Survey of Bacterial and Fungal Seedborne Diseases in Imported and Domestic Potato Seed Tubers

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Potato seed tubers are imported to Israel from northern Europe and planted in spring; tubers harvested early from the spring crop are used as seed for the autumn crop. Although only seed lots registered as certified are imported, a previous survey (1984–1994) indicated that most imported lots were affected by latent or active infections caused by *Erwinia carotovora, Streptomyces scabies, Rhizoctonia solani, Fusarium* spp. and *Spongospora subterranae.* The survey was extended until 1998, and included additional pathogens: *Ralstonia solanacearum, Helminthosporium solani, Colletotrichum coccodes* and *Verticillium dahliae.* Most of these pathogens were also monitored in domestic seed tubers, and are reported for the first time. Brown rot was not observed in any of the imported lots. Blackleg and soft rot caused by *Erwinia* spp. were detected in most of the imported lots; however, less than 7% of the lots were contaminated at high levels, while approximately 65% were contaminated at moderate levels. Common scab was detected in most of the imported lots; 51% of the imported lots were contaminated at moderate or high levels, whereas only 6.5% of the domestic seed lots were contaminated at these levels. Black scurf was detected in most of the imported lots; on average, 47.3%, 44.2% and 1.4% of the lots were contaminated at low, moderate and high levels, respectively, and only 7.1% were disease-free. In contrast, most of the domestic lots were either disease-free (45.4%) or had a low disease incidence (37.3%). Only 16.7% of the lots were moderately infected and 0.2% were highly contaminated. Silver scurf was observed in most of the imported lots during all years of the survey, with no differences among the producing countries; on average, 22.7%, 66.1% and 7.5% of the lots were contaminated at low, moderate and high levels, respectively, and only 3.7% were disease-free. Most of the domestic lots (76%) were disease-free and only 6.6% were infected at moderate or high levels. Black dot was observed in a considerable portion of the shipments from Holland during all years of the survey, particularly in 1998, when 34% of the lots were infected. The shipments from France and Germany were infected at low levels, except in 1998, when 19% and 11% of the lots, respectively, arrived infected. In shipments from Scotland and Ireland low incidences of the disease were observed in 1994 and 1995. In the domestic lots, black dot incidence was low (<2.4%) except in 1996, when 11% of the lots were infected. *V. dahliae* was monitored only in domestic seed tubers. The incidence of disease-free lots was 56–64%, whereas in 20–30% of the lots the level of infection was <5%, and in 6–16% of the lots the level was >5%. The survey findings demonstrate transmission of seedborne pathogens; most of these pathogens can become established in the soil and eventually cause severe outbreaks of disease in potatoes grown in Israel.

KEY WORDS: *Erwinia carotovora; Streptomyces scabies; Ralstonia solanacearum; Rhizoctonia solani; Helminthosporium solani; Colletotrichum coccodes; Fusarium* spp.; *Spongospora subterranae; Verticillium dahliae*.

Received Dec. 8, 1998; received in final form March 28, 1999; http://www.phytoparasitica.org posting May 16, 1999.

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INTRODUCTION

Each year, Israel imports approximately 14,000 tons of potato seed tubers (from northern Europe, particularly Holland [70%]) (19) for the spring season. Progeny tubers of this season are harvested early and planted during the following autumn–winter, mainly for export to Europe as fresh potatoes. Although in their country of origin the seed tubers (most defined as grade A) had been tested and certified, monitoring carried out in Israel during 1984–94 (14,19) indicated that bacterial and fungal pathogens, either latent or active, were carried in/on the tubers. The tubers become infected during their growth in Europe; however, some diseases become apparent only after the tubers are washed, or following shipment or the storage period. Moreover, some latent infections can be identified only by microbiological testing or sensitive serological methods, which were not performed in the exporting countries (with the exception of Holland, which tested for brown rot).

Infected seed tubers are a major source of disease development during the growing season, and are also a source for soil infestation (1,3,7). Minimizing soil infestation is of major importance in Israel, where short crop rotation cycles (2–3 years between crops) are employed due to land shortage. The short rotation cycle leads to extensive use of soil fumigation and entails the risk of enhanced aggressiveness of seedborne pathogens, due to a reduction of competition by other organisms eliminated by the fumigation (4). Thus, planting contaminated tubers in fumigated soil could cause severe outbreaks of disease.

Monitoring for diseases transmitted by tubers facilitates the identification of ‘cleaner’ lots, which can be earmarked for seed production, reducing the risk of disease establishment and extensive soil infestation. It may also indicate if seed and/or soil treatments are required in each case.

