Seedlings Damping-off of *Chenopodium quinoa* Willd.

Monika DŘÍMALKOVÁ and Karel VEVERKA

Department of Plant Protection, Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic

Abstract

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The causal agents of damping-off of quinoa seedlings were determined in greenhouse experiments. *Ascochyta caulina*, *Fusarium avenaceum*, *Fusarium* spp., *Alternaria* spp. and *Pythium* spp. were isolated from infected parts of quinoa seedlings. The most frequent *Pythium* sp. was *P. aphanidermatum*. Pathogenicity tests confirmed that *P. aphanidermatum* and *F. avenaceum* were the causal agents of damping-off of quinoa seedlings under greenhouse conditions. A comparison of the reaction of quinoa with that of other susceptible plants (spinach, cabbage, sugar beet) showed that quinoa is most susceptible to the pathogen before emergence, during germination till the end of the stage of the first pair of true leaves. Germinable quinoa seeds seemed to have a lower ability to emerge from the soil. This serious problem is caused not only by pre-emergence damping-off from pathogens but more so by a complex of several adverse factors during germination when quinoa is most sensitive.

Keywords: seedlings damping-off; *Chenopodium quinoa*; germination; emergence; *Pythium* spp.; *Fusarium* spp.

Quinoa (*Chenopodium quinoa* Willd.) has been cultivated in the Andean region for thousands of years, and has been introduced elsewhere as a source of starch and high quality proteins. The high nutritional value of the grains supports recent interest in cultivation in the USA and European countries (Jacobsen 1997). Under both Andean and European conditions problems with germination of quinoa have been observed (Jacobsen & Bach 1998).

At present, the Czech Republic is an importer of quinoa products. Appropriate genotypes of quinoa were tested and selected by the Research Institute of Crop Production in Prague-Ruzyně within the project The American and European Test of Quinoa. In 1999 quinoa stands were severely damaged by damping-off and by flea beetles *Chaetocnema* spp.

There is little information about diseases of quinoa under conditions of other European regions. Most attention is paid to downy mildew, caused by *Peronospora farinosa* (Fr.: Fr.) Fr. 1849. This disease is consistently present in Peru, Bolivia, Ecuador and Columbia and is regarded as endemic (Danielsen *et al*. 2003), but has also been detected in Canada (Tewari & Boyetchko 1990) and Europe (Mujica *et al*. 2000).

There is scant knowledge about other quinoa pathogens that infect seeds or seedlings and lead to decreased crop production. These diseases are less widespread than downy mildew but are still considered potential production constraints, particularly when the crop is introduced in areas outside its traditional growing regions (Danielsen *et al*. 2003). From quinoa seeds of Czech origin these fungi were isolated: *Alternaria* spp., *Ascochyta caulina*, *Fusarium avenaceum* and *Fusarium* spp. which can be causal agents of damping-off of quinoa seedlings (Dřímalková 2003).

The main aims of this study were, therefore, to identify seedborne and soilborne pathogens that

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infect quinoa seedlings during germination and emergence, and compare damping-off on quinoa with that on other plants susceptible to seedlings damping-off.

MATERIAL AND METHODS

Comparison of damping-off of quinoa seedlings to that of other plants. The tests were carried out in the greenhouse (20 ± 5°C). Germination of *C. quinoa* and of cultivars of spinach, sugar beet and cabbage that are susceptible to damping-off of seedlings was determined according to the International Rules for Seed Testing (ISTA) (Table 1).

100 seeds of each variant were equidistantly (1.5 × 2.0 cm) sowed into a garden soil in pots placed in a randomised block design with four replicates. The soil was watered to keep it moist. The number of emerged or dying seedlings per pot were counted at three developmental stages: formation of cotyledon leaves, of the first pair of true leaves, and of the second pair of true leaves. Dying seedlings were removed from each pot to identify the cause of damping-off. They were washed in distilled water, surface-treated with 0.5% NaClO for 15 s, rinsed twice in sterile distilled water (SDW), and placed into Petri dishes with wet filter paper and water agar. Determination of isolated fungi was based on descriptions by Brandenburger (1984), Fassatiová (1979), Kröber (1985) and Gerlach and Nirenberg (1982). The tests were analysed by analysis of variance and mean separation was according to Tukey's test.

