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Gnomonia fragariae, a cause of strawberry root rot and petiole blight

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Abstract

Gnomonia fragariae has been occasionally listed among the fungi associated with diseased strawberry plants. However its pathogenicity has not been established. During the investigation on strawberry decline in Latvia and Sweden, a fungus was repeatedly recovered from discoloured root and crown tissues of severely stunted plants. Attempts to induce sporulation of the isolates grown on several agar media resulted in the formation of mature ascomata only on potato carrot agar and oatmeal agar. On morphological grounds and comparisons with reference herbarium specimens these isolates were identified as *Gnomonia fragariae*. The pathogenicity of the fungus was evaluated initially in the detached leaf assay and subsequently in three bioassays on strawberry plants. All the bioassays showed that *G. fragariae* was pathogenic on strawberry and capable of causing severe root rot and petiole blight. The symptoms that developed in the greenhouse experiments closely resembled those observed in the fields. The fungus did not cause rapid plant death but growth and development of inoculated strawberry plants was severely affected. To our knowledge this is the first time when pathogenicity of *G. fragariae* as a root rot pathogen has been clearly established. Our study shows that *G. fragariae* is one of the serious pathogens involved in the root rot complex of strawberry in Latvia and Sweden.

Introduction

Strawberry is a susceptible host to several diseases that can affect the root system, crown, and the basal part of the petioles, causing serious damage to the host and considerable reduction of the yield. Among these, red stele and crown rot caused by *Phytophthora* spp., *Verticillium* wilt, and disorders caused by *Colletotrichum* spp. are known as severe and specific diseases in strawberry cultivation world-wide (Anon., 1996; Maas, 1998). However, field-infected strawberries often present a complex disease profile. A very common and increasing problem in perennial strawberries is a disease generally named 'black root rot' or 'root rot complex', which results in stunted plants and poor production of berries. These types of disorders are not well defined. Several fungi belonging to *Pythium*, *Rhizoctonia*, *Cylindrocarpon*, and *Fusarium*, as well as nematodes can be involved, and the disease can be favoured by abiotic stresses (Chen and Rich, 1962; laMondia and Martin, 1989; Yuen et al., 1991; Wing et al., 1994, 1995; Anon., 1996).

Two species of the genus *Gnomonia* have also been listed among the fungi which may play role in the root rot complex of strawberry. The first, *G. comari*, is a world-wide strawberry pathogen which causes leaf blotch, fruit rot and stem end rot (Alexopoulos and Cation, 1948; Shipton, 1967; Bolay, 1972; Gubler and Feliciano, 1999). This fungus is, however, mostly considered as a weak

pathogen that rarely causes considerable losses (Bolay, 1972). It has also been shown to induce petiole blight (van Adriechem and Bosher, 1958) and root rot, particularly in a synergic interaction with nematodes (Kurppa and Vrain, 1989). The second species, *G. fragariae* is an apparently limited in distribution to Europe, and was first reported on petioles of dead leaves of strawberry by Klebahn (1918). *Gnomonia fragariae* has also been listed among the fungi associated with a severe root rot of strawberry in Finland (Parikka, 1981). However, the pathogenicity of this fungus was not evaluated.

During a survey on the possible causes of strawberry decline in Latvia and Sweden, a fungus was repeatedly recovered from discoloured root and crown tissues of severely stunted plants. This fungus was considered as one of the possible pathogens, and further studies were carried out for its identification and pathogenicity on strawberry.

Materials and methods

Sampling and isolation

Twenty-one fields in Latvia and four fields in Sweden with severely stunted and wilting strawberry plants were surveyed, and diseased plants showing root and crown rot symptoms were sampled from May to November in the years 2001–2004. Plant parts were first washed in running tap water. Stem bases, crowns and roots showing disease symptoms were then surface-sterilised in 1.25% sodium hypochlorite for 2 min (younger roots and petioles) and 3 min (crowns), washed three times in sterile distilled water (SDW), and blot-dried. Fragments ca. 0.5 cm long were cut from the margins of healthy and diseased tissues of roots and petioles, and plated on potato-dextrose agar (PDA) (Oxoid), half strength PDA, and water-agar (WA) (Oxoid) media. Crowns were cut in slices and plated in the same way. To detect a possible *Phytophthora* infection, non-surface-sterilised tissues from diseased roots and crowns were plated on a *Phytophthora* selective medium (Tsao and Ocana, 1969), and they were also placed in water to induce sporangia formation. The growing fungal colonies were transferred onto PDA and maintained at 4 °C both as test-tube slants on PDA, and as 4 mm diam mycelial plugs in SDW.

