical handling at harvest and postharvest.

Seed potato crops are classified into different seed grades according to several criteria, including the level of roguing and blackleg, from nil to a given percentage, depending on national certification scheme criteria. Seed crops are usually subjected to field inspections twice during the growing season in most seed-producing countries in Europe. Infected crops can be downgraded to a lower seed category or rejected from the market. Harvested progeny tubers (future-generation seed) can be tested also with molecular and serological methods to detect latent infections. However, this is not yet an obligatory part of testing programmes. As tubers from disease-free crops are frequently contaminated, laboratory testing can help to detect latently infected tubers. In contrast to other bacterial diseases of potato where there is often zero tolerance, some contamination of seed tubers can be allowed, in particular in low-grade seed. Therefore, the use of a detection procedure which allows estimation of both the density of bacteria and the incidence of contaminated tubers is desirable.

The relationship between seed health status and its contamination level has not been fully evaluated. It involves several steps, namely, collection of representative tuber samples from large quantities of seed lots, preparation of tuber tissue for testing, use of a quantification method and, last but not least, interpretation of the results in terms of blackleg risk assessment (Pérombelon, 2000). An additional compounding factor is the high cost involved in testing for contamination level. One possibility is to restrict testing to the highest seed grades where tolerance level could be zero. Testing of tubers should include the peel to detect lenticel and wound infections, and the stolon end, including the vascular tissue.

When it was demonstrated that the seed (mother) tuber is an important source of the soft rot bacteria, attempts were made to produce pathogen-free progeny (next-generation) tubers. Initially, potato stem cuttings were used (Graham & Harper, 1967), later axenically produced microplants and currently *in vitro*-produced minitubers, which should yield bacteria-free progeny tubers (Stead, 1999). Minitubers are grown in a controlled pathogen-free environment, using aeroponic and hydroponic cultures or in artificial soil systems in order to prevent contamination with soft rot bacteria (Ranalli *et al.*, 1994; Ali *et al.*, 1995; Rolot & Seutin, 1999; Farran & Mingo-Castel, 2006). Testing of approximately 100 seed lots of minitubers per year during 4 consecutive years showed that minitubers were consistently free of blackleg-causing bacteria (Velvis & van der Wolf, 2009).

In large-scale seed potato production, multiplication of the initial minitubers in the field is necessary for economic reasons. However, this has led, even after only two or three field generations, to *c.* 30% contamination with *Dickeya* spp. and 10% with Pa (Velvis & van der Wolf, 2009). Similar results were found in previous studies in Scotland, which showed that an initially bacteria-free potato stock became progressively more contaminated after the third year in the field. Interestingly, contamination occurred at the time that mechanical handling at harvest and grading in store became necessary (Pérombelon *et al.*, 1980). Therefore, it is likely that initial contamination came from machines already contaminated, although contamination by airborne bacteria cannot be ignored. In an attempt to overcome this problem, the number of generations from bacteria-free initial propagative material is restricted to a set number before loss of seed status in order to reduce buildup of contamination during seed-stock multiplication.

Studies carried out in the 1980s on the ecology of the bacteria identified several sources of the bacteria to contaminate seed crops before and after harvest (Pérombelon, 1992). This knowledge has allowed a more focused approach to reducing risks of introducing the bacteria at different stages of seed production. For example, it is desirable that crops should be dedicated as seed or ware, since the tolerance levels for blackleg are different, as well as harvesting time. However, when, for economic reasons, dual-purpose crops are sometimes grown, harvested late to maximize yield, and the seed-size fraction separated later from the ware, it is unavoidable that quality suffers (Van Der Zaag & Horton, 1983). Use of well-drained fields reduces the risk of tubers being surrounded by a water film that can result in anaerobiosis and consequent tuber decay in the field (Pérombelon, 1992). Late harvesting allows bacterial multiplication on leaves and in debris left on the ground following haulm flailing. This may result in contamination of progeny tubers underground during wet weather conditions (Burgess *et al.*, 1994). Monitoring tuber contamination during bulking in crops derived from stem cuttings over 5 years on five seed-producing farms in Scotland showed that farms which regularly applied hygienic measures consistently produced cleaner seed than the others (Pérombelon *et al.*, 1980). Washing and disinfection of machines used when planting, spraying, haulm flailing, harvesting and grading in store no doubt help to reduce risks of introducing soft rot bacteria in a pathogen-free crop (Pérombelon & Kelman, 1980; Pérombelon, 2002). Spreading and smearing of the bacteria in a seed lot can be reduced by removal of rotten tubers during harvesting and grading. Avoidance of wounding by correct machinery adjustment during harvesting and grading is important to reduce the risks of wounding, as bacteria can survive after wound healing (Pérombelon, 1992; van Vuurde & de Vries, 1994). Use of mature tubers with a well-developed periderm will also reduce risks of wounding.

