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Postharvest treatments of potato tubers for the removal of potato cyst nematodes

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Potato cyst nematodes (PCN) (*Globodera* species) are a significant pest of potatoes worldwide. These nematodes are quarantine pests and their movement is regulated by many countries. The eggs of PCN are protected within cysts that can survive for several years and may be transported in soil attached to tubers. The bodies of female PCN are transformed into cysts after maturity, and become detached from potato roots, stolons or tubers into the soil at harvest. Cysts of PCN remain in the soil after tuber harvest and since this soil may remain attached to potato tubers, they pose serious threats of spread of the pest (along with tubers) into areas (including countries) where PCN has not been reported.

The risk of spreading PCN with tubers is greatest if soil is not removed using some pre-plant treatment. Seed and fresh potatoes destined for export, need to be certified as free of adhering soil and cysts of PCN. In this respect any postharvest treatments of tubers that destroy PCN cysts without harming the tubers would not only minimise the risk of PCN spread but also remove non-tariff barriers in potato export markets and reduce the market access cost.

A literature search was carried out to find reports of any postharvest treatments that are likely to completely remove the adhered soil from potato tubers to mitigate the risk of PCN spread. This has shown that brushing and thorough washing of tubers can completely remove the adhered soil. However, tubers with deep and narrow cracks resulting from mechanical damage during harvesting and grading should be removed from export lines, as they could retain soil and PCN cysts. Modern PCN detection methods could possibly be used as part of certification of freedom from the pest for tubers destined for export.

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1 Introduction

Potato cyst nematodes (PCN) (*Globodera* species) are the most damaging pest of potato crops worldwide, especially where potatoes are intensively grown. Two species of PCN, *Globodera rostochiensis* and *G. pallida*, which are genetically different, are recognised as serious threats to potato production globally. These pests are subject to strict quarantine regulations in many countries. These nematodes can cause severe crop damage and considerable yield reduction (Trudgill et al. 1998), with 100% yield loss under severe infestation (Brodie & Mai 1989) and very high cost of management (Marshall 1998).

Once established, PCNs are very difficult to eradicate, due to their long survival (20–30 years) as cysts in field soil, even in the absence of suitable host plants (Turner 1996). Their ability to produce cysts that are transported for long distances when soil adheres to tubers has made PCN the most highly regulated nematode pests (Sullivan et al. 2007). Because of the difficulty in eradicating PCNs, management practices such as surveillance and quarantine are widely implemented to prevent their spread to potato-growing areas where the nematodes are not widespread (Lehman 2004). Hygiene measures have also been put in place for using PCN-free seed tubers and table potatoes.

Natural spread of nematodes through soil has been calculated as 1 to 2 m per year (Hunt et al. 2001). Thus, the key form of PCN dispersal over the long distances is by humans and movement of contaminated soil adhering to table potatoes and seed tubers, to cultivation and harvesting equipment, or to the roots or bulbs of plants grown in contaminated fields (Mai 1977). Since eradication of PCN is very expensive and virtually impossible, decontamination of potato tubers, particularly seed tubers, is considered a prerequisite for market access of potatoes for any purpose.

A review of scientific and related literature was undertaken to ascertain if there were any reported efficient postharvest treatments for the removal of soil and PCN cysts from potato tubers that do not harm seed tuber quality (viability).

2 Origin of potato cyst nematodes

Both the species of PCN, *G. rostochiensis* and *G*. *pallida*, are biotrophic plant parasites, and can only grow and complete their life cycles on host plants. There is general consensus that PCN originated from the Andean highlands of South America, where they co-evolved with their host plants belonging to the family Solanaceae. Both the nematode species were accidently introduced into Europe from South America, probably in soil adhering to seed potato tubers (Hockland et al. 2012). Potato varieties currently grown in Europe were developed from two early introductions, one to Spain in 1570 and one to Britain in 1588 (Evans et al. 1975). The cultivated Andean tetraploid subspecies of potato (*Solanum tuberosum* subsp.*andigena*) was probably introduced on both occasions (Turner & Evans 1998). Brodie (1984) concluded that Europe was the main source of potatoes, which spread throughout the world from these two early introductions.

PCN were probably introduced to Europe around 1850 (Evans et al. 1975) when potatoes from South America were taken to Europe as part of a search for late blight resistance. This was in response to the Irish famine as a consequence of late blight epidemics in the 1840s. It is most probable that PCN arrived into Europe in soil adhering to seed potatoes (Hockland et al. 2012), and that the nematodes established in Europe after this introduction. Subsequently, Europe probably acted as the secondary distribution centre of PCN, which was again carried to other countries in soil adhering to seed potatoes (Evans & Rowe 1998; Turner & Evans 1998). This conclusion is based on similarities in the virulence characteristics between European PCN populations and those in the rest of the world outside South America. This conclusion is supported by several molecular studies (Madani et al. 2010; Skantar et al. 2007; Pylypenko et al. 2005).