Freshly collected petioles and roots of diseased plants collected from several locations were examined for the presence of fruiting bodies. Further, the infected petioles and roots were also incubated in moist chambers for up to one month for the formation of fruiting bodies.

Morphological examination and identification

The isolates were grown on several agar media including PDA, potato carrot agar (PCA) (Dhingra and Sinclair, 1995), corn meal agar (CMA) (Oxoid), oatmeal agar (OA) (Difco), and water agar, incubated at room temperature and examined under the light microscope. To stimulate sporulation isolates were also inoculated on PCA plates containing autoclaved toothpicks.

Pathogenicity tests

Initially, the pathogenicity of the isolates was tested in a detached leaf assay. Leaflets of strawberry cultivar 'Honeoye' plants grown in a greenhouse were surface-sterilised in 1.25% NaOCl for 20 s, washed three times in SDW and placed on moistened filter paper in Petri plates (two leaflets per plate). The test was performed on unwounded and wounded leaflets with a sterile needle to enhance infection. The leaflets were inoculated by mycelial plugs (5 mm diam) taken from 5 day-old fungal colonies grown on PCA. Each leaflet had two inoculation sites. A control set of strawberry leaflets was inoculated with sterile plugs of PCA. The Petri plates were then sealed by parafilm, incubated at room temperature and the leaflets were checked daily for the development of necrotic lesions. After two weeks the mycelial plugs were removed and the leaflets were sterilised in 70% ethanol for 20 s, washed three times in SDW and blot dried. Small pieces cut from the margins of rotted and healthy leaf tissues were transferred onto PDA to recover the fungus. The assay was carried out twice with two replicates per each isolate. Seventeen isolates were examined in this biotest. Pathogenicity of the isolates on strawberry plants was further evaluated in three separate bioassays as described below:

Pathogenicity test No.1

Fungal inoculum was prepared in 0.5 l flasks on 15 g perennial ryegrass (*Lolium perenne*) seeds which were soaked in distilled water and autoclaved at 121 °C for 20 min. Each flask was inoculated by five mycelial plugs (5 mm diam) from 7 day-old fungal cultures grown on PDA, and incubated at room temperature for 17 days. Strawberry runner plants (Stiftelsen Trädgårdsodlingens Elitplantstation, Sweden) of cv. 'Honeoye' were planted in plastic pots each containing 800 g of potting soil mixed with 0.5% (w/w) of the inoculum. The potting soil consisted of 50/50 (v/v) mixture of the commercial garden soil (Hasselfors Garden AB, Sweden) and washed building sand. This mixture was steam-treated at 80 °C for 24 h and then cooled to 20 ° C before the inoculum was added. Six isolates coded S1, S4, S7, M1, UN20, and UN35 were tested in this greenhouse assay. Potting soil without inoculum was used for control plants. Each treatment contained four replicates and the experiment was arranged in a complete randomised design. Additional light in the greenhouse was set for 12 h regime and the temperature varied from 13 to 25 °C. Symptoms and dead plants were recorded once a week. After six weeks plants were removed from pots and roots were carefully washed under tap water. Disease severity (DS) was recorded according to the following scale: 0 = plant well developed, no disease symptoms; 1 = no visible symptoms on above ground parts, $\leq 25\%$ of roots discoloured; 2 = plant slightly stunted, black necrosis on petiole bases, 26–50% of roots discoloured; 3 = plant stunted, black necrosis on petiole bases, yellowing and death of outer leaves, 51–75% of roots discoloured; 4 = plant severely stunted, outer leaves collapsed, younger leaves bluish-green and wilting, > 75% of roots discoloured; 5 = plant dead. The re-isolation was done from root and crown tissues on PDA and WA following the same procedure as for the initial isolation.