Pathogenicity test. Two isolates of *Pythium aphanidermatum* (Edson) Fitzp. 1923, *Ascochyta caulina* (P. Karst.) v. d. Aa & v. Kest. 1979, *Fusarium* sp., *F. avenaceum* (Fr.: Fr.) Sacc. 1886 and *Alternaria* sp. were used to verify their pathogenicity on quinoa seedlings through an inoculation technique (Brantner & Windels 1998), with modification for quinoa. The soil mix in 10 cm clay pots consisted of field soil, sand and peat moss (3:1:1 by volume). The pots were autoclaved for 1 h on each of two consecutive days. Inoculation was done at four times: simultaneously with sowing, at the formation of cotyledon leaves, of the first pair of true leaves, and of the second pair of true leaves. In the first case, five square sections (0.125 cm^3) of a 3 day old culture of *P. aphanidermatum* and a 10 day old culture of the others mentioned pathogens were placed on the soil surface and covered with soil; 25 quinoa seeds of cv. Cochabamba were surface-treated with 1% NaClO for 10 min and equidistantly placed on the soil surface and covered with soil. The controls consisted of soil with agar containing no pathogen. In the case of inoculation after sowing, 10 quinoa plants were left standing in each pot. A 3 day and 10 day old culture of a pathogen were cut into square sections and attached to the basal part of the seedling stems by aluminum foil. In the controls the agar was without pathogen. The pots were placed in incubators (25 ± 1°C, 12 h photoperiod) in a randomised block design with four replicates. The soil was watered to keep it moist. Emergence and number of dying seedlings were evaluated every 4 days until 4 weeks after planting. Dying seedlings were removed from each pot and assayed to verify the presence of the appropriate pathogen. Seedlings were washed in distilled water, surface-treated with 0.5% NaClO for 15 s, rinsed twice in SDW and placed into Petri dishes with wet filter paper and water agar for determination of pathogens. The test was analysed by analysis of variance and mean separation was according to Tukey's test.

Table 1. List of cultivars used to compare the effect of damping-off on quinoa seedlings to that on other susceptible plants

Quinoa	*Chenopodium quinoa* Willd.	cv. Cochabamba	VURV
Quinoa	*Chenopodium quinoa* Willd.	Johny White	VURV-GB-1
Spinach	*Spinacia oleracea* L.	cv. Matador	commercial seeds
Cabbage	*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC.	cv. Sláva	commercial seeds
Sugar beet	*Beta vulgaris* L. var. *altissima* Döll.	cv. Eureka	commercial seeds

Johny White – breeding material selected in Czech Republic from breeding material of fodder quinoa imported from KVL, Denmark; VURV-GB-1 – Gene Bank of the Research Institute of Crop Production in Prague-Ruzyně

RESULTS

Comparison of seedlings damping-off of quinoa to that of other plants

The emergence in spinach, cabbage and sugar beet was significantly (probability ≤ 0.01) higher than in quinoa. Differences in percentage of emergence between the former plants and quinoa were greater than expected solely from the differences in their germination (Figure 2). Seedlings damping-off was significantly ($P \leq 0.01$) highest during the phase of cotyledon leaves in spinach and sugar beet in contrast to quinoa and cabbage (Figure 1). Seedling damping-off of quinoa was significantly ($P \leq 0.05$) higher at the phase of the first pair of true leaves, but the sum of post-emergence damping-off was significantly ($P \leq 0.05$) lower than in sugar beet and higher than in cabbage (Figures 1 and 2).

Fungi isolated from infected parts of dying quinoa seedlings were identified as *Fusarium* spp., *F. avenaceum*, *Pythium* spp., *P. aphanidermatum*, *Alternaria* spp. and *A. caulina*. *Fusarium* spp. was isolated from spinach, cabbage and sugar beet, *Pythium* spp. from spinach and cabbage, *Alternaria* spp. from spinach and sugar beet and *Fusarium*