Storage in bulk, or preferably in one-tonne boxes in the case of seed, in well-ventilated stores at low temperatures will avoid condensation on tuber surfaces, which in turn will prevent multiplication of the blackleg pathogen. If the tubers remain wet long enough, tuber decay can ensue, resulting in further spread of the bacteria when tubers are graded, and sometimes massive tuber decay (Pérombelon, 2000). It is critical to dry the tubers rapidly by forced ventilation with warm air to favour wound healing, followed by cooler air to control sprouting and for long-term storage (Wale & Robinson, 1986; Wale *et al.*, 1986). Good storage management is of importance, not only to prevent tuber decay, but also to avoid increasing the tuber inoculum load, which would result in greater subsequent disease risks.

Reduction in the incidence of tuber infections with blackleg and soft rot bacteria can be achieved by using true seeds instead of seed tubers. Such seeds, derived from sexual crosses, are believed to be free from blackleg and soft rot bacteria (Pérombelon & Kelman, 1980). Although soft rot bacteria are not easily transferred to true seeds via vascular tissue, some reports suggest that true seeds may also become externally contaminated with low populations of the bacteria (Colyer & Mount, 1983). However, this externally sited inoculum can be removed by hot-water or chlorine treatments (Colyer & Mount, 1983).

For the majority of developing countries, seed potato classification schemes have failed or are not yet available, while imported seed potatoes are often too expensive (Van Der Zaag & Horton, 1983; Chujoy & Cabello, 2007). The use of true seeds may be an attractive and low-cost alternative to the use of seed tubers in these countries in particular. The main advantage of using true seeds is that they can be easily produced in large numbers (Chujoy & Cabello, 2007). Above all, botanical seeds do not require cold storage facilities. They can be kept in simple stores for a long time, which is of considerable importance in hot regions in developing countries (Wiersema, 1986). However, the major drawback is the genetic diversity of the progeny, which requires that every generation has to be carefully selected for desirable traits to ensure a stable and as uniform a crop as possible (Chujoy & Cabello, 2007).

Effect of nutrition on plant resistance to blackleg and soft rot

Plant nutrition is believed to be an important component of natural disease resistance. Nutrition affects the growth of plants and their interactions with pathogens and other microorganisms, and in general is important for plant fitness status (McGovern *et al.*, 1985). Deficiency of essential elements will often result in an increased susceptibility to diseases.

Calcium plays an important role in the resistance of plants against bacterial pathogens (Bateman & Miller, 1966; Berry *et al.*, 1988). A high calcium content in crops is often positively related to increased resistance against bacterial diseases, including potato blackleg (Platero & Tejerina, 1976; McGuire & Kelman, 1984; Berry *et al.*, 1988). Calcium ions improve the structure and integrity of plant cell wall components, resulting in higher resistance to diseases involving tissue maceration.

Calcium fertilization is known to reduce soft rot caused by *Pectobacterium* spp. in Chinese cabbage (Park, 1969) and in bean (Platero & Tejerina, 1976). McGuire & Kelman (1984) showed, both in *in vitro* and field experiments, that bacterial soft rot caused by Pa was negatively correlated with calcium concentration in tubers. Resistance to blackleg and tuber soft rot appeared to be related to calcium concentration in seed tubers, but the results varied between the cultivars tested and over the 3 years of field experimentation (Pagel & Heitefuss, 1989). Calcium is not equally distributed inside plant parts and potato tubers in particular often have a low calcium level (Dunn & Rost, 1945; Collier *et al.*, 1980). Soils naturally low in Ca can be amended by adding $CaSO_4$ (gypsum) to increase resistance, not only to blackleg, but also to soft rot of progeny tubers (Bain *et al.*, 1996).

The level of nitrogen seems to be another factor that can affect susceptibility to soft rot pathogens. High levels of nitrogen, between 1050 and 1700 p.p.m., significantly reduced bacterial leaf blight of *Philodendron selloum* caused by *Dickeya* spp., but resulted also in a significant deterioration of plant growth (Haygood *et al.*, 1982). The effect of nitrogen levels on blackleg and soft rot in potato has not been explored, apart from a study by Graham & Harper (1966), who showed that blackleg incidence caused by Pa was lower in field plots treated with high than with low levels of N fertilizers.

A balanced fertilization of potato plants and an increase of the calcium content in soils alone will not provide sufficient control of blackleg and soft rot pathogens. It may, however, be a part of an integrated control strategy.

Breeding for resistance

Traditional breeding

Commercial potato cultivars that are naturally immune to blackleg and soft rot caused by *Dickeya* and *Pectobacterium* species do not exist, but some cultivars show partial resistance (Lyon, 1989). Attempts to breed potato cultivars with increased levels of resistance have only been partially successful and have never resulted in immune cultivars, probably because of the narrow range of genetic diversity in parental breeding material used (Tzeng *et al.*, 1990). In any event, breeding for soft rot resistance has not been given a high priority in most breeding programmes relative to other diseases and desirable agronomic traits. At best, cross progeny have sometimes been screened for resistance at advanced selection stages and the results possibly taken into consideration in the final selection.