Pathogenicity test No.2

This bioassay was performed by using micropropagated strawberry plants (Pure Horticultural Research Station, Latvia) of cv. 'Syriuz'. Plants were carefully removed from tissue culture tubes, rinsed in distilled water and planted in plastic pots containing 120 g potting soil which consisted of peat amended with limestone flour to obtain pH 6.5, and mixed 2/1 (v/v) with river sand. The potting soil was steam-treated as described above and mixed with 1% (w/w) of inoculum on perennial ryegrass seeds. Non-inoculated soil was used as the control. Two isolates (UN22 and UN35) were used in this assay. The experiment was set up in a complete randomised design with 10 replicates (plants) per treatment in a growth chamber with 14 h light and 25 ± 3 °C. The assessment of symptoms, DS and re-isolation of pathogen was done according to the procedures described above.

Pathogenicity test No.3

Micropropagated strawberry plants (Stiftelsen Trädgårdsodlingens Elitplantstation, Sweden) of cv. 'Zefyr' were planted in plastic pots containing 500 ml of soil mixture. The assay was performed as described in pathogenicity test No.1. The treatments included 0.5%, 1.0% and 2.0% (w/v) of inoculum on perennial ryegrass seeds in the soil, three mycelial plugs (5 mm diam) placed near the roots, roots dipped in ascospore suspension for 2 h and the control with no inoculum applied. The ascospore suspension was prepared from a 2 month-old culture of *G. fragariae* on PCA. The culture was flooded with sterile water 2 h prior to scraping ascomata with a loop. The suspension was filtrated through two layers of miracloth, washed twice in sterile water and collected by centrifugation. Ascospore suspension was diluted to 1×10^6 spores ml^{-1}. The isolate coded as UN22 was used and the experiment was arranged in a complete randomised design with five replicates per treatment. Pots were placed in the greenhouse for 11 weeks with additional light for 12 h, temperature range from 13–25 °C. Plants were watered with 10% Knop's solution (Hohryakov, 1976) during the experiment. DS and symptoms were evaluated as described above. The fresh and dry weight of roots and shoots was measured. A re-isolation was performed from all tested plants following the same procedure described above.

Data analyses

Data from the pathogenicity tests were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SAS computer software (SAS Institute Inc. Cary, NC). Mean values were compared by Duncan's multiple range test at $P = 0.05$ significance level. Descriptive

statistics on data of dimensions of spores, appendages and asci of studied specimens/isolates of *G. fragariae* were performed with Minitab Statistical Software (Minitab Inc., State College, Pennsylvania).

Results

Disease symptoms and isolation

Strawberry plants from which the studied fungus was isolated showed various symptoms such as collapsed outer leaves, rotted petiole bases, root and crown rot. The diseased plants were found singly interspersed among visually healthy ones, as well as in patches in low and wet parts of the fields. Infected plants were stunted and often one side of the plant was dead. Younger leaves of these plants were frequently wilted and bluish-green coloured, but the older ones were either dead or red and yellow coloured. The petioles of the coloured, older leaves often had black rot developing upwards at their bases. On the necrotic bases of petioles collected in May, June, July, September and November numerous black *Gnomonia*-like ascomata were found. The root system of infected plants was, in general, poorly developed, with very few lateral roots. Structural roots could be entirely black but infected lateral roots were often found with irregular shaped black lesions which also developed from root tips upwards. Crowns cut lengthwise showed distinct reddish-brown discolouration of tissues at different parts of the rhizome. Thirty six isolates of *Gnomonia* were obtained from eight strawberry fields in Latvia and 32 isolates from three fields in the central and southern part of Sweden. They were isolated alone or associated with other known pathogenic fungi such as *Cylindrocarpon*, *Fusarium*, *Verticillium* and *Rhizoctonia* from strawberry roots, crowns and petioles from 2–4 year-old plants mostly in farms with long histories of strawberry production.