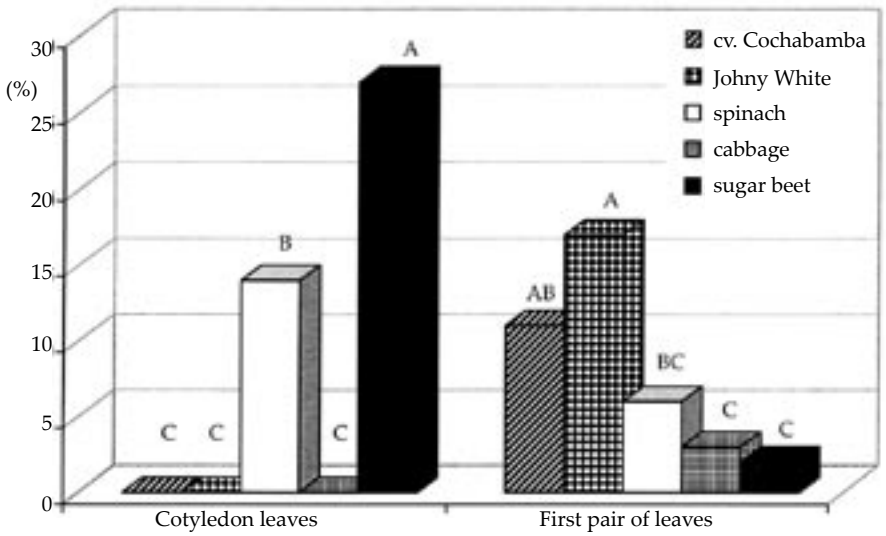

Figure 1. Percent of seedlings post-emergence damping-off at the stage of the cotyledon leaves ($P \leq 0.01$) and the first pair of true leaves ($P \leq 0.05$)

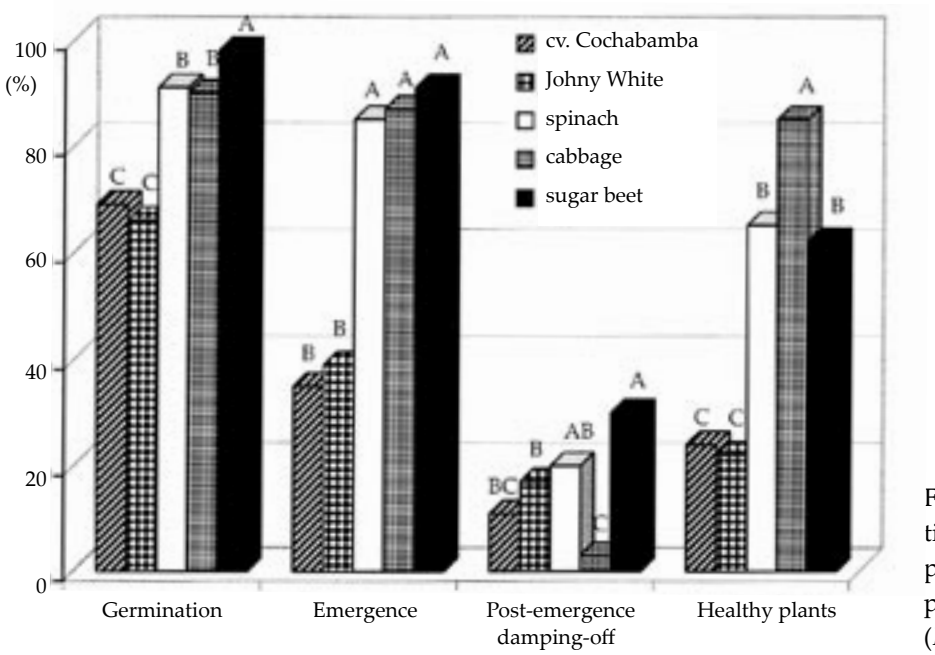

Figure 2. Percent of germination, emergence and healthy plants ($P \leq 0.01$) and seedlings post-emergence damping-off ($P \leq 0.05$)

Table 2. Fungi isolated from infected parts (stem and root) of seedlings with damping-off

Quinoa	cv. Cochabamba	*F. avenaceum*, *Fusarium* spp., *Pythium* spp., *P. aphanidermatum*, *A. caulina*, *Alternaria* spp.
Quinoa	Johny White	*F. avenaceum*, *Fusarium* spp., *Pythium* spp., *P. aphanidermatum*, *Alternaria* spp.
Spinach	cv. Matador	*Fusarium* spp., *F. equiseti*, *Pythium* spp., *Alternaria* spp.
Cabbage	cv. Sláva	*Fusarium* spp., *Pythium* spp.
Sugar beet	cv. Eureka	*Fusarium* spp., *Alternaria* spp.

equiseti (Corda) Sacc. 1886 was isolated only from infected seedlings of spinach (Table 2).