Furthermore, screening for resistance is not straightforward. Cultivar resistance/susceptibility to blackleg and tuber soft rot are not always correlated; rather, different combination patterns are common (Pérombelon & Salmond, 1995). Several methods of screening for blackleg and soft rot resistance in breeding lines have been described. Ranking of cultivars for resistance to tuber soft rot caused by Pa varied according to inoculation method, presence or absence of oxygen and laboratory and field assessments (Bain & Pérombelon, 1988; Łojkowska & Kelman, 1994). Moreover, resistance/susceptibility ranking of cultivars varied from one season to another (Pérombelon & Salmond, 1995). Ultimately, field experimentation is the only reliable way to evaluate resistance of potato lines against blackleg and soft rot diseases (Allefs *et al.*, 1995).

The relatively low resistance to blackleg and soft rot in cultivars can be strengthened by utilizing the high levels observed in wild potato species (Dobias, 1977; van Soest, 1983; French & de Lindo, 1985; Corsini & Pavek, 1986). More than 200 wild *Solanum* species present in North and South American and European potato collections contain a large reservoir of useful genetic material, including resistance to soft rot bacteria (Hijmans & Spooner, 2001). The majority of these species are diploids which facilitate hybridization with *S. tuberosum* compared to *Solanum* spp. tetraploids (Watanabe *et al.*, 1994). These include *S. canasense* and *S. tarijense* (Carputo *et al.*, 1997) and *S. tuberosum* subsp. *andigena*, believed to be the direct ancestor of the common potato, which showed relatively high levels of tuber and stem resistance to both *Pectobacterium* spp. and *Dickeya* spp. (Hidalgo & Echandi, 1982). Sexual hybrids between *S. tuberosum* and *S. phureja* commonly used in breeding programmes also displayed relatively high resistance, but tuber yields were reduced (Rousselle-Bourgeois & Priou, 1995). When the wild *Solanum* species *S. commersonnii*, known for its resistance to frost, nematodes and fungi, was crossed with *S. tuberosum*, the hybrids showed high resistance to both *Pectobacterium* spp. and *Dickeya* spp. when screened on potato slices and in greenhouse experiments and field trials (Laferriere *et al.*, 1999). Similarly, hybrids of commercial potato and *S. stenotomum* resulted in lines with a higher resistance to blackleg and soft rot bacteria (Fock *et al.*, 2001). Sexual hybrids of *S. tuberosum* with *S. chacoense*, *S. sparsipillum* or *S. multidissectum* also showed higher resistance to blackleg than commercial *S. tuberosum* cultivars, but at the same time had a higher glycoalcaloid content, hence a higher toxicity to humans and animals (Carputo *et al.*, 1997).

Solanum brevidans is a diploid wild *Solanum* species that does not naturally produce tubers but bears resistance to several potato viruses and frost. Somatic hybrids obtained by fusion of protoplasts of *S. tuberosum* and *S. brevidans* showed a high level of resistance to blackleg and soft rot. Their resistance was attributed to the higher degree of esterification of cell-wall-binding pectin (McMillan *et al.*, 1994). Resistance to *Dickeya* and *Pectobacterium* bacteria was stable and could be sexually transferred to the progeny in the F_1 and F_2 generations as well as in backcrosses with *S. tuberosum* cultivars (Zimnoch-Guzowska & Łojkowska, 1993; Zimnoch-Guzowska *et al.*, 1999). This indicates that even relatively distantly-related *Solanum* species can be used to create hybrids with resistance to blackleg and soft rot (Austin *et al.*, 1988).

So far, traditional breeding has not succeeded in producing potato cultivars immune to *Pectobacterium* and *Dickeya* spp. Although none of the above results have been pursued further in breeding programmes, they indicate that classical (traditional) breeding for resistance against blackleg and soft rot can result in potato lines more resistant to *Pectobacterium* and *Dickeya* (Glendinning, 1983). However, breeding is usually a long process taking more than 10 years of screening and trialling to ensure that the selected material does not carry over undesirable traits. None of the hybrid lines have been commercialized at present.

Genetically modified (GM) potato plants

Genetic engineering is a promising alternative to traditional plant breeding, which is limited to closely related species and is time-consuming. An already established cultivar could be modified to increase resistance without the need to undergo time-consuming field trials for other traits. In Europe, however, introduction of genes into crops from noncrossable species to improve their quality is not readily accepted by society. For a long time, there was a moratorium on the cultivation of GM (transgenic) crops and only in March 2010 was the first GM potato cultivar with increased starch content for industrial use allowed in Europe (http://www.nytimes.com/2010/06/11/world/europe/11sweden.html).