Potato cyst nematodes were first discovered in New Zealand in 1972 in the intensive potato growing district of Pukekohe, near Auckland in the North Island (Dale 1972). The particular infested potato crop was brought to the notice of the Ministry of Agriculture and Fisheries (MAF) because of its poor growth, wilting and reduced yield. This infestation from Pukekohe was identified as *G. pallida* (Wouts 1976). A national PCN survey programme was initiated by MAF as PCN was considered a pest of quarantine importance to the export of New Zealand produce. A number of PCN infestations were indentified outside the Pukekohe area in other parts of New Zealand including Christchurch and Dunedin in the mid 1970s, and in Pukekawa, Opiki and Oamaru in the late 1980s. Each year from 1973 to 1988 one quarter of the potato crops throughout New Zealand were surveyed (Marshall 1993) by examining living plants in the field (Wood et al. 1983). MAF revoked all the PCN controlling regulations within New Zealand in 1988, and any future control of PCN became the responsibility of potato growers.

3 Biology of potato cyst nematodes (PCN)

Like many other plant parasitic nematodes, PCNs are slender, microscopic, eel-shaped worms. Juveniles of PCN undergo the first moult in the eggs and second-stage juveniles (J2) emerge in response to the stimulus of exudates released from host roots. Freshly emerged second-stage juveniles are very active and penetrate host roots just behind the root tips. They enter roots by puncturing the cell walls with their stylets. They migrate through root cortices for short distances and start feeding by withdrawing root cell contents. Once the nematodes become sedentary, they undergo a series of three moults to become fourth-stage juveniles, which is the adult stage. When they emerge, adult males are very active, vermiform and about 1 mm long. The males migrate from roots to fertilise the females. Adult females swell, rupture the host root cortex and eventually protrude from root surfaces and become visible. Only the thin anterior portion of each female remains embedded in the root. After the fourth moult the females fill with eggs and die at full maturity. The body cuticles of females become hardened and transformed into cysts each containing 200–500 eggs. At this stage the cysts generally detach from the surface of roots into the soil. PCNs are capable of infecting stolons and newly formed tubers, but their life cycle remains the same as on roots. Cysts of the nematode develop outside of stolon and tuber tissues, exposed to the soil and detach from the plant tissue surface.

Within the cysts, the eggs and the J2 individuals are protected by a sugar solution from extreme climatic conditions. Eggs protected in this way can remain viable for 25–30 years. PCNs complete their life cycles during the potato cropping period, and there is generally only one generation per crop. Under some environmental conditions, however, there is evidence that a second nematode generation may occur in each crop (Evans & Stone 1977; Greco et al. 1988; Perez et al. 2009).

Potato cyst nematodes, introduced into new areas with potato tubers or other contaminated plant material or soil, are likely to establish during long cycles of potato crops. In the absence of potato, PCN can infect eggplants and tomatoes, which are equally good hosts of these pests. Even in the absence of host crops, PCN can become established at low population densities because they may reproduce on any of several wild hosts (Sullivan et al. 2007) present in the area around fields or in non-potato crops.

4 Postharvest treatments of potato tubers for removal of soil or PCN cysts:

Very few postharvest treatments are reported in the literature for disinfecting seed potato tubers from PCN and adhered soil. Some of the treatments necessary to kill PCN cysts have proved to be harmful to the potato tubers (Mabbott 1956), human beings and the environment, and are also very costly. These include fumigation with highly toxic compounds. To facilitate market access of New Zealand potato tubers, it is important to ensure tubers are free from soil material that may be contaminated with PCN cysts. In this respect the pioneering work done by Wood and Foot (1975, 1977) from New Zealand was an important contribution to the production of clean seed tubers. They showed that 1% sodium hypochlorite solution disinfected potatoes by destroying the PCN cysts and eggs in 30–45 minutes. Their work was successfully replicated in other countries to disinfect seed potato tubers from cysts of PCN (Manoharan et al. 1978; Brzeski & Rogala 1984). Recently, however, sodium hypochlorite treatment of seed potato tubers has not been recommended due to the phytotoxic and corrosive effects of high concentrations of available chlorine (Gardener et al. 2006).

Machmer (1946) and Mabbott (1956 and 1960) demonstrated that washing could remove the soil adhering to potatoes grown in PCN infested soil, and that the potatoes were free of cysts when examined after washing. Dry brushing and washing removed 80% of the nematode cysts, while jet washing removed more than 90% of the cysts and soil from potato tubers harvested from soil heavily infested with PCN. The efficiency of jet washing to remove soil from tubers to minimise the risk of PCN spread has increased since Machmer (1946).

It has been found that, for efficient soil removal from harvested tubers, washing should be done within 1 or 2 days after lifting. If the tubers harvested from heavy clay soils are “clamped” (stockpiled) before washing then it may be difficult to remove the firmly adhered soil. In the Netherlands, pre-soaking before washing of tubers with adhered dried soil was found to be useful (Mabbott 1960).