Morphological examination and identification

Isolates of the studied fungus grew easily but slowly in culture, attaining on PDA, for example, 30 mm diam after 6 days. On PDA colonies appeared as thick radiating mycelial strands, yellow when young, becoming tawny or grimy brown with age, and having distinct irregular margins (Figure 1). Hyphae were mostly submerged, profusely branched, with pronounced swellings at most of the septa resulting in dumbbell-shaped or pyriform cells. These hyphal swellings resembled those of *Rosellinia necatrix*. The colonies were grey to olive green on PCA, but white and with more aerial mycelium on OA. No sporulation was observed in young colonies. When grown on PDA under continuous near-UV light the isolates did not form spores. Numerous ascomata with long and curved necks, characteristic for *Gnomonia* were formed in cultures on PCA and OA after several weeks of incubation at room temperature. Asci were unitunicate with a distinct apical ring and contained eight ascospores which were biseriate (Figure 2a). Ascospores were straight to slightly curved, hyaline, with a median septum, often somewhat constricted at the septum, with 1–3 oil droplets in each cell. The ascospores had typical filiform gelatinous appendages which in some cases were longer than 25 μm (Figure 2b). The length of appendages varied among the examined specimens and even spores lacking appendages were found. Table 1 presents the mean values of dimensions of asci, ascospores and appendages of the studied specimens. On morphological grounds our isolates were identified as *Gnomonia fragariae*. They were compared with the descriptions given by Klebahn (1918), Bolay (1972) and Monod (1983) and with the authentic specimens of *G. fragariae* obtained from the herbarium at Cantonal Museum and Botanical Garden, Lausanne. Attempts to find any specimens of *G. fragariae* deposited or identified by Klebahn were not successful.

Pathogenicity tests

Pathogenicity of *G. fragariae* was initially confirmed using the detached leaf assay. Clear necrotic and black lesions developed around mycelial plugs 2–3 days after inoculation on wounded leaflets, and after 3–4 days on unwounded leaflets in all the 17 isolates tested. Later, the lesions enlarged and produced irregular, black areas on the leaflets. Necrotic lesions were not observed on any of control leaflets. A few weeks after inoculation mature ascomata were formed on the necrotic lesions of inoculated leaves. The fungus was re-isolated from surface-sterilised infected tissues.

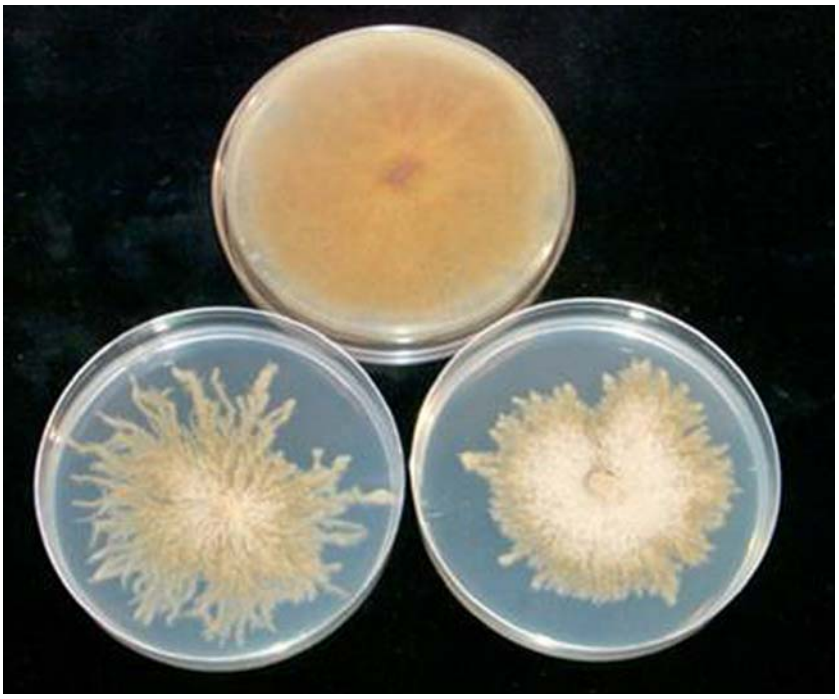

Figure 1. Gnomonia fragariae colony appearance on potato dextrose agar: typical irregular margin (bottom) and yellow pigmented reverse of colony (top).