Only a limited amount of work has been done so far using genetic modifications to increase resistance against bacterial pathogens. In the case of blackleg and soft rot bacteria, work has involved only *in vitro* plants and none of the transgenic potato lines have been exploited commercially. At present, single genes used to improve resistance are those coding for proteins that are bactericidal, impede pathogen multiplication or prevent pathogen–host or pathogen–pathogen interactions. Little if any of the transgenic resistance in tubers was verified under anaerobic conditions essential for rotting initiation.

Lysozymes are enzymes that lyse bacterial cells by degradation of their cell wall. T4 and chicken lysozymes are well-described proteins showing broad bactericidal activity. Potato plants modified to produce T4 or chicken lysozyme showed an increased resistance to Pa in greenhouse assays (Düring, 1996; Serrano *et al.*, 2000). However, introduction of these genes in potato plants may be harmful to naturally present beneficial bacterial populations of the potato rhizoplane and rhizosphere. It was observed that in transgenic potato plants, T4 lysozyme is released from roots into the soil and is able to kill *Bacillus subtilis* (Ahrenholtz *et al.*, 2000) and probably also other bacteria present on the root surface.

Peptides and proteins such as attacin and cecropin, that were found in the humoral immune response in insects, also showed antibacterial activity (Arce *et al.*, 1999). Cecropins and their synthetic analogues showed antibacterial activity in *in vitro* tests, and potato plants transformed with genes coding for attracin and cecropin analog SB-37 were generally more resistant to blackleg than the untransformed control strains, but the results were variable.

When invading plant tissue, blackleg and soft rot pathogens produce large quantities of pectolytic enzymes that degrade plant cell wall components (Hugouvieux-Cotte-Pattat *et al.*, 1996). Degradation of plant tissue cell wall generates polygalacturonate products that are used by the bacteria as a substrate for energy and biosynthesis but may also act as positive signals for the further production of pectolytic enzymes, depending on the length of the galacturonate chain (Lyon, 1989). Production of pectolytic enzymes is induced mainly by the presence of unsaturated digalacturonates released from the polygalacturonic polymers. Weber *et al.* (1996) proposed that expression of enzymes such as pectate lyases that can degrade these signals in transgenic potato plant tissue should inhibit the synthesis of large quantities of enzymes responsible for tissue maceration. Transgenic potato plants modified to produce pectate lyase were found to be resistant to infection caused by Pc in *in vitro* experiments and *in planta* tests (Wegener *et al.*, 1996; Wegener, 2001).

Many secondary plant products, including flavonoids (e.g. anthocyanin), steroidal alkaloids and saponins, show antibacterial activity against plant pathogenic bacteria (Hirotani *et al.*, 2000). Their synthesis and modification in plant tissues are controlled by different enzymes, among which glucosyltransferases seem to be important. Lorenc-Kukula *et al.* (2005) produced transgenic potato plants with increased 5-*O*-glucosyltransferase content. The ectopic over-expression of 5-*O*-glucosyltransferase improved resistance of potato tubers against *P. carotovorum* in *in vitro* experiments. The resistance to soft rot was at least twofold higher in transgenic lines than in non-transformed control tubers (Lorenc-Kukula *et al.*, 2005).

Bacteria sense their population density by a cell-to-cell communication mechanism in which particular genes are expressed only when the threshold bacterial density (quorum) is reached (von Bodman *et al.*, 2003). This mechanism, known as quorum sensing, controls diverse biological processes in human, animal and plant pathogenic bacteria, including virulence. Communication between cells occurs via small, diffusible signal molecules and in Gram-negative bacteria it is mediated mainly by acyl homoserine lactones (AHLs) (Fuqua *et al.*, 2001). AHLs regulate virulence gene expression in *Pectobacterium* and *Dickeya* species, possibly to ensure that infection will start only if the bacterial density is large enough to overwhelm the plant's response (Reverchon *et al.*, 1998; Andersson *et al.*, 2000). Bacteria of the genus *Bacillus* possess an AHL lactonase gene which blocks the quorum sensing mechanism by enzymatic degradation of signal molecules. When that gene was cloned and expressed in commercial potato cultivars, the transgenic plants showed a high level of resistance against *P. carotovorum*. Either symptom expression was entirely blocked or symptom development was significantly reduced (Dong *et al.*, 2001).

Objection to GM plants could be overturned by producing cisgenic rather than transgenic potato plants. In a cisgenic approach, recipient plants are modified with resistance genes from cultivars or lines of the same or a sexually compatible (crossable) species. The advantage of cisgenic over transgenic plants is that use of a gene of interest already present in the species for centuries does not alter the gene pool and/or provides no additional traits (Schouten *et al.*, 2006). Current research is looking at the potato–soft rot bacteria interaction at a molecular level. Bacterial proteins active during infection provide clues on resistance responses in potato clones with known different resistance levels and markers suitable for marker-assisted breeding could be developed. It is hoped that this would allow rapid introgression of resistance into cultivars (Phipps & Park, 2002; König *et al.*, 2004). To date, no resistance genes have been identified to control blackleg and soft rot of potato for use in a cisgenic approach. It is likely that more than one gene would be involved, which would complicate the task.