The following diseases were monitored: (*a*) Brown rot caused by *Ralstonia solanacearum* is a quarantine disease. Each lot arriving from Europe is tested for this disease, and tubers may be planted only if they have been certified to be free of this disease. Brown rot symptoms include wilting of young plants, stunting, chlorosis, discoloration of vascular bundles in tubers, and a beige-gray bacterial exudate. *R. solanacearum* is a soilborne pathogen that can infect other hosts as well (8). Monitoring of this pathogen in imported seed lots began in 1995, upon the detection of the disease in Holland in fields designated for seed production. (*b*) Blackleg and soft rot caused by *Erwinia carotovora* subsp. *atroseptica* (Van Hall) (*Eca*) and *E.c.* subsp. *carotovora* (Jones) (*Ecc*), respectively, become apparent in early spring, and are manifested by blackening of the stem and stunting. As the season progresses, rot appears in the below-ground stems, and desiccation of the plant is common (10,11,13). Several surveys conducted within the last decade indicate that most shipments of commercial seed tubers are infected with *Erwinia* (1,19). Moreover, a correlation was observed between the level of seed contamination by *Eca* and blackleg incidence in the field (2,13). (*c*) Common scab, caused by *Streptomyces scabies* (Thaxter), affects mainly tuber quality, due to the appearance of tan to brown corky lesions of various sizes developing at random across the tuber surface (14). (*d*) Black scurf, caused by *Rhizoctonia solani* (Kühn), appears as sclerotia on the tubers, thus reducing the quality of progeny tubers. The pathogen is also responsible for stem canker and stolon pruning (15). (*e*) Silver scurf, caused by *Helminthosporium solani* (Dur. & Mont.), appears as round grayish spots on the tuber’s surface; in severe infections the spots spread, covering considerable portions of the surface and damaging tuber quality. Affected tubers may shrivel during storage, and lose weight (8). *H. solani* was monitored only during the extension (1994–98) of the survey. (*f*) Black dot caused by *Colletotrichum coccodes* (Wallr.) is characterized by the development of small black sclerotia on senescent and dead plant tissue, on decaying roots and stems, and on stolons and daughter tubers (8). Yield reduction may occur, especially in the autumn or under stress conditions (18). Monitoring of *C. coccodes* was started in 1994, following the first outbreak of the disease in Israel. (*g*) *Fusarium* dry rot affects sprouting and emergence at the beginning of the season, results in yield loss, and damage to the quality of daughter tubers, especially during storage (8). (*h*) Powdery scab, caused by *Spongospora subterranea* (Wallr.), occurs as pustules (2–20 mm in diameter) on the tuber surface which develop beneath the epidermis and erupt upon emergence. When disease is severe, wart-like outgrowths appear (8). The disease was first identified in Israel in 1982. Field experiments proved that diseased imported seed tubers produced progeny that was infected at high levels, particularly under arid conditions (9). (*i*) Verticillium wilt caused by *Verticillium dahliae* (Kleb.) causes leaf chlorosis, stunting, and early dying of the plant, and thus substantially decreases yields (14). The pathogen may be transmitted *via* latent infection of the vascular bundles of the tuber. Monitoring of *V. dahliae* was carried out only in domestic seed tuber lots. Such monitoring is important because *V. dahliae* has already caused severe outbreaks of disease in Israel and is also known to be a multi-host pathogen. For several years we had examined imported seed tubers from Holland and found them to be free of this pathogen. Weather conditions in northern Europe, where the imported potato seed tubers are produced, are probably not favorable for *V. dahliae*.

MATERIALS AND METHODS

Defining lots

Lots imported from Holland have a number assigned to each grower, even when the tubers of one cultivar are harvested from several fields. For potatoes from other European countries and for domestic ones, a separate lot number was assigned to each field and cultivar. Each lot consisted of five to 100 tons.

Sampling

Seed tubers are usually imported to Israel during November and December in bulk (large bags of 1000 kg), or in sacks of approximately 50 kg. Officials of the Ministry of Agriculture, Plant Protection and Inspection Services, took samples for brown rot tests. Testing for brown rot was done prior to the shipment's release from the port, on samples of 200 tubers per 25 tons (statistically there is a 87% chance of detecting an infected tuber if the infection rate is 1%). For the other diseases a single random sample was taken from each lot directly from bags or during grading. Each sample consisted of 120–200 tubers taken at random from each imported lot (statistically there is a 99% chance of detecting an infected tuber if the infection rate is 5%). Domestic tubers (120 tubers per lot) were sampled in the field, in W or X pattern, 7–10 days after defoliation, or during grading before storage.

Testing methods

Testing for *R. solanacearum* infection was done in accordance with the protocols of the Plant Protection and Inspection Services, and the usual inspections as specified by the

European Market and the EPPO. Samples from the stolon end of the tubers, where the bacteria commonly occur, were extracted in sulphate buffer (0.05M), filtered, centrifuged and plated on the following modified semi-selective medium: 0.1% casamino acids (Difco), 1% peptone (Difco), 1.7% agar (Difco), 0.5 ml glycerol, 5 mg/*l* crystal violet, 100 mg/*l* polymixin B sulphate, 25 mg/*l* bacitracin, 5 mg/*l* chloramphenicol, 0.5 mg/*l* penicillin, 50 mg/*l* tetrazolium salts, for isolation and diagnosis of *R. solanacearum* (5). Suspect colonies were tested with more sensitive and specific methods (ELISA, fatty acid profiling, PCR and bioassay).

Tuber contamination by *Eca* and *Ecc* bacteria was monitored according to Pérombelon (12). Four composite sub-samples of 20 tubers each were peeled in a commercial potato peeler by dry abrasion for 3–5 minutes. The sap was extracted from the resulting peel pulp, decimal dilutions were prepared after adding dithiothreitol (0.05%), and 0.1 ml per dilution was plated on four replicated plates of Crystal Violet Pectate medium. Two plates were incubated at 27°C for 48 h and the other two at 33.5°C for 24 h. The bacteria were identified and counted on the basis of the cavity formation pattern of colonies and their numbers at the two temperatures: *Eca* forms pits only at 27°C and *Ecc* at both 27 and 33.5°C. *Erwinia* contamination was expressed as number of cells per gram peel. Levels of 10^3–10^5 bacteria/g peel are considered to be moderate; levels higher than 10^5 bacteria/g peel have been reported to damage potato crops under the growing conditions in Israel (13).

