Eur J Plant Pathol (2012) 132:59–70
DOI 10.1007/s10658-011-9848-5

The influence of *Aphanomyces cochlioides* on selected physiological processes in sugar beet leaves and yield parameters

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Accepted: 8 August 2011 / Published online: 19 August 2011

Abstract Alterations in some physiological processes in source leaves of sugar beet—such as chlorophyll and carbohydrate concentrations, stomatal conductance, rate of net photosynthesis and transpiration, and activity of the photosynthetic apparatus during root interaction with *Aphanomyces cochlioides,* were investigated. The influence of time of infection on plant health, yield quality and quantity was also examined. Plants were infected at different times of their growth period: on the sowing day and 4 or 8 weeks after sowing. A variation treatment, with non-pelleted seeds infected on the sowing day, was also analyzed. The experiment showed that development of disease symptoms depends on the time of infection and seed protection. A significant root yield decrease was observed in case of late infection, as compared to the yield of plants infected on the sowing day. The fresh weight of leaves was significantly increased where there was late infection. The infected plants showed a lower content of K^+, Na^+ and α-amino-N than did the controls. Infection by *A. cochlioides* induced chlorophyll degradation mostly in older leaves with the occurrence of natural senescence processes. Chlorophyll fluorescence parameters indicated that the photosynthetic apparatus of younger leaves was more sensitive to pathogen infection, when compared to older ones. The photochemical efficiency of photosystem II was reduced in young leaves mainly due to disturbance of the water-splitting system. In plants grown from non-pelleted seeds a strong impairment of PSII was observed only in those leaves which developed during early pathogen infection. In young leaves of plants infected in the fourth week after sowing, inhibition of the rate of net photosynthesis was correlated with the increase in intercellular CO_2 concentration, indicating some disturbance in the carbon assimilation phase. In mature leaves of late infected plants the reduction of photosynthesis net rate was associated with a decrease of stomatal conductance and an increase of diffusion resistance to CO_2 and H_2O, which was also the cause of the transpiration rate inhibition. When the leaves developed during early infection, an increase of specific leaf weight and accumulation of carbohydrates was observed. In mature leaves of non-protected plants infected on the sowing day, the recovery of all physiological processes was observed together with a diminution of disease symptoms.

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Key words *Aphanomyces cochlioides* infection · *Beta vulgaris* · Photosynthesis · Transpiration · Yield

Introduction

Soil-borne diseases can have a major impact on sugar beet at all stages of crop development. They infect seedlings, leading to poor crop establishment, or damage the roots of more mature plants (Luterbacher et al. 2005). *Aphanomyces cochlioides* Drechsler is one of the main pathogens that infect sugar beet seedlings, leading to a damping-off disease complex. The consequence of soil infestation is reduction of yield and stand density. This pathogen infects also the roots of spinach, cockscomb and other species of *Chenopodiaceae* and *Amaranthaceae* through biflagellate motile zoospores and causes root rot and damping-off diseases (Islam et al. 2001). The zoospores of phytopathogenic *Aphanomyces* spp. are believed to orient to the host surface by chemotaxis and then encyst, germinate and finally penetrate the host tissues (Horio et al. 1992; Islam et al. 2003). Sugar beet infection by *A. cochlioides* primarily occurs 1–3 weeks post-emergence; seedling hypocotyls are invaded, eventually reducing the stem to a black and thread-like structure (Duffus and Ruppel 1993). *A. cochlioides* infection is favoured by high temperature and high soil moisture content. Thus the damage is greatest when sugar beet is sown late into warm, damp soils. In such conditions total crop failure can occur (Williams and Asher 1996). More commonly, infected seedlings survive but are stunted and less vigorous (Duffus and Ruppel 1993).

A. cochlioides can also cause a root rot in more mature sugar beet plants although this is generally considered less important than seedling infection, particularly in Europe (Duffus and Ruppel 1993). The pathogen has been controlled by a mixture of tiram and hymexazol applied in the seed pellet (Payne and Williams 1990). The persistence of chemicals in the pellet lasts approximately one month; after this time the root is not protected and the occurrence of favourable weather conditions for *A. cochlioides* can result in late infections and pathogen development. The pathogen is frequently a reason for a chronically-expressed disease by a continued attack on the root system (Payne et al. 1994). In some cases the yield of roots is strongly reduced. In spite of the fact that the foliage is less affected (Harveson and Carlson 2003) *A. cochlioides* is commonly encountered on sugar beet throughout the world (Hall 1989) and is often considered the most important damping-off pathogen affecting the crop (Walker 2002). In the UK the infection of this pathogen has been identified on 39% of sugar beet fields (Payne et al. 1994). The USDA estimated losses caused by *A. cochlioides* as 1% of the entire crop (Papavizas and Ayers 1974).

Until now there has been very little information about changes in leaf physiology of host plants during an infection with oomycetes, in particular with *A. cochlioides*.

The aim of this work was: (i) to study the influence of sugar beet infection by *A. cochlioides* on some physiological processes in leaves, e.g. gas exchange parameters, relative content of chlorophyll, activity of photosynthetic apparatus and carbohydrate concentration; and (ii) to examine the influence of time of *A. cochlioides* infection on the health, yield quantity and quality of sugar beet.

Materials and methods

The experiment was carried out in a greenhouse in controlled conditions. The soil humidity was kept at 85% of the soil capacity. Experimental pots were automatically watered daily according to the tensiometric measurement. Commercially pelleted and non-pelleted seeds of a variety Lolita susceptible to *A. cochlioides* were used in the test. Three variations in infection time were applied as treatments in this test: infection on the sowing day (P-I); infection 4 weeks after the sowing day (P-EI); infection 8 weeks after the sowing day (P-LI); and a non-infected control (P-control). A variation treatment, with non-pelleted seeds infected on the sowing day (NP-I), was also applied. Each treatment contained 10 pots. Pots (4 dm^3) were filled with steam-treated soil mixed with sand and peat (60:20:20) at pH=7, sown with three seeds in each pot. The experiment was carried out with four replications.