Although promising results have been obtained, there is a long way to go before GM potato plants resistant to soft rot bacteria become commercially available. Research has been restricted to laboratory or greenhouse studies and none were conducted under field conditions to test the performance of resistant lines in the field or to assess potential environmental risks of release of the GM plants. At present, it is likely that future research will focus on identification of resistance genes which could be used in a cisgenic approach.

Physical seed tuber treatment

The traditional method to conserve seed tubers in good health has been storage in ventilated stores at low temperatures (*c.* 5°C). These conditions are easily met in temperate countries when temperatures in stores can be regulated using cold outside air over the winter until planting time in the spring. However, in tropical and subtropical regions, unless expensive refrigerated stores are available, storage is a real problem. At high ambient temperatures, not only is dormancy restricted, but also, infection by pests, fungi and particularly soft rot bacteria can cause serious losses. This is an important factor limiting potato production in these regions.

Disease can develop before cold storage or in transit to the market, and specific treatments become useful. Physical control, mainly by heat, is recognized as competitive to biological and chemical methods as it does not require registration and may be effective against a broad range of pathogens. However, physical procedures affect not only superficially located pathogens but also beneficial microorganisms and may negatively influence tuber emergence and health. Most of the information that is available on physical control of plant pathogens in potato comes from the control of *Pectobacterium* spp. under postharvest storage conditions. There is only limited information concerning *Dickeya* spp. The physical factors applied in controlling tuber soft rot infection are those involving hot water, steam (Robinson & Foster, 1987; Shirsat *et al.*, 1991), dry hot air (Bartz & Kelman, 1985) and UV and solar radiation (Ranganna *et al.*, 1997; Bdliya & Haruna, 2007).

Hot water treatment of potato tubers to control soft rot bacteria contamination was first applied in 1983 (Mackay & Shipton, 1983). Pcc and Pa could not be detected in tuber peel after dipping naturally infected potato tubers for 10 min in water at 55°C. In field experiments, no blackleg was observed in plants grown from treated tubers. Similar results were obtained by Wale & Robinson (1986) and Shirsat *et al.* (1991), who showed that incubation in water at 44·5°C for 30 min or at 56°C for 5 min significantly reduced the periderm and lenticel contamination of seed potatoes and consequently blackleg incidence in the field (Wale & Robinson, 1986; Shirsat *et al.*, 1991). However, failure to dry large quantities of the tubers rapidly could result in multiplication of any surviving bacteria and even rotting. This difficulty was overcome by a continuous hot water treatment in which 50-kg batches were continuously treated for 5 min at 55°C followed by drying under forced ventilation with air knives. Cooling of the water when a large number of tubers is immersed is avoided and any residual moisture evaporated by the latent heat still in the tubers (Pérombelon *et al.*, 1989a). Effective blackleg control was obtained in field experiments with both vacuum-infiltrated and naturally contaminated tubers. Moreover, the treatment led to the control of several fungal pathogens causing gangrene, skin spot, silver scurf and black scurf (Dashwood *et al.*, 1991). The temperature/time combination used is critical, more so in bulk tuber dipping than in the continuous treatment. However, several side effects can adversely affect growth and have to be taken into consideration: depending on the cultivar used, tuber physiology can be altered resulting in delayed sprouting or even tuber death, and as a result, yield can be affected (Robinson & Foster, 1987; Pérombelon *et al.*, 1989a).

Steam was also tested as an alternative to hot water treatment to remove fungi and bacteria, especially Pc and Pa present superficially in the tuber periderm. The use of steam treatment reduced infestation of tuber periderm from 26–59% to 1–3% (Afek & Orenstein, 2002).

Bartz & Kelman (1985) reported that external but not internal populations of *Pectobacterium* spp. can be eliminated from washed tubers by application of hot dry air at 50°C. Hot dry air also dries the tubers and stimulates wound healing without interfering with tuber sprouting as much as hot water treatment. However, heat transmission by air is generally less effective than by water, necessitating a longer incubation time, which could adversely affect tuber physiology.

Ranganna *et al.* (1997) tested the efficacy of UV radiation for controlling Pcc in potato tubers. When tubers were inoculated by vacuum infiltration 6 h before radiation, bacteria were totally eliminated by a relatively low UV dose of 15 kJ m^{-2}. Vacuum-infiltrated tubers infected with Pcc and exposed to direct sunlight for at least 180 min did not develop soft rot symptoms, probably more because of an increase in the temperature of tuber superficial tissues than the action of UV energy, which is unable to penetrate tuber tissue to reach the pathogens. However, the practical value of these methods such as steaming, hot dry air and UV radiation is doubtful when applied on a large scale involving several tonnes of tubers.