To evaluate common scab, black scurf and silver scurf, tubers were washed in running tap water and examined visually. The percentage of contaminated tubers at different disease severities was determined on a four-grade scale (nil, low [<3% of the tuber surface affected], moderate [3–25%] and high [>25%]) (6).

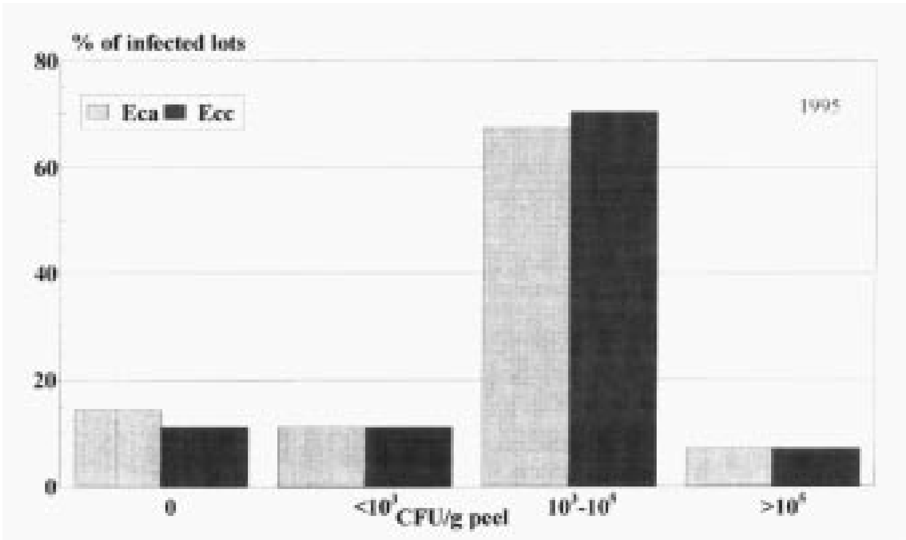

Fig. 1. Percentage of imported seed lots from Holland (1995) infected by *Erwinia carotovora* subsp. *atroseptica* (Eca) and *E. carotovora* subsp. *carotovora* (*Ecc*), and level of contamination within samples, expressed as colony forming units (CFU) per gram peel extract.

Black dot, Fusarium dry rot and powdery scab levels were determined as the percentage of infected tubers in a 200-tuber sample. Microscopic examination was carried out on at least five typical powdery scab lesions per lot, to verify the presence of the pathogen.

When Fusarium dry rot, silver scurf or black dot were observed, isolations from the lesions on symptomatic tubers were made on potato dextrose agar plates, and the identification of pathogens was confirmed under a microscope.

Only domestic seed tubers were monitored for latent infection with *V. dahliae* and *C. coccodes*, which are carried in the vascular bundles of the tuber. Three segments were taken from the vascular bundles and plated on a growth medium of 2% sorbose, 1.5% agar and 100 ppm streptomycin (16). One hundred and twenty tubers were sampled from each lot.

RESULTS AND DISCUSSION

Screening for brown rot in imported seed lots began in 1995, following a report on the disease in Holland. Despite extensive testing of all imported Dutch seed lots, *R. solanacearum*, the causal agent of brown rot, was not detected in any of the imported lots (17).

