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SHORT NOTES

Fungi from symptomless strawberry plants in Switzerland

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Summary. Forty symptomless strawberry plants (*Fragaria* × *ananassa* Duch.) belonging to different cultivars were examined for fungal flora. All the plants had originally been imported as frigo plantlets from the same nursery. Assays were performed on frigo plantlets from one batch and on plants grown under field conditions from another batch. Fungal isolations were taken from different parts of the plants (roots, crowns, petioles, leaves, flowers and fruits). Some 40 different fungal species were isolated in all, about 20 from in frigo plantlets and 30 from field-grown plants (with some overlap). About half the fungi isolated were common fungal strawberry pathogens in Switzerland. This paper outlines the problems inherent in the large-scale import of certified planting material containing potential pathogenic fungi that are not detected by routine phytosanitary inspection.

Key words: latent infections, potential pathogens.

Introduction

Swiss soft fruit cultivation covers an area of about 630 ha, representing 0.7% of the arable surface. Despite this relatively small cropping area, the growing of soft fruits is economically important and is expanding because of new production systems which aim at producing fruits all the year round. Strawberry (*Fragaria* × *ananassa* Duch.) is the major soft fruit, comprising over 70% of the total area under berry cultivation. In 2001, 5000 tons of strawberries were picked, covering 30% of domestic consumption (Mariéthoz, 2002).

Strawberry planting material for Swiss growers is imported mainly from Italy, France and Hungary. Healthy, certified plants are required according to the European Community regulations of integrated production, following which imported plants should be free of pathogenic microorganisms such as the bacterium *Xanthomonas fragariae* Kennedy and King, and the fungi *Colletotrichum acutatum*, *Gnomonia comari, Pezizella oenotherae*, *Verticillium* sp., *Phytophthora fragariae* and *P. cactorum*. Though certified, however, imported symptom-free strawberry plants can be found to be infected by some of these organisms (Bosshard and Schwindt, 1997; Bosshard *et al*., 1998). Of these, *P. fragariae*, *C. acutatum* and *X. fragariae* are defined as quarantine organisms in Switzerland, which means that any imported material to be used for planting must be free of them (EPPO/CABI, 1997). Phytosanitary inspection is done routinely by symptom observation. Isolation on cultural

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media, treatment with paraquat (Gindrat and Pezet, 1994), ELISA (Bosshard and Schwindt, 1997) and PCR using specific genetic markers (Sreenivasaprasad *et al*., 1996; Lacourt *et al*., 1997; Rigotti *et al*., 2002) are also used to detect several important pathogens that occur as latent infections.

In the present paper traditional isolation techniques were used to test small samples of symptomless plantlets and field-grown plants for the presence of pathogenic fungi as epiphytes or as latent infections. The risk posed by the fungi found is discussed.

Materials and methods

Plant material

Strawberry plants were imported in April 1999 and in April 2000 from a single nursery in Italy in the form of frigo plantlets, deriving from open-grown microcuttings of strawberry runners.

Fungal isolations were made from 24 symptomless frigo plantlets, 10 of the cv. Madelène and 14 of the cv. Raurica. The plantlets belonged to the batch imported in April 2000, and were examined after five (Madelène) and seven (Raurica) months of cold storage at -2 °C, RH 80–90%.

Fungal isolations were also carried out on 16 symptomless field-grown plants (cv. Elsanta, Kimberly, Madelène, Marmolada and Raurica). The plants, belonging to different batches imported in April 1999, were planted in July 1999 and collected, including their roots, in May and October 2000. They had not been treated with fungicides to allow unhindered expression of any pathogens present.