Inoculum of *A. cochlioides* was prepared according to the method given by Windels (2000). Each inoculation was done using a zoospore solution obtained after a 3-day incubation of *A. cochlioides*. Incubation was for 2 days in a broth containing Sigma type IV peptone (20 g l^{-1}) and glucose (5 g l^{-1}) in deionised water, and after that washed three times in 1.5 h periods using Mineral Salts solutions and than left undisturbed overnight (Windels 2000). Pots were infested by zoospores at a rate of 3×10^5 per pot. After inoculation, pots were watered. Plants in pots were counted every 2–3 days during the test. After 1 month some plants were uprooted, and then only one, the most vigorous plant was left in each pot. During the test, the health condition of sugar beet was observed using a scale: 0—healthy, 1—weak disease symptoms, 2—severe disease symptoms, 3—dead plant (seedling). An infection coefficient (I_p) was calculated using the formula (Burgieł 1980):

$$I_p[\%] = \left[\sum_{k=0}^{k=\max} (n_k \cdot k)/N \cdot k_{\max} \right] \cdot 100\%,$$

where k is a scale degree (for estimation of a health; given above), N is the total number of observed plants, and n_k is the number of diseased plants observed in each degree of the scale.

At the end of the experiment (23 weeks) some plants were taken to the laboratory to check the microorganisms present on diseased roots. A 9-degree scale was used for the final estimation of the health condition of roots (9, a healthy root; 1, a completely rotten root). The fresh weight of roots and leaves and the dry weight of tap-roots for each treatment were measured, as well as parameters indicating the quality of the yield: sugar content (%), α-amino-N content, K^+ content, Na^+ content. Chemical analyses were made by means of an automatic analytical system for sugar beets (Venema).

Physiological analyses were made in the following experimental treatments: not infected control sown with pelleted seeds (P-control); inoculated by *A. cochlioides* on the fourth week (P-EI) or on the eighth week (P-LI) after sowing with pelleted seeds; and inoculated during sowing with non-pelleted seeds (NP-I).

The analyses were performed twice at 56 and 105 days after sowing, about 1 month after early (P-EI) or late (P-LI) soil infestation with *A. cochlioides*. All analyses were made on young, mature and/or old leaves. The relative content of chlorophyll was measured using SPAD-502 (Minolta, Japan). The activity of the light phase of photosynthesis by fluorescence parameters was done with the use of Handy-PEA fluorometer (Hansatech Instruments Ltd., UK). The leaves differing in their development phase were kept for 30 min in the dark and then the minimal fluorescence (Fo) was detected. The maximal fluorescence (Fm) was determined by saturation pulse mode (1 s and 3000 µmol m^{-2} s^{-1} photon flux density). Other fluorescence parameters were automatically calculated: Fv=Fm−Fo=variable fluorescence; Fv/Fm=maximal quantum yield of PSII; Fv/Fo=activity of donor side of PSII; P.I. = ratio of leaf vitality, Tfm—half time for increase of fluorescence value from Fo to Fm.

Carbon exchange (Pn), transpiration (Tr), leaf stomata conductance (gs) and intercellular CO_2 concentration (C_I) were measured using a portable photosynthesis system Li-6400 (LI-COR, USA). The measurements were made on young and mature leaves under artificial irradiation (blue and red light) at 600 µmol m^{-2} s^{-1} photon flux density (PPFD),

Fig. 1 The influence of infestation time of *A. cochlioides* on the sugar beet root infection coefficient (I_p); [explanations: **P-control**-pelleted and not infected seeds; **P-I**-pelleted seeds, infection on the sowing day; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

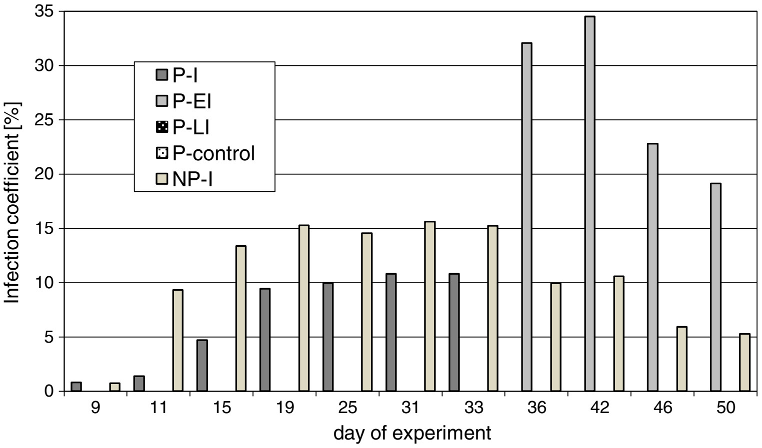


Table 1 Influence of infection time on disease development on the sugar beet roots

Treatment	Disease development (1 to 9° scale**)
Pelleted seeds—not infected, (P-control)	9.0 b*
Pelleted seeds—sowing day (P-I)	9.0 b
Pelleted seeds −4 weeks after sowing (P-EI)	8.5 a
Pelleted seeds −8 weeks after sowing (P-LI)	8.8 b
Nonpelleted seeds—sowing day, control (NP-I)	8.9 b

values followed by the same letter do not differ significantly ($P>0.95$); **1 means a completely rotten root, 9 a healthy root

with temperature 25°C, and concentration of CO_2 at 350 μmol mol^{-1}. Water use efficiency (WUE) was calculated as a ratio of carbon exchange (Pn) to transpiration (Tr) and WUE_I as a ratio of carbon exchange rate (Pn) to stomatal conductance value (gs). The specific leaf weight (SLW) was determined as the ratio of leaf dry weight to leaf area.