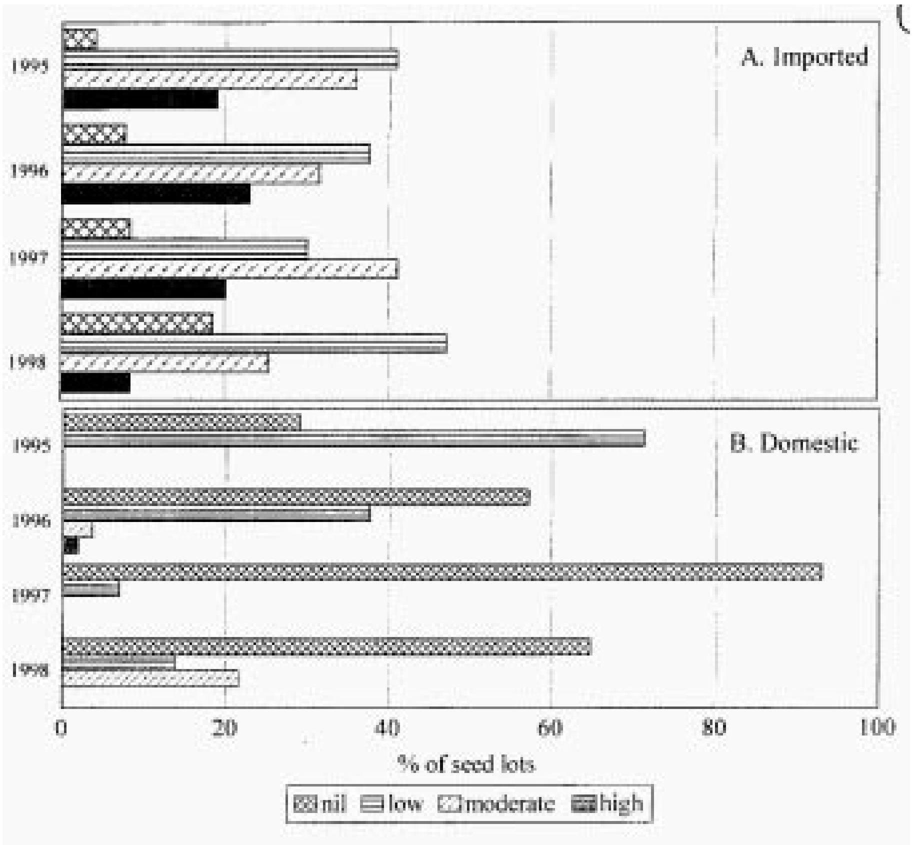

Fig. 2. Distribution (%) of common scab (*Streptomyces scabies*) infection and disease severity in imported and domestic seed lots surveyed during 1995–98.

Latent contamination with *Erwinia* was tested during 2 years of the survey in the imported seed lots. Similarly to our previous findings (19), approximately 65% of the lots were contaminated at moderate levels (10^3–10^5 bacteria/g peel), with no difference between subspecies (*Eca* and *Ecc*) and country of origin. This level of contamination might affect yield under certain weather conditions or if seed tubers are cut before planting. Up to 7% of the lots were contaminated at high levels ($>10^5$ bacteria/g peel). Representative data from lots imported from Holland in 1995 are shown in Figure 1.

Common scab was detected in imported seed lots each year (Fig. 2A), irrespective of the country of origin. On average, 10% of the lots were disease-free, 39% were contaminated at low levels, 33% at moderate levels, and 18% at high levels. In domestic seed lots the incidence of disease-free lots was significantly higher (Fig. 2B). On average, 61% of domestic lots were disease-free, 32% were contaminated at low levels, 6% at moderate levels, and 0.5% at high levels. Low levels of infection in Israel can be attributed to horticultural practices. All Israeli potato fields are irrigated, a practice which affects disease expression by preventing development of the drought conditions that can affect European fields during dry summers.

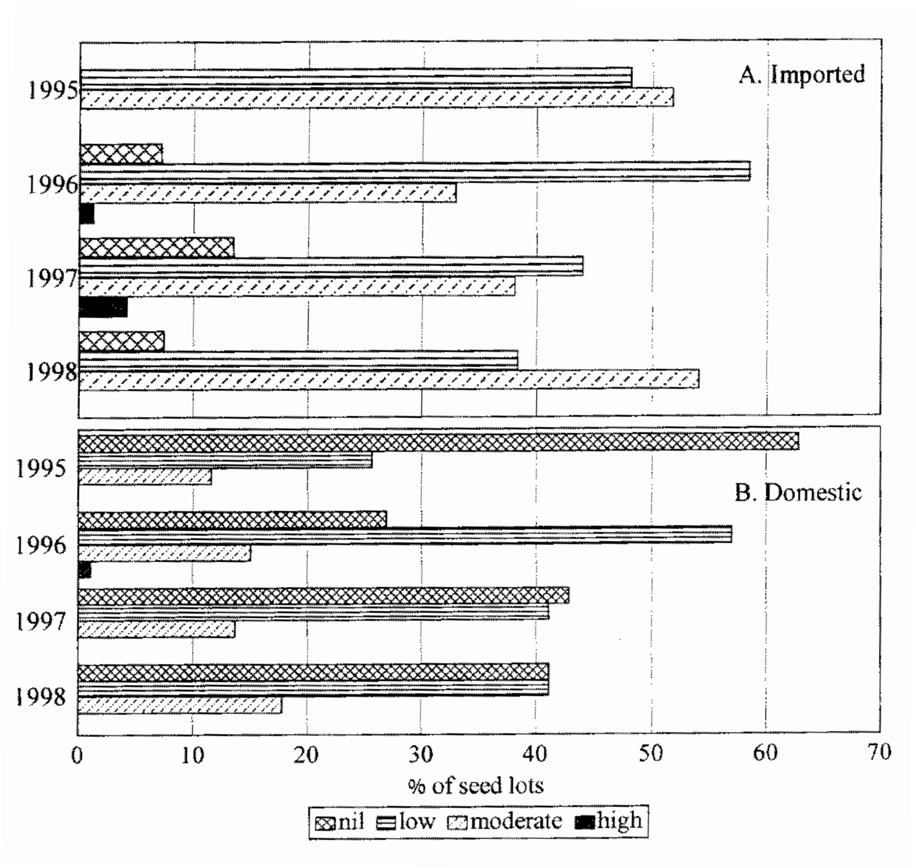

Fig. 3. Percentage of imported and domestic seed lots infected by black scurf (*Rhizoctonia solani*) at different disease severities (1995–98).

The importance of the blemish diseases caused by *R. solani, H. solani, C. coccodes* and *Fusarium* spp. has increased recently, mainly due to changes in marketing. A greater proportion of potatoes is being washed before marketing, causing skin blemishes to be more apparent and less tolerated by the consumer.

