WASHING POTATO TUBERS GROWN IN SANDY LOAM SOILS COMPLETELY DECONTAMINATES THEM FROM *GLOBODERA* CYSTS

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Summary. Potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, are quarantine organisms that can severely affect quality and quantity of potato production and can easily be disseminated on infested tubers and in soil debris adhering to tubers. Because eradication of PCN from a field is virtually impossible, decontamination of tubers is considered a prerequisite for marketing potatoes for any purpose. We demonstrated that a procedure combining washing and brushing of tubers successfully removed all PCN cysts from tubers of the potato cultivars Lizeta and Marfona produced in sandy loam soils severely infested with PCN. This would allow subsequent transportation and use of potato tubers without the risk of spreading nematode cysts.

Key words: Potato cleaning machine, potato cyst nematodes.

Potato is one of the main components of the Mediterranean diet and the most common side dish within the everyday Greek meal. Moreover, it is one of the most profitable crops in Greece and the main source of income for many farmers. For example, on the Cyclades islands, especially the island of Naxos, a traditional potato growing region, potato fields cover ca. 1400 hectares and produce a high quality crop of about 14,000 tons per annum, according to the 2005 Statistics of the Hellenic Ministry of Rural Development and Foods. Moreover, Naxos island has a special system for growing potatoes: the crop is grown in spring, harvested around June and planted again in autumn or winter, which is not common for other potato growing regions of Greece. This production is not exported but is for domestic use only. Moreover, despite the fact that Lizeta is a rather common cultivar in the international markets, its seed cannot be imported as it is not available anywhere else at this time of the year as seed potato tubers.

In Greece, potato is affected by several pests and pathogens, but one of the main problems is that posed by potato cyst nematodes, which can cause considerable yield losses (Trudgill *et al.*, 1998). Published information on the distribution of these pests in Greece is scarce and limited to only a few reports on *G. rostochiensis* being present in only a small number of potato growing areas, and *G. pallida* has never been reported except for the island of Crete (Vovlas and Grammatikaki 1989; Vlachopoulos, 1991, 1994; Tzortzakakis *et al.*, 2004). In a preliminary sampling conducted on the island of Naxos, in February 2007, twelve out of thirteen samples were found infested (0, 10, 10, 20, 30, 50, 70, 150, 155, 200, 200, 200, 267 cysts/kg soil), in six of which the population was extremely high. In addition, since 2002, the majority of the soil samples analyzed at the Benaki Phytopathological Institute have been found to be infested with PCN, indicating that these pests are quite common.

Globodera pallida Stone and *G. rostochiensis* (Woll.) Skarbilovich are quarantine organisms and infested fields within the European Community are, according to the Council Directive for the control of PCN (2007/33/EC), subject to quarantine and management regulations. These regulations include the use of resistant cultivars, tillage, soil solarization, three-year crop rotation schemes, fallow and nematicide treatments. In Greece, crop rotation and fallow are not economically feasible, mainly because most of the potato producers have only small holdings. The use of resistant cultivars is not yet possible because PCN pathotypes in Greece have not yet been identified. Also, their frequent use may select for new pathotypes.

As seed potato crops cannot be planted in fields infested with PCN, the cultivation of seed potato tubers in Greece is expected to be banned in the near future. This will inevitably have a strong economic impact in Greece and especially on Naxos island. Therefore, we decided to test whether seed potato tubers produced in infested fields can be freed from adhering PCN cysts before transportation, in order to avoid contamination of PCN-free areas. This had been demonstrated earlier by Machmer (1946) and Mabbott (1956, 1960), but not confirmed with adequate experimental replicates. Also, Wood and Foot (1975, 1977) disinfested potato tubers using 1% sodium hypochlorite, but the use of high concentrations of chlorine should be considered carefully as this chemical has phytotoxic and corrosive effects. Finally, in a more recent publication (Gardner *et al.*, 2006) it was concluded that washed potato tubers do present a low risk of carrying PCN cysts, and this is not

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Table I. Potato cultivars, base level of field infestation and soil type of the experimental fields (SL: Sandy loam, LS: Loamy sand, L: Loam).

Test	Trial	Potato cv.	Infestation level (cysts/kg soil)	Soil type
1	1	Lizeta	2236	SL
	2	Lizeta	1664	SL
2	3	Lizeta	267	SL/LS
	4	Lizeta	200	SL
	5	Marfona	50	L

Table II. Numbers of cysts recovered in the two trials. C: control (base level of tuber infestation = number of cysts recovered from the controls).

Test	Trial	Sack	Number of cysts on		
			Tuber	Paper	Floor
1	1	1+2 (C)	0	56	0
		3+4+5+6+7	0	312	0
	2	1+2 (C)	0	12	3
		3+4+5+6+7	0	94	0
2	3	1+2 (C)	0	6	0
		3+4+5+6+7	0	0	0
	4	1+2 (C)	0	5	0
		3+4+5+6+7	0	2	0
	5	1+2 (C)	0	2	0
		3+4+5+6+7	0	1	0

acceptable for seed potato tubers. However, during the latter study, the tubers were simply washed with water using a washing plant without brushes and, in addition, the work was conducted with tubers of potato cv. Trent grown in peaty clay soil, conditions that do not conform to the situation in Greece.