Pathogenicity test

In the pathogenicity test carried out by inoculation simultaneously with sowing, *P. aphanidermatum* caused significantly ($P \leq 0.01$) lower emergence than the other tested fungi and the uninoculated control (Figure 3). Pre-emergence damping-off caused by *P. aphanidermatum* was higher than post-emergence damping-off. *F. avenaceum* and *P. aphanidermatum* caused significantly ($P \leq 0.01$) higher post-emergence damping-off in the stand when inoculated at formation of the cotyledon leaves (Figure 4). *F. avenaceum* caused 59% post-

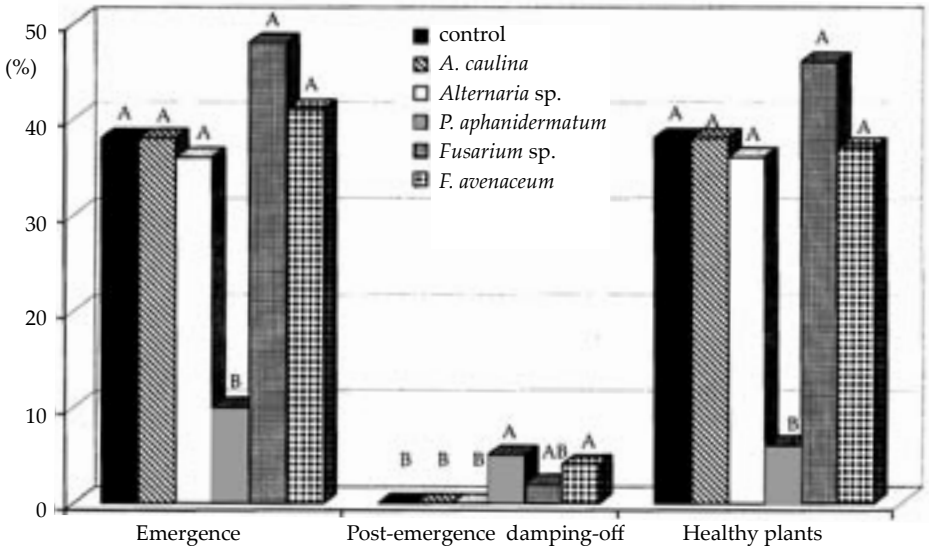

Figure 3. Percent of emergence, seedlings post-emergence damping-off and healthy plants after inoculation simultaneously with sowing ($P \leq 0.01$)

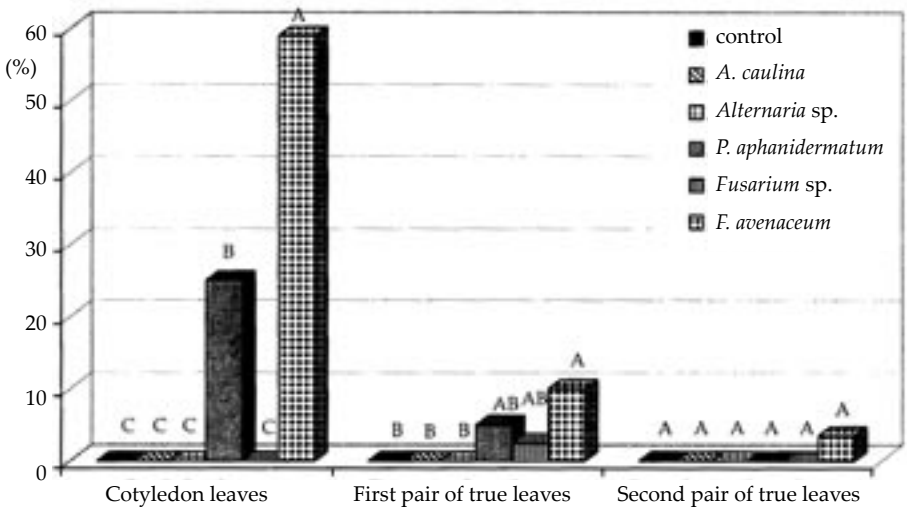

Figure 4. Percent of seedlings post-emergence damping-off after inoculation at the stage of the cotyledon leaves formation ($P \leq 0.01$) and at the stage of first and second pair of true leaves ($P \leq 0.05$)

emergence damping-off and *P. aphanidermatum* 22% post-emergence damping-off of quinoa seedlings. Only *F. avenaceum* caused significantly ($P \leq 0.05$) higher post-emergence damping-off in the stand when inoculated at the stage of the first pair of true leaves than the uninoculated control. There were no significant differences after inoculation at the stage of the second pair of true leaves (Figure 4). Nor were there any significant differences between isolates of each of the tested fungi.