Considerable portions of imported seed lots were found to be contaminated with *R. solani* each year (Fig. 3A), irrespective of country of origin. On average 7.1% were disease-free, 47.3% were contaminated at low levels, 44.2% at moderate levels, and 1.4% at high levels. By comparison, 45.4% of the domestic seed lots were free of the disease, 37.3% were infected at low levels, 16.7% at moderate levels, and only 0.2% at high levels (Fig. 3B). The lower level of *R. solani* infection might be explained by the different growth conditions. Conditions in late spring in Israel are less favorable for black scurf development than the growing conditions in Europe, due to summer rains there.

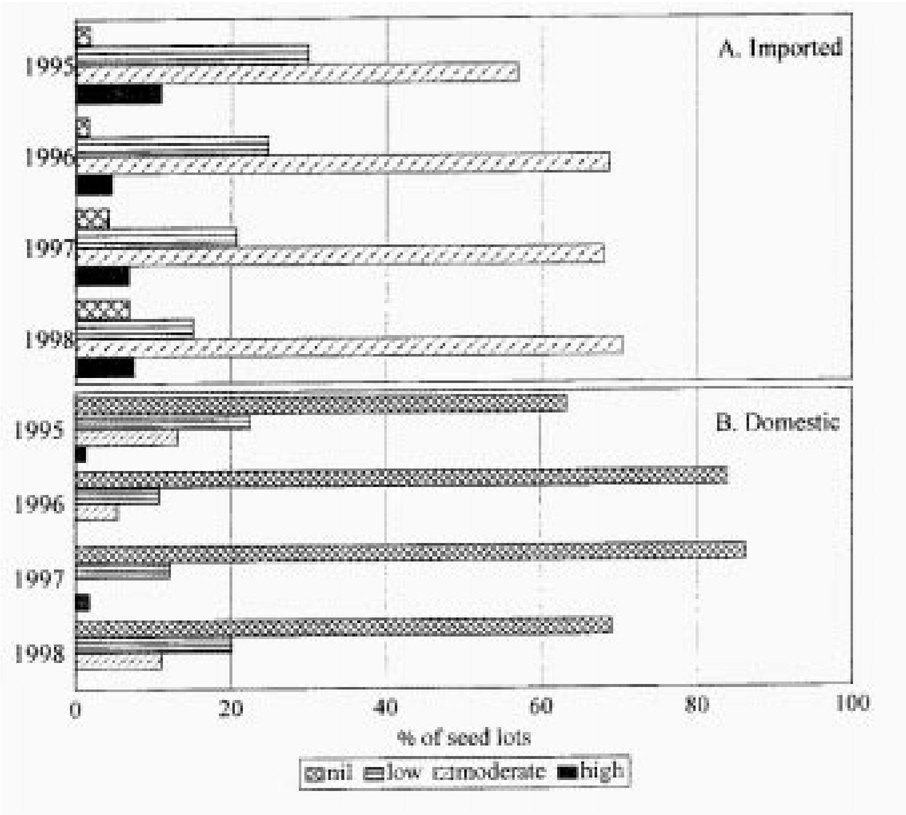

Fig. 4. Percentage of imported and domestic seed lots infected by silver scurf (*Helminthosporium solani*) at different disease severities (1995–98).

Helminthosporium solani was detected in imported seed lots during each year of the survey (Fig. 4A), irrespective of country of origin. On average, 3.7% of the lots were disease-free, 22.7% were infected at low levels, 66.1% were moderately contaminated, and 7.5% of the lots were infected at high levels. In contrast, most of the domestic seed lots were disease-free (Fig. 4B). On average, 76% of the lots were disease-free, 17.2% were infected at low levels, 6.4% at moderate levels, and 0.6% at high levels. The low disease incidence in domestic seed can be attributed to the fact that monitoring was implemented immediately upon harvest, whereas monitoring of imported seed tubers was done after storage and shipping. Silver scurf is known to become more severe during storage; therefore, if monitoring of domestic seed had been implemented closer to the time of planting (following 3 months of storage), a higher disease incidence might have been observed.

Colletotrichum coccodes was observed in imported seed tubers during each year of the survey (Fig. 5). In 1998, high percentages of the lots were contaminated (34%, 19% and 11% from Holland, France and Germany, respectively). In the previous years of the survey (1994–97), a high incidence of contaminated seed lots was observed only in shipments from Holland (13.6% and 23.7% in 1995 and 1996, respectively). In shipments from other countries, the incidence of contaminated seed lots was low (0–4%). In domestic seed lots, visual inspection detected low incidences of contamination (<2.4%) in most years (except 1996, when 11% of the lots were infected) (Fig. 6). Domestic seed lots were also screened for latent contamination of the vascular bundles, and a considerable portion of the lots (7–22%) was found to be infected (Fig. 6). Latently infected seed tubers might cause soil infestation, yield reduction and damage to the quality of daughter tubers. Furthermore, latent infection cannot be controlled by chemical seed treatment. Screening imported and domestic seed tubers for surface and latent infection with *C. coccodes*, and use of pathogen-free lots are the best strategy to eliminate introduction and spread of this pathogen.