Fungal isolation and identification

Fungal isolations were done from different parts of the frigo plantlets (roots, crowns, petioles, leaflets) and of the field-grown plants (roots, crowns, petioles, leaves, flowers, fruits). Plant organs were washed under tap water for 1–2 hours. Entire mature fruits were placed directly in a closed box on wet blotting paper, RH 100%. Ten pieces (about 2×2 mm) were cut out from each plant part of each frigo plantlet, and fifteen pieces per plant part from each field-grown plant. The fragments were rinsed three times for 30 s in sterile distilled water, air-dried on sterile blotting paper and placed on agar in Petri dishes. Corn meal agar (CMA, Difco, Basel, Switzerland) and Potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) containing 12.5 ppm aureomycin to suppress bacteria were used to isolate oomycetes and other fungi respectively. Dishes were kept at 21 °C with a 12 h day. Any colonies that formed were individually transferred to fresh CMA or PDA plates. Non-sporulating colonies were placed under near-UV light to induce spore production. Each defined plant part was considered a replicate. Fungal species were recorded once for every plant part examined. Identification of fungi to the genus level, and when possible to the species level, was based on the usual determination keys (IMI, 1964–2001; Sutton, 1980; Von Arx, 1981). Identified fungi were stored at 4 °C in tubes containing CMA or PDA.

Results and discussion

Fungi belonging to about 40 genera (some of which were identified to species level) were isolated from either frigo- or field-grown strawberry plants. More than half the fungi isolated from imported certified frigo plantlets (Table 1, column A) were potential pathogens. Besides ubiquitous fungi such as *Botryotinia fuckeliana* (predominant in the roots and petioles), *Fusarium* spp. (in all organs) and *Pythium* spp. (mainly in the crowns), pathogens more specific to strawberry like *Rhizoctonia fragariae, Gnomonia comari* and *Pezizella oenotherae* were identified. These last two species have already been reported by Bosshard and Schwindt (1997) on imported strawberries. In addition, the tests on frigo plantlets revealed a number of fungal genera, including species pathogenic to flowers and fruits such as *Mucor* spp., *Aspergillus* spp., *Botrytis* spp., *Cladosporium* spp. and *Penicillium* spp. (Maas, 1998).

Fifteen potential pathogens were also isolated from symptomless field-grown plants (Table 1, column B). The same fungi were isolated from plants collected in both spring and autumn, except *Coniella fragariae*, *Gnomonia comari* and *Rhizopus stolonifer*, which were only detected in plants sampled in spring. This was probably because of the small size of the sample and because the viability of some of these species, such as *R. stolonifer* was impaired by low temperatures (IMI, 1964–2001; Maas, 1998). In addition to the isolated fungi, powdery mildew (*Sphaerotheca aphanis* [Wallr.] U. Braun) was regularly observed. Furthermore, 20

Table 1. Fungi isolated from different parts of symptom-free strawberry plants, expressed as a percentage of (A) 24 frigo plantlets and (B) 16 field grown plants.