The pathogenicity tests of *G. fragariae* on strawberry plants, performed in the three experiments, clearly showed that all the isolates tested were pathogenic and capable of causing severe root rot and petiole blight while none of the control plants showed such disease symptoms. The mean values of disease severity in experiments No. 1 and No. 2 are shown in Table 2. Five out of the six isolates tested in experiment No. 1, at the low inoculum level of 0.5%, caused significant damage to inoculated plants. However isolate S7 showed a low level of pathogenic activity. The infected plants in this test also showed crown rot symptoms similar to those observed on the diseased plants in the field. However, discolouration in crowns did not appear as a consistent symptom in the other experiments (data not shown). In the second pathogenicity test, the level of disease severity was lower in the case of strain UN35 and it remained at the same level in the case of strain UN22 despite a higher dose of

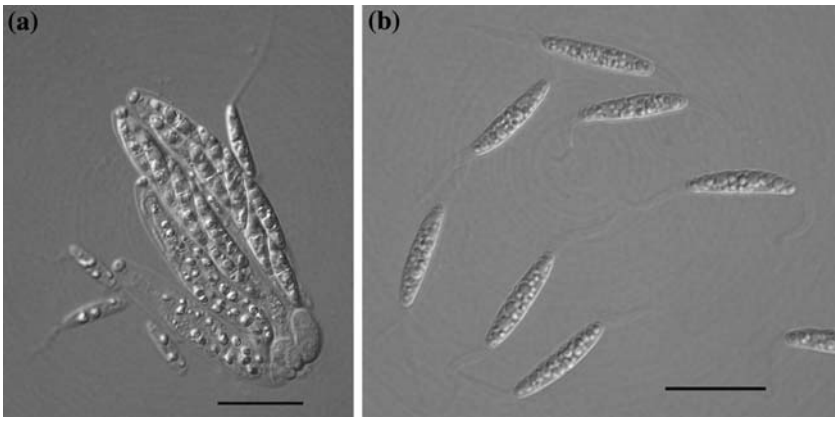

Figure 2. Gnomonia fragariae asci (a) and ascospores (b) from culture on potato carrot agar. Bars represent 20 μm.

Table 1. Mean values of dimensions of spores, appendages and asci of studied specimens/isolates of *Gnomonia fragariae*

Isolate/specimen	Ascospore length × width (μm)	SD	Appendage length (μm)	SD	Asci length x width (μm)	SD
1[a]	15.8 × 3.3	1.4 × 0.4	5.4	0.8	–	–
2[a]	17.7 × 3.9	1.7 × 0.3	–	–	–	–
4[a]	15.8 × 3.4	1.2 × 0.5	5.5	1.8	–	–
5[a]	16.4 × 3.9	1.1 × 0.2	–	–	–	–
6[a]	15.4 × 3.9	1.3 × 0.2	4.7	0.8	–	–
PR2[b]	14.4 × 3.2	1.3 × 0.3	4.5	0.9	54.6 × 7.3	6.0 × 0.7
EL[b]	16.5 × 3.6	1.8 × 0.4	13.2	5.6	58.3 × 7.3	5.0 × 0.8
PR1[b]	16.1 × 3.6	1.6 × 0.4	11.6	4.3	52.0 × 7.1	5.2 × 0.8
F5[b]	16.8 × 3.9	1.7 × 0.3	11.5	4.4	65.1 × 7.4	5.1 × 0.9
L56[c]	16.6 × 3.4	1.5 × 0.4	8.0	2.7	59.5 × 7.5	4.5 × 1.0
M1[c]	14.7 × 3.2	1.4 × 0.3	9.9	0.4	56.4 × 7.3	4.2 × 0.7
S2[c]	15.4 × 3.1	1.5 × 0.3	12.6	8.0	55.3 × 6.6	5.0 × 0.8