The total carbohydrate concentration in mature leaves was also determined. About 1 g of fresh weight (FW) leaf tissue was collected in 5 cm^{-3} of ethanol (96%) and stored under light until chlorophyll had broken down. The ethanol was evaporated and leaf tissue was homogenised and centrifuged at 15000×g for 15 min. Supernatant was collected and total contents estimated. Starch was hydrolysed in 0.5 cm^{-3} of supernatant at 100°C for 3 h by using 0.5 cm^{-3} of 2 MHCl. After that, 0.5 cm^{-3} of 1 MNaOH was added and the content of reducing sugars was measured spectrophotometrically at 560 nm according the method given by Waffenschmidt and Jaenicke (1987). Total carbohydrate concentration was expressed as glucose concentration (μmol of glucose per g of leaf fresh weight). All physiological measurements were done in five replications for one leaf from a different plant in each experimental treatment.

Statistical analyses were done using analysis of variance method. The significance of mean separation was calculated by Duncan's multiple range test ($P>0.95$).

Results and discussion

The experiment demonstrated that development of disease symptoms depends on the infection time and the type of seed protection (Fig. 1, Table 1). The most effective infection was observed in case of early inoculated (P-I) and not-protected plants (NP-I), but severe symptoms were observed on the plants inoculated 4 weeks after sowing (P-EI), starting from the 36th day of the experiment, due to a short persistence of hymexazole contained in seed coatings (Heijbroek and Huijbregts 1995). Disease symptoms on roots were not observed on beets infected 8 weeks after sowing (P-LI), but the infestation affected some physiological processes in leaves (Fig. 1).

A. cochlioides infection influenced the yield of sugar beets depending on the time of infection (Table 2). Significantly greater fresh weight of leaves was observed in case of late infection (8 week) when disease symptoms were not visible on roots (Fig. 1). Root yield was not significantly affected by infection, but some decrease in fresh weight was observed in early and late infected plants (Table 2). Significant

Table 2 The influence of the infection time on the fresh weigh of leaves, tap-roots and dry weigh content of sugar beet tap-roots

Treatment	Fresh weight (g)[a]		Dry weight content in tap-roots (%)[a]
	leaves	tap-roots	
Pelleted seeds—not infected (P-control)	4397.85 c	1687.65 ab	20.3 ab
Pelleted seeds—sowing day (P-I)	4194.35 bc	1928.25 b	21.7 b
Pelleted seeds −4 weeks after sowing (P-EI)	4114.00 bc	1367.65 a	21.1 ab
Pelleted seeds −8 weeks after sowing (P-LI)	4782.50 d	1496.90 ab	19.9 a
Nonpelleted seeds—sowing day, control (NP-I)	4154.25 bc	1978.00 b	21.7 b

[a] data indexed by the same letter do not differ significantly in columns (P>0.95)

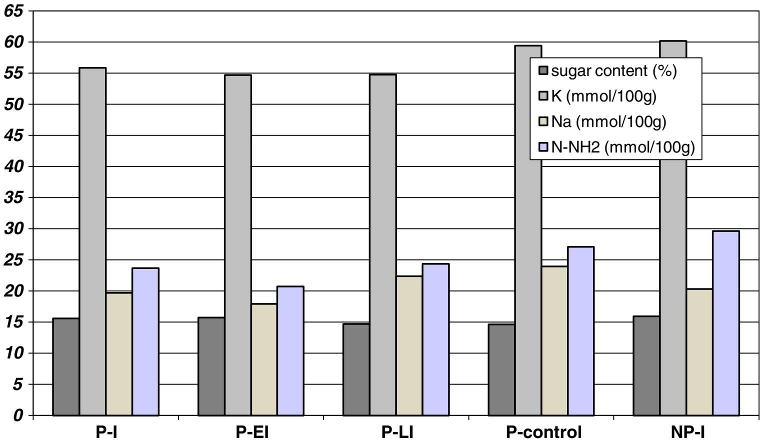


Fig. 2 The influence of infestation time of *A. cochlioides* on the yield quality; [explanations: **P-control**-pelleted and not infected seeds; **P-I**-pelleted seeds, infection on the sowing day; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

root yield decrease was observed in early infection when compared to the yield of plants infected on sowing day (P-I, NP-I). In an experiment carried out with *Aphanomyces*-resistant sugar beet cultivar Cristal 205 and plants infected at sowing time, Harveson and Carlson (2003) did not observe negative effects of *A. cochlioides* infection on leaf fresh weight and area but found a significant decrease in tap-root yield. In this experiment the most vigorous plant was left in each pot and this was perhaps the reason why no great differences between treatments were found; because of this, severe disease symptoms, especially in early infected plants, were not found.