In conclusion, physical control methods, especially hot water treatment, are environmentally friendly and allow some control of blackleg caused *Pectobacterium* spp. as well as of several superficial fungal pathogens simultaneously. Their limitations, however, are the inability to kill plant pathogenic bacteria located deep inside the tubers (vascular level) without a negative effect on plant growth, the substantial operational costs and the difficulty of expanding for large-scale use.

Chemical seed treatment

Chemical control strategies used against bacterial diseases are based on the eradication of the pathogen and/or the creation of unfavourable environmental conditions (e.g. low or high pH, etc.) for disease development. Once disease has been initiated, disease control is limited because of rapid bacterial multiplication and spread, and the inability of the chemicals to penetrate the inner tissues (Bartz & Kelman, 1985). Therefore, disease control has focused on latently infected tubers rather than blackleg-affected plants. A wide range of chemical compounds has been tested to reduce infection on or inside tubers by *Pectobacterium* spp. and *Dickeya* spp.

Most compounds used contain antibiotics (mainly streptomycin and its derivatives), inorganic and organic salts or combinations of these compounds. For a long time, streptomycin was considered a promising control agent against blackleg and soft rot diseases in potato. Immersion of seed tubers in a mixture of streptomycin and oxytetracycline hypochloride or streptomycin and mercury compounds before planting reduced the incidence of blackleg in the field and tuber decay in storage (Bonde & de Souza, 1954). Similar results were obtained when kasugamycin or virginiamycin was substituted for streptomycin (Wyatt & Lund, 1981; Bartz, 1999). However, although treatments with antibiotics showed promise, larger-scale field studies are no longer allowed because of the risks of introducing resistance to bacterial pathogens of humans or animals.

As an alternative to antibiotics, a wide range of potential bactericides have been tested, more often in small laboratory-scale experiments than in the field. Thus, organic compounds such as hydroxyquinoline and 5-nitro-8-hydroxyquinoline were effective for control of soft rot in wounded potato tubers (Harris, 1979). Similar results were obtained with chlorine-based compounds, bronopol (2-bromo-2-nitropropane-1,3-diol) and the synthetic bactericide, 7-chloro-1-methyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinic carboxylic acid (Bartz & Kelman, 1986). Immersion of potato tubers in citric, acetic, ascorbic or malonic acids also reduced rotting by Pcc in freshly vacuum-infiltrated potato tubers without affecting sprouting in *in vitro* conditions (Bartz & Kelman, 1986). Mills *et al.* (2006) showed that certain inorganic and organic salts, including aluminium acetate, sodium metabisulphate, propyl paraben, sodium benzoate, alum (hydrated potassium aluminium sulphate), potassium sorbate, calcium propionate, sodium hypochloride, sodium bicarbonate, aluminium chloride and copper sulphate, could inhibit the growth of Pcc and Pa *in vitro*. Some of these salts have already been approved as food preservatives and consequently their use to control soft rot bacteria would need limited additional registration testing. The activity of organic and inorganic salts may be attributed to the presence of cationic ions released from the salts that inhibit bacterial cell membrane protein functions or by modulation of the environmental pH by the anion moiety (Mills *et al.*, 2006).

Synthetic antimicrobial peptides were evaluated as they are a group of antibacterial agents that by interacting with the bacterial cell membrane increase its permeability (Gabay, 1994). Kamysz *et al.* (2005) reported that the synthetic peptide CAMEL (KWKLFKKIGAVLKVL, a hybrid peptide derived from two naturally present antibacterial peptides, cecropin A and melittin (Oh *et al.*, 2000)) gave greater protection to potato tubers against Pa and *Dickeya* spp. than streptomycin, protecting tuber tissue from rotting.

These chemical treatments of tubers to control blackleg and tuber soft rot are far from being straightforward. First, there is the problem of reaching the bacteria, which are usually well protected in lenticels, suberized wounds and the vascular system. Even systemic bactericides, if available, would fail if applied postharvest because there is no vascular activity in harvested tubers. A gaseous bactericide might be more successful, but penetration in tubers is likely to be poor and can be phytotoxic, as found by Eckert & Ogawa (1988) in the case of chlorine gas. The apparent success mentioned above can be explained by the fact that freshly harvested tubers with unsuberized lenticels and wounds were used. It may also be that testing was done on cut seed tubers, the use of which is common practice in some countries (Eckert & Ogawa, 1988). In addition, treating large quantities of tubers after harvest with a liquid bactericide would require efficient drying of the tuber surface to prevent multiplication of the bacteria and rotting, depending on storage method. For example, one option would be to treat freshly harvested washed tubers at the last rinse with hypochlorite solution to reduce superficial inoculum load, then dry them by forced ventilation using air knives to minimize the risks of rotting during storage in plastic bags in supermarkets.