[a]Herbarium specimens: (1) *Fragaria* spp., Chailly–sur–Clarens, Vaud, Switzerland, 17 June 1958, Bolay; (2) *Fragaria vesca*, La Conversion sur Lutry, Vaud, Switzerland, 22 May 1958, Bolay; (3) *Fragaria* spp., cultivar 202, Vouvry, Valais, Les Barges, Switzerland, 25 June 1958, Bolay; (4) *Potentilla anserine*, Torgon, Valais, Switzerland, 11 October 1961, Corbaz; (5) *Fragaria spp*., cultivar 342, Miex, Valais, Switzerland, 29 August 1961, Corbaz.
[b]On cultivated *Fragaria* spp. petioles: PR1, PR2, EL – collected in Latvia; F5 – collected in Sweden.
[c]Living isolates originated from cultivated *Fragaria* spp. roots and crowns in Latvia.

inoculum applied in the soil (Table 2). In Pathogenicity test No. 3 in which fungal inoculum mixed with soil at three doses, mycelial plugs of the fungus placed near the roots, or roots dipped in ascospore suspension were treatments all led to significant disease severity and the reduction of shoot and root dry weights (Figure 3). In all of the pathogenicity tests early symptoms of inoculated plants appeared as delay of growth in one to two weeks after inoculation in comparison with the control. Infected plants became severely stunted (Figure 4a) within 4–6 weeks and in some cases this resulted in death. Yellowing of outer leaves and particularly upwardly developing black rot on petiole bases which resulted in collapse of affected leaves, resembled the symptoms observed in field-infected plants. Bluish-green colour and wilting of younger leaves was also observed on infected plants of cv. 'Zefyr'. Roots of inoculated strawberries were, in general, poorly developed; they were entirely black or covered with black lesions which developed also from the root tips (Figure 4b). No fruiting bodies of *G. fragariae* were found on roots or petioles of inoculated plants in the greenhouse but they were formed within two months when infected tissues were incubated in the moist chambers. *Gnomonia fragariae* was recovered from roots and petioles of all the plants that developed characteristic disease symptoms.

Table 2. Mean values[a] of disease severity caused by *Gnomonia fragariae* on strawberry plants in two biotests

Isolate	Pathogenicity test No.1[b]	Pathogenicity test No.2[c]
S1	2.8 AB	–
S4	4.5 A	–
S7	1.5 B	–
M1	4.3 A	–
UN22	2.8 AB	2.8 A
UN35	4.3 A	2.8 A
Control	1.0 B	0.1 B

[a]Means in a column followed by the same letters are not significantly different according Duncan's multiple range test (P = 0.05).
[b]Runner plants of strawberry cv. 'Honeoye' were used for inoculation, inoculum 0.5% w/w was mixed in the soil, disease severity was evaluated six weeks after inoculation according the scale 0–5 (0 = no disease symptoms; 5 = plant dead).
[c]Micropropagated strawberry plants of cv. 'Syriuz' were used for inoculation, inoculum 1.0% w/w was mixed in the soil, disease severity was evaluated six weeks after inoculation according the scale 0–5, (0 = no disease symptoms; 5 = plant dead).

Discussion

The results of our study show that *G. fragariae* is a serious pathogen involved in root rot complex of strawberry in Latvia and Sweden. To our knowledge this is the first time when pathogenicity of *G. fragariae* on strawberry roots has

been demonstrated. Parasitic species belonging to the genus *Gnomonia* usually attack aerial parts of the host plants (Barr, 1978), although *G. radicicola* was described as a cause of severe root rot of roses (Noordeloos et al., 1989; Amsing, 1995). The morphologies of ascomata, asci and ascospores of our isolates on agar media and those found on naturally infected plant tissues were, in general, in agreement with the characteristics of *G. fragariae* described by Klebahn (1918), Bolay (1972) and Monod (1983). When our collection was compared with herbarium specimens collected by Bolay (1972) also examined by Monod (1983) no considerable morphological differences were observed. The only exception was that the ascospore appendages of our isolates and specimens were longer and showed greater variation in length. These differences may be due to examination methods used and age of specimens examined. *Gnomonia comari* which has often been confused with *G. fragariae*, has smaller asci and ascospores with a submedian septum and no appendages (Barr, 1978).