There is still a risk of PCN spread with washing and brushing for soil removal, because 90% elimination of soil from tubers is unlikely to be sufficient where 100% freedom from PCN is required under strict quarantine regulations.

Complete freedom from PCN can be guaranteed if consignments from land declared free (using appropriate sampling methods) are thoroughly washed (Mabbott 1956). However, the results of Machmer (1946) and Mabbott (1956 and 1960) were based on non-replicated trials so these may not be verifiable.

More recently, Gardener et al. (2006) reported that all PCN cysts were removed from tubers of potato ‘Trent’, harvested from PCN-infested peaty clay field, when they were washed (three washes) until less than 5% of the tubers retained small visible soil patches. The results of this study were confirmed in four validation tests of three wash treatments. No cysts were detected in any of the 25 replications, consisting of a total of 2500 tubers, although statistical analysis showed that one wash was sufficient to remove most of the PCN cysts. On the basis of these results, it was concluded that tubers washed to this standard would have an acceptably low risk of carrying PCN cysts on potatoes for processing. Other jurisdictions may require higher levels of risk mitigation provided by conditional testing. This could include pre-planting soil tests (using traditional and/or molecular detection protocols) to establish tuber production occurred in fields designated “PCN-free”. Gardener et al. (2006) further reported that certification based on the described standard of washing was used to approve interstate movement of washed tubers

from fields in the quarantine area where no PCN was detected to a processing factory in Queensland, Australia. No PCN cysts were isolated from soil and waste from these potato tubers using standard PCN extractions techniques (Fenwick 1940; Turner 1991).

A greater level of risk mitigation than simple thorough washing may be required. Karanastasi and Kormpi (2011) reported that combining washing and brushing of the tubers was very efficient, and successfully removed all PCN cysts from potato tubers, for ‘Lizeta’ and ‘Marfona’ grown in sandy loam soil heavily infested with PCN. Mechanised brushing and washing was performed in a specially designed machine and all the washed tubers were found free of soil and PCN cysts, although significant numbers of PCN cysts were found on control (untreated) tubers. The washing and brushing machinery consisted of an inclined delivery elevator and a platform equipped with a series of rotating cylinders bearing brushes and an exit container. Water was supplied by a series of three nozzles positioned over the rotating brush platform. Each series of nozzles was followed by a series of brushes with which the tubers were in direct contact while they were passing over the platform. Karanastasi and Kormpi (2011) suggested that following this protocol would allow the transportation and use of potato tubers without the risk of spreading PCN.

Modern potato washing systems are widely used for preparation of potato tubers for fresh market sale. These systems very efficiently remove soil to present the tubers in excellent and clean condition. The machinery could equally be used for seed or table potatoes bound for export.

Care should be taken to completely dry the washed potato tubers to avoid the infection by fungi and bacteria. Karanastasi and Kormpi (2011) further emphasised that treating washed tubers with copper would protect them from fungal and bacterial infection. Brushing of soil, particularly from deep-eyed varieties, followed by thorough washing would be beneficial to further mitigate the risk of tubers carrying PCN cysts.

5 Molecular detection of potato cyst nematodes

Modern molecular methods for detection could be used for routine assessment of PCN contamination of soil and tubers to reduce the risk of any accidental introduction of this pest. Different molecular tests are available which can be used along with the visual assessment after washing of potato tubers. One of the PCR diagnostics tests was developed by Bulman and Marshall (1997) in New Zealand and recommended by the European and Mediterranean Plant Protection Organization (EPPO) (Anonymous 2007) as a standard for the diagnosis of PCN. This is a multiplex PCR test using species specific primers based on sequences of18S and internal transcribed spacer 1 (ITS1) ribosomal RNA genes. This test is used routinely for diagnostics to confirm the identification of cysts as part of a commercial service.

6 Conclusion

PCN is a highly regulated organism in many countries. This pest is considered as a serious threat to potato production worldwide, and the main cause of spread to new locations is through PCN-contaminated soil adhering to potato tubers. Based on current knowledge, table or seed potatoes free from PCN contamination can be exported to countries without the pest if:

- All the soil adhering to the tubers is completely removed by efficient washing and brushing;
- Potato tubers are harvested from land declared free of PCN contamination, after confirmation with appropriate testing protocols for detection of the pest.

Potato tubers with unusual protuberances, narrow cracks and holes due to harvesting or grading damage or from pests should be removed as there could be a chance of soil retention in these cavities.

The washed tubers should be completely dried after washing to avoid fungal and bacterial infection during storage and export. Brushing and washing would mitigate the risk of tubers carrying PCN cysts in all potato cultivars in general and in deep-eyed varieties in particular.

Modern PCN detection methods could possibly be used as part of certification of freedom from the pest for seed tuber or table potato lines destined for export.

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