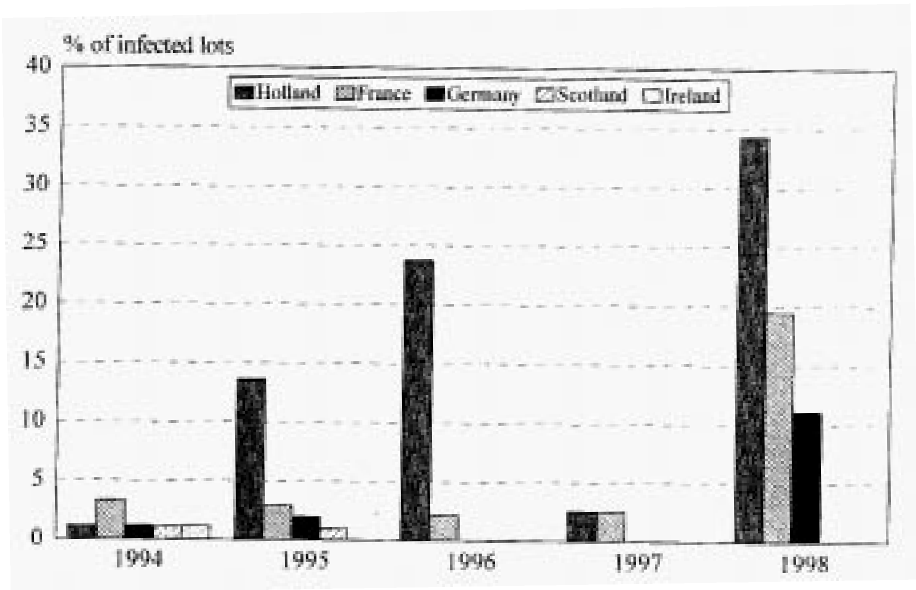

Fig. 5. Percentage of seed lots imported from different countries (1994–98) and infected by black dot (*Colletotrichum coccodes*).

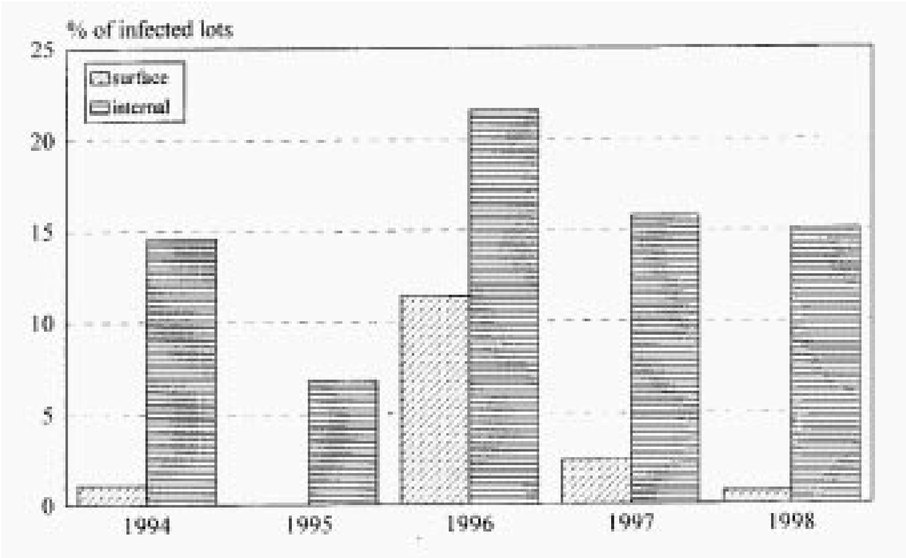

Fig. 6. Percentage of domestic seed lots infected by black dot *(Colletotrichum coccodes)*, on the surface and internally in the vascular bundles of the tuber (1994–98).

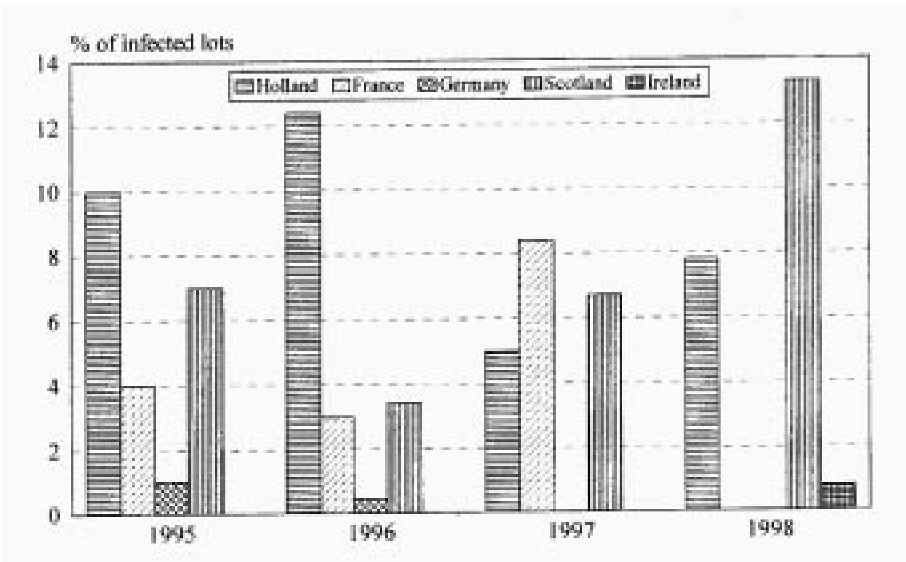

Fig. 7. Percentage of seed lots imported from different countries (1995-98) and infected by Fusarium dry rot.