During the present work, we applied washing in combination with brushing, the most important innovation in this study, in a procedure that is repeated three times. The washing/brushing plant used for this study was designed specifically for the study, *i.e* to ascertain whether or not washing, in combination with brushing, of tubers of potato cvs Lizeta and Marfona, both susceptible to PCN, grown in sandy soil fields heavily infested with cyst nematodes, can remove the risk of spreading the pest. Lizeta is a commonly planted cultivar, whereas Marfona is less popular but was selected for testing as it has a lot of indents on its surface and may be more liable to carry adhering cysts and difficult to clean.

The aim of this work was to find a way that allows potato growing areas, such as in Greece, considered as PCN-infested for the purposes of the EU Council Directive, to continue potato cultivation without being totally dependent on imported seed.

MATERIALS AND METHODS

The experiment consisted of two tests. The first test was performed with potato tubers collected and packed directly as they come from the field, so that a large quantity of soil would have remained attached to the tubers (Trials 1 and 2 in Table I). The second test was performed with potato tubers that had followed the traditional procedure of harvesting, windrowing[1], quality/size sorting and packing (Trials 3-5 in Table I). The tubers from both tests were immediately transferred to the washing/brushing plant.

Site and crop details. The fields, from which the tubers were collected, were all located on the island of Naxos. The details of soil type and level of infestation of each field are summarized in Table I. Potatoes were planted at the end of February 2009 and harvested at the end of May 2009. Soil sampling was conducted prior to planting potatoes, according to the instructions given by the Council Directive 2007/33/EC, and cysts were extracted using the Fenwick can (Fenwick, 1940).

For the first test, six fields were sampled to choose the two that were more heavily infested, whereas for the second test, thirteen fields were sampled to choose the three more heavily infested.

[1] Harvested tubers are stacked in piles and covered with straw for a period of up to 20 days so that possible wounds are healed and any fungal or bacterial infections show symptoms. Also during windrowing, the stimulation of high temperatures in combination with high humidity prepares the tubers for hibernation termination.

Fig. 1. The first component of the washing/brushing plant: the inclined delivery elevator.

Fig. 2. The inclined delivery elevator is followed by this platform equipped with rotating cylinders bearing brush hairs. Water is supplied by three series of nozzles positioned over the rotating brush platform.

Tuber sampling. Each trial utilised seven sacks of tubers, each containing 50 kg of potatoes (ca. 400 tubers). For each trial, two of the sacks were treated as controls and the remaining five were treated in the washing/brushing plant.

For the first test, the tubers were collected randomly from the field, whereas for the second, sacks were chosen randomly from the lot, after harvest, windrowing, sorting and packing.

Washing/brushing procedure. Control tubers were hand washed immediately after sampling to avoid the attached soil becoming dry and hard to remove. The base level of attached PCN cysts was determined by thorough scrubbing of the tubers with the aid of a fine-celled sponge. The whole process was performed in a large bucket of water so that no soil debris or cysts would be lost. The water suspension collected in the bucket, with all contents, was sieved through a 200 µm sieve and the catch on the sieve transferred onto a white filter paper, left to dry for one day, so that examination of debris would be easier, and then examined under a stereomicroscope at 10× magnification for the presence of cysts. The sponge used for washing was examined after washing of each sack of tubers for any cysts remaining attached. Also, immediately after processing the control, the sacks were washed, the floor where they had stood was thoroughly swept and the refuse examined under a stereomicroscope for the presence of cysts.

The other five sacks of tubers were transferred to the washing/brushing plant immediately after sampling, so that attached soil would not dry out and become difficult to remove.

The washing/brushing plant consisted of an inclined delivery elevator (Fig. 1), followed by a platform equipped with a series of rotating cylinders bearing brushes (Fig. 2) and an exit container. Water was supplied by three series of 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 as they passed over the platform. The tubers advanced progressively towards the exit container, passing slowly through the three series of nozzles and brushes. The exit container was filled with water so that tubers would undergo a final rinse. A drainage pipe was connected to the spinning platform, and this allowed the water from the nozzles to drain into a tank after passing through a sieve of 200 µm aperture. In addition, a sieve of 1 cm aperture was placed between the brush platform and the drainage pipe to catch any larger debris and to avoid blocking the drainage pipe.

Each trial was processed separately and, when finished, the plant was thoroughly cleaned. The water from the exit container was also drained through the 200 µm aperture sieve and the tubers were packed in fresh sacks. The catch of each sieve was transferred onto white filter paper as described above and taken to the laboratory for further examination.