Biological control

Biological control of plant pathogenic bacteria could be an alternative to chemical and physical control and breeding for resistance. Biocontrol strategies comprise the use of antagonists affecting pathogen populations directly, or via antibiosis, competition for nutrients or induction of plant systemic resistance (Howarth, 2003). Although several attempts have been made to control *Pectobacterium* spp. and *Dickeya* spp. on potato using biological control agents, most were restricted to *in vitro* overlay studies, potato slice assays or *in vitro*-cultured potato plants; few included field experiments to check for consistency of results. Only the more recent work will be discussed here.

It has long been shown that bacteria isolated from the potato rhizosphere or those isolated from potato tuber periderm can be used successfully to protect potato tubers from *Dickeya* and *Pectobacterium* infections in laboratory conditions (Kloepper, 1983; Rhodes & Logan, 1986; Jafra *et al.*, 2006). Initial selection of the control agent was based on random occurrence of bacteria that inhibited growth of soft rot bacteria in *in vitro* overlay studies. Further selection was based on characters likely to be inimical to soft rot bacteria. The agent is usually applied to seed tubers to control blackleg and rarely to control soft rot in stores.

In general, soil fluorescent and non-fluorescent *Pseudomonas* spp. obtained by *in vitro* screening have shown to be potential candidates for biological control of blackleg and soft rot diseases (Kastelein *et al.*, 1999). They are able to survive in the potato rhizosphere and in soil (Kloepper, 1983; Azad *et al.*, 1985; Gross, 1988; Loper & Henkels, 1999) and produce a variety of secondary antibacterial metabolites (Weller, 1988) including mainly siderophores, antibiotics and surfactants (Kloepper *et al.*, 1980; Cronin *et al.*, 1997; Compant *et al.*, 2005). Fluorescent *Pseudomonas* spp. applied to tubers were able to reduce populations of blackleg and soft rot bacteria on potato roots and inside progeny tubers (Kloepper, 1983). They could also apparently control soft rot on potato when applied as a bacterial suspension directly to the tuber periderm (Colyer & Mount, 1984). Cronin *et al.* (1997) used *Pseudomonas fluorescens* strain F113 producing 2,4-diacetylphloroglucinol (DAPG) to control Pa *in vitro* and on potato tubers. The wild-type strain F113 was able to inhibit growth *in vitro* and colonization of tubers by Pa, whereas a F113 mutant unable to produce DAPG was not effective, indicating that biocontrol occurred via antibiosis.

Kastelein *et al.* (1999) used strains of *Ps. fluorescens* to protect wounds and cracks on tubers from colonization by Pa. Application of individual and combinations of strains reduced the contamination of potato tuber peel by 85% and 60–70%, respectively, indicating the potential of *Pseudomonas* spp. for controlling soft rot caused by Pa.

Lactic acid bacteria are commonly found on fresh fruits, vegetables and milk products and pose no risk to human or animal health. *Lactobacillus plantarum*, *La. acidophilus*, *La. buchneri*, *Leuconostoc* spp. and *Weissella cibaria* isolated from fresh fruits and vegetables showed *in vitro* antagonistic activity towards Pcc in overlay assays which was attributed to the production of hydrogen peroxide and acidification of the medium (Trias *et al.*, 2008). In general, lactic acid bacteria possess different modes of action, mainly the production of organic acids, hydrogen peroxidase and siderophores, which can be effective for biocontrol. Lactic acid bacteria are able to inhibit more than one phytopathogen, thus *La. plantarum*, *W. cibaria* and *La. acidophilus* also inhibit the fungus *Botrytis cinerea*. They have a wide range of growth temperatures, ranging from 8 to 45°C, providing possibilities for broad applications (Trias *et al.*, 2008).

Gram-positive *Ba. subtilis* BS 107, which was selected for its broad antibiotic activity towards different plant pathogenic bacteria and fungi, was used as a biocontrol agent against soft-rot- and blackleg-causing bacteria (Sharga & Lyon, 1998). The strain was active in overlay assays against not only human pathogenic or opportunistic *Ps. aeruginosa*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Staphylococcus aureus* and *Escherichia coli*, but also against plant pathogenic Pcc, Pa and *Dickeya* spp., *Ps. syringae* and *Xanthomonas campestris*, which indicates that it is a potentially powerful agent to control different plant diseases. Cladera-Olivera *et al.* (2006) reported a bacteriocin-like substance produced by *Bacillus licheniformis* P40 that was bactericidal to Pcc. This substance interacted with cell membrane lipids, provoking lysis of Pcc cells. It was also effective in protecting potato tubers against soft rot under standard storage conditions (Cladera-Olivera *et al.*, 2006).