Fusarium dry rot was found in 0.4–13% of the imported seed lots (Fig. 7). A high incidence of contamination was found in lots from Holland (5.0–12.4%), France (0–8.4%) and Scotland (3.4–13.3%). Domestic seed lots were disease-free in 1997 and 1998, whereas

11.6% and 1.3% of the lots were contaminated in 1995 and 1996, respectively (data not shown).

Powdery scab was found in lots imported from Scotland throughout the survey (5–17%). The disease was detected in lots imported from Holland in 1995 and 1996 (1.0% and 0.9% of the lots, respectively) and from Germany in 1997 (0.9%). No contamination was observed in lots imported from France and Ireland (Fig. 8). Domestic seeds were disease-free (data not shown).

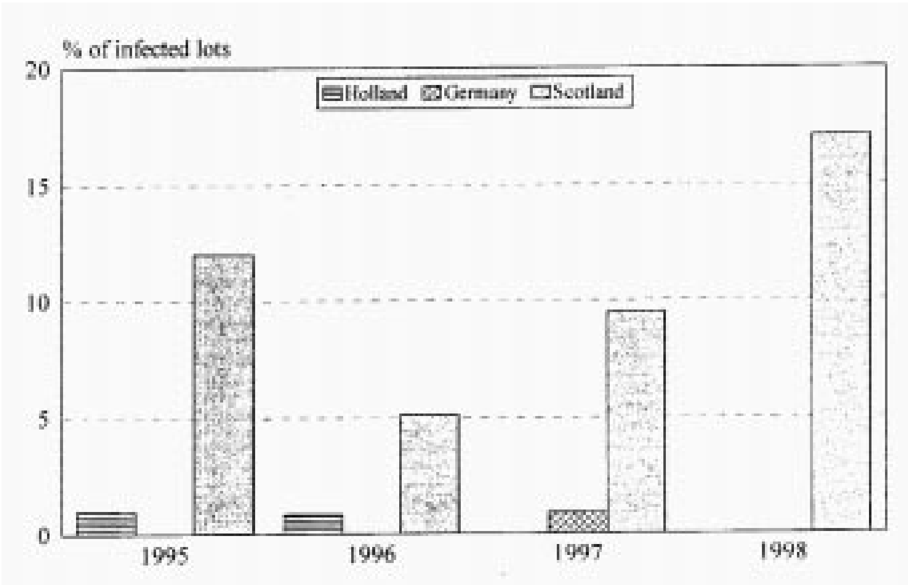

Fig. 8. Percentage of seed lots imported from different countries (1995–98) and infected by powdery scab *(Spongospora subterranea)*.

Screening seed tubers for the presence of *V. dahliae* within the tubers is important in order to prevent soil contamination by diseased tubers. In domestic seed, various levels of contamination were found throughout the years of the survey. Seed lots that were determined by laboratory testing to be contaminated at levels >5% were rejected for use as seed tubers. The incidence of disease-free lots had been 56–64%; 20–30% of the lots were contaminated at levels <5% (within a lot), and 6–16% of the lots were contaminated at levels >5% (Fig. 9). The relatively high incidence of infected seed lots suggests that soil fumigation prior to planting is of limited effectiveness in reducing inoculum levels.

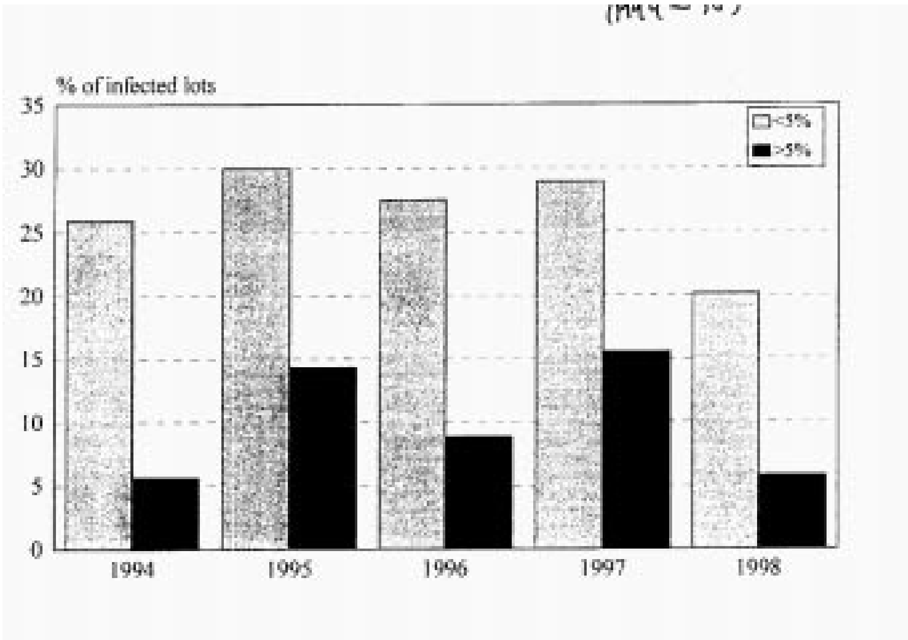

Fig. 9. Percentage of domestic seed lots contaminated with *Verticillium dahliae* below and above 5% level of infection within a lot (1994–98).