The ratio of dry weight to fresh weight was least in roots infected on the eighth week (P-LI), suggesting that in these roots water content was greater than in roots from other treatments (Table 2). The greater water content in plant tissues can be the reason for a lower level of infection (Rubin and Arcichowska 1971), as was observed in this experiment (Fig. 1, Table 1). Roots of early infected plants (treatments P-I, NP-I) as well as roots infected on fourth week (P-EI) had higher dry weight content, and disease symptoms were observed (Table 2, Fig. 1)

Yield quality showed a small difference in sugar concentration with roots from early infected treatments (P-I, P-EI, NP-I) showing about one per cent more sugar yield (Fig. 2). It has been shown that sugar content decreases when the foliage is infected by the leaf pathogens *Ramularia betae* and *Cercospora beticola* (Šikalčik and Botjan 2002). On the other hand, in tobacco leaves inoculated by *Phytophthora nicotianae,* carbohydrates were immediately retained at the infection site (Scharte et al. 2005). Until now it is not known how *A. cochlioides* infection influences the photosynthesis and carbohydrate metabolism of sugar beet leaves, especially if symptoms of the disease are not visible. More evident

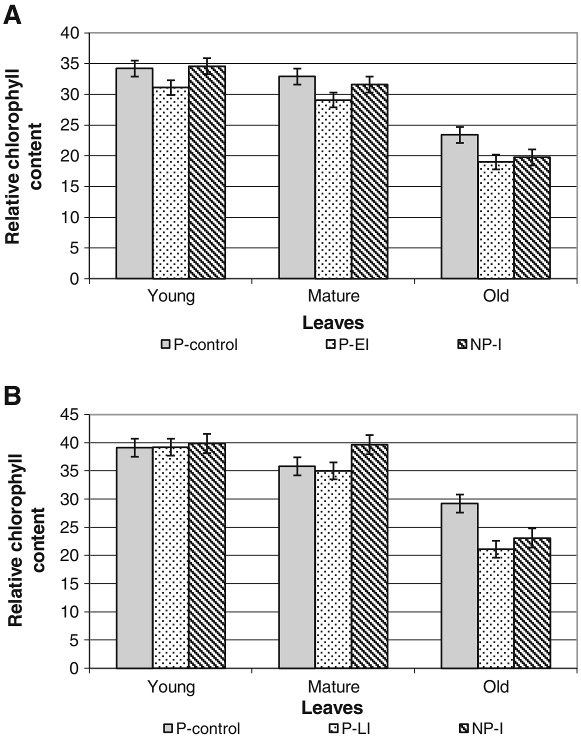


Fig. 3 The influence of *A. cochlioides* infection on a relative chlorophyll content in sugar beet leaves [inoculation on the fourth (**a**) or on the eighth (**b**) week after sowing; **P-control**-pelleted and not infected seeds; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

Table 3 Chlorophyll *a* fluorescence parameters measured in young and mature leaves of sugar beet plants at 56 days after sowing

Treatment	Young leaves			Mature leaves		
	Fo	Fm	Tfm	Fo	Fm	Tfm
Pelleted seeds—not infected (P-control)	648 a[a]	3802 a	143 a	745 a	3893 a	202 a
Pelleted seeds −4 weeks after sowing (P-EI)	790 b	3729 a	258 b	726 a	3802 a	226 a
Nonpeletted seeds—sowing day (NP-I)	704 a	3774 a	178 a	702 a	3573 a	160 a

[a] data indexed by the same letter do not differ significantly in columns ($P>0.95$); Fo-minimal fluorescence, Fm-maximal fluorescence, Tfm-half time for increase of fluorescence value from Fo to Fm

differences were observed in the case of K^+, Na^+ and α-amino-N concentrations. Infected plants showed a lower content of these elements than did the controls (P-control, NP-I) (Fig. 2).

Plant defence responses against some pathogens are orchestrated by various signaling pathways. As well as plant growth regulators such as salicylic acid, jasmonic acid and ethylene (Howe 2001; Pieterse et al. 1998; Thomma et al. 1998), hydrogen peroxide, NO and sugars (Bolwell 1999; Neill et al. 2002; Scharte et al. 2005) have been implicated in plants defence responses as signaling molecules. Some of these mobile signals are transported systemically throughout the plant from the infected to the non-infected tissues where re-programming of gene expression and changes in cell metabolism could be induced. Until now there is little information about changes in leaves primary metabolism during a root infection with oomycetes. Here, we examined alternations in some physiological processes in source leaves of sugar beet, such as chlorophyll and carbohydrate concentrations, stomatal aperture, photosynthesis, transpiration and activity of photosynthetic apparatus, during the roots interaction with *A. cochlioides*.

A. cochlioides infection considerable reduced the relative chlorophyll $a+b$ concentration in all examined leaves but only when disease developed in the earlier phase of plant development (Fig. 3a). At both the earlier (Fig. 3a) and later (Fig. 3b) times of infection, the most significant decrease of chlorophyll concentration was observed in older leaves. Pathogen inoculation on the eighth week of plant growth did not change chlorophyll concentration in younger leaves. In plants grown from non-pelleted seeds (NP-I) a decrease of chlorophyll content was observed by *A. cochlioides* infection only in old senescencing leaves, but in both phases of sugar beet growth (Fig. 3a and b). This suggests that in plants grown from non-pelleted seeds without fungicides, leaves were senescing much earlier than when grown from pelleted seeds, but only during the early phase of growth. Much less root disease was caused by *A. cochlioides* after the 50th day of vegetation (Fig. 1). At that time, regeneration processes in leaves of infested plants (NP-I) were induced because the chlorophyll content of mature leaves was even higher in comparison with plants grown from pelleted seeds.