DISCUSSION

Ascochyta caulina, *F. avenaceum*, *P. aphanidermatum*, *Pythium* spp., *Alternaria* spp. and *Fusarium* spp. were isolated from infected stems of quinoa seedlings grown in the greenhouse. *A. caulina*, *F. avenaceum* and in addition *Alternaria* spp. were also isolated from quinoa seeds of Czech origin. *Ascochyta hyalospora* (Cooke & Ellis) Boerema, S. B. Mathur & Neerg. 1977 is reported as seed-borne pathogen causing leaf spot of quinoa on the fields in the highlands of Peru (Boerema *et al*. 1977). *A. caulina* that had been isolated from both infected stems and seeds of quinoa is considered a potential mycoherbicide against *Chenopodium album* L. The experiments on disease development by *A. caulina* on species of the genera *Chenopodium* L., *Atriplex* L. and *Spinacia* L. have shown that *C. quinoa* and *S. oleracea* were susceptible to this pathogen (Kempenaar *et al*. 1996).

Our subsequent pathogenicity tests confirmed that the isolated *P. aphanidermatum* and *F. avenaceum* were the causal agents of the damping-off of quinoa seedlings. Quinoa was highly susceptible to *P. aphanidermatum*, especially during germination till the end of the cotyledon leaves formation. However, it then became more susceptible to *F. avenaceum* at the stage of cotyledon leaves and the first pair of true leaves. *Fusarium* spp. and *Pythium* sp. are also reported as soilborne pathogens causing seedling damping-off of quinoa from other regions. *Fusarium* spp. was isolated, together with *Rhizoctonia solani* Kühn 1858, from a field of quinoa at the International Potato Center in Peru (Danielsen *et al*. 2003). In Japan, *Pythium zingiberum* Takah. 1954, known as the pathogen that causes rhizome rot in ginger, was determined as the causal agent of damping-off of quinoa seedlings (Ikeda & Ichitani 1985). However, in a greenhouse experiment, an unidentified *Pythium* sp. killed emerging amaranth seedlings, particularly at high moisture levels, whereas quinoa seedlings remained healthy (Aufhammer *et al*. 1994). Another pathogen causing pre- and post-emergence damping-off and seed rot of quinoa is *Sclerotium rolfsii* Sacc. 1911, which was first reported in California (Beckman & Finch 1980).

The comparison of the reaction of quinoa with that of other susceptible plants (spinach, cabbage, sugar beet) showed that quinoa is most sensitive to attack by the pathogen(s) before emergence during germination till the end of the stage of the first pair of true leaves. Post-emergence damping-off is higher at the stage of the first pair of true leaves than at the stage of the cotyledon leaves, but is lower than pre-emergence damping-off. Germinable quinoa seeds seemed to have a lower ability to emerge from the soil. This serious problem is caused not only by pre-emergence damping-off pathogens but more so by a complex of factors. This complex includes effect of sowing depth, soil condition, effect of harvest time and temperature on the germination and emergence of quinoa (Jacobsen & Bach 1998). Low soil moisture content as well as crusty topsoil strongly reduced percent emergence. Per contra high soil temperature and abundant moisture, identified as the two most important factors for infection by *Pythium* spp. (Hendrix & Campbell 1973).

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Souhrn

Dřímalková M., Veverka K. (2003): **Padání klíčních rostlin** *Chenopodium quinoa* Willd. Plant Protect. Sci., **40**: 5–10.

Původci padání klíčních rostlin *Ch. quinoa* byli určováni ve skleníkových pokusech. Z infikovaných částí rostlin byly izolovány druhy: *Ascochyta caulina*, *Fusarium avenaceum*, *Fusarium* spp., *Alternaria* spp. a *Pythium* spp. Nejvíce izolátů rodu *Pythium* tvořilo *Pythium aphanidermatum*. Test patogenity potvrdil jako původce padání klíčních rostlin *Ch. quinoa* druh *P. aphanidermatum* a *F. avenaceum*. Testy patogenity a srovnání padání klíčních rostlin *Ch. quinoa* s jinými náchylnými plodinami (špenát, zelí, cukrová řepa) ukazují, že *Ch. quinoa* je nejnáchylnější k napadení v době před vzejitím, tedy v průběhu klíčení, a to až do konce fáze prvního páru pravých listů. Ve srovnání s ostatními testovanými plodinami se klíčivá semena quinoy vyznačují mnohem nižší schopností vzcházet. To však není způsobeno pouze vlivem preemergentních patogenů, nýbrž komplexem mnoha nepříznivých faktorů působících zejména ve fázi klíčení.

Klíčová slova: padání klíčních rostlin; *Chenopodium quinoa*; klíčení; vzcházení; *Pythium* spp.; *Fusarium* spp.

Corresponding author:

Prof. Ing. Karel Veverka, DrSc., Mendelova zemědělská a lesnická univerzita v Brně, Agronomická fakulta, Ústav ochrany rostlin, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 133 043, fax: + 420 545 212 044, e-mail: veverka@mendelu.cz