Use of disease-free potato seed tubers (imported as well as domestic) can prevent yield loss and quality reduction. A short-term advantage is increased profitability of the potato crop, while a long-term advantage is prevention of soil contamination. Data from this survey indicate that tuber infection with bacterial and fungal pathogens is generally less prevalent in domestic seeds than in imported seeds. However, higher levels of viral infections can occur in domestic than in imported seeds, due to local climatic conditions that foster a massive presence of vectors; this is the major difficulty in seed production in Israel. Prevention of soil infestation is now an important objective, since methyl bromide, which is widely used for soil fumigation, is to be phased out within a few years. Seed tuber disinfection treatments are used to reduce seedborne diseases; however, organic mercury, which has been used for this purpose, has been banned, and the alternative agents available are effective against only some of the pathogens. Moreover, an increasing number of pathogens are found to be unaffected by any of these treatments (*e.g.* *V. dahliae* and *C. coccodes*, which can be carried in the tuber's vascular bundles). Determining the exact sanitary profile of seed tuber lots might help the grower to select 'healthier' lots for domestic seed production, and to choose the most effective combination of seed and soil treatments; this would minimize the spread of diseases *via* propagation material.

REFERENCES

1. Adams, M.J., Hide, G.A. and Lapwood, D.H. (1980) Relationships between disease levels on seed tubers, on crop during growth and in stored potatoes. 1. Introduction and black scurf. *Potato Res.* 23:201-214.

2. Bain, R., Pérombelon, M.C.M., Tsror, L. and Nachmias, A. (1990) Epidemiology and etiology of blackleg. *Plant Pathol.* 39:125-133.
3. Bang, U. (1986) Effects of planting potato tubers attacked by *Phoma exigua* var. *foveata* on yield and contamination of progeny tubers. *Potato Res.* 29:321-331.
4. Chen, Y., Gamliel, A., Stapelton, J.J. and Aviad, T. (1991). Chemical, physical and microbial changes related to plant growth. pp. 103-129. *in:* Katan, J. and DeVay, J.E. [Eds.] Soil Solarization. CRC Press, Boca Raton, FL, USA.
5. Englebrecht, (1994) Modified SMSA selective medium for isolation and diagnosis of *Pseudomonas solanacearum.* Bacterial Wilt Newsletter No. 10, pp. 3-5. ACIAR, Canberra, Australia.
6. Forsund, E. (1987) Black scurf (*Rhizoctonia solani* Kühn) on tubers, roots, stems and stolons of the potato plants. *in:* Potato Diseases – Assessment Keys. European Association for Potato Research, Section for Pathology, Wageningen, the Netherlands. pp. 39-48.
7. Hide, G.A., Griffith, R.L. and Adams, M.J. (1977) Methods of measuring the prevalence of *Phoma exigua* on potatoes and in soil. *Ann. Appl. Biol.* 87:7-15.
8. Hooker, W.J. (1981) Compendium of Potato Diseases. The American Phytopathological Society, St. Paul, MN, USA.
9. Nachmias, A. and Krikun, J. (1988) Etiology and control of powdery scab of potato in a semi-arid region of Israel. *Phytoparasitica* 16:33-38.
10. Pérombelon, M.C.M. (1972) The extent and survival of contamination of potato stocks in Scotland by *Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora. Ann. Appl. Biol.* 71:111-117.
11. Pérombelon, M.C.M. (1992) Blackleg development and control in the Mediterranean region. *Proc. Symp. on Advanced Potato Production in Hot Climates* (Nahal Oz, Israel), pp. 46-54.
12. Pérombelon, M.C.M., Lumb, V.M. and Hyman, L.J. (1987) A rapid method to identify and quantify soft rot erwinias on seed potato tubers. *EPPO Bull.* 17:25-35.
13. Pérombelon, M.C.M., Lumb, V.M. and Zutra, D. (1987) Pathogenicity of soft rot erwinias to potato plants in Scotland and Israel. *J. Appl. Bacteriol.* 63:73-84.
14. Powelson, M.L., Johnson, K.B. and Rowe, R.C. (1993) Management of diseases caused by soilborne pathogens. *in:* Rowe, R.C. [Ed.] Potato Health Management. The American Phytopathological Society Press, St. Paul, MN, USA. pp. 149-156.
15. Scholte, K. (1989) Effects of soilborne *Rhizoctonia solani* Kühn on yield and quality of ten potato cultivars. *Potato Res.* 32:367-376.
16. Tsror (Lahkim), L., Erlich, O., Aharon, M. and Nachmias, A. (1998) Potato seed-borne diseases in Israel. *Israel Agres.* 9:127-139 (Hebrew, with English summary).
17. Tsror (Lahkim), L., Erlich, O., Chen, C., Ben-Ze'ev, I., Kritzman, G. and Zutra, D. (1996) Detection of brown rot in imported potato seeds. *Phytoparasitica* 24: 136 (abstr.).
18. Tsror, L., Erlich, O., Hazanovski, M., Nachmias, A. and Perets, I. (1994) *Colletotrichum* in potato – Is it a new disease? *Phytoparasitica* 22: 89-90 (abstr.).
19. Tsror (Lahkim), L., Nachmias, A., Erlich, O., Aharon, M. and Pérombelon, M.C.M. (1993) A 9-year monitoring study of diseases on potato seed tubers imported to Israel. *Phytoparasitica* 21:321-328.