It could be concluded that, mostly in older leaves with the occurrence of natural senescence, some processes of chlorophyll degradation can be induced by *A. cochlioides* infection. It has been known that changes in chlorophyll *a* fluorescence emissions

Table 4 Chlorophyll *a* fluorescence parameters measured in young, mature and old leaves of sugar beet plants 105 days after sowing

Treatment	Young leaves			Mature leaves			Old leaves		
	Fo	Fm	Tfm	Fo	Fm	Tfm	Fo	Fm	Tfm
Pelleted seeds-not infected (P-control)	645a[a]	3962a	182a	633a	3528a	298a	686a	3513a	222a
Pelleted seeds-8 weeks after sowing (P-LI)	612a	3163b	372b	640a	3482a	276a	588a	3080b	196a
Nonpeletted seeds-sowing day (NP-I)	570a	3756a	204a	561a	3416a	247a	785b	3435a	191a

[a] data indexed by the same letter do not differ significantly in columns ($P>0.95$); Fo-minimal fluorescence, Fm-maximal fluorescence, Tfm-half time for increase of fluorescence value from Fo to Fm

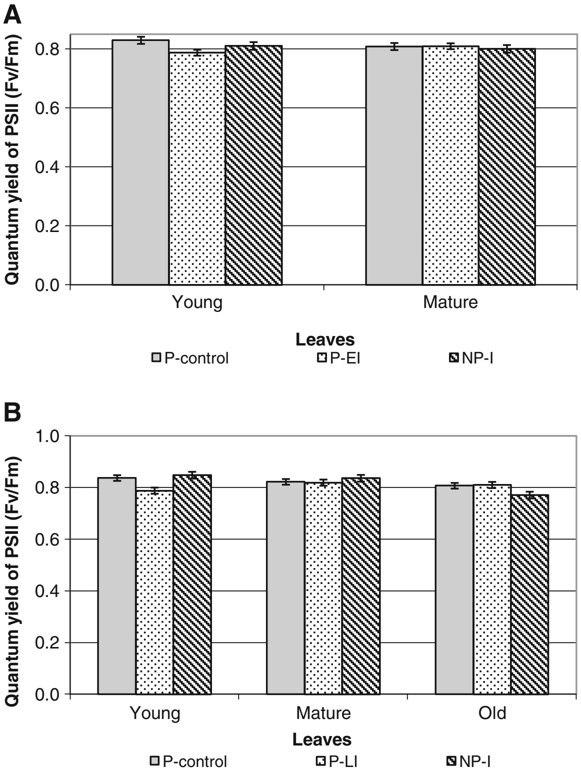


Fig. 4 The influence of *A. cochlioides* infection on quantum yield of PS II in sugar beet leaves [inoculation on the fourth (**a**) or on the eighth (**b**) week after sowing; **P-control**-not infected and pelleted seeds; **P-EI**-pelleted seeds, infection on fourth week after sowing; **P-LI**-pelleted seeds, infection on eighth week after sowing; **NP-I**-nonpelleted seeds, infection on sowing day, served as a control]

frequently indicate a change in the activity of photosystem II (PSII) induced by stress conditions. The most popular technique involves the detection of fluorescence induction kinetics occurring upon exposure of the dark-adapted photosynthetic system to light (Baker and Rosenqvist 2004).

The initial fluorescence (Fo) represents emission by excited antenna chlorophyll *a* molecules. The value of Fo and the half-time from Fo to Fm (Tfm) in the youngest leaves of the plants infested at earlier term (P-EI) significantly increased when compared to control plants (Table 3). The levels of Fo, Fm and Tfm in mature leaves were not affected by *A. cochlioides* infection. Some moderate increase of the Fo of young leaves indicates disturbance of the excitation energy transfer between antenna pigments and from those to reaction centres of PSII as an effect of pathogenesis (Krause and Weis 1984). A high value of Tfm suggests that the amount of active pigments associated with PSII decreased after the pathogen infection only in younger leaves. The initial or maximal fluorescence emission and Tfm of both analysed types of leaves were not changed in plants grown from non-pelletted seeds (NP-I) in comparison to the controlled ones (Table 3).

Fm is defined as the maximal fluorescence yield of the dark adapted leaf with all PSII reaction centres reduced by saturated light (Krause and Weis 1991). It is clear that in plants later infected this parameter was strongly reduced by the pathogen infection in young and the oldest leaves (Table 4). The decrease in Fm indicates alternations to the thylakoid structure influencing the electron transfer through PSII (Baker and Rosenqvist 2004). A high value of Tfm demonstrated in younger leaves, as an effect of pathogenesis, suggested smaller antennae size of PSII, a block at

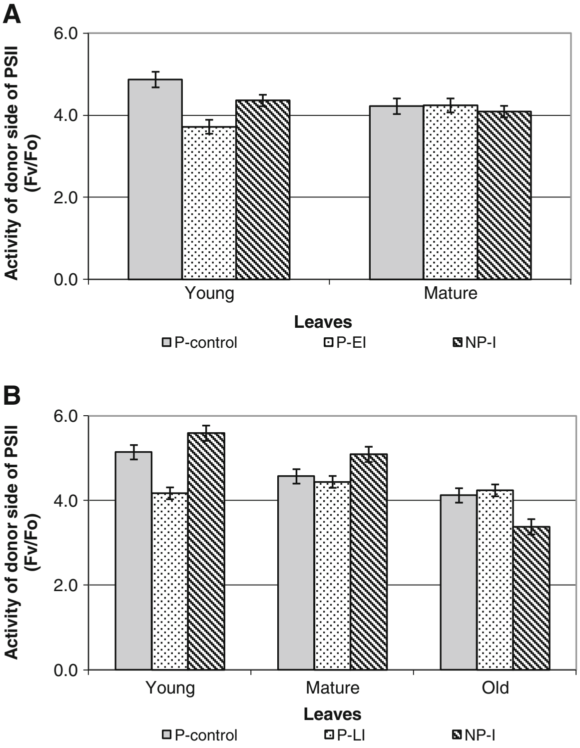


Fig. 5 The influence of different time of *A. cochlioides* infection on activity of donor side of PS II in sugar beet leaves [inoculation on the fourth (**a**) or on the eighth (**b**) week after sowing; **P-control**-pelleted and not infected seeds; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

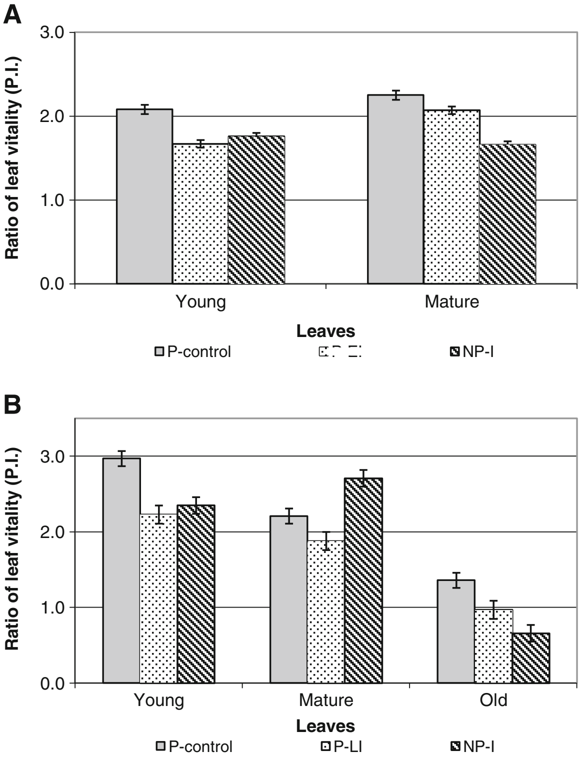


Fig. 6 The influence of different time of *A. cochlioides* infection on the vitality ratio (P.I.) of sugar beet leaves [inoculation on the fourth (**a**) or on the eighth (**b**) week after sowing; **P-control**-not pelleted and not infected seeds; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

the level of D_1 protein and/or the size of plastoquinone pool (Krause and Weis 1984). In the case of plants grown from seeds without fungicide protection, only some changes in the energy transfer within the antennas at the oldest leaves, expressed as a high level of Fo, were observed as an effect of infection.

Maximal quantum yield in photochemistry of PSII calculated as the ratio Fv/Fm (Baker and Rosenqvist 2004) was decreased in the youngest leaves as a response to *A. cochlioides* treatment, independently of the time of soil inoculation (Fig. 4a and b). In the non-pelleted treatment (NP-I), only the oldest leaves responded to pathogen infection by inhibition of the quantum yield of PSII. Photochemical efficiency of photosystem II was reduced in those leaves mainly due to disturbance among water-splitting system, shown by the decrease in the Fv/Fo ratio (Fig. 5a and b). The decrease in the maximum quantum efficiency of PSII photochemistry has also been observed in tomato leaves during the late stage of disease development induced by *Fusarium oxysporum* (Nogués et al. 2002). In tobacco leaves inoculated with *Phytophtora nicotianae* a drastic decline in the whole of the electron transport chain and in particular, in the electron transfer to oxidized PSI reaction centres was demonstrated (Scharte et al. 2005) with concomitant moderate photoinhibition of PSII at the infection site.

The leaf vitality ratio (P.I.) demonstrates the efficiency of the energy transfer within the whole photosystem, beginning from the light absorption by antennas pigments through the activity of the PSII centres and the rate of electron transport. This ratio was reduced in almost all examined leaves of the infected plants (Fig. 6a and b). It was only in the non-pelleted (NP-I) treatment that mature leaves demon-

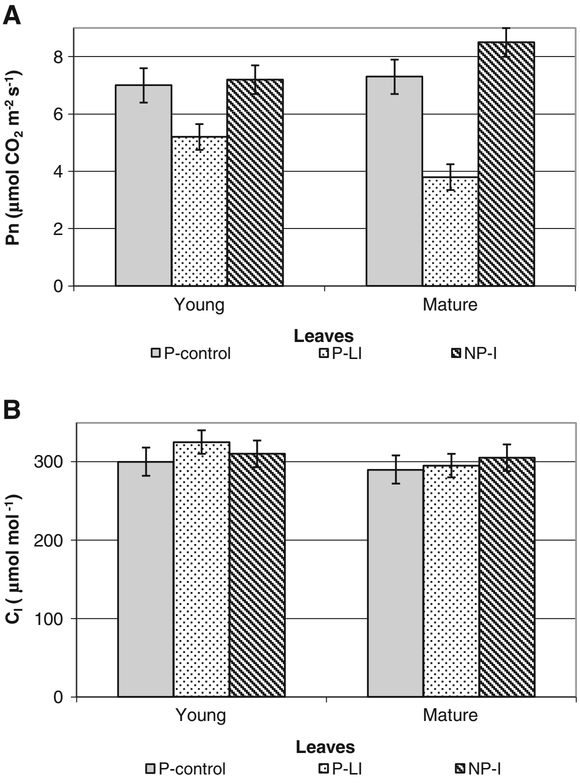


Fig. 7 The influence of *A. cochlioides* infection on the intensity of net photosynthesis—Pn (**a**) and intercellular CO2 concentration—Ci (**b**) of sugar beet leaves [inoculation on the eighth week after sowing; **P-control**-pelleted and not infected seeds; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

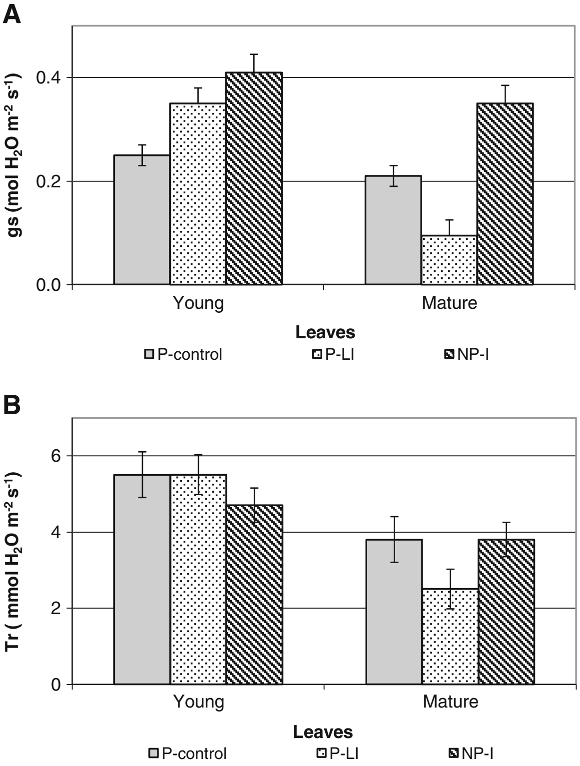


Fig. 8 The influence of *A. cochlioides* infection on the stomatal conductance—gs (**a**) and the intensity of transpiration—Tr (**b**) of sugar beet leaves [inoculation on the eighth week after sowing; **P-control**-pelleted and not infected seeds; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

strated a higher value of P.I. than the controls (Fig. 6b). This observation suggests that some regeneration processes in photosynthetic apparatus were induced in those leaves.

In conclusion, the photosynthetic apparatus of younger leaves was more sensitive to *A. cochlioides* infection. In the case of plants grown from non-pelleted seeds, pathogen inoculation caused a strong impairment of PSII only in the oldest leaves, those which were developed during an early pathogen infection. In those plants, the efficiency of light phase of photosynthesis in fully developed leaves was even higher than in the controls, indicating the induction of regeneration processes.

In plants infected on the eighth week of their growth, carbon exchange (Pn) was reduced by *A. cochlioides* infection in both young and mature leaves (Fig. 7a). In young leaves inhibition of Pn was correlated with the increase of intercellular CO_2 concentration (C_I) indicating some impairment in a carbon assimilation phase (Fig. 7b). In mature leaves of late infected plants the reduction of Pn was connected with the decrease of stomata conductance (gs) (Fig. 8a) and increase of diffusion resistance to CO_2 and H_2O which was also a result of transpiration rate (Tr) inhibition (Fig. 8b). In NP-I plants the rate of net photosynthesis of mature leaves was even higher than in controls, suggesting that in those leaves some compensation on carbon assimilation processes was observed (Fig. 7a). Plant-pathogen interactions are usually associated with increased demands for energy, reducing carbon skeletons for biosyntheses. Whereas respiration, the pentose phosphate pathway, and the shikimic acid pathway are usually enhanced in infected tissue (Scheideler et al. 2002), photosynthesis is often depressed at the later stage of an infection

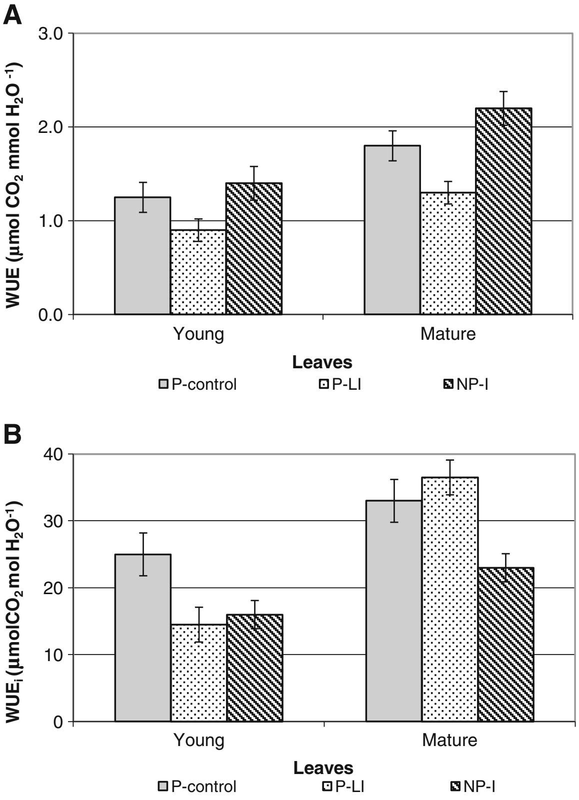
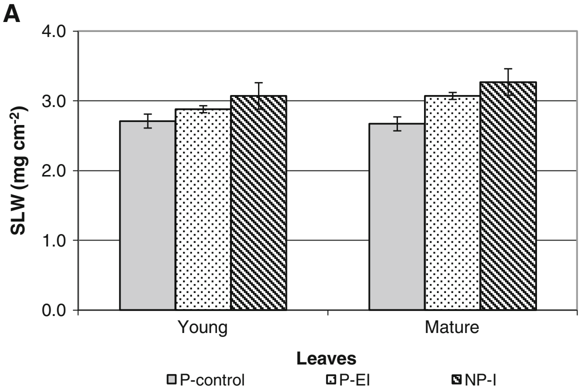


Fig. 9 The influence of *A. cochlioides* infection on the water use efficiency—WUE (**a**) and internal use efficiency—WUEi (**b**) of sugar beet leaves [inoculation eight weeks after sowing; **P-control**-pelleted and not infected seeds; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]



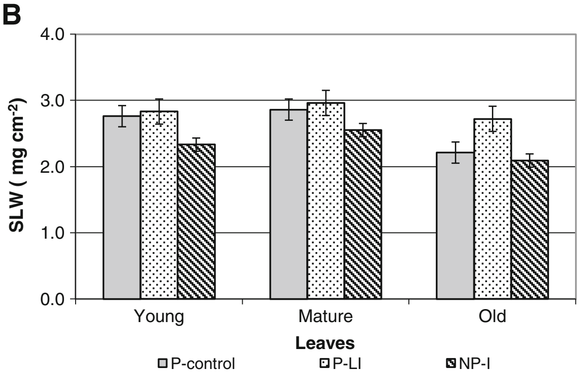
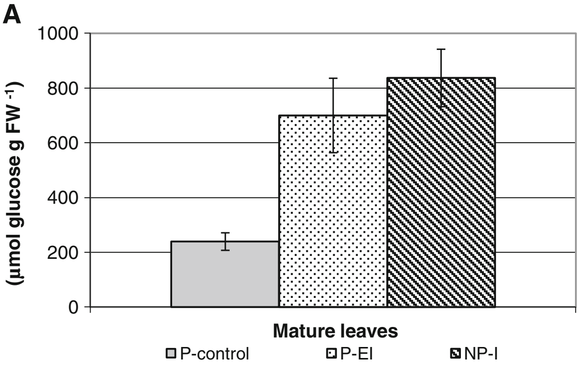


Fig. 10 The influence of different time of *A. cochlioides* infection on the specific leaf weight—SLW of sugar beet leaves [inoculation on the fourth (**a**) or on the eighth (**b**) week after sowing; **P-control**-pelleted and not infected seeds; **P-EI**-pelleted seeds, infection on the fourth week after sowing; **P-LI**-pelleted seeds, infection on the eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

(Bassanezi et al. 2002; Noguès et al. 2002; Herbers et al. 2000).

Water use efficiency (WUE) was reduced in young and mature leaves in plants infected by *A. cochlioides* on the eighth week of their growth (Fig. 9a). This result indicates a stronger inhibition of carbon exchange (Pn) than transpiration rate (Tr) induced by pathogen infection. Internal water use efficiency (WUEi), describing Pn change on a background of stomata conductance (gs), was decreased as an effect of *A. cochlioides* infection only in young leaves (Fig. 9b). This demonstrates that the decrease of the net rate of photosynthesis in those leaves was unaffected by the level of diffusion resistance to CO_2, but probably affected by the activity of the Calvin cycle. It could be pointed out that WUE (Fig. 9a) and stomata conductance (gs) (Fig. 8a) values of the mature leaves in plants of NP-I variant were higher than in the controls.

In the plants infected on the fourth week after sowing, specific leaf weight—SLW of young and mature leaves was increased in comparison to the controls (Fig. 10a); although much less change was observed in plants infected at the later phase of their growth, when SLW of only older leaves was higher in P-LI variant than in the controls (Fig. 10b). These results suggest that accumulation of assimilates in those leaves and inhibition of their export could be one reason for the carbon exchange (Pn) reduction by *A. cochlioides* infection. This was confirmed by the carbohydrates concentration data (Fig. 11). The accumulation of carbohydrates was observed in mature leaves both in plants infected early and late (Fig. 11a and b). In tobacco leaves four days after



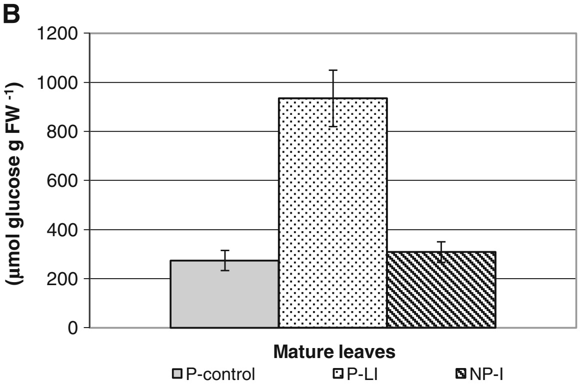


Fig. 11 The influence of different time of *A. cochlioides* infection on the carbohydrate concentration (µmol glucose) of mature sugar beet leaves fresh weight (FW) [inoculation in the fourth (**a**) or in the eighth (**b**) week after sowing; **P-control**-not infected and pelleted seeds; **P-EI**-pelleted seeds, infection on fourth week after sowing; **P-LI**-pelleted seeds, infection on eighth week after sowing; **NP-I**-nonpelleted seeds, infection on the sowing day, served as a control]

potato virus infection, an accumulation of soluble sugars and a concomitant decrease of leaf photosynthesis was also observed (Herbers et al. 2000). In the case of plants grown from non-pelleted seeds, pathogen inoculation caused an increase of SLW (Fig. 10a) and carbohydrate accumulation (Fig. 11a) in mature leaves developed during the first 50 days of the vegetation. Nevertheless, in those plants the SLW of all examined leaves was lower (Fig. 10b), but carbohydrates concentration of mature leaves was unchanged (Fig. 11b). It seems that the export of assimilates from the source—leaves to sinks—was stimulated during a recovery period.

In conclusion, infection of sugar beets by *A. cochlioides* was significantly higher in spite of the pelleted treatment when it was made 4 weeks after sowing. Sugar beets protected by hymexazole and infected on the sowing day were less infected than the controls and in this case the infection developed about 1 week later. We observed that roots of sugar beets (pelleted) were most sensitive to infection in the beginning of the second month of growth. *A. cochlioides* infection affected chlorophyll degradation mainly in older leaves when the natural senescence processes had already commenced. Both the light and biochemical phase of photosynthesis processes in the youngest leaves of sugar beet plants were affected by the pathogen. Their photosynthetic apparatus was the most sensitive to *A. cochlioides* infection. A pathogen-induced change in photochemistry of PSII was smaller in mature leaves, but with disease development their net photosynthesis rate was reduced mainly due to stomata closure. In the leaves that developed during early pathogen infection, some disturbance in the source-sink relationship was observed. The recovery mechanisms of all analyzed physiological processes in mature leaves were induced during disease recovery.

Acknowledgements The research was financially supported by KWS Polska and Südzucker Polska.

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