Pathogenicity experiments showed that *G. fragariae* was indeed the causal agent of severe rotting of roots and petiole bases which eventually resulted in the collapse of outer leaves. The re-isolation of the fungus from diseased tissues confirmed the pathogenicity. Klebahn (1918) discovered *G. fragariae* for the first time on dead petioles of diseased strawberries in Germany. However he did not establish its pathogenicity and considered this fungus as a saprophyte. The disease symptoms developed in our greenhouse experiments strongly resembled those observed on infected plants in the field. The fungus did not cause rapid plant death but growth and development of inoculated strawberry plants were severely affected. This also corresponded to our observations from affected fields in that rapid death was not immediately apparent but plants became severely stunted over a longer period of time.

The discolouration of rhizome tissues, collapse of plants from one side, red or yellow coloured older leaves and bluish-green colour of younger leaves (Nickerson, 1998; Seemüller, 1998) indicated the possible presence of *Phytophthora* in strawberry fields. Contrary to expectations, *Phytophthora* was not isolated or were any sporangia formed when diseased tissues of roots and crowns were incubated in the moist chambers or in water. Symptoms such as wilting and bluish-green colour of younger leaves also developed in plants inoculated with *G. fragariae* in greenhouse experiments. And in many cases, this fungus was isolated from symptomatic rhizome tissues from field-infected plants.

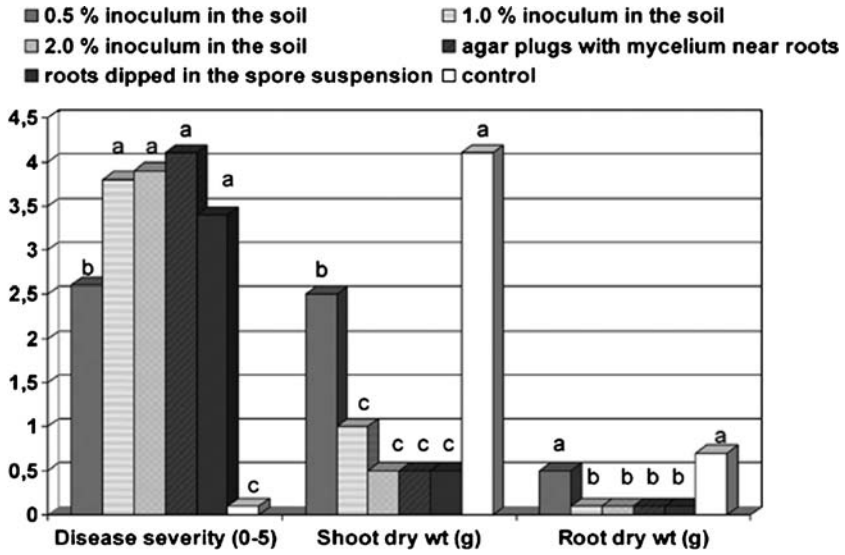

Figure 3. Disease severity (0 = plant healthy, 5 = plant dead) and effect on shoot and root dry weights caused by *Gnomonia fragariae* on strawberry plants cv. 'Zefyr' 11 weeks after inoculation. Means topped with the same letter for each variable are not significantly different according to the Duncan's Multiple Range Test ($P = 0.05$).

These facts suggest that the symptoms observed in the surveyed fields were caused by *G. fragariae* or may be caused by different pathogens.

A severe strawberry petiole blight caused by *G. comari* was reported by van Adriechem and Bosher (1958). Symptoms described in that study, particularly yellowing and collapse of outer leaves and black discolouration girdling the bases of petioles, are very similar to those caused by *G. fragariae* and observed during our work. They also found numerous ascomata formed on affected petiole bases in the field. However in that study inoculation of *G. comari* on roots did not cause any disease symptoms. This fungus appeared to be pathogenic on roots in synergetic interactions with nematodes (Kurppa and Vrain, 1989). During our survey *G. comari* was found only twice on diseased petioles together with *G. fragariae* on the same plants in Latvia. This indicates that in the field both *Gnomonia* species can attack strawberry petioles and cause similar symptoms but *G. fragariae* is also capable of affecting the roots. In a survey on strawberry root rot in Finland, described with similar disease symptoms to those observed in our study, *G. fragariae* was also listed among the pathogens found (Parikka, 1981). Nevertheless confusion remains on the identity of that fungus since the references listed by the author were related to *G. fructicola*, a synonym of *G. comari*. Unfortunately, no specimens of the fungus