Fungus		Frequency (%)									
		Underground parts				Foliage				Flowers	Fruits
Class	Genus or species	Roots		Crown		Petioles		Leaves			
		A	B	A	B	A	B	A	B	B	B
Oomycetes	*Pythium* sp.	4	0	17	0	0	0	0	0	0	0
Zygomycetes	*Mucor* spp.	33	6	0	13	0	19	0	0	0	6
	Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	0	0	0	0	0	0	0	0	0	25
	Other Mucorales	25	31	4	38	17	19	0	31	0	0
Ascomycetes[a]	*Botryotinia fuckeliana* (de Bary) Whetzel (*Botrytis cinerea* Pers.: Fr.)	21	19	0	31	25	56	8	31	13	38
	Gnomonia comari P. Karst. (*Zythia fragariae* Laib.)	0	0	0	6	4	6	4	0	0	0
	Nectria radicicola Gerlach & Nilsson (*Cylindrocarpon destructans* [Zinssm.] Scholten)	0	25	0	25	0	0	0	0	0	0
	Nectria sp. (*Acremonium* sp.)	0	0	0	6	0	0	0	0	0	0
	Neocosmospora sp. (*Acremonium* sp.)	0	0	0	13	0	0	0	6	0	0
	Pezizella oenotherae (Cooke & Ellis) Sacc. (*Hainesia lythri* [Desm.] Höhn.)	50	0	46	0	71	0	67	0	0	0
	Pleospora herbarum (Pers.) Rabenh. (*Stemphylium botryosum* Wallr.)	0	0	0	0	0	0	0	6	0	0
	Sclerotinia minor Jagger	0	0	0	0	0	0	0	0	0	6
	Sordaria sp.	0	0	0	0	4	0	0	0	0	0
Anamorphic fungi	*Acremonium* spp.	17	0	33	38	4	25	8	6	0	13
	Alternaria spp.	63	19	42	19	67	56	58	69	6	13
	Arthrinium sp.	0	0	0	6	0	6	0	19	0	0
	Ascochyta sp.	0	0	0	0	0	0	0	6	0	0
	Aspergillus spp.	4	0	0	6	4	6	0	0	0	6
	Beauveria bassiana (Bals.) Vuill.	0	0	0	0	0	0	4	0	0	0
	Bipolaris sp.	0	0	0	0	0	0	4	0	0	6
	Botrytis spp.	8	6	0	0	4	13	0	19	13	0
	Cladosporium spp.	42	44	29	13	42	50	38	63	0	6
	Coniella fragariae (Oudem.) B. Sutton	0	0	0	6	0	6	0	0	0	0
	Drechslera sp.	0	0	0	0	0	0	4	0	0	0
	Epicoccum purpurascens Ehrenb.: Schlecht.	13	6	8	25	38	44	38	69	0	0
	Fusarium spp.[b]	25	25	42	44	29	25	29	19	0	0
	Gliocladium roseum (Link) Bainier	0	0	0	6	0	6	0	0	0	0
	Metarrhizium sp.	0	6	0	0	0	0	0	0	0	0
	Penicillium sp.	42	19	33	38	29	56	50	69	6	25
	Pestalotiopsis guepinii (Desm.) Stey.	38	0	25	0	8	13	4	0	0	0
	Phialophora cinerescens (Wollenw.) v. Beyma	0	0	0	0	0	6	0	0	0	0
	Phoma spp.	21	0	29	25	46	13	42	19	6	6
	Pithomyces sp.	0	0	0	0	0	0	0	6	0	0
	Rhizoctonia fragariae Husain & McKeen	13	0	25	25	4	0	0	0	0	6
	Robillarda sp.	4	0	0	0	4	0	0	0	0	0
	Sporendonema sp.	0	0	0	0	0	0	0	0	6	0
	Stilbella sp.	0	0	0	6	0	0	0	0	0	0
	Trichoderma sp.	4	0	8	0	4	0	13	0	0	0
	Trichurus goronifer Bainier	0	0	0	6	0	6	0	6	0	6
	Truncatella sp.	0	0	0	6	0	0	0	0	0	6

[a] Teleomorphs; anamorphs cited in parentheses.
[b] *F. acuminatum* Ellis & Everh.; *F. avenaceum* (Corda) Sacc.; *F. oxysporum* Schlecht.; Fr., *F. solani* (Mart.) Sacc., *Fusarium* spp.

to 50% of fruits showed visible infection with *B. fuckeliana* at harvest. No quarantine pathogens such as *Colletotrichum acutatum* or *Phytophthora fragariae* var. *fragariae* were detected.

The routine detection of latent pathogens is difficult in symptomless plant material, whichever the method used. Moreover, disease symptoms are extremely hard to find in large batches of plantlets. Therefore, phytosanitary precautions, starting with a thorough inspection of the production fields, should be a priority along the entire chain of strawberry plantlet production and marketing.

In this study, only a limited number of imported, symptomless strawberry plantlets were examined. Nevertheless, a broad range of fungi including potential pathogens were isolated. These results show that various fungal pathogens occur on strawberry plantlets, both be ubiquitous fungi (e.g. *B. fuckeliana*) and fungi specific to the host (e.g. *G. comari*). The methods used in this study did not allow endophytes to be distinguished from epiphytes. However, any epiphytic, endophytic or latent pathogen poses a hazard to the strawberry crop (Andrews *et al*., 1982; Nathaniels and Taylor, 1983; Kulik, 1984; Davis and Fitt, 1990; Sinclair, 1991).

The preliminary findings of the present study create a framework of reference in which experiments can be repeated yearly on greater numbers of strawberry plants imported from different nurseries, in order to obtain more representative results for statistical analyses. A comparison of infection rates between strawberry cultivars does not seem useful because none of the cultivars are resistant to fungal pathogens. The choice of a cultivar is essentially based on fruit quality. However, a comparison between frigo plantlets and later field-grown plants originally from the same batch would make it possible to trace the evolution of the fungal flora, and to give some indication about the origin of the infection, whether at the propagating stage, or at the field-grown stage.

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