The sacks used for packing were not kept for examination as they are burnt after transportation; however, the area where they were kept was thoroughly swept and the refuse examined under a stereomicroscope for the presence of cysts.

Examination. All washed tubers (control and test) were examined under a stereomicroscope at 10× magnification for the presence of any adhering soil or cysts, taking care not to exclude the areas around the eyes, the stolon ends or other indentations. All skin blemishes, fungal and insect scars or other indentations of the tubers were also examined thoroughly.

RESULTS

Background level of cysts in the soil and on unwashed tubers. The background level of cyst infestation in the

field before experimentation was very high, especially in the case of Test 1 (Table I). The numbers of cysts found on the control and test tubers, the sponge and floor for both tests are shown in Table II.

Test 1. Before treatment, all tubers appeared quite dirty, covered with large patches of soil. After washing, they were virtually completely clean around the eyes, stolon ends and other indentations.

Examination of the controls showed that a significant number of cysts adhered to the tubers; however, no cysts were recovered from the tubers after hand scrubbing. Despite the large numbers of cysts on the unwashed tubers, none was trapped on the sponge, but a very small number of cysts was recovered from the floor (Table II).

With regard to the test tubers, the number of cysts recovered from the water passing through the drainage pipe was 312 for trial 1 and 94 for trial 2. No cysts were recovered from the tubers after washing or from the exit container.

Test 2. Examination of the controls showed that a very small number of cysts remained adhering to the tubers after windrowing, quality/size sorting and packing; only 6, 5 and 2 cysts were recovered from each of the two sacks of trials 3, 4 and 5, respectively. No cysts were found on the tubers either after hand scrubbing or after processing in the washing/brushing plant. Also, no cysts were found trapped on the sponge or recovered from the floor.

With reference to the test tubers, the number of cysts recovered from the water of the drainage pipe was zero for trial 3, two for trial 4 and one for trial 5. No cysts were recovered from the tubers after the process or from the exit container.

DISCUSSION

The results of this study indicate that the washing/brushing procedure was very efficient in removing PCN cysts adhering to potato tubers produced in heavily infested sandy loam soils. After washing, all tubers were found free of cysts, despite the high level of soil infestation and the significant numbers of cysts found on the control tubers. However, tuber transport to the washing plant may involve a possible, small risk of spreading cysts, as a small number was found on the floor where the sacks were left until use. Specifically, three cysts were recovered from the fourteen sacks of trial 2, whereas no cysts were recovered from trials 1, 3, 4 and 5. Also, the possibility of spreading cysts before washing applied only to the first test, in which the tubers did not follow the normal procedure followed with seed potatoes on Naxos island. In a normal situation, after harvest and before packing, Naxos seed potato tubers are left for ca. 20 days on the field (windrowing) and subsequently undergo a quality/size sorting that is performed manually rather than automatically.

During the period of windrowing, the attached soil dries out and, due to its sandy consistency, most of it falls off the tubers. Then, while sorting, the remaining soil is removed as workers scrub the tubers by hand to detect any insect, fungal or bacterial contaminations, cracking, shattering or other damage. Nevertheless, to overcome any possible problems, the authors suggest using closed containers during transportation. Additionally, the sacks used for packing should not be made of hessian cloth but of other material, for example plastic, that makes it more difficult for any cyst possibly present to get caught amongst its net. Also, plastic sacks have smaller pores that do not allow cysts to exit from the sack easily. However, we recommend the use of non-reusable paper sacks instead of plastic, whose destruction by burning would release dioxins into the atmosphere. Finally, all containers, forklift trucks etc, used during transportation, should be thoroughly cleaned after each use and all trash and sacks burnt.

To avoid contaminating the area surrounding the washing plant, all drainage pipes and exits should be equipped with 200 μm sieves so that cysts are trapped, and the catch of each sieve should be destroyed either by burning or by decontamination at a minimum of 56 °C for 2 hrs. Based on our study, and the fact that the soil on Naxos island is sandy and easily removed from the tubers during harvesting, windrowing, quality/size sorting and packing, it can be concluded that Naxos seed potato tubers have only a very low risk of carrying PCN cysts, even when they are not washed. However, cleaning in specially made washing/brushing plants can completely remove PCN cysts from the tubers. Care should be taken to completely dry the tubers after washing, so that they do not favour development of fungi or bacteria. Alternatively, the exit container that is used to provide the final rinse to the tubers could be filled with an appropriate disinfectant. Treating seed potato tubers with copper, as practised on Naxos island, would protect them from bacterial infections.

This present work may not include an impressive amount of results but it does describe the construction of a massive and important machine. The problem emphasized herein and the typical system of potato production are not confined to the island of Naxos but are representative of the whole coastal area of the Mediterranean basin. The proposed machine could be useful for many small farmers all around Europe and also for central Chile, where potatoes are cultivated during September-December, February-June and June-September, while certified seed potato tubers are available only in September.

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