Jafra *et al.* (2006) focused on bacteria able to degrade quorum-sensing signal molecules produced by *Pectobacterium* spp. and *Dickeya* spp., which is a useful and effective strategy for the control of the bacteria by preventing the secretion of large quantities of pectolytic enzymes to macerate tuber tissue. The result of this work was a selection of several bacterial isolates (e.g. *Delftia* spp., *Ochrobactrum* spp., *Rhodococcus* spp.) able to control pectinolytic bacteria by the quorum-quenching mechanism in which infection of potato plants by target *Dickeya* and *Pectobacterium* bacteria was attenuated.

Predatory bacteria are ubiquitous in nature, present in different environments and able to invade and consume other bacteria (Stolp & Starr, 1963). *Bdellovibrio bacteriovorans* is a motile δ-proteobacterium that preys on Gram-negative bacteria (Rendulic *et al.*, 2004). Epton *et al.* (1990) tested different strains of *B. bacteriovorans* to control Pa on potato. However, only limited control of soft rot was obtained in potato slice assays when co-inoculated with *P. atrosepticum* and *B. bacteriovorans*. The main difficulty in using *B. bacteriovorans* as a biocontrol agent is that interaction with the prey bacterial cells is ruled by a specific predator–prey relationship in which the populations of both microorganisms may fluctuate without complete eradication of the target bacteria (Crowley *et al.*, 1980). Thus, Varon & Zeigler (1978) estimated that *Bdellovibrio* spp. were efficient as predators only when large populations of target bacteria were present. The minimum population density required for biocontrol of the target bacterium is about 10^5–10^6 CFU mL^{-1}, which, in the case of *Dickeya* and *Pectobacterium* spp., may already be high enough to establish infection in plants under conditions favourable to disease (Varon & Zeigler, 1978). Finally, as *B. bacteriovorans* feeds on *Pectobacterium* spp., it is impossible to use *Bdellovibrio* spp. to prevent contamination in tubers free of soft rot bacteria.

Another possibility for controlling bacterial diseases of plants is the use of bacteriophages. Bacteriophages are viruses that infect and lyse bacterial cells. They are specific to their hosts and do not infect other microorganisms. They are self replicating, persistent in the environment and safe to use, as they cannot infect humans or animals. It has already been shown that bacteriophages possess the potential to control plant pathogenic bacteria (e.g. *Erwinia amylovora*, *Agrobacterium tumefaciens*) (Jones *et al.*, 2007). However, their use is limited as they are not motile and the target bacteria tend to become rapidly resistant. Until now, little attention has been paid to the use of bacteriophages to control soft rot and blackleg bacteria in potato, but since Ravensdale *et al.* (2007) succeeded in reducing soft rot incidence on calla lily tubers inoculated with Pcc by up to 50% in greenhouse trials, there has been greater interest in this approach.

To date, no commercial biocontrol agents active against blackleg and soft rot bacteria have been produced. In fact there are few instances of this approach being successful in other crop–pathogen systems. The main difficulty is the requirement for the antagonist to satisfy several criteria. It has to reach its target, which in the case of potato would be located in lenticels, suberized wounds and the vascular system, sites not readily available at all times. Then, to be active, the agent needs to survive and multiply, preferably becoming established in the tuber and in the rhizosphere microflora. Another requirement is the preparation of a stable formulation. Too often, previous attempts have failed because some of the above criteria were not met. Moreover, transfer from small-scale to large-scale field testing can be difficult because of annual variations in the weather, resulting in lack of consistency in the results. Finally, there is the costly and time-consuming registration of biological control agents which would require expensive large-scale field experiments and ecotoxicological studies (Weller, 1988).

A possible approach which takes into account most of the above requirements is the application of the selected antagonist bacteria, preferably spore-forming, at the initial stage in seed-stock multiplication. Inoculation of microplants producing minitubers or of the microtubers before planting could allow establishment of the agent which could persist in later generations in the field. Protection of the first generations of seed crops is crucial, as control at that stage would reduce the risks of multiplication and spread of the pathogens at a later stage, at least in the high-grade seed lots.

Conclusions and perspectives

Although many different control strategies against *Pectobacterium* spp. and *Dickeya* spp. have been employed, effective control of blackleg and soft rot diseases has not yet been achieved. Until highly resistant cultivars become available, disease control measures will continue to rely primarily on avoidance of contamination in the production of healthy certified seed. This is primarily based on seed derived from bacteria-free minitubers, the use of rigorous seed certification schemes and strict hygienic practices. Knowledge of the pathogen sources and contamination pathways should justify the application of hygienic measures, especially at harvest and postharvest. Control strategies can be supported also by tuber treatments, as discussed above. Only an integrated disease control strategy is likely to succeed in reducing blackleg and soft rot incidences effectively in seed and thence ware crops. The recent presence of apparently more virulent forms of *Dickeya* spp. relative to *Pectobacterium* spp. should give a new urgency to research, notably on diagnostics, initial crop contamination and breeding for resistance.

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