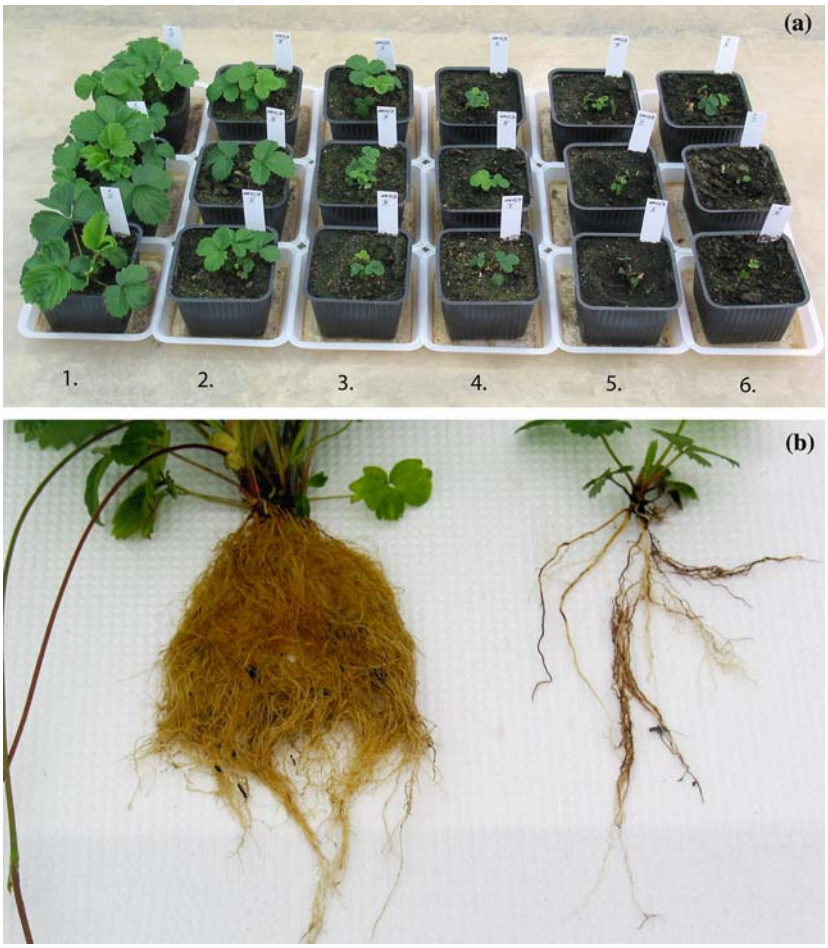

Figure 4. Symptoms on strawberry plants cv. 'Zefyr' caused by *Gnomonia fragariae* in the greenhouse: (4a) Severe stunt of plants 6 weeks after inoculation with following treatments: (1) control, no inoculum applied; (2) 0.5% of inoculum in the soil; (3) 1% of inoculum in the soil; (4) 2% of inoculum in the soil; (5) mycelial plugs placed near roots; (6) roots dipped in an ascospore suspension; (4b) Affected roots (right) control (left) 11 weeks after inoculation.

described in that study were available on our request for examination (P. Parikka, MTT Agrifood Research, Finland, pers. comm.). The misapplication of the name of *G. fragariae*, in the literature, probably referring to *G. comari* has already been pointed out by Bolay (1972), Maas (1998), and Farr et al. (1989).

Our work suggests that *G. fragariae* could be a widely distributed pathogen in strawberry fields but it has been ignored or misidentified in other studies. The reasons could be the relatively slow growth of the fungus and its sterility on ordinary agar media such as PDA. As was noticed in our study, colonies of *G. fragariae* growing out from the field-infected plant roots and crowns appeared 3–5 days after plating on agar media and they were often overgrown by faster-growing fungi. The differentiation from associated fungi and isolation in pure culture in many cases required careful microscopic examination. *Gnomonia fragariae* was therefore possibly present in other surveyed strawberry fields in Latvia but was overlooked during initial isolations.

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