

Differentiation of the Disease Caused by *Aphanomyces cochlioides* and Girth Scab on Sugar Beet Roots – a Review

EWA B. MOLISZEWSKA

Opole University, Department of Natural Sciences and Technology, Opole, Poland

Abstract

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Severe symptoms of root rot on sugar beet have been observed in Poland and Germany since 2001. The symptom classification suggested girth scab as it was mistakenly classified on the basis of current classification, e.g. in LIZ. However, the cause of the disease was *Aphanomyces cochlioides* infection, not *Streptomyces* spp. According to these findings we cannot call the symptoms caused by *A. cochlioides* as 'girth scab'. The typical scab (girth scab) symptoms can be promoted by *A. cochlioides* infections. In many cases, *A. cochlioides* developed at the beginning of the season, during the seedling stage. Its further development was due to rainfall and was not routinely recognised in disease-changed tissues if in the middle of the summer the rainfall level was reduced. According to the described findings, renewed description and differentiation of the girth scab symptoms caused by *Streptomyces* spp. and root rot caused by *A. cochlioides* on sugar beet roots are suggested. Typical symptoms of the disease caused by *A. cochlioides* occur mainly on the upper part of the root but can also occur on its lower part, if weather conditions are favourable for the pathogen. The coexistence of *A. cochlioides* and *Pythium* spp. in the same niches is also possible. Currently the illustrations showing these symptoms are included in the 'girth scab' descriptions.

Keywords: description; *Pythium*; *Aphanomyces* root rot; *Streptomyces*; symptoms

Sugar beet belongs among the major crops in the European Union, especially in Poland or in Germany. The quality of its yield is strongly dependent on pathogens existing in the field soil and possible infections of roots in all stages of their development (ASHER 1992; BEALE *et al.* 2002; DYER *et al.* 2004). The crop can be affected by soil derived pathogens, such as *A. cochlioides*, *Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp. (MOLISZEWSKA 2000; PISZCZEK 2004; MOLISZEWSKA & PISZCZEK 2008). As NECHWATAL *et al.* (2012) stated, the disease frequency and severity depend on some environmental factors, such as weather conditions and structure of the soil microorganism community, soil water content, degree of soil compaction, temperature, plant age, alternative hosts and other as-yet unknown factors (WILLIAMS & ASHER 1996; KNUDSEN *et al.* 2002; MOLISZEWSKA & PISAREK 2004; SZYM CZAK-NOWAK 1987; PEARSSON & OLSSON 2005; NECHWATAL *et al.* 2012), although MOLISZEWSKA (2008a) believed that soil fungal communities could not influence

the level of the infection of sugar beet seedlings (damping-off) or the particular infections caused by pathogens such as *A. cochlioides*, *Fusarium* spp., *Rhizoctonia* spp. or *Pythium* spp. (MOLISZEWSKA 2008a). However, the population of the pathogen in the soil is an important factor as MOLISZEWSKA (2008a) evidenced by the pot test correlation with field infection.

Girth scab as a factor suspected of root damage

Throughout the last dozen years or more, sugar beet crops have been affected by root rot with symptoms recognisable as a girth scab disease described in phytopathological literature (PISZCZEK 2002; MÄRLANDER *et al.* 2003; PETERSEN & SCHLINKER 2003; PISZCZEK 2004). These symptoms are illustrated as a root rot. E.g. BENADA *et al.* (1984) suggested in the description of the disease, printed in the handbook of sugar beet pests and diseases, the causal agents

of girth scab are *Streptomyces scabies*, *S. nigricans*, and *S. intermedius*; HINFNER and HOMONNAY (1966) suggested that unfavourable soil conditions are the cause of the disease. Symptoms classified as girth scab (Figure 1) have frequently been observed within Poland and Germany since 2001, although severe symptoms with such diagnosis were noticed in Bavaria also in 2002 and 2010 (PI SZCZEK 2002, 2004; PETERSEN & SCHLINKER 2003; MOLISZEWSKA 2009; NECHWATAL *et al.* 2012). The year 2001 was critical due to rainfall – the yearly amount of rainfall was 662.3 mm (304.5 mm/June–August) in some parts of Poland (MOLISZEWSKA 2009). PETERSEN and SCHLINKER (2003) reported that at that time, in Germany, the girth scab was a causal agent of about 50% yield reduction of sugar beets. Yield losses were dramatically huge also in Poland, so sugar companies decided to start investigations to resolve the problem. It was pointed out that the level of the yield losses was untypically severe, what was not probable for the case of *Streptomyces* infection. Simultaneously PI SZCZEK (2002) informed that *A. cochlioides* was isolated in Poland for the first time from mature roots of sugar beet. PI SZCZEK (2002) discovered the occurrence of *A. cochlioides* on all developmental stages of sugar beet, and the symptoms observed by him were comparable to those classified as girth scab. In 2001–2002 no investigations on the reasons causing the girth scab were yet conducted in Poland.

Starting the search for girth scab causal agents

The research on girth scab started in Poland in 2003 and continued until 2006 (MOLISZEWSKA 2009). PETERSEN and SCHLINKER (2003) found that unquestionably the favourable reason for girth scab was wet weather. These kinds of conditions were observed in Poland and in Germany at that time. They believed that causal agents of this disease were *Actinomycetes*, but they also found *Fusarium* spp. and bacteria of the genus *Bacillus* in the diseased tissues. At that moment, they concluded that severe *Actinomycetes* infection was probable due to earlier infection by *A. cochlioides*. In fact, at that time, most disease diagnoses were based on the available phytopathological descriptions (Figure 1A–C) (HINFNER & HOMONNAY 1966; BENADA *et al.* 1984). The recognition of the disease depended on the individual qualifications. The role played by *A. cochlioides* was unknown, it was undecided when girth scab

started and changed to root rot, which microorganism was the main pathogen or if they were able to act together. Indeed, there was a great demand to clearly distinguish girth scab on sugar beet roots from root rot caused by *A. cochlioides*, especially when the scabby symptoms are generally attributed to *Actinomycetes* (namely *Streptomyces*). However, it is worth noticing that symptoms are difficult to distinguish when both pathogens are present. This is important in the case of the qualification of the disease in field conditions.

Aphanomyces cochlioides on sugar beet in Central Europe

The last investigation on *A. cochlioides* in Poland was done by SZYMCZAK-NOWAK (1987) in the eighties of the twentieth century. At that time it was known in Poland only as a pathogen of seedlings and young plants (black root/black leg) but not as a pathogen of mature roots. The lack of investigations in the previous years is also visible in the references of the article of NECHWATAL *et al.* (2012). On the other hand, the root rot due to *A. cochlioides* on sugar beet is clearly known in the USA (HARVESON *et al.* 2002, 2009; HARVESON 2006) and in Great Britain as well as in Belgium (ASHER 1992; FRANCIS 2005), but it should be pointed out that the weather conditions in those countries are quite different from those in Poland and in Germany. It is possible that the climate conditions were favourable for better and faster development of the pathogen in Great Britain and Belgium and led to successive colonisation in the other European countries.

A. cochlioides occurrence in the field is distributed spatially (patch-like). Like in other soil-borne diseases, its control is difficult. Crop protection should usually be based on seed treatment with fungicides, use of cultivars with higher levels of resistance, management of soil moisture, long rotations with non-host crops and weed control (SZYMCZAK-NOWAK 1987; ASHER 1992; BEALE *et al.* 2002; PEARSSON & OLSSON 2005; CHOŁUJ & MOLISZEWSKA 2012; NECHWATAL *et al.* 2012).

Investigation of the sugar beet root rot

MOLISZEWSKA (2009) started detecting a causal agent of girth scab in Poland in greenhouse and field

experiments in 2003, which started from the investigation of seedling diseases. Sugar beet seedlings taken from two field locations were analysed in the laboratory. *A. cochlioides*, *Fusarium* spp., *R. solani* and other non-infectious species were found in necrotic spots. The frequency of *A. cochlioides* was 77.5–87.5% in the first location depending on the cultivar, and 0–25% in the second one. The frequency of *F. oxysporum* was 55.6–75%. As roots aged, symptoms observed in the field showed scab-like spots starting from the end of June up to harvest time. From this kind of symptoms on mature roots, *A. cochlioides* was occasionally isolated, while *Fusarium* spp. was predominant in them. The spots observed were belt-like, with the corky tissue on the surface; they were on ½ or on the whole girth of the root but no rot was observed. Summer 2003 was not rainy (sum of rainfall 421.2 mm), with an average temperature of 21–22°C (max. 34°C) (MOLISZEWSKA 2009). The next year, 2004, was not favourable for oomycete organisms either; the sum of rainfall was 504.6 mm, and maximum temperatures in June, July and August were 21.7–25.7°C (average 18–20°C). The spectrum of the main pathogens was changing according to the date of isolation. In May, the predominant pathogen was *A. cochlioides* in one experimental site (71% frequency) and *Rhizoctonia solani* in the second experimental site (58.4% frequency); however, in June and July, *A. cochlioides* was not detected. It was observed next time in August with only 17.6% frequency and in only one (the same as earlier) experimental site. Detection of *A. cochlioides* followed the soil moisture conditions and thus the pathogen's activity. Simultaneously, with classical isolation of fungi from scabby spots, the procedure for the isolation of *Actinomyces* was introduced (the method described by SCHAAD *et al.* 2001). This resulted in the collection of 151 *Actinomyces* isolates in 2003 and 49 in 2004. They were compared macroscopically with *Streptomyces scabies*, kindly obtained from the KWS laboratory, and divided into several groups according to morphological characters. Based on this division, the most frequently isolated strains were chosen for greenhouse pathogenicity tests in which two strains of *Streptomyces* were included together with *A. cochlioides*. The experiment showed that they can significantly promote disease severity if they were applied together with *A. cochlioides*, but *Streptomyces* alone without *A. cochlioides* could not develop any disease symptoms. As it was not sure that strains chosen for the test were pathogens, in the

next step a mixture of the most frequent *Streptomyces* isolates was prepared on the perlite soaked with the medium and used as an inoculum. These tests confirmed previous observations, i.e. *Streptomyces* could cause only slight 'scab' symptoms and they were observed only once in the variant with a high level of irrigation. After about six weeks, these symptoms were almost invisible (roots were bigger). The way of *Streptomyces* action was confirmed also in a laboratory experiment (for different from previous strains of *Actinomyces*). This experiment showed their inability to cause the disease and also their inhibitory properties against *S. scabies* (MOLISZEWSKA 2009, 2011). NECHWATAL *et al.* (2012) pointed out that all *S. stelliscabiei* strains which they obtained, lacked a pathogenicity factor responsible for virulence against plants. In the pathogenicity test with a *Streptomyces* strain they conducted, no damage to the root periderm was observed. They also made tests with isolates of *A. cochlioides*, *Pythium sylvaticum*, *P. recalcitrans*, and *P. salpingophorum*, which were used for soil infection under controlled greenhouse conditions, including two flooding events. They showed that plants infested with *A. cochlioides* displayed severe symptoms actually known as 'girth scab' on the roots. Diseased beet roots showed large, darkly discoloured necrotic patches and were usually fissured, cracked, and constricted in the middle part of the beet ('belt-like'). The inner tissues of beets were not affected by the rot; foliage also remained healthy. Leaf and root mass were significantly reduced as compared to the controls. In the case of experimental variants with *A. cochlioides* infection, the pathogen could be easily re-isolated from infected tissue samples, while in the *Pythium* spp. variants the pathogens were not recovered (NECHWATAL *et al.* 2012). The coexistence of *A. cochlioides* and *Pythium* spp. in the same niches was reported also by PAYNE *et al.* (1994) and MOLISZEWSKA (2008b, 2009). This observation was supported by FRÖHLICH and SMALLA (2014), because they did not find any *A. cochlioides* DNA in the total microbial community DNA obtained from girth scab lesions of sugar beet.

The experiments in the greenhouse with simulated irrigation were also included. In the greenhouse experiment soils from two fields were used. They were taken from fields on which severe symptoms of girth scab were noticed in 2001–2002. This showed that the oomycete pathogens were frequently isolated when the watering of soil in pots was applied. In this group, *Pythium ultimum*, *P. irregulare*, and

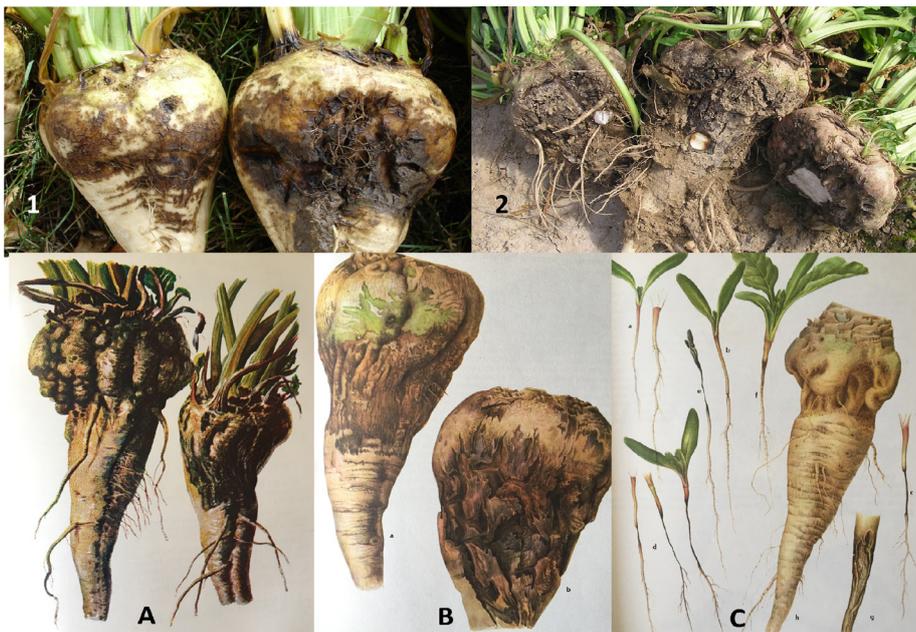


Figure 1. Symptoms of *Aphanomyces* root rot (1, 2) and of girth scab given by (A) BENADA *et al.* (1984), (B) by HINFNER and HOMONNAY (1966), and (C) symptoms of seedling damping-off given by BENADA *et al.* (1984) – all of them showing typical for *A. cochlioides* distortions

P. aphanidermatum as well as *A. cochlioides* were identified. In the next step, infectious tests with *A. cochlioides* were carried out in a greenhouse. The results produced symptoms comparable with those given by NECHWATAL *et al.* (2012). Pathogen re-isolation allowed detecting *A. cochlioides* in 62% and 71% of diseased plants obtained from coated and non-coated seeds, respectively, after having infected them on the sowing day. Another experimental combination, consisting in infecting the plants with *A. cochlioides* four weeks after sowing, gave 78% and 85% recovery of the pathogen, depending on the irrigation level. The symptoms were comparable with those known for girth scab from phytopathological descriptions (Figure 1A–C) (HINFNER & HOMONNAY 1966; BENADA *et al.* 1984; LIZ 2005).

The greenhouse and field experiments completed in 2003–2004 resulted in prospects which might help to resolve the problem of ‘girth scab’, but they were not undisputable, so experiments continued in the field throughout 2005–2006. The year 2005 was rather hot and dry with maximum temperatures of 29–36°C and 32.2–61.7 mm of rainfall in the period June–August, so interesting experimental data was obtained only on the irrigated part of the field. Symptoms observed during that year on irrigated plots were as those known from old literature as ‘girth scabs’ with cracks and rot in the inner tissues. The colour of rotten tissues was light brown for small spots localized on the surface to dark brown for deeper ones. The upper part of the root showed folds and distortions with cicatrices composed of

corky tissue. Water-soaked rot spots were possible (Figure 1-1, 1-2). This kind of symptom description for *A. cochlioides* infection on sugar beet roots was also given by ASHER (1992), WINDELS and LAMEY (1998), WINDELS (2000), FRANCIS (2003), and HARVESON *et al.* (2009). *A. cochlioides* was rarely isolated from tissues by conventional phytopathological methods (2.7–7%), but the detection of this pathogen by methods used by WINDELS (2000) enabled its identification in 62–75% of diseased tissues. Isolation on agar plates revealed *Pythium ultimum* (28.6%) and a white coenocyte thallus with no fructification structures (14.3%) in diseased tissues analysed in October. This suggested that organisms other than *A. cochlioides* are possible as causal agents of symptoms observed on diseased sugar beet roots. The observations were confirmed in the following year’s experiment. This experiment was conducted in the same field location, so the field inoculum of pathogens was cumulated. *A. cochlioides* was detected throughout the whole summer: 27.5–46.7% in June–July and 44.7% (not irrigated) to 73.7% (irrigated) in August. *P. ultimum* was denoted in June (37.5%) and in July (3.3%), but simultaneously a coenocyte thallus was present in 30% of the analysed tissues in June and 6.7% in July. The weather conditions for oomycete pathogens were most favourable in that year. *A. cochlioides* could develop easily in the non-irrigated part of the experiment due to the high amount of rainfall in May (57.1 mm) and July (71.4 mm) 2006 and due to the pathogen accumulation throughout the previous year (MOLISZEWSKA 2009). Sugar beet cultivars

showed different susceptibility to the damage caused by *A. cochlioides* (MOLISZEWSKA 2009). CHOŁUJ and MOLISZEWSKA (2012) proved that the severity of the disease increases in conditions favourable for the pathogen (hot and wet conditions, the experiment was realised in a greenhouse). The disease develops if the pathogen inoculum is at an appropriate level in a period of about one month after sowing, even if the seed protection was applied. Pathogens can affect the photosynthetic apparatus even if the disease symptoms are not visible; and in mature leaves, *A. cochlioides* causes chlorophyll degradation (CHOŁUJ & MOLISZEWSKA 2012).

Distinguishing the symptoms

The symptoms of girth scab are stripes of corky tissue around the root surface: the stripes are light brown to brown, sometimes with callus protuberances (Figure 2). Small to big, round or irregular spots made of corky tissue are also possible (Figure 2B) (MOLISZEWSKA 2009). In the case of *A. cochlioides* infection, light brown to dark brown rot spots are observed on some parts of the root tissues, instead of this sometimes a thick layer of cork develops. In some cases, only folds without rotten parts can be observed on the root surface (Figure 1-1, 1-2). If the weather is not favourable for *A. cochlioides*, only scars on mature roots can be observed as traces of its

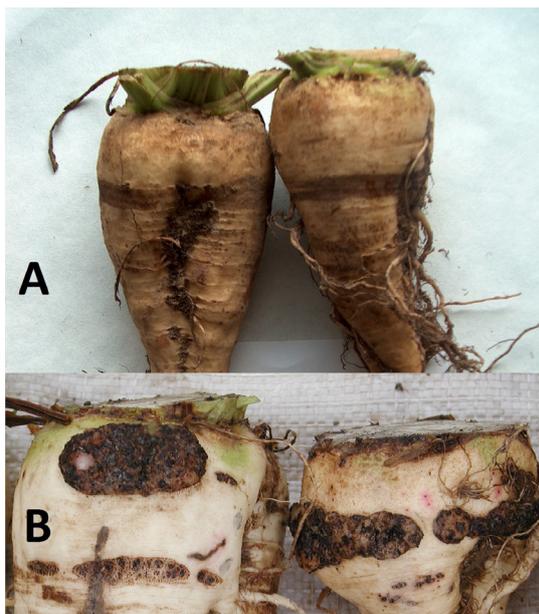


Figure 2. Symptoms of girth scab: (A) flattened form and (B) protuberant form

prior activity symptoms (Figure 1C). The pathogen is not uniformly distributed in rotten tissues and usually it is not easy to isolate it by conventional phytopathological methods. The best way to detect it is the method described by WINDELS and LAMEY (1998) and WINDELS (2000) or molecular methods of identification by the PCR protocol could be applied (HOVI & NIHLGARD 2005). The pathogen is more easily isolated and detected from seedlings or young plants during spring than from mature roots. Probably, because of these problems with isolation from diseased tissues of *A. cochlioides*, the first descriptions of 'girth scab' included only *Streptomyces* spp. as causative agents of the disease. For the same reason *A. cochlioides* was known in Poland for a long time only as a pathogen of seedlings and young plants (black root/black leg) (SZYM CZAK-NOWAK 1987). The same can be said about pathogens of the genus *Pythium*, because their developmental demands are the same as those of *A. cochlioides*. This was shown by MOLISZEWSKA (2009) in a monograph based on long-term investigations including a 3-year test under conditions provocative for *A. cochlioides* and by NECHWATAL *et al.* (2012). NECHWATAL *et al.* (2012) clearly showed that in symptoms actually recognised on sugar beets as 'girth scab', oomycete (*A. cochlioides* and *Pythium* sp.) organisms were involved. According to these findings we cannot call the disease caused by *A. cochlioides* as 'girth scab'. The problem is that in Europe the 'girth scab' disease is still illustrated by *Aphanomyces* root rot symptoms (Figure 1).

Until recently, both types of symptoms described above were known in the phytopathological literature only as 'girth scab' (Figure 1) (HINFNER & HOMONNAY 1966; BENADA *et al.* 1984; LIZ 2005). Additionally MOLISZEWSKA (2009) pointed out that the rotting tissue was never observed in the case of evident scab symptoms, even when roots with such scabby spots were stored in a refrigerator or at room temperature for three months.

The results of the field experiments completed by MOLISZEWSKA (2009) also showed that weak injuries on the sugar beet root surface, just as those indicated as scab or delicate 'girth scab', can be conducive to an increase in sugar content in roots (MOLISZEWSKA 2009).

The distribution of *A. cochlioides* in soils in Poland has recently been unknown. As it was mentioned above, the most complete investigation in the theme was done by SZYM CZAK-NOWAK (1987) in the 80s of the twentieth century. Now we can only predict

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that its occurrence coincides with the sugar beet fields, those of contemporary and past time. The observations from the sugar beet fields show that *A. cochlioides* can survive in the field soil even fifteen years without sugar beet cultivation (MOLISZEWSKA, personal observation, data not published).

CONCLUSION

According to the described findings, the proposed conclusion is therefore to make a new description of ‘girth scab’ disease of sugar beets caused by *Streptomyces* (Figure 2). Another conclusion is to rename the disease described actually in manuals as ‘girth scab’ and use the name ‘scab’ for symptoms caused by *Streptomyces* spp. In fact, descriptions and illustrations from the phytopathological manuals of sugar beet diseases present symptoms characteristic of ‘*Aphanomyces* root rot’ as ‘girth scab’ ones (see Figure 1). Additionally, there is a need to describe the ‘*Aphanomyces* root rot’ disease related to root rot caused by *A. cochlioides*. Typical symptoms of the disease caused by *A. cochlioides* occur mainly on the upper part of the root when they are due to infections on hypocotyls of younger plants, but can occur on the lower part of the root when infections occur once roots are already larger, depending on the soil moisture. The root surface becomes distorted, cracked, and constricted below the crown. Light-brown or brown to black water-soaked lesions can occur. In severe cases, the whole lower part of the root is damaged and rot is developed (Figure 1). The disease is enhanced by rainy (approximately 500–600 mm of rainfall in the period of June–August) and hot weather (close to 30°C), and then it can develop during summer until harvest (ASHER 1992; WINDELS & LAMEY 1998; WINDELS 2000; HARVESON *et al.* 2002; FRANCIS 2003; MOLISZEWSKA 2009). The ‘girth scab’ disease or ‘scab’ disease should be identified only as consisting of typical surface lesions with stripes of corky tissue which can cover the whole root or only a part of the root girth (Figure 2). It should be pointed out that typical girth scab symptoms can be promoted by *A. cochlioides* infection. Protection against girth scab caused by *Actinomycetes* should include soil management, proper crop rotation with avoiding potatoes as a pre-crop. On this background protection against *A. cochlioides* is difficult and should be based on seed treatment with fungicides, resistance to *A. cochlioides*, management of soil

moisture, long crop rotations with non-host crops and control of weeds.

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Corresponding author:

EWA MOLISZEWSKA, Opole University, Department of Natural Sciences and Technology, ul. Kominka 6A, 45-032 Opole, Poland; e-mail: ewamoli@uni